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The various subjects covered in this report are listed in the Table of Contents. Abstracts of the individual investigators are included on the DD Form 1498 introducing each work unit report.

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FOREWORD

IN CONDUCTING THE RESEARCH DESCRIBED IN THIS REPORT, THE INVESTIGATORS ADHERED TO THE "GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS" AS PREPARED BY THE COMMITTEE ON CARE AND USE OF LABORATORY ANIMALS OF THE INSTITUTE OF LABORATORY ANIMAL RESOURCES, NATIONAL RESEARCH COUNCIL.

SUMMARY

THE VARIOUS SUBJECTS COVERED IN THIS REPORT ARE LISTED IN THE TABLE OF CONTENTS. ABSTRACTS OF THE INDIVIDUAL INVESTIGATIONS ARE INCLUDED ON THE DD FORM 1498 INTRODUCING EACH WORK UNIT REPORT, AND NAMES OF THE INVESTIGATORS ARE GIVEN AT THE BEGINNING OF EACH REPORT.

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IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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23. (U) The objective of this work unit is to elucidate the mechanisms of protective immunity to leishmania. Leishmaniasis is endemic in Africa, the Mid East and Indian Subcontinent and South America, posing a significant potential threat to military operations in these areas.

24. (U) The approach is to cultivate human monocytes in vitro, infect them with Leishmania, examine the processes of entry into the cell and intracellular replication, and examine the effect of immunomodulatory agents on these processes.

25. (U) 82 10 - 83 09 Human monocytes have been infected with Leishmania tropica and donovani. Replication of parasites in monocytes has been documented. Studies with high-titer L. donovani-immune serum indicated that antibody does not facilitate entry of parasites into cells, and does not inhibit intracellular replication. L. tropica parasites exposed to fresh normal human serum, however, were rapidly killed. Such complement-killed organisms did not enter human monocytes. Thus, entry of Leishmania into their human host cell largely depends on active participation by the parasite. Further studies are in progress to clarify the mechanisms of parasite participation in the entry process. Understanding of such mechanisms may permit therapeutic intervention aimed at disrupting the interactions of monocytes with extracellular parasites, thereby preventing spread of organisms from one cell to another. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 1982 - 30 Sep 1983.

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PROJECT 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 095 Mechanisms of Human Mononuclear Cells for Killing of Intracellular Parasites

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Problems and Objectives:

Leishmania species cause skin and visceral disease throughout the tropics. No effective strategy has been developed to prevent acquisition of disease by troops deployed in endemic areas. In animal models, cellular immunity is believed to control established disease. Murine macrophage activated by product of stimulated lymphocytes in vitro, for example are resistant to infection by Leishmania and destroy those parasites that do enter the host cell. If similar processes govern human macrophage-parasite interactions, enhancement of human host defenses by immunomodulating agents such as gamma interferon might be possible. Construction of an effective immuno-therapeutic strategy requires the development of a system to allow analysis of the interplay between immuno-stimulated human monocytes macrophages and parasites. The following problems must be addressed in the design of such a system: (1) What factors regulate parasite entry into the host cell? (2) Can human monocytes support intracellular replication of the parasite in vitro? (3) If so, can monocytes respond to immune mediators, especially interferons, to inhibit such replication and kill intracellular parasites? Evidence from the murine system indicates that immature mononuclear phagocytes are readily infected with Leishmania and support its replication, but respond poorly to immune mediators to kill it. Mature macrophages, however are responsive. (4) If human monocytes also prove to be relatively refractory to activation by immunomodulators to kill intracellular parasite, can such refractoriness be overcome by providing additional priming signals or by targeting mediators to the cell (e.g., by encapsulation in liposomes or by attachment of mediators to monocyte-specific antibody)?

Progress:

We have separated monocytes from normal human blood and have harvested peritoneal macrophages from healthy women undergoing tubal ligation. We have established conditions for the maintenance of these cells *in vitro*, and have exposed them to *Leishmania* amastigotes. Amastigotes of both *L. tropica* and *L. donovani* infect freshly harvested human monocytes and peritoneal macrophages and survive for at least 72 hr in *in vitro* culture. Treatment of *L. donovani* amastigotes with heat-inactivated high-titer *L. donovani* immune serum did not alter entry of parasites into human monocytes; % infected monocytes and the number of amastigotes per 100 macrophages were unchanged following immune serum treatment of amastigotes. When amastigotes were treated with fresh serum alone (*L. tropica*) or fresh serum plus immune serum (*L. donovani*), parasites failed to enter monocytes. The reduced infectivity of amastigotes for their host cell was related to reduction in parasite viability effected by human serum complement. These data suggest that the entry of amastigotes into human monocytes is a dynamic process that requires active participation of the parasite.

Following ingestion by monocytes, *L. tropica* replicated, with a least 2-fold increase in parasite numbes in 72 hrs. Human cells did not kill *L. tropica* after treatment with supernatants of mitogen-stimulated leukocyte cultures or with gamma interferon. The cells did, however, kill extracellular tumor cell targets. These studies suggest that, although competent to express one effector function (tumor cell killing) human monocytes do not kill intracellular *L. tropica* after treatment with this particular lymphokine signal. This selective expression of one effector function rather than another may be a consequence of the immaturity of the blood monocyte.

We have also initiated studies of lymphokine delivery to macrophages via encapsulation in liposomes. Preliminary work indicates that liposomes inhibit the induction by lymphokines of intracellular microbicidal activity, possibly by interfering with an early priming step. This successful modulation of the activation sequence holds great promise for further studies aimed at enhancing the ability of lymphokines to induce microbicidal effector activity.

Recommendations:

We will investigate the following problems in activation of human macrophages and monocytes to kill Leishmania: (1) Do human monocytes fail to develop anti-leishmanial microbicidal activity after lymphokine treatment because they do not recognize signals mediating that activity? (2) Can the application of different signals or alterations in the timing or mechanism of delivery of signals (e.g., by liposome encapsulation) enhance the responsiveness of monocytes? (3) Can more mature or more differentiated human macrophages kill Leishmania after lymphokine treatment? (4) Do humoral factors (antibody and complement) enhance lymphokine-induced microbicidal activity?

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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	96 WWGO
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Immunochemistry of Non-toxic O-specific Polysaccharide Antigens

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	2.0	50
FISCAL YEAR CURRENT 84	2.0	55

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

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Div. of CD&I

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Formal, S B

TELEPHONE: (202) 576-3344

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Seid, R

NAME: Schneider, H POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Enteric diseases; (U) O-specific Polysaccharide; (U) Lipopolysaccharide; (U) Recombinant DNA

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) Development of non-toxic O-specific polysaccharide (O-Ps) antigens as immunizing agents against infections of military importance.

24. (U) O-Ps antigens will be isolated from mutants lacking genes for complete lipopolysaccharide (LPS) synthesis or from recombinants infected with plasmids encoding for O-Ps synthesis. For the latter, endonuclease-digested DNA encoding O-Ps from appropriate pathogenic strains will be cloned into Escherichia coli using genetic vectors. Recombinants will be detected by replica blot immunoassays. The O-Ps will be extracted with hot phenol water and purified by biochemical techniques. The structures of these antigens will be elucidated by nuclear resonance and other analytical methods. The toxicity will be measured by the Limulus lysate assay. Non-toxic antigens will be tested for immunogenicity in laboratory animals.

25. (U) 82 10 - 83 09 A Shigella O-Ps, extracted from the cell walls of a Salmonella typhi-Shigella transconjugant carrying a S. sonnei plasmid, was purified to homogeneity by biochemical techniques. It did not contain core-lipid A and was non-toxic in the Limulus assay. By NMR and chemical analyses, this Shigella O-Ps was shown to consist of a repeating disaccharide unit of 2-acetamido-4-amino-2,4-trideoxygalactose and 2-acetamido-2-deoxyaltruronic acid. During gel electrophoresis, it migrated slower than parental S. sonnei LPS and had an estimated molecular weight between 14-20 Kdal. A replica blot radioimmunoassay was developed to detect E. coli recombinants that have inherited endonuclease-digested DNA encoding Shigella O-Ps. (For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sept 83).

*Available to contractors upon originator's approval

DD, FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 96: Immunochemistry of Nontoxic O-specific Polysaccharide Antigens

Investigators:

Principals: Samuel B. Formal, Ph.D.
Robert C. Seid, Jr., Ph.D.
Herman Schneider, Ph.D.

Associates: Dennis J. Kopecko, Ph.D.
Louis S. Baron, Ph.D.
Thomas Larry Hale, Ph.D.
COL Jerald C. Sadoff, M.D.

Objectives

The objectives are (1) to isolate and to purify lipid A free, O-specified polysaccharide (O-Ps) antigens from hybrid enteric organisms, (2) to compare their physicochemical properties with parental lipopolysaccharide (LPS) antigens, and (3) to determine their immunogenicity in laboratory animals. Salmonella typhi strain 5076-1C, a potential live, oral vaccine for protection against typhoid fever and shigellosis due to Shigella sonnei, serologically expresses the Shigella sonnei form I antigen and normal Salmonella typhi somatic antigens. To assess the cell surface architecture of the major protective antigens of this genetically-derived strain (Formal, S.B., et al (1981) Infect. Immun. 34, 746-750), polysaccharide components were purifed and chemically analyzed.

Progress

The Salmonella typhi 5076-1C cells, which cannot produce complete (i.e., smooth) lipopolysaccharide (LPS) without added galactose due to a galE mutation, were grown with galactose (+gal) and without (-gal) and subsequently treated with hot phenol water to release the somatic antigens. Ultracentrifugation of the aqueous layer from (+gal) cell resulted in a pellet of complete LPS having the classical core-linked S. typhi O-antigen but no core-linked form I antigen; the pellet from (-gal) cells consisted of incomplete (i.e., rough) S. typhi LPS. Regardless of cell growth conditions, the form I antigen did not sediment during ultracentrifugation but was recovered from the supernatant by alcohol precipitation

and purified by DEAE ion exchange chromatography. Unlinked form I and S. typhi O-polysaccharide antigens, both present in the (+g) supernatant, were further separated by gel filtration. Chemical analysis revealed the form I antigen to be a polymer (M_r, 14-20 Kdal) having O-dissaccharide repeating units comprised of 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose and 2-acetamido-2-deoxy-L-altruronic acid. Unlike parental S. sonnei form I LPS, the 5076-1C derived form I antigen lacked core-lipid A, displayed low Limulus amoebocyte lyste gelatin activity, had low phosphorus content, and migrated with lower mobility on SDS gels. These data indicate that in strain 5076-1C the form I antigen is noncovalently bound to core-lipid A, but exists as a polymerized cell surface entity. Moreover, in contrast to the current theory of LPS assembly in Enterobacteriacease, the form I antigen of strain 5076-1C does not require translocation to core-lipid A at the cytoplasmic membrane for transport to the outer membrane layer.

Future Plans

This project needs to be continued in the following areas: (1) to compare the immunogenicity of the transconjugant derived "nontoxic" Shigella O-Ps with that of native Shigella LPS, (2) to develop methods to enhance the immunogenicity of nontoxic O-Ps, and (3) to study O-Ps production in E. coli-Shigella recombinants carrying endonuclease-digested DNA encoding for Shigella O-Ps synthesis.

Bibliography

1. Seid, R.C., Formal, S.B., Schneider, H., and Sadoff, J.C. (1983) "Elaboration of shigella polysaccharide Antigen by Salmonella-shigella recombinants" Federation Proceedings, Vol 34, Abstract No. 2377, page 2164.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300521	83 10 01	DD-DH&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. LIBR'N MISTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. CHANGE	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	97 WWGU
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Immunochemistry on Binding Domain Peptides of Bacterial Pili

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
82 10	CONT	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 83	2.0	35
CURRENT FISCAL YEAR 84	2.0	40

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Formal, S B

TELEPHONE: (202) 576-3344

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Seid, R

NAME: Chung, R

21. GENERAL USE

Foreign Intelligence Considered

DOC: DA

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Immunochemistry; (U) Pilus protein; (U) Peptide Sequencing; (U) Synthetic Vaccine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code)

23. (U) Development of peptides encompassing the receptor binding domains of Escherichia coli (EC) and Gonococcal (GC) pili as immunizing agents against diarrheal and gonococcal infections. These two diseases are major health problems to military personnel.

24. (U) Pili will be enzymatically and chemically cleaved to yield peptides, which will be isolated and characterized by biochemical techniques and tested for binding activity by immunological assays. Chemical and genetic sequencing techniques will be utilized to delineate the primary structure of peptide(s) encompassing the common domain. Cloning of the appropriate gene fragments into other bacterial systems will be attempted to generate a large quantity of GC pili as well as to prepare synthetic vaccines. To enhance immunogenicity of peptides, covalent coupling to adjuvant molecules will be developed.

25. (U) 82 10 - 83 09 GC pilus has been cleaved with CnBr into three fragments that were resolved by gel filtration. The largest fragment reacted with anti-pilus antibody. Pilus digested with trypsin, yielded about 25 peptides which were grouped into 3 categories: polar, neutral, and hydrophobic by liquid chromatography. All three groups reacted with antipili antisera. A GC DNA fragment encoding pilus synthesis has been packaged by cosmid vectors into E. coli. A replica blot immunoassay was developed to detect recombinants expressing GC pili. Gel electrophoresis of EC pili, obtained from the Department of Gastroenterology, revealed two protein bands of 17.8 and 15.5 Kdal molecular weights (MW), respectively. The lower MW protein was purified by gel permeation in 30 percent acetic acid and was still antigenic as evidenced by Western blot using naturally immune human sera. The 17.8 Kdal MW protein remains to be purified. (For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sept 83).

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 97: Immunochemistry on Binding Domain Peptides of Bacterial Pili

Principals: Samuel B. Formal, Ph.D.
Robert C. Seid, Jr., Ph.D.
MAJ Raymond C.Y. Chung, M.D.
Dennis J. Kopecko, Ph.D.

Associates: COL Edmund C. Tramont, M.D.
MAJ John Boslego, M.D.
Herman Schneider, Ph.D.
COL Jerald C. Sadoff, M.D.
LTC Edward Boedecker, M.D.
LTC Robert Reid, M.D.
Charles A. Hooper, B.S.

Objectives

The objectives of this long term work project are (1) to delineate the "common" binding peptide domains of pilus proteins among antigenically diverse strains of Neisseria gonorrhoeae (GC) and Escherichia coli (EC) and (2) to develop these peptides as synthetic vaccines, either by themselves, or after conjugation to adjuvant carriers.

Progress

(1) GC pilus has been cleaved with CnBr into three fragments that were resolved on a gel column in 30% acetic acid. The largest of the CnBr fragments reacted with anti-pilus antibody. Pilus, digested with trypsin, yielded about 25 peptides, as evidenced by liquid chromatography. These peptides have been grouped into 3 categories: polar, neutral, and hydrophobic. All three groups reacted with antipilus polyclonal antiserum.

(2) A GC DNA fragment encoding pilus synthesis has been packaged by cosmid vector into E. coli. Recombinants expressing GC pilus protein have been detected by an in situ replica blot immunoassay using a monoclonal antibody as a probe.

(3) A CFA II protein preparation was subjected to physicochemical analyses including amino acid determination, N-terminal analysis, and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Acid hydrolyzed CFA II protein gave no histidine, cysteine, or methionine. The absence of methionine precludes the use of cyanogen

bromide cleavage. Edman degradation released two prominent N-terminal residues, alanine and valine, suggesting that this CFA II preparation contained two polypeptides. Further evidence for the presence of two polypeptides was obtained by SDS-PAGE. Two protein bands of 17.8 and 15.5 Kdal molecular weights (MW), on SDS-gel were revealed by staining with Coomassie blue dye. The lower MW protein was purified by gel permeation on Sephadex G-100 column equilibrated in 20% acetic acid. This 15.5 Kdal MW protein, eluting behind the void volume, retain antigenicity as evidenced by Western blot using naturally immune human sera. Tne 17.8 Kdal MW protein remains to be purified.

(4) A rapid microanalytical technique of the Dorman chloride determination was developed to monitor both t-BOC deprotection and amino acid coupling reaction during solid phase peptide synthesis. The procedures include (a) an altenate preparation of pyridium hydrocholride salt of the peptide, (b) use of chloridometer to titrate the amount of chloride ions released, (c) the weighing of 2 to 3 mg of dried substituted resin, and a single tube preparation of the resin for chloride extraction. This analytical technique has been employed during the solid-phase synthesis of an octapeptide tumor surface antigen and will be utilized in the future for preparing synthetic peptides of EC and GC pili.

Future Plans

The funding ($35K) allocated to this long term project was used to purchase a solid phase sequenator. The installation date has been tentatively set around October, 1983. This instrument will be used to determine the amino acid sequence of both the enzymatic and chemical-cleaved peptides of GC pilus and the *E. coli* CFA II protein. Future work will also involve nucleotide sequencing of the DNA fragment encoding GC pilus synthesis to define the common peptide region, further biochemical characterization of EC CFA II protein preparation and development of chemical methods to link peptide fragments to carriers to enhance immunogenicity.

Bibliography

1. Kopecko, D.J.. Schneider, H., Seid, R., Zollinger, W., and Wohlieter, J.A. (1983) "Cosmid Cloning and Expression in *Escherichia coli* of *Neisseria gonorrhea* Pili Genes", 83rd Annual Meeting of the Amer. Soc. Microbiol., Abstract D°, page 60.

2. Hooper, C.A., Reid, R.H., Seid, R.C., and Marnane, W.G. (1983) "Rapid Microanalytical Chloride Monitoring and Amino Acid Analysis During the Solid-Phase Synthesis of an Octapeptide Tumor Surface Antigen", Federation Proceedings 34, Abstract 39, page 1763.

3. Hooper, C.A., Reid, R.H., and Seid, R.C. (1983) "Synthesis and Conformatinal Analysis of an Octapeptde Tumor Surface Antigenic Site Common to Human Ductal Carcinoma (Breast) Cells", 8th Amer. Peptide Symposium, Abstract 1-24, page 31.

4. Hooper, C.A., Reid, R.H., Marnane, W.G., and Seid, R.C. (1983) "Monitoring Solid-Phase Peptide Synthesis Deprotection and Amino Acid Coupling Efficiency by a Rapid Microanalytical Chloride Determination", 8th Amer. Peptide Symposium, Abstract 8-13, page 199.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300[illegible]	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPN INSTR'N	9. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	98 WWJJ
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Dynamic Regulation of Neurotransmitter Systems

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 012900 Physiology Neuroanatomy [illegible] Psychology [illegible] Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATE/EFFECTIVE: EXPIRATION
b. NUMBER:
c. TYPE d. AMOUNT
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	PREVIOUS FY	A. PROFESSIONAL MAN YRS	B. FUNDS (In thousands)
FISCAL YEAR	83	1.0	60
	CURRENT FY 84	2.0	80

19. RESPONSIBLE PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
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20. PERFORMING ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER

RESPONSIBLE INDIVIDUAL
NAME: Russell, P K
TELEPHONE: (202) 576-3551

21. GENERAL USE
Foreign Intelligence Considered

ASSOCIATE INVESTIGATORS
NAME: Holaday, J W
NAME: Tortella, F C

FOC: DA

22. KEYWORDS (Precede each with Security Classification Code):
(U) Nervous System; (U) Receptor; (U) Autoradiography; (U) Immunocytochemistry

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide brief explanatory paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Although the existence of discrete cholinergic neurotransmitter systems is well known, still to be accomplished is a careful synthesis of physiological, biochemical and morphological data which would indicate how these systems function normally, during development, in aged animals and in response to chemical or electrolytic lesions. By employing sophisticated biochemical and morphological methodologies, we will investigate the baseline parameters for the cholinergic system and characterize the dynamic alterations which attend its operation under a variety of conditions. The effect of nerve growth factor and other neuropeptides on several parameters of cholinergic function will be tested in normal adults and under the conditions to be described. There is military relevance in this research.

24. (U) In the first series of experiments neonatal, adult and aged rats will receive intracerebroventricular injections of nerve growth factor (NGF). This protein will be prepared from the submaxillary glands of adult male mice. Injections will follow surgical preparation of animals by the aseptic placement of intracranial burr holes. Following injection, animals will be sacrificed at intervals for determination of choline acetyltransferase (ChAT) activity, high affinity acetylcholine uptake, autoradiographic confirmation of acetylcholine receptors and immunocytochemical staining for ChAT. In subsequent experiments, these same measures will be determined after exposure of normal rats to acetylcholinesterase inhibitors and electrolytic lesions of cholinergic pathways.

25. (U) 82 10 - 83 09 NOTE PRIOR TITLE: DYNAMIC REGULATION OF OPIOID NEUROTRANSMITTER SYSTEMS. NGF was shown in neonatal animals to markedly enhance the activity of the cholinergic neurotransmitter enzyme ChAT. This change occurred in stepwise fashion and was first apparent 4 days after the first injection. The cholinergic neuronal groups which participated in the ChAT increase included all those of the basal forebrain as well as the caudate. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 67 AND 1498-1, 1 MAR 68 FROM ARMY USE ARE OBSOLETE.

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 98 Dynamic Regulation of Neurotransmitter Systems

INVESTIGATORS:

Principal:	Mobley, W.C., MAJ, MC
Associates:	Holaday, J.W., GM-15 and Tortella, F.C., GS-12
Assistants:	Rogers, Otis R., SP6, Robles, Lydia, SP6, and Cardenales, Lysaida, SP5

Problem:

The following problems are being investigated:

(1) What is the normal anatomy of the cholinergic cells of the basal forebrain? The studies are aimed at characterizing these cells and their fibers at the light and ultrastructural levels.

(2) What biochemical parameters characterize these cells? The studies include determination of the specific neurotransmitter enzyme choline acetyltransferase (ChAT), high affinity choline uptake, and acetylcholine receptor analysis.

(3) What anatomical and biochemical features describe this system during normal development and normal aging?

(4) What changes result from administration of a potential trophic factor, nerve growth factor (NGF)? What is the role of other neuropeptides in these changes?

(5) What changes occur after intoxication with anticholinesterase agents and electrolytic lesions, and do such treatments alter the response of cholinergic neurons to NGF? This work may indicate a role for NGF in the treatment of cholinergic injuries, including those of military personnel exposed to acetylcholinesterase inhibitors.

Importance:

A variety of animal and human studies indicate a key role for the cholinergic neurotransmitter system in learning and memory. Essential to an understanding of the system is a full description of its anatomical and biochemical characteristics. The observations to be made will provide important insights into the functioning of these neurons and may provide clues as to how to enhance their function in normal as well as in lesioned or intoxicated subjects. Of special military relevance is the involvement of the cholinergic neurotransmitter system in battlefield exposures to toxic chemical agents.

Approaches:

(1) Anatomical studies will employ immunocytochemical techniques with a monoclonal antibody directed against ChAT. Cholinergic cell bodies and their fibers will be mapped.

(2) Enzyme levels of ChAT and acetylcholinesterase will be determined in the basal forebrain and in cortical regions. Standard procedures will be used to measure choline uptake. Acetylcholine receptor density will be determined autoradiographically.

(3) NGF will be prepared from mouse submaxillary glands. The protein will be injected intracerebroventricularly and, in subsequent experiments, directly into specific nuclear groupings. Iodinated NGF will be prepared, and determinations will be made of NGF receptors and of specific NGF retrograde flow.

(4) Acetylcholinesterase inhibitors will be administered intravenously. Electrolytic lesions will be placed stereotaxically.

Results:

(1) Immunocytochemical studies with monoclonal anti-ChAT antibody have revealed quite specific nuclear groupings of cholinergic cells. In agreement with other investigators, we have described four major groupings in the basal forebrain of the rat. These are the medial septal nucleus, the vertical and horizontal limbs of the diagonal band of Broca and the nucleus basalis of Meynert. Anatomical studies of neonatal and aged animals are in progress.

(2) Neonates receiving an intracerebroventricular injection of NGF demonstrate dramatic increases of ChAT activity in basal forebrain nuclei. These increases are specific to ChAT, and occur in stepwise fashion with increases first noted 4 days after the first injection. Other biochemical and anatomical studies of this NGF effect are in progress. The response to NGF of normal and lesioned adult animals will be evaluated.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 300525	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	99 WWN6
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Pathogenesis of Campylobacter jejuni in Laboratory Animals

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology 005900 Environmental Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
82 10	CONT	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	1.0	30
	CURRENT 84	1.0	20

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)
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TELEPHONE: (202) 576-3019/2071
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Brendle, J J Sims, R E
NAME: Grate, S J POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Campylobacter fetus subsp jejuni; (U) Pathogenesis; (U) Antibiotic Resistance; (U) Plasmids; (U) Monoclonal Antibody

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The technical objectives of this work unit are to determine whether passage of Campylobacter jejuni isolates can be made under experimental conditions and if passage is successful, to determine any pathogenesis in the animal model obtained. Isolates from humans, bush dogs, maned wolves, aotus, baboons, and domestic dogs will be investigated. C. jejuni occurs in a large number of species from all areas of the world and has been implicated in disabling gastroenteritis which has been noted as an obstacle to fielding of the rapid deployment force.

24. (U) Isolates will be characterized by antibiotic sensitivity, plasmid presence, and serology prior to infecting laboratory animals. Infectivity and pathology will be correlated with said characteristics. Monoclonal antibodies will be developed against cell wall portions to aid in understanding the bimorphism (spiral and cocci) of the organism at different stages of the growth cycle and as a means of determining significant antigenic portions of the cell surface.

25. (U) 8210-8309 Over 120 isolates of C. jejuni have been identified. Plasmid extractions have been done on over 100 of these isolates, finding close correlation between the low molecular weight plasmids and sulfonamid resistance in porcine isolates. Endotoxin studies using primary quail cells and newborn mice have been done on several isolates with negative results in newborn mice and minor cpe in quail cells. Colonization of C. jejuni in nude mice, coturnix quail and dwarf rabbits was successful. For technical report see WRAIR Annual Progress Report, 1 Oct 82 - 30 Sep 83.

Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 99 Pathogenesis of *Campylobacter jejuni* in Laboratory Animals

INVESTIGATORS:

Principal: Curtis R. Bartz, MAJ, VC
Associates: James J. Brendle, DAC; Robert E. Sims, DAC; Stephen J. Grate, CPT(P), VC
Assistants: SP5 Germaine Flores; PFC Naomi Capozza

OBJECTIVES:

The objectives of this work unit were to:

a. Collect fundamental data on *Campylobacter jejuni*
b. Determine the suitability of different animals as models of Campylobacteriosis in humans

IMPORTANCE:

Basic information is required on animal reservoirs, antibiotic resistance patterns and plasmid content of *C. jejuni* for empirical treatment regimes and prophylaxis. An animal model of disease is required for study of the pathogenesis of *C. jejuni* enteritis, of which little is known.

ACCOMPLISHMENTS:

Over 120 isolates of *C. jejuni* have been collected from laboratory animals at WRAIR, captive nondomestic animals at the National Zoo, and human patients at the Bethesda Naval Hospital. Plasmid extractions have been completed on over 100 of these isolates, finding close correlation between the low molecular weight plasmids and sulfonamid resistance in porcine isolates. Procedures to cure isolates of specific plasmids to correlate with tetracycline resistance have been begun. Tests to identify endotoxin production by *C. jejuni* have been done using newborn mice for in vivo studies and primary embryonic quail cells for in vitro studies. Pathology has been found in the newborn mice but the specific assay being used for fluid accumulation in the gut has been negative. Colonization of *C. jejuni* in nude mice, coturnix quail, and dwarf rabbits was successful.

INTERPRETATION:

Over 85% of *C. jejuni* isolates were found in conjunction with other enteric pathogens or stress. Neither the antibiotic resistance patterns nor the plasmid profiles could be used to identify the animal species from which the isolate was obtained. Bacterial colonization in coturnix quail, nude mice and dwarf rabbits may make these animals useful in studying the pathogenesis of disease caused by *C. jejuni*.

FUTURE:

The work initiated will continue under this work unit. Clinical isolates will be characterized by antibiotic resistance and plasmid profile. Plasmids will be cured from isolates obtained, allowing correlation with specific characteristics, i.e., antibiotic resistance and pathogenicity. Additional laboratory animal species and in vitro assays will be investigated for usefulness as an assay in evaluating individual C. jejuni isolates. The nude mice, dwarf rabbits, and coturnix quail will be further investigated as models to study the pathogenesis of this disease.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	100 EWP8
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Oral Vaccination of Peyer's Patches and Mucosal Surfaces

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 10	CONT	DA	C. In-house

17. CONTRACT GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	1.0	87
	CURRENT 84	1.0	90

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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NAME: Russell, P K

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20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Pathology

ADDRESS: Washington, D. C. 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)

NAME: Roy, [illegible]

TELEPHONE: (202) 576-3053

SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

Foreign Intelligence Considered

ASSOCIATE INVESTIGATORS

NAME: Tseng, [illegible]

NAME: Reid, [illegible]

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Microfold cells; (U) Peyer's patches; (U) Mucosal Surfaces; (U) Oral immunization; (U) Gastrointestinal immunity; (U) Secretory immunity

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23(U) The primary objectives are to study the processes of antigen sampling, attachment, uptake and presentation within the follicle-associated epithelium (FAE) of Peyer's patches in the small intestine. This will be accomplished by studying the derivation and characteristics of the cells involved and the dynamics of antigen trapping and processing in FAE. Knowledge derived from these studies will be used to manipulate the mucosal immune system by enhancing antigen attachment and presentation, thereby promoting oral immunization while preventing establishment of oral tolerance. The military relevance of this research is prevention of respiratory and gastrointestinal infections.

24(U) Cell surface receptors on FAE microfold (M) cells will be characterized on the basis of lectin, immunoglobulin and antigen binding capabilities. Leukocytes of the FAE, grouped on the basal surface of M cells, will be classified as T cells, B cells, macrophages or a combination of these cell types, on the basis of well defined surface and cytoplasmic markers. Possible binding of oral antigens by FAE leukocytes will be determined, also. Immunocytochemistry and autoradiography will be used as basic techniques.

25(U) 82 10 - 83 09. A lectin from Ulex europaeus (UEA) binds exclusively to M cells in rabbit gut-associated lymphoid tissues, as shown by lectin cytochemistry. Mature epithelial cells were localized in lymph nodes draining mucosal tissues. M cells were selectively dissociated from these tissues using EDTA and collagenase. UEA will be used to further purify M cells, M cells will be cultured and moroclonal antibodies will be prepared to M cell surface proteins. This represents progress toward the selective immunization of mucosal surfaces. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 100: Oral Vaccination of Peyer's Patches and Mucosal Surfaces

Investigators:
Principal: Michael J. Roy, Ph.D.
Associates: Jeenan Tseng, Ph.D.
LTC Robert H. Reid, MC

Problem:

More effective methods are needed to selectively deliver antigens to cells involved in the afferent arm of secretory immunity. Infections of the gastrointestinal, respiratory and genital tracts result in a substantial loss of military manpower. Effective oral vaccines have not been developed for most pathogens that traverse or infect mucosal tissues, in large part because traditional methods of immunization result in poor secretory immune responses.

Approach:

The mucosa of gut associated lymphoid tissues consists of three cell types: M cells, columnar enterocytes and lymphocytes. M cells are actively involved in the uptake of antigens and the subsequent transport of these materials to underlying lymphoid tissues. It is hypothesized that these events are crucial for effective oral immunization to occur. Glycoproteins are likely involved in antigen binding and transport. Therefore, M cell surface molecules will be studied in more detail using three experimental approaches: 1) searching for lectins or neoglycoproteins that bind exclusively to M cells; 2) isolating and culturing M cells so that antigen binding capabilities can be studied, in vitro; and 3) producing monoclonal antibodies reactive with the M cell surface glycoproteins involved in antigen binding and uptake.

Progress:

Morphologic studies on rabbit sacculus rotundus and appendix indicate that these organs are an unusually rich source of M cells. Lymphoepithelial cells, including M cells, were dissociated from sacculus rotundus by sequential incubation with EDTA and collagenase. Mice were immunized with these cells and hybridoma production and screening for anti-M cell antibodies will begin soon. The lectin UEA, from Ulex europaeus, binds to mature cells of ectodermal or endodermal origins and to M cells, indicating that M cells are well differentiated epitheleal cells and suggesting that M cells have biochemical properties different from adjacent immature enterocytes.

Future Objectives:

Continue ILIR. Enrichment of M cells from populations of dissociated lymphoepithelium will be attempted using centrifugation on discontinuous density gradients and UEA-dependent agglutination or chromotography. Isolated M cells will be cultured and monoclonal antibodies will be produced to antigen receptors on the surface of M cells. Achievement of these goals will represent steps toward the selective presentation of immunogens to mucosal surfaces in a way that will stimulate strong, long lasting secretory immune responses to infectious diseases.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300531	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	101 WW08
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Effects of Thai Medicinal Plant Preparations on in vitro Development of Plasmodium falciparum

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	0.2	55
	CURRENT 84	0.5	50

19. RESPONSIBLE DOD ORGANIZATION
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20. PERFORMING ORGANIZATION
NAME: AFRIMS
ADDRESS: Bangkok, Thailand
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: WEBSTER, H
TELEPHONE:
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: PAVANAND, K.
NAME: POC: DA

21. GENERAL USE
Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Malaria; (U) Plasmodia; (U) Medicinal plants; (U) Infectious Diseases

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The technical objective is to determine whether selected Thai medicinal plants and their chemically isolated components exhibit an inhibitory effect on the in vitro growth of P. falciparum. There is a military requirement for new therapeutic agents to prevent and treat malaria.

24. (U) The approach involves chemical preparation of crude plant extracts for in vitro screening; followed by isolation and purification of components for in vitro antimalarial activity confirmation, structure determination and mechanism of action studies.

25. (U) 82 10-83 09 We have identified two Thai plants with impressive antimalarial activity. Four active compounds were isolated from the whole fruit of Brucea javanica (L.) Merr. Two of these compounds (BJ/A and BJ/B) were obtained as pure crystals in good yield. These two compounds showed activity in vitro against multi-drug resistant strains of P. falciparum comparable to that observed for mefloquine in parallel tests. The structure of BJ/A was determined as quassinoid bruceolide - bruceine A. In addition a pure compound was isolated from the chloroform extract of the dry root of Plumbago indica L. This compound also demonstrated good activity against P. falciparum. The Plumbago compound has the structure of a naphthoquine. At present 3 other plants that have shown antimalarial activity in their crude extracts are undergoing chemical fractionation for continued study. Work is currently underway to assess the mechanism of action of the bruceolide and naphthoquine compounds. These are the first studies in Thailand to confirm the antimalarial properties of Thai medicinal plants used in the traditional treatment of human malaria. For technical report see Walter Reed Army Institute of Research Progress Report, 1 Oct 82 - 30 Sept 83.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 72 AND 1498 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 101: Effects of Thai Medicinal Plant Preparation on in vitro Development of Plasmodium falciparum

Principal Investigator: MAJ H. K. Webster, MSC

Evaluation of Thai Medicinal Plant Preparations for Antimalarial Activity Against Drug - Resistant Strains of Plasmodium falciparum

PROBLEM: Antimalarial drug resistance in Thailand is a major health problem that continues to intensify. There is an urgent need to identify new compounds effective against malaria parasites resistant to chloroquine and to pyrimethamine-sulfonamide combinations. Thai medicinal plants with putative antimalarial activity offer a unique source for biological and chemical study to elucidate active antimalarial compounds for use against drug-resistant P. falciparum. Botanical preparations are of special interest to Thailand because they represent a natural resource with considerable economic potential.

PROGRESS: We have identified two Thai plants with impressive antimalarial activity. Four active compounds were isolated from the whole fruit of Brucea javanica (L.) Merr. Two of these compounds (BJ/A and BJ/B) were obtained as pure crystals in good yield. These two compounds showed activity in vitro against multi-drug resistant strains of P. falciparum comparable to that observed for mefloquine in parallel tests. The structure of BJ/A was determined as a guassinoid bruceolide - bruceine A. In addition a pure compound was isolated from the chloroform extract of the root of Plumbago indica L. This compound also demonstrated good activity against P. falciparum. The Plumbago compound has the structure of a naphthoquinone. At present 3 other plants that have shown antimalarial activity in their crude extracts are undergoing chemical fractionation for continued study. Work is currently underway to asess the mechanism of action of the bruceolide and naphthoquinine compounds. These are the first studies in Thailand to confirm the antimalarial properties of Thai medicinal plants used in the traditional treatment of human malaria.

FUTURE OBJECTIVES: These studies should be continued. Further work is needed to complete the structural characterization of the bruceine group of compounds. Once structure determination and isolation procedures have been completed in vivo studies using an animal malaria model should be undertaken.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESS		2. DATE	3. CONTROL SYMBOL
A		83 10 01	DD DR&E(AR)636

4. DATE PREV SUMRY	5. KIND OF SUMMARY	6. SUMMARY SCTY	7. WORK SECURITY	8. REGRADING	9. DISP'N INSTR'N	9A. SPECIFIC DATA CONTRACTOR ACCESS	10. LEVEL OF SUM
82 10 01	D. Change	U	U		XE	☒ YES ☐ NO	A. WORK UNIT

11. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	102 WW19
b. CONTRIBUTING				
c. CONTRIBUTING				

12. TITLE (Procede with Security Classification Code)

(U) Pharmacokinetics of Atropine and L-hyoscyamine

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology 012900 Physiology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
82 12	Cont	DA	C. In-House

18. CONTRACT GRANT

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT

19. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (in thousands)
FISCAL YEAR	PRECEDING 83	1.5	20
	CURRENT 84	1.5	33

20. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Considered

22. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

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Washington, D.C. 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: SMALLRIDGE, R C

TELEPHONE

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: VERMA, P S

NAME: WHORTON, J E

FOC: DA

23. KEYWORDS (Precede EACH with Security Classification Code)

(U) Pharmacokinetics; (U) Atropine; (U) L-hyoscyamine; (U) Drug metabolism; (U) Human volunteer

24. TECHNICAL OBJECTIVE. 25. APPROACH. 26. PROGRESS (Furnish individual paragraphs identified by numbers. Precede text of each with Security Classification Code.)

23.(U) To develop radioimmunoassays for atropine and L-hyoscyamine. To determine the pharmacokinetics and tissue distribution of atropine and its active form, L-hyoscyamine in animals. To determine the pharmacokinetics of atropine and L-hyoscyamine in humans. Atropine is the single most important drug used to treat casualties from exposure to nerve agents. There is a paucity of information on drug metabolism of atropine in normal subjects, and no information on its fate under conditions known to alter an individual's physiologic response to the drug. There is no information on the metabolism of L-hyoscyamine.

24.(U) Sensitive and specific radioimmunoassays for (a) atropine and (b) L-hyoscyamine will be used. After either intramuscular or intravenous administration, drug metabolism will be determined in animals and humans by measuring drug concentrations for kinetic analysis. Tissue concentrations of both drugs will be measured in animals, particularly with respect to the degree to which they penetrate the blood-brain barrier.

25.(U).8210-8309, An antibody for atropine has been raised in rabbits. A highly sensitive radioimmunoassay for measurement of atropine in biologic fluids has been developed, using a tritiated tracer of high specific activity. Detection limit of the assay is 15 pg. This assay has been validated for use in seven animal species, including man. A protocol has been written for determination of atropine kinetics in normal humans, and two large animal protocols are currently being prepared. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 to 30 Sep 83.

* Available in units from organization's approved

DD FORM 1498 [illegible]

Project: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit: 102 Pharmacokinetics of Atropine and L-hyoscyamine

Investigators:
Principal: Robert C. Smallridge, LTC MC
Associate: Pritam S. Verma, CPT, MSC
Nancy E. Whorton, GS-11
Carolyn Umstott, GS-7
Henry Fein, MAJ MC

Atropine is the first-line drug used to treat combat casualties from exposure to chemical warfare nerve agents, and it is issued to soldiers in auto-injectors. There is considerable information about the physiologic responses to selected doses of atropine (1-4), and it is recognized that a variety of independent variables may alter one's response to a fixed dose (e.g., extremes of climate, exercise, co-administration of other drugs, protective garments). However, there is scant information on the absorption, distribution, and elimination of atropine in normal individuals (5-7), and no studies of drug metabolism in situations which might be affecting drug metabolism. It is quite possible that the reason why a specific dose of atropine is well tolerated under certain conditions, yet can physically incapacitate a soldier under other circumstances, is due to alterations in the metabolism of the drug. To assess this possibility, and, thence, to determine the appropriate dose for treatment under given conditions, knowledge of the pharmacokinetics of the drug is required. This information is currently unavailable. The objectives of this Work Unit are to: (1) develop radioimmunoassays to measure small quantities of atropine and L-hyoscyamine (the active component in atropine) in blood, urine, and tissues; and (2) determine the rates of absorption and elimination of atropine from the body, based on serial blood levels of atropine after administration.

This was a new Work Unit in FY83. Progress to date has been primarily the development of a highly sensitive radioimmunoassay for atropine. The atropine antibody was produced in the following manner. Atropine was conjugated to bovine serum albumin by initially conjugating the diazotized p-amino-benzoic acid to atropine and then forming an amide linkage between the amine groups of the protein and the carboxyl group of benzoic acid. Four male New Zealand white rabbits were immunized with this atropine-BSA immunogen. The rabbits were first bled two months after the initial immunization procedure and antibodies were detectable at that time. To obtain optimum sensitivity, the antibodies generated were used in the assay at a final dilution of 1:9000. Pre-immunization serum failed to bind any tritiated atropine. The standard curve is linear up to 2 ng of atropine. Identical standard curves were obtained using human plasma, serum,

urine, or phosphate-buffered saline, pH 7.5, indicating the lack of interfering substances in these body fluids. The assay reliably detects as little as 15 pg of atropine, and a 50% inhibition of binding of the ^{3}H-atropine ligand to the antibody is attained with 140 pg of atropine. The antibody recognizes both L- and D- forms of the drug, the L- form being slightly preferable. The atropine hydrolysis products, tropine and tropic acid, do not cross-react with the antibody. The interassay and intraassay coefficients of variation are 13.5% and 8.3%, respectively, and recoveries range from 94-102%. Excellent displacement curves and recoveries have been documented in a variety of species, including man, monkey, dog, cat, sheep, goat, rabbit, rat, guinea pig, and chicken. Thus, the assay is adaptable for use in a number of animal models.

A human use protocol for determination of atropine pharmacokinetics in normal men is near completion, as are two animal protocols. All three studies should be conducted in FY84.

Recommendations for the future first involves an assessment of atropine and L-hyoscyamine pharmacokinetics in normal situations. This should be followed by similar studies under conditions known to alter the dynamic responses to a fixed dose of atropine. From these studies should emerge guidelines for optimum drug dosages under a variety of situations, based upon pharmacokinetic principles.

References

1. Cullumbine, H., W.H.E. McKee, and N.H. Creasey, The effects of atropine sulfate upon healthy male subjects. Quart J Exp Physiol 40:309. 1955.

2. Morton, H.J.V., and E.T. Thomas, Effect of atropine on the heart rate. Lancet 2:1313, 1958.

3. Craig, F.N., Effects of atropine, work and heat on heart rate and sweat production in man. J Applied Physiol 4:826, 1952.

4. Headley, D.B., A review of the effects of atropine sulfate and pralidoxime chloride on visual, physiological, performance, subjective, and cognitive variables in man. Mil Med 147:122, 1982.

5. Wurzburger, R.J., R.L. Miller, H.G. Boxenbaum, and S. Spector, Radioimmunoassay of atropine in plasma. J Pharmacol Exp Therap 203:435, 1977.

6. Adams, R.G., P. Verma, A.J. Jackson, and R.L. Miller, Plasma pharmacokinetics of intravenously administered atropine in normal human subjects. J Clin Pharmacol 22:477, 1982.

7. Berghem, L., U. Bergman, B. Schildt, and B. Sorbo, Plasma atropine concentrations determined by radioimmunoassay after single-dose I.V. and I.M. administration, Br J Anaesth 52:597, 1980.

Formal Presentations

1. Verma, P.S., R.G. Adams, and R.L. Miller, Reactivity of plasma kallikrein-kinin system during sickle cell crisis. In International Congress on Pediatric Lab Med Toronto, Canada, May 29-June 2, 1983.

2. Verma, P.S., R.F. Hoyt, Jr., A.J. Jackson and Y.Y. Phillips, Radioimmunoassay (RIA) for desmosine and study of its pharmacokinetics. Fed Proc 42:1298, 1983. Presented at the Federation of American Societies for Experimental Biology, Chicago, Il.

3. Verma, P.S., D.E. Butkus, and R.G. Adams. Inhibition of rat kidney angiotensin converting enzyme by arginine vasopressin Fed Proc 42:1948, 1983. Presented at the American Society of Biological Chemists, San Francisco, CA

4. Verma, P.S., R.G. Adams, and R.L. Miller, Activation of plasma kallikrein-kinin system in patients during sickle cell crisis. Clin. Chem 29:1266, 1983. Presented at the American Society of Clinical Chemists, New York City, NY.

Bibliography

1. Verma, P.S., C. Umstott, and R.C. Smallridge, Development of an atropine radioimmunoassay suitable for use in a variety of animal models (submitted for presentation).

2. Adams, R.G., P.S. Verma, A.J. Jackson, and R.L. Miller, Plasma pharmacokinetics of intravenously administered atropine in normal human subjects. J Clin Pharmacol 22:477, 1982.

3. Miller, R.L., P.S. Verma, and R.G. Adams, Studies of the kallikrein-kinin system in patients with sickle cell anemia. J Nat Med Assoc 75:551, 1983.

4. Verma, P.S., R.G. Adams, and R.L. Miller, Reduced plasma kininogen concentration during sickle cell crisis. Res Comm Chem Pathol and Pharmacol 41:313, 1983.

5. Verma, P.S., R.F. Hoyt, Jr., A.J. Jackson, and Y.Y. Phillips, Pharmacokinetics of intravenously administered desmosine in sheep. Conn Tissue Res (In Press).

6. Moore, J., J.A. Gagnon, P.S. Verma, G.E. Sander, and D.E. Butkus, Plasma kinin levels in acute renovascular hypertension in dogs. Renal Physiol (In Press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 500703	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
83 01 18	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	103 WWHB
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code):

(U) Membrane Fusion and Diffusion of Receptors in Cells

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

002300 Biochemistry 002600 Biology 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 01	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER:

c. TYPE: d. AMOUNT

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	0.5	25
FISCAL YEAR CURRENT 84	0.5	25

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Doctor, B P

NAME: Gordon, R K POC: DA

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code):

(U) Receptors; (U) Enzymes; (U) Membranes; (U) Lipid Bilayers

23. TECHNICAL OBJECTIVE; 24. APPROACH; 25. PROGRESS (Furnish individual paragraphs identified by number. Provide text of each with Security Classification Code.)

23. (U) The object of this work unit is to study the fusion and diffusion of receptors of cells reconstituted into phospholipid bilayers introduced to other cell types as studied biochemically or by fluorescence microscopy. There is military relevance in this research.

24. (U) Research focuses on the characterization and isolation of receptors of cells. The receptors studies are mainly muscarinic and nicotinic receptors of the following cell lines; N4TG1 neuroblastoma and NG108-15 neuroblastoma x glioma hybrid cells. After initial characterization of the receptors in the cell lines, their isolation will be carried out from either the cell lines or brain tissues. The ultimate goal is to purify and reconstitute the receptors, and to introduce the latter into phospholipid bilayers. For example, the fusion of muscarinic receptors into a noncholinergic cell allows the testing of the muscarinic functions in that cell. Whether the cell with new receptors inserted can exhibit muscarinic functions will be tested by radioactive ligand binding (such as [^{3}H]QNB), cyclic GMP coupling effect, and phosphatidyl inositol turnover. Fluorescence microscopy will also be used to monitor the diffusion and translocation of receptors into cells.

25. (U) 83 01 - 83 03: A more reliable procedure has been developed to assay the muscarinic receptors of N4TG1 neuroblastoma cells [^{3}H]QNB (quinuclidinyl benzilate) binding. Compounds structurally related to QNB have been tested for their ability to inhibit the binding of QNB, and minimal binding requirements have been characterized. For technical [illegible] Walter Reed Army Institute of Research Annual Progress Report, [illegible]

*Available in [illegible] unless otherwise approved

DD FORM 1498 [illegible]

PROJECT: 3A161101A91C BASIC RESEARCH ON MILITARY DISEASES

WORK UNIT: 103 Membrane Fusion and Diffusion of Receptors in Cells

INVESTIGATORS:

Principal: Peter K. Chiang, Ph.D.
Associate: Richard K. Gordon, Ph.D.; B. P. Doctor, Ph.D.
Assistant: F. Y. Chang, Ph.D.,; SFC Evelyn Moore, SP4
Felipe N. Padilla; Gregory R. Lattanze

The objective of this work unit is to study, characterize and isolate receptors of cells. The ultimate goal is to re-constitute the isolated receptors into lipid bilayers and to introduce them to cell types lacking those receptors and to follow the fusion and diffusion of the receptors in the host cells. The following investigations were conducted:

Importance:

1. Mapping the muscarinic receptors of N4TG1 neuroblastoma cells.

2. Developing an assay of pipecolate receptors, which may be involved in the anticonvulsion of animals.

1. Mapping of the muscarinic receptors of N4TG1 neuroblastoma cells:

A new method for assaying the muscarinic receptors of cells has been developed. The assay involves the measurement of the binding of radioactive quinuclidinyl benzilate ([^{3}H]QNB) to the muscarinic receptors. Typically, 0.5 x 10^6 cells, representing about 1 mg of protein, were incubated in the assay medium containing 2 nM [^{3}H]QNB. After 20 min of incubation at 22°, the cells were layered over 500 µl of silicone oil (General Electric Versilube F50) in an Eppendorf tube and pelleted by centrifugation for 30 sec at 10,000xg. After removel of the aqueous (top) and oil (bottom) layers, the cell pellet in the Eppendorf tip was cut off and solubilized in 1.0 ml of 1% Triton X-100, 10 ml of scintillation fluid, and then counted. This method is far superior to the conventional method using filters, in having workable lower blanks.

By Scatchard plot analysis of [^{3}H]QNB binding, there are 2 x 10^5 muscarinic sites/cell with a K_D of about 10 nM in both the N4TG1 neuroblastoma cells, and the NG108-15 neuroblastoma x glioma hybrid cells. A group of compounds structurally related to QNB have been examined for their ability to cmpete for binding to the muscarinic receptors. Using this structure-inhibition relationship, the functional groups of the muscarinic ligands necessary for binding were partially characterized. It was found that the quinuclidinyl ring structure of QNB can be substituted by either alkane, H, or pyrrolidine at the N without loosing their ability to bind. Additions to the quinuclidinyl ring increases the bulk of the structure and decreases binding. The diphenyl portion can be replaced by quinidines or tricyclic structures, and still retain inhibitory activity. Like the benzilate in QNB, a similar hydrophobic structure is apparently required for the binding.

2. Identification and Characterization of the Pipecolate Receptors:

Convulsant and anticonvulsant drug binding sites are believed to be related to GABA-regulated chloride ion channels. Based on *in vivo* observation of the anticonvulsant activity of lysine and its metabolites against pentylenetetrazole- and picrotoxin- induced convulsion, it was speculated that lysine metabolites may exert their anticonvulsant effect through the chloride ion channels, which may be either associated with or distinct from the GABAergic neurotransmmission.

Effects have been devoted mainly to the development of an acceptable assay technique for the binding of DL-[^{3}H]pipecolic acid, which is a lysine metabolite, to the rat cerebral cortex and synaptic membranes. Due to the high non-specific binding of this labeled metabolite to the glass fiber filter and other types of membrane filters, an alternative assay method was developed instead. This is based on the adaption of the centrifugation technique described for the muscarinic receptors, as described in the previous paragraph.

Preliminary results suggest that there is a specific binding of [^{3}H] pipecolic acid to the synaptic membranes, even though there is a high non-specific binding. Attempts to solubilize the receptor protein from the membrane preparations are now underway.

PUBLICATONS

Chang, F. -Y., Lattanze, G. R., Carroll, F. I., Gordon, R. K., and Chiang, P. K. (1983) Mapping of muscarinic receptors of N4TG1 Neuroblastoma Cells. The Pharmacologist 25, 256.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300022	83 10 01	DD-DR&E/AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	104 WUH8
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Development of Monoclonal Antibody - Producing Hybridomas

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-house

17. CONTRACT GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER

c. TYPE: d. AMOUNT

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	1.0	85
	CURRENT 84	1.0	85

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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20. PERFORMING ORGANIZATION

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Division of Biochemistry

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Gemski, P

TELEPHONE: (202) 576-2594

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Gentry, M A

NAME:

21. GENERAL USE

Foreign Intelligence considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Hybridoma; (U) Monoclonal antibody; (U) Protein; (U) Enzyme; (U) Toxin;

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The technical objective is to produce hybrid cell lines which secrete monoclonal antibodies by use of cell fusion techniques in the mouse-mouse hybridization system. Antigens include viruses, bacterial LPS antigens, toxins, enzymes, macrophage suppressor factors and peptides. There is military relevance in this research.

24. (U) Spleen cells will be exposed to antigens either in vivo or in vitro and subsequently fused with mouse plasmacytoma cells. Fused cell products will be screened for production of antibodies and selected for cloning and further characterization on the basis of their reactivity on serological and biochemical tests.

25. (U) 82 10 - 83 09. Antibodies against a mixture of (17 + 13)s, 11s, and 5.6s forms of acetyl cholinesterase have been isolated. One antibody exhibits preferential binding for the 5.6s species, while another appears to be directed against the collagen like tail of (17 + 13)s form. Monoclonal antibodies prepared against the four prototype strains of dengue have been used to define epitopes on the dengue - 2 virion. Monoclonal antibodies prepared aginst dengue - 4 virus have identified a new subtype of this virus isolated from a recent Carribean epidemic. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available in unclassified upon originator's approval

DD FORM 1498 [illegible]

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 104 Development of Monoclonal Antibody Producing Hybridomas

INVESTIGATORS:
Principle: Peter Gemski, Ph.D.
Associates: Mary K. Gentry, M.S.; SP4 Karla K. Kopec, B.S.

In collaboration with: W.E. Brandt, Ph.D. (DCD & I); B.P. Doctor, Ph.D; CPT E.A. Henchal, Ph.D., MSC (DCD & I)

DESCRIPTION:

The development of hybridoma cell lines which produce monoclonal antibodies to different antigens of importance to the military provides an excellent source of monospecific antibody reagents required for the improved rapid detection and control of infectious agents which affect military personnel operating in areas of poor sanitation. Also, antigens and virulence determinants of importance to pathogeneses can be recognized and characterized by use of these tailor-made monoclonal antibody preparations. The investigation of host antibody responses to such microbial antigens provides important basic information needed to facilitate development of new synthetic vaccines of military relevance.

A. Monoclonal Antibodies to Dengue Virus

1. Antigenic and Biological Variation Occuring Among Dengue Type 4 Viruses.

A new subtype of dengue type 4 (DEN-4) virus was identified among dengue isolates obtained during the 1981-1982 epidemic season in the Caribbean. Sixteen isolates were identified as DEN-4 virus in a complement fixation test by the Caribbean Epidemiologic Centre (Trinidad). We also identified the isolates as DEN-4 virus by an indirect immunofluorescence assay using non-neutralizing serotype-specific monoclonal antibodies. However, cross neutralization assays (NA) showed that while hyperimmune fluids prepared against mouse adapted Caribbean DEN-4 virus neutralized both the prototype (H241) and Caribbean viruses, reference antibodies to the H241 virus neu-tralized homologous virus to a higher titer. Both antibody preparations were able to neurtralize a variety of 1973-1980 DEN-4 virus isolates from the South Pacific. This new virus strain induces higher virus yields (2-3 $\log_{10}$) in misquito cell culture and suckling mouse brain, and produces plaques on LLC-MK2 (monkey kidney) cell monolayers twice the diameter of the prototype. The results of this study suggest that the Caribbean DEN-4 may be a better reference strain. With no other assay for detection of variation in the neutralization determinant, the NA remains the sole method for identifying antigenic variations of public health significance.

B. Monoclonal Antibodies to Acetylcholinesterase

1. Evidence for Structural and Antigenic Differences between Hydrophobic Dimer and Asymmetric Forms of Acetylcholinesterase Torpedo Californica.

Acetylcholinesterase is found in two molecular forms: 5.6S hydrophobic species, a simple dimer of catalytic subunits, and (17 + 13)S, an asymmetric form in which several catalytic subunits are linked to collagenous and non-collagenous structural subunits. Treatment of the (17 + 13)S species with trypsin removes the structural subunits yielding an 11S species. Monoclonal antibodies against the (17 + 13)S, 11S and 5.6S forms have been isolated and purified. Of the eleven antibodies, one (4E7) shows an 80-fold more binding to the 5.6S species than other species. This difference is retained following denaturation. A second antibody (4F3) reacts only with (17 + 13)S species and appears to be directed to the collagenous subunit. Using HPLC, all the tryptic peptides obtained from the catalytic subunits of the 11S and 5.6S enzymes have been separated. Comparison of the two maps shows at least 4 different peptides. The active site peptide, the cystine-containing peptides and some of the glycopeptides have been identified by radioactive labeling and lectin chromatography. Our findings demonstrate that the catalytic subunits of the 11S and 5.6S species show antigenic and structural differences.

Publications:

1. B. P. Doctor, Shelly Camp, Mary Kay Gentry, Susan S. Taylor, and Palmer Taylor: Antigenic and structural differences in the catalytic subunits of the molecular forms of acetylcholinesterase. Proc. Nat. Acad. Sci. USA, 80 5767-5771, 1983.

2. B. P. Doctor, Shelly Camp, Mary K. Gentry, Susan S. Taylor, and Palmer Taylor: Evidence for structural and antigenic differences between hydrophobic dimer and asymmetric forms of acetylcholinesterase from Torpedo Californica. Federation Proceedings, 42: 2024, 1983

3. E. A. Henchal, J. M. McCown, W. E. Brandt, and M. K. Gentry: Antigenic and biological variation occuring among Dengue type 4 viruses. Abstracts of the Annual Meeting of the American Society for Microbiology, 1983, p. 306.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300 [illegible]	83 10 01	DD-DR&E/AR(6)6

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORG'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	105 WWJH
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Neuropharmacology of Performance and Fatigue

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012900 Physiology 016200 Stress physiology 010340 Psychology 000000 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER:

c. TYPE: d. AMOUNT

e. KIND OF AWARD: f. CUM. AMT.

	18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING	82	2.0	60
FISCAL YEAR CURRENT	84	2.0	80

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute or Research

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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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TELEPHONE: (202) 576-3027

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Holaday, J W

NAME: Tortella, F C

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Performance; (U) Pharmacology; (U) Stress; (U) Stimulant; (U) Fatigue; (U) Sleep; (U) Perseveration

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To continue ongoing studies to evaluate the role of endogenous and exogenous neuromodulators in performance, fatigue, and sleep. Stimulant drugs which are presently available (e.g. amphetamine) have the adverse consequence of severely impairing judgement while providing their stimulant effects. Insights into the body's own systems for producing arousal, sleep, and other behaviors relating to vigilance and performance have suggested the use of novel substances which may act either directly or indirectly to enhance endogenous stimulants and depress endogenous sedatives and thus improve battlefield performance.

24. (U) Experimental animals will be entrained on an eight arm maze, and performance will be assessed with and without prior pharmacological treatment. This general paradigm will be complemented with direct analysis of locomotor activity. Established drugs and treatments [including amphetamine, thyrotropin releasing hormone (TRH), prochlorperizine and the application of electroconvulsive shock (ECS)] will be employed alone and in combination. Depending upon results, descriminative operant tasks will be evaluated to compare stereotypic responses relative to general stimulant actions. Correlations among behavioral findings, analysis of biochemical substances and assay of receptor changes will allow for interactive evaluations with the hope of determining cause and effect. Additionally, recent acquisition of equipment enables direct comparisons of electroencephalographic responses to these substances.

25. (U) 82 10 - 83 09 Electroconvulsive shock (ECS) was shown to activate endogenous opiates whose actions are mediated via delta receptors; a selective increase in delta opioid receptor numbers was shown. At present, specific brain regions involved, as well as general neuroendocrine responses to ECS are under analysis. Other selective opioid peptides were shown to interact with brain arousal by altering seizure susceptibility. TRH, an excitatory neuropeptide found in the brain, has stimulant properties which increased locomotor activity and, unlike amphetamine, did not alter correct responses. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

Available [illegible] upon originator's approval

DD FORM 1498 1 MAR 68 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE [illegible] AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 105 Neuropharmacology of Performance and Fatigue

INVESTIGATORS:

Principal:	Belenky, G.L., LTC MC
Associates:	Holaday, J.W., GM-15 and Tortella, F.C., GS-12
Assistants:	Robles, Lydia, SP6 and Cardenales, Lysaida, SP5

Problem:

To derive an understanding of the mechanisms by which endogenous and exogenous neuromodulators regulate performance, fatigue and sleep through complementary behavioral, biochemical and electroencephalographic studies in rodents. Such information will enable novel approaches to the development of neuropharmacological agents which will rely on manipulations of endogenous biochemicals to enhance performance, diminish fatigue and prevent sleep.

Importance:

Stimulant drugs which are presently available (e.g. amphetamine and related substances) have the adverse consequence of impairing judgement. Thus, their utility as stimulants in military and non-military applications is severely limited. If insights into the body's own systems for producing arousal, sleep, and other behaviors relating to vigilance and performance are elucidated, it may be possible to enhance substances which elicit arousal or, conversely, to specificially inhibit those substances which have sedative properties. Even without the demonstration of endogenous involvement, the testing of various novel combinations of old drugs as well as neuropeptides which have recently become available may provide pharmacological tools by which to improve performance, decrease fatigue, and delay sleep while minimizing adverse side effects.

Approaches:

Prior work has involved evaluation of performance of an eight-arm maze to include speed and accuracy; and complementary experiments are directed at analysis of locomotor activity as another index of stimulant effects. Both of these experimental procedures are evaluated prior to and following administration of substances - amphetamine, prochlorperizine, TRH, cholecystokinin and opioids - which may be linked to states of arousal. Discriminatory operant tasks will compare stereotypic responses and relate them to these various stimulant actions. In addition to drug administration, studies with electroconvulsive shock (ECS) as a model of stress-induced alteration of performance and vigilance will be continued. Biochemical experiments are planned to determine possible mechanisms by which endogenous systems regulate behavior to include assaying brain and plasma concentrations of neuropeptide substances, as well as changes in receptor numbers and affinities for various endogenous ligands (e.g. opioid peptides, TRH, etc.). In addition, recently acquired equipment will allow

for a direct measurement of electroencephalographic responses during these experiments. These pharmacological, behavioral, and electroencephalographic approaches will make it possible to elucidate fundamental neuropharmacological mechanisms underlying performance, fatigue, and sleep in rats with the hope of extrapolating results to the clincial domain.

Results:

Prior work has shown that thyrotropin-releasing hormone (TRH), a neuropeptide with stimulant properties, failed to alter maze running time or accuracy in contrast to amphetamine, which decreased the number of correct responses. Additional studies in rats demonstrated that the endogenous narcotics (the endorphins) were significantly activated by electroconvulsive shock, and increase in numbers of delta opioid receptors was shown. The involvement specific brain regions in this response is currently being analyzed. Other selective opioid peptides were shown to interact with brain arousal by altering seizure susceptibility. The studies provide potentially novel stimulant drugs, namely TRH and opiate antagonists, which will be further evalutated along with other potentially useful substances in paradigms as outlined above.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6469	83 09 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	H. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161102A91C	00	106
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Include with Security Classification Code): Metabolism of the Malaria-Infected Erythrocyte and Host Cell Interactions (Red Blood Cell Metabolism)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002602 Biology; 012600 Physiology

13A. START DATE	13B. ESTIMATED COMPLETION DATE	14. FUNDING AGENCY	15. PERFORMANCE METHOD
77 10		DA	C. In-house

16. CONTRACT/GRANT
a. DATES/EFFECTIVE: — EXPIRATION:
b. NUMBER:
c. TYPE: — d. AMOUNT:
e. KIND OF AWARD: — f. FROM AMT:

17. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PREVIOUS: 82	2.0	
FISCAL YEAR — CURRENT: 83	2.0	

18. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
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SOCIAL SECURITY ACCOUNT NUMBER

20. GENERAL USE

ASSOCIATE INVESTIGATORS
NAME: Power, Wesbitt, Dr.
NAME:

21. KEYWORDS (Provide DATA with Security Classification Code): (U) Red Blood Cell (Erythrocyte); (U) Malaria; (U) Metabolism

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide individual paragraphs identified by number. Provide text of DATA with Security Classification Code.)

23.(U) To establish an integrated concept of intermediary metabolism in the malaria-infected human erythrocyte. Studies are directed towards understanding parasite specific metabolic pathways that may influence the normal metabolic functions of the erythrocyte. In addition, identification of parasite-specific metabolic pathways may suggest biochemical targets for the development of new antimalarial chemotherapy that is effective against resistant strains of malaria. Development of new antimalarial chemotherapy is of major military importance because of mobility of military personnel in regions where malaria is endemic.

24.(U) Laboratory studies include measurement of (1) intermediates and enzyme levels of the purine and pyrimidine salvage and interconversion pathways; (2) intermediates and enzyme levels of glycolysis, the pentose cycle, the Krebs cycle and fatty acid synthesis; and (3) intermediates and enzyme levels of membrane lipid metabolism.

25.(U) 82 10 - 83 09 Studies on the intermediary metabolism of human red blood cells infected with malaria parasites have shown that inhibitors of ornithine decarboxylase, an enzyme which mediates polyamine biosynthesis, interfere with malaria parasite growth and development in vitro, that the heterocyclic inhibitor of protein synthesis, homoharringtonine, has anti-malarial activity in vitro with P. falciparum resistant strains, that inhibitors of methylation pathways and S-adenosyl-homocysteine analogues also markedly inhibit malaria parasite growth in vitro. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 31 Sep 83.

* Available to contractors upon originator's approval

DD FORM 1 MAR 88 1498 — PREVIOUS EDITIONS ARE OBSOLETE AND 1498 1 MAR 88 FOR ARMY USE ARE OBSOLETE

Project 3A161101A91C: IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 106 Red Blood Cell Metabolism

Investigators LTC June Whaun, MC; Dr. Nesbitt Brown, GS-13 (Div. of Biochemistry);

Description

The goal of this work unit is to study the intermediary metabolism of normal red blood cells (RBC) and of red blood cells that have been infected with malaria parasites. Malaria is a major world health problem that is of particular importance to the military. The stationing of U.S. military personnel in areas that are endemic for malaria (essentially all tropical and subtropical regions of the world) poses a serious threat to the health of individual soldiers and to tactical/strategic unit preparedness. The emergence of drug resistant strains of malaria (especially P.falciparum) significantly compounds the medical problem of malaria, and there is a consequent need for novel approaches to the development of new antimalarial chemotherapies that are effective against resistant strains. Purine metabolism is an appropriate focus for studies of host-parasite interactions which occur in malaria infected red blood cells, for purines are essential to the synthesis of nucleic acids, proteins and folates as well as to energy metabolism (ATP), enzyme co-factors and regulators of intermediary metabolism that are critical both for normal RBC function and for parasite differentiation and proliferation. Our objectives are to define the major pathways of purine metabolism in human RBC infected with malaria (P.falciparum) using novel in vitro RBC culture techniques, to determine whether there are parasite specific pathways of purine metabolism, whether P.falciparum is capable of any de novo purine synthesis under conditions of continuous in vitro RBC culture, whether specific inhibitors of purine metabolism can be used to interfere with the growth and development of drug-resistant malaria strains, and whether there are differences in purine metabolism in drug-resistant and drug-sensitive strains of P.falciparum, and to evaluate the biochemical effects of malaria infection upon host RBC and its implications for host defenses. Similar focus has been given to pyrimidine metabolism and to polyamine metabolism in the malarious red cell.

Progress

In the laboratory set up in the Dept. of Hematology, WRAIR, various biochemical pathways related to red cells, both with and without intracellular malaria parasites have been examined:

1. Studies on polyamines and enzymes of the polyamine biosynthetic pathways, such as ornithine decarboxylase, the first enzyme for biosynthesis of polyamines;

2. Studies on purines and salvage synthesis pathways of purines;

3. Studies on methylation pathways, metabolites and enzymes.

These have been studied with the use of appropriate metabolic inhibitors. Logistical considerations have required collaborative work with scientists at WRAIR in Div. of Experimental Therapeutics and Div. of Biochemistry as well as scientists in other institutions, (Sloan-Kettering Institute, NYC).

Studies on polyamines have been undertaken with N.D. Brown, of the Div. of Biochemistry. Dansylated deproteinized extracts have been analyzed by an ultrasensitive automated HPLC method. Ornithine decarboxylase has been measured by the amount of labelled trapped radioactive carbon dioxide formed from 1- ^{14}C -ornithine. Purine nucleotides, nucleosides and bases have been determined by analysis of deproteinized, neutralized biological extracts by HPLC. Salvage synthesis enzymes have been determined in collaboration with M.E. Balis and L. Yip, Dept. of Biochemistry, Sloan-Kettering Institute, NYC. Studies of the methylation pathways, both metabolites and enzymes, have been done in collaboration with Dr. Peter Chiang at WRAIR. Specifically, he has helped in the analysis of S-adenosylmethionine decarboxylase levels. We have measured the activity of methylthioadenosine phosphorylase, the enzyme which catabolizes methylthioadenosine, by a modified spectrophotometric method of Pegg and Williams-Ashman, (Biochem. Pharmacol. 30:189-199 (1981).

Examination of selective new chemotherapeutic agents has been to determine their efficacy as antimalarials and their mode of action. Methylation inhibitors in particular appear to offer a chance for selectively targetted chemotherapy in the malaria-infected red cell in vitro system, that we are using for study.

Specific observations include:

1. The combination of ornithine decarboxylase inhibitor, difluoromethylornithine and an S-adenosylmethionine decarboxylase inhibitor, methylglyoxal bis(guanylhydrazone), a spermidine analogue, is effective in perturbing polyamine metabolism and parasite growth in vitro. This combination inhibited spermine production as well as spermidine levels 30%, while inhibiting parasite growth 75%. Further in vivo studies are planned with Div. of Experimental Therapeutics regarding this novel drug combination.

2. The known antimetabolite, homoharringtonine (NSC-141633) is an effective antimalarial in vitro against three strains of P. falciparum with no cross resistance to chloroquine. This agent, a large heterocyclic molecule, is an inhibitor of protein synthesis, and has a history in herbal folk medicine as an anti-leukemic agent. Its range of action against strains FCR-3/Gambia, Smith/Vietnam, and Camp/Malay was 1.89, 1.47, and 1.35 ng/ml, respectively. This is similar in dose to the effective dose of chloroquine against sensitive strains of P. falciparum.

3. We have examined novel inhibitors of methylation pathways, adenosine analogues, 5'-deoxy-5'-isobutylthio-3-adenosine (SIBA) and 3-deaza-SIBA. These drugs perturb polyamine biosynthesis and purine metabolism and inhibit parasite growth 96% at 0.3 mM for SIBA, and 74% at 0.1 mM for

deaza-SIBA. Very high spermidine levels were found with both drugs which were reversible with micromolar concentrations of hypoxanthine. Hypoxanthine at that dose appeared to reverse toxicity which was characterized morphologically by pyknosis of nuclei and arrest at the ring stage of the intracellular parasite. These findings are currently being investigated through HPLC examination methylation metabolites.

S-adenosylmethionine decarboxylase levels have been assayed and were decreased -- 59% residual activity with deaza-SIBA, 0.2 mM, and 75% residual activity with SIBA, 0.1 mM. In addition, parasitized erythrocytes exposed to drug showed pronounced echinocyte formation which suggested disturbed adenine nucleotide levels. Further studies are ongoing as to the nature of these changes in the RBC, and on the effect of SIBA metabolites on methylation reactions.

Future Plans

Work will continue along the lines of research carried out during the past 3 years in this work unit with a basic goal of continuing to identify metabolic targets in the malaria-infected RBC for design of new antimalarial chemotherapy and to explore the link between polyamine synthesis and turnover, purine metabolism and methylation reactions in regulating the growth and differentiation of Plasmodia within the human RBC host.

Abstracts

1. Whaun, J.M., Brown, N.D. and Webster, H.K.: Studies of host-parasite interaction identify biochemical pathways for chemotherapy targets. J. Cell. Biochem. suppl. 7A. 12th Annual UCLA Symposia "Molecular Biology of Host-Parasite Interaction", Park City, UT, p. 17, 1983.

2. Whaun, J.M., Brown, N.D., and Chiang, P.K.: Antimalarial activity of methylation inhibitors. 36th annual meeting of the Society of Protozoologists, New York, p. 30, 1983.

3. Whaun, J.M., Brown, N.D. and Chiang, P.K.: Methylation inhibitors in malaria. 6th International Conference on Red Cell Metabolism and Function, Ann Arbor, Michigan, 1983.

Publication

1. Whaun, J.M., Rittershaus, C. and Ip, S.H.C.: Rapid identification and detection of parasitized human red cells by automated flow cytometry. Cytometry, in press.

2. Whaun, J.M. and Clarke, H. deC: Daunomycin and its effect on platelets *in vitro*. In review. (Cancer Chemotherapy and Pharmacology).

3. Whaun, J.M.: The effects of aspirin-containing serum in the continuous culture of P. falciparum. In review. (J. Protozoology).

4. Whaun, J.M. and Brown, N.D.: Ornithine decarboxylase inhibition and the malaria-infected red cell: a model for polyamine metabolism and growth. In review.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303 [illegible]	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	106 WWJI
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Acquisition of NCO Skills for Stress Casualty Prevention

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 83	0.0	00
CURRENT FISCAL YEAR 84	1.0	50

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Neuropsychiatry

ADDRESS: Washington, D.C. 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)

NAME: INGRAHAM, L H

TELEPHONE: (301) 427-5210

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME

NAME

POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Adjustment; (U) Psychiatric Illness; (U) Epidemiology; (U) Behavioral Dysfunction; (U) Psycho-Social Factors

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code.)

23. (U) Recent data on combat stress casualties among Israeli troops in Lebanon have confirmed the WW II finding that the single most critical element in the prevention and management of these casualties is the quality of interpersonal relations in the face-to-face work groups. This work unit investigates the way in which NCOs, the central figures in the creation and maintenance of this work group climate, come to learn their role in the process.

24. (U) The method is that of the oral historian: recording career histories of senior non-commissioned officers. Through recall of concrete events and actual people, a data base can be established focusing on what NCOs at various levels actually do, what aspects of those activities can be learned in school, and how do they develop and apply standards of conduct, discipline, and morale in actual practice.

25. (U) New.

*Available for restrictive upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NOV 73 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303105	10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. GRP'D UNDER	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		CX	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	107 WWL6
b. CONTRIBUTING				
c. CONTRIBUTING	None			

11. TITLE (Include Security Classification Code):

(U) Neogut

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

0616 Physiology 0602 Bioengineering

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
83 10	CONT	DA			C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE	A. PROFESSIONAL MAN YRS	B. FUNDS (In Thousands)
PRECEDING FISCAL YEAR 83	0.0	00
CURRENT FISCAL YEAR 84	3.0	30

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H Jr

TELEPHONE: (202) 576-3551

20. GENERAL USE

Foreign Intelligence Considered

H

19b. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Surgery

ADDRESS: Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide last name, first name, middle initial)

NAME: HARMON, J W

TELEPHONE: (202) 576-3791

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. KEYWORDS (Provide Key Words with Security Classification Code):

(U) small bowel; (U) nutrition; (U) short gut syndrome; (U) rats; (U) Lab Animals

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide individual paragraphs identified by number. Provide text of each with Security Classification Code.)

23(U) The primary objective is to study means to augment the absorptive area of the small intestine to provide nutrition for individuals with "short gut syndrome." Currently if the small bowel is lost secondary to trauma, internal hernia or diseases such as Crohn's disease, enteral nutrition is impossible. Such patients must be maintained forever on intravenous infusion pumps which limits activity, is expensive and difficult and sometimes technically impossible. There is military relevance in this research.

24(U) The approach will be to transplant intestine from fetal Fisher rats into the subcutaneous space of adult Fisher rats. Such intestine becomes vascularized, enlarges, and may develop functions of normal intestine.

25(U) None.

* Available in contractor's speed memorandum approved

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NOV 88 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303 106	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	108 WWIIC
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Inhibitors of Methylation as Potential Therapeutic Agents

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 002600 Biology 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
83 10	CONT	DA			C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	0.0	00
FISCAL YEAR CURRENT 84	0.5	20

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Biochemistry

ADDRESS: Washington, D.C. 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Chiang, P K

TELEPHONE: (202) 576-1361

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Gordon, R K

NAME: Doctor, B P

POC: DA

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Methylation; (U) S-adenosylhomocysteine; (U) S-adenosylmethionine

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective of this work unit is to study the role of inhibitors of methylation reactions as potential therapeutic agents: in viral, parasitic infection and as inducers of cellular differentiation. There is military relevance in this research.

24. (U) The potential chemicals to be screened for their ability to inhibit methylation are nucleosides; specifically, nucleosides that are potent inhibitors of S-adenosylhomocysteine hydrolase. Nucleosides that are potent inhibitors and/or alternative substrates of S-adenosylhomocysteine hydrolase will be screened for their ability to raise the cellular level of S-adenosylhomocysteine and/or S-nucleosidylhomocysteine. In the event of the latter, S-adenosylmethionine will also be increased as a consequence of the inhibition of methylation reactions caused by the appearance of large amounts of S-adenosylhomocysteine and S-nucleosidylhomocysteine. The changes in the cellular levels of these metabolites will be analyzed by high pressure liquid chromatography. The specific biological targets to be looked at will be viruses, Plasmodium cultures amd cell lines that can be induced to undergo differentiation. The cell lines to be examined are 3T3-L1 fibroblasts that can under differentiation to become fat cells, and HL-60, which are human promyelocytic leukemia cells that can differentiate into more mature granulocyte-like cells. Correlative changes in DNA methylation, RNA methylation, lipid methylation, and protein methylation will be studied.

25. (U) New.

* Available in accordance with originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 MAN 68 (FOR ARMY USE) ARE OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 303 107	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161102AC35	00	109 WWHD
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Include with Security Classification Code)

(U) Elucidation of Antigenic Determinants

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 012100 Organic Chemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
83 10	CONT	DA			C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER

c. TYPE d. AMOUNT

e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	0.0	00
FISCAL YEAR CURRENT 84	0.8	80

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Biochemistry

ADDRESS: Washington, D.C. 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Gentry, M K

TELEPHONE: (202) 576-3527

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Rush, R

NAME: Doctor, B P POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Monoclonal; (U) Peptide; (U) Antibody; (U) Determinants

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective of this project is to elucidate antigenic determinants of infletious agents and other macromolecules against which monoclonal antibodies are directed so that synthetic peptide vaccines can be produced and tested for bioefficacy. There is military relevance in this research.

24. (U) Monoclonal antibody against peptide or protein antigens will be generated by accepted techniques. Those antigens which are difficult to obtain in sufficient quantities will be used in In Vitro immunization techniques using serum free media. The hybrid cells will be grown and the antibody secreted in culture media will be purified. The purified antibody will be coupled to Protein A sepharose using Sublimate reagent. The stable solid support-antibody thus obtained is known to have Fab region free on its surface to bind with the specific antigenic sites that it recognizes. The protein antigens will be digested with trypsin or chymotrypsin. One half of this proteolytic digest will be subjected to HPLC to obtain peptide maps. The other half will be applied to protein A sepharose-antibody solid support. The effluent from this chromatography will be subjected to HPLC to obtain the differential peptide maps. The missing peptide peaks in the second map will be isolated from original HPLC map of proteolytic digest. These peptides will be sequenced. A series of peptides containing various combinations will be synthesized and tested for their antigenic property and eventually for its protective activity.

25 (U) New.

* Available for instructions upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND USER MAY BE FOR AWAY USE ARE OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2531	83 09 30	DD DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. CHRON MASTER	8B. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	B. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	110 WRH6
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code):

(U) Genetic Basis of Virulence of Bacterial Pathogens

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	83 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER:

c. TYPE: d. AMOUNT

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2.0	93
FISCAL YEAR CURRENT 83	2.0	100

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20307

RESPONSIBLE INDIVIDUAL

NAME: Russell, P K

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Biochemistry

ADDRESS: Washington, D.C. 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)

NAME: Gemski, P

TELEPHONE: (202) 576-2594

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Lazere, J

NAME:

21. GENERAL USE

Foreign Intelligence Considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Gene; (U) Virulence; (U) Antigens; (U) Plasmids; (U) Chromosome

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective is to study the chromosomal and plasmid genes controlling virulence determinants of enteric pathogens and to alter by genetic manipulation such virulence factors so as to define their role in patho-physiological and invasive steps of diseases and to develop attenuated vaccines and improved methods to prevent and treat enteric disease in military personnel operating in areas of poor sanitation.

24. (U) The approach is to prepare mutants, chromosomal hybrids, plasmid transconjugants and transformants in strains of invasive intestinal pathogens which are altered in genes for somatic antigens, toxins and other factors and to assess the impact of such alterations on virulence.

25. (U) 82 10 - 83 09: This work unit has been terminated after a 3 year period as a 91C project. Further studies are being continued in other work units. Colonial characteristics have been correlated to presence and expression of virulence plasmid function. Virulence and phenotypic characterization of Y. enterocolitica isolated from humans in the USA has revealed that no single assay correlates with virulence in Y. enterocolitica. Molecular homology comparisons of the 82 and virulence plasmid showed little, if any relationships. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 110 Genetic Basis of Virulence of Bacterial Pathogens

INVESTIGATORS:
Principle: Peter Gemski, Ph.D.
Associate: George Fanning, B.S., M.S, Janet Lazere, B.S., Tim Merriweather, SP5 and Dana H. Wells, B.S., SP4.

In collaboration with: A.D. Cross, M.D. (DCD & I); K. Wachsmuth, Ph.D., (DCD & I); J.A. Wohlhieter, Ph.D. (DCD & I); W. Zollinger, Ph.D. (DCD & I).

DESCRIPTION:

Studies on the pathogenesis of enteric infections have established that some organisms evoke diarrheal disease by an invasive mechanism by which the pathogen penetrates and replicates within gastrointestinal tissue. Without doubt, several bacterial attributes must function in concert to allow expression of enteric pathogens.

An understanding of chromosomal and plasmid genes associated with invasive properties of enterics will provide basic information needed to facilitate the development of (1) live attenuated vaccines and (2) improved methods for prevention and treatment of intestinal infections in military personnel operating in areas of poor sanitation. Although such diseases are temporary, they are sufficiently devastating to interfere seriously with military activities.

Mutants, chromosomal hybrids, plasmid derivatives, transformants and transconjugants of Shigella, Yersinia, Salmonella and E. coli which are altered in antigens, toxins and other factors associated with virulence are being prepared and analyzed biochemically, genetically and immunologically to assess the impact of such alterations on virulence. Various small animal models of infection are also employed.

Progress:

A. Studies of the Virulence and Plasmids of Yersinia enterocolitica

In our study of the virulence and plasmid properties of Y. enterocolitica, we have found that plasmids are associated with the invasiveness of strains. Furthermore, we have revealed that strains harboring such plasmids are calcium dependent when grown in vitro at 37C. Such a temperature dependent calcium deficiency in Y. pestis has been correlated to the production of the V and W antigen complex, an important virulence determinant of plague bacilli. Two plasmid types have been identified and designated as Vwa and 82.

1. Molecular Homology Among Plasmids of Yersinia enterocolitica.

We have shown previously that Y. entercolitica contain plasmids associated with virulence. Vwa virulence plasmids, 41 - 46 Mdal in size, are associated with calcium dependency and production of the V and W antigen complex. Another plasmid type, about 82 Mdal in size, is found with the Vwa plasmid in certain serotypes of Y. enterocolitica and appears to be associated with disseminating lethal infections in mice. We have examined the molecular homology among these plasmids isolated from serotypes 0:3, 0:8 and 0:20. All Vwa plasmids had a high degree of genetic relatedness, sharing over 50% DNA sequence homology at optimum reassociation temperatures (60°C). Comparison of an 82 Mdal plasmid with representative Vwa plasmids revealed less than 30% homology. The similarity between 82 and Vwa plasmids was further studied by comparing DNA fragmentation patterns following treatment with restriction nucleases. Such enzyme digests of the 82 plasmid were distinct from those of the Vwa class. Our results show that the 82 plasmid is in a DNA hybridization group distinct from the Vwa plasmids.

2. Virulence and Phenotypic Characterization of Yersinia enterocolitica Isolated from Humans in the United States.

Yersinia enterocolitica was recently reclassified into Yersinia enterocolitica sensu stricto and three additional species. With this new classification, it was of interest to reexamine pathogenicity previously ascribed to Y. enterocolitica. All available clinical isolates of Y. enterocolitica sent to the Centers for Disease Control from 1970 through 1980 were selected for characterization and comparison. One-hundred such strains had been submitted, from 21 states. Most (85%) were biotype 1, and 0:8 was the most common of the 24 serotypes encountered. All strains were examined by several virulence assays. Two strains caused conjunctivitis in guinea pigs, 7 were lethal for mice, 54 invaded HEp2 cells, 18 produced a heat-stable enterotoxin, 9 were calcium dependent, 20 autoagglutinated, and 34 had a distinctive colonial morphology at 37°C. Ten isolates of each of the new species that had previously been grouped with Y. enterocolitica (Y. kristensenii, Y. intermedia, and Y. frederiksenii) were characterized and were generally negative in all assays. This study points out pathogenicity differences among Yersinia species, confirms the complex nature of virulence in Y. enterocolitica, and confirms that no single current assay correlates with virulence in Y. enterocolitica.

3. Association of Colony Morphology with virulence of Yersinia enterocolitica.

Our studies of Y. enterocolitica have revealed a correlation between colonial characteristics and Vwa plasmid related functions. Strains of serotype 0:8 which produce small opaque (O) colonies when cultivated at 37°C were found to possess Vwa plasmids, expressing calcium dependency, autoagglutinate and cause invasive disseminating infections in mice. Translucent (T) colonies which are segregants from O derivatives were found to have lost the Vwa plasmid and its associated virulence properties. A similar colonial variation was also seen among the serotype 0:3 strains that we screened. We characterized the virulence of T and O

derivatives of serotype O:3 strains by scoring for production of diarrhea. T colony segregants of serotype O:3 strains which had lost the Vwa plasmid, calcium dependency and autoagglutinating characteristics, also failed to provoke diarrhea in mice.

The physiological basis for O and T colony characteristics remains obscure. Expression of an O colony morphology is dependent however on the presence of Vwa plasmid and cultivation at 37°C. No colonial distinctions were observed when the strains were grown at 26°C.

We have found examination of colony morphology to be a simple and rapid method for isolating isogenic virulent/avirulent derivatives of Y. enterocolitica. Recent screening studies of other Y. enterocolitica isolates have now established similar colony variation in serotype O:5, O:7, O:8, O:13, O:20, and O:40. The use of colony morphology however as a reliable screening step for determining possible virulence of Y. enterocolitica isolated from clinical and food sources awaits now to be examined.

B. Studies of K1 Antigen as a Virulence Factor

1. Evaluation of Immunotherapeutic Approaches for the Potential Treatment of Infections Caused by K1-Positive Escherichia coli.

Levels of antibody to the K1 polysaccharide capsule were examined in sera from patients naturally infected with K1-positive Escherichia coli, in sera from volunteers vaccinated with a group B meningococcal vaccine, in immune globulin prepared for intravenous use, and in a preparation of murine IgM monoclonal antibody to group B meningococci. In a phagocytic assay, the monoclonal antibody in mouse ascites fluid killed K1-positive E. coli to a final dilution of 1:250,000 (48 ng of antibody protein/ml); no killing was observed with any of the other antibody sources. This monoclonal antibody, which required human complement and exhibited a prozone when high concentrations of antibody (>6 ug/ml) were used, killed all K1-positive strains but none of the K1-negative strains of E. coli tested. Levels of K1-binding antibody in the sera of vaccinated volunteers exceeded antibody levels resulting from natural infection or present in commercially prepared immunoglobulin and were less than those obtained in mouse ascites fluid containing the monoclonal antibody.

C. Future Projections:

This work unit has been merged with work unit 124, DA OC 644, page 163 on Biochemical Research on Military Diseases.

Publications:

1. Cross, A., W. Zollinger, R. Mandrell, P. Gemski and J. Sadoff. Evaluation of Immunotherapeutic Approaches for the Potential Treatment of

Infections Caused by K1-positive E. coli J. Inf. Diseases, 147: 68 - 75

2. Kay, B.A., Kaye Wachsmuth, P. Gemski, J.C. Feeley, T.J. Quan and D.J. Brenner. 1983. Virulence and Phenotypic Characterization of Yersinia entero-
colitica Isolated from Humans in the United States. J. C Microbiol. 17: 128 - 138.

3. Lazere, J. and P. Gemski. 1983. Association of Colony Morphology with Virulence of Yersinia denterocolitica. FEMS Microbiol., 17: 121 - 126.

Abstracts:

1. Fanning, G.R., J.R. Lazere, J.N. Coulby, J.A. Wohlheiter and P. Gemski. 1983. Molecular Homology Among Plasmids of Yersinia enterocolitica. Am. Soc. Microbiol., Abst. B78.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA 363 108 | 2. DATE OF SUMMARY: 83 10 01 | REPORT CONTROL SYMBOL: DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPOSITION	8A. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	[illegible]	[illegible]		111
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE: (U) Studies on the Interactions between Prostaglandins and Glucocorticoids to Improve Chemotherapeutic Measures for Radioprotection, Radiation Disease

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 012600 Pharmacology; 014100 Radiobiology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	[illegible]	DA	C. In-House

17. CONTRACT/GRANT: a. DATES/EFFECTIVE: ; EXPIRATION: ; b. NUMBER: ; c. TYPE: ; d. AMOUNT: ; e. NO OF AMND: ; f. CUM. AMT:

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR FREEZING 83	0.0	00
FISCAL YEAR CURRENT 84	2.0	13

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL
NAME: [illegible], F B
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of Experimental Therapeutics
ADDRESS: Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide name if U.S. Academic Institution)
NAME: HEIFFER, M H
TELEPHONE: (301) 427-5393
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: NIELSEN, C
NAME: WRIGHT, N POC:DA

21. GENERAL USE: Foreign Intelligence Considered

22. KEYWORDS: (U) Radioprotectants; (U) Prostaglandins; (U) Glucocorticoids; (U) Metabolism

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS: (Provide brief paragraphs identified by number. Precede text of each with security classification code.)

23. (U) The purposes of this project are to further the understanding of how current antiradiation drugs function and to develop some of the scientific information necessary to design improved drugs and treatment regimens for radioprotection. The focus of the effort will be on better understanding and control of the vasoactive prostaglandins the production of which is stimulated by radiation of animal tissue. The ultimate goal is to improve the pharmacological protection of military personnel faced with the threat of radiation from nuclear weapons employment. It is anticipated that this information will also be of value to other military medical needs such as the treatment of radiation disease and prevention of irreversible shock.

24. (U) The first phase of this work will be to examine the effects of current radioprotective drugs on prostaglandin synthesis and on the controls of that synthesis rendered by glucocorticoids. This will be conducted in cellular, subcellular, and whole animal preparations. The next phase will be to examine how the effects of radioprotectant drugs can be modified by supplementary drugs that affect prostaglandin synthesis i.e., steroids, antisteroids, aspirin, and indomethacin. Finally, the information obtained from these studies will be tested in whole animals irradiated under controlled conditions.

25. (U) [illegible].

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498A 1 NOV 69

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | DA 303 109 | 83 10 01

3. DATE PREV SUMM	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. SUMM SECURITY	7. RESTRICTED	8a. INSTITUTION	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	112 WW111
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Proceed with Security Classification Code):

(U) Effects of Ionizing Radiation of Intestinal Motility and Flora

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

010100 Microbiology 012900 Physiology 014100 Radiobiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGCY.	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
	83	0.0	00
	84	1.2	30

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Division of Medicine
Washington, D.C. 20307

PRINCIPAL INVESTIGATOR (Civilian or Military)

NAME: Sjogren, R W

TELEPHONE: (202) 576-3530

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Dubois, A

NAME: Boedeker, E C

NAME: Sjogren, M H

POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Intestinal Motility; (U) Radiation; (U) Hepatitis; (U) Diarrhea

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief explanatory statements in numbered sequence for each with Security Classification Code)

23. (U) Ionizing radiation is a major non-conventional, anti-personnel measure resulting in serious gastrointestinal manifestations including vomiting, diarrhea, hepatitis, enteric infection, sepsis and death. Our objective is to describe the effects, mechanisms and medical countermeasures of ionizing radiation on gastrointestinal and hepatic structure and function. There is military relevance in this research.

24. (U) An initial descriptive study in rhesus monkeys with implanted intestinal electrodes will describe the effects of a single, lethal exposure of ionizing radiation on intestinal motility, morphology and flora and on hepatic morphology. Subsequent studies will assess the mechanisms and pharmacologic modulation of these effects and possibly the effects of repeated sublethal radiation exposures on these parameters and on intestinal immune function.

25. (U) New.

* Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND MAY BE USED UNTIL EXHAUSTED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | DA 303 110 | 83 10 01

3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. KIND SECURITY	7. GRADE	8. CONTRACTOR INTERACTION	8a. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	(X) YES ( ) NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	113 WUIN
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Effect of Anticholinesterases on Gastrointestinal Motility

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012900 Physiology 012600 Pharmacology 002400 Bioengineering 004200 Computers

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING	16. PERFORMING METHOD
84 01	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE	EXPIRATION			
b. NUMBER				
c. TYPE	d. AMOUNT			
e. KIND OF AWARD	f. CUM. AMT			

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
FY PRECEDING	0.0	00
FY 84	0.5	30

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Division of Medicine
Washington, D.C. 20307

PRINCIPAL INVESTIGATOR (Current Year if DoD Academic Institution)

NAME: Sjogren, R W

TELEPHONE: (202) 576-3530

SOCIAL SECURITY ACCOUNT NUMBER

RESEARCH INVESTIGATORS

NAME: Van Albert, S

POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede each with Security Classification Code)

(U) Anticholinesterases; (U) Diarrhea; (U) Gastrointestinal Motility; (U) Cholinergic Nervous System; (U) Signal Analysis

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by item number. Precede text of each with Security Classification Code.)

23. (U) Both chemical warfare agents and their medical countermeasures are generally active against the nervous system, especially the cholinergic nervous system, and both may result in serious gastrointestinal disturbances including vomiting, abdominal pain, malabsorption and diarrhea. Our objective is to describe the nervous control of gastrointestinal function and the effects and interactions of potential chemical defense treatments on these functions. There is military relevance in this research.

24. (U) An initial pilot study will establish a model (probably rabbit or/and rat) to record GI motility from unrestrained animals and will develop computer programs to interpret the motility patterns recorded. Subsequent studies will describe motility patterns, investigate their control mechanisms and study the effects and/or interactions of commonly used drugs and potential chem defense treatments on these patterns. Potentially, the effects of these motility patterns on gastrointestinal flora, immune function and/or fluid and electrolyte transport could be investigated.

25. (U) New.

* Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND WILL NOT BE USED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303 112	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
A. PRIMARY	61101A	3A161101A91C	00	114 WWSA
B. CONTRIBUTING				
C. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Antigenic Epitopes of Dengue Viruses

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

A. DATES/EFFECTIVE: EXPIRATION:

B. NUMBER:

C. TYPE: D. AMOUNT:

E. KIND OF AWARD: F. CUM. AMT.

18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	B. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	0.0	00
	CURRENT 84	2.0	150

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Division of CD&I
Washington, DC 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: BANCROFT, W H

TELEPHONE: (202) 576-3757

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: FEIGHNY, R J

NAME: BRANDT, W E POC: DA

21. GENERAL USE:

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Arbovirus; (U) Dengue Virus; (U) Antigen; (U) Immunology

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To characterize the important antigenic epitopes of dengue viruses by isolation and purification of the envelope glycoprotein. This is an essential preliminary step to the development of alternative vaccines to the attenuated, live virus vaccines. Vaccines are the only feasible means for preventing epidemic dengue fever in American soldiers.

24 (U) Dengue viral envelope proteins will be purified in natural and denatured forms from large volume cell culture harvests. Purification will utilize high speed centrifugation, isoelectric focusing and affinity chromatography. Epitopes will distinguished by their reactivity with monoclonal antibodies and characterized by amino acid sequencing.

25 (U) New.

*Available for instructions upon originator's approval

DD FORM 1 MAR 86 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 86 (FOR ARMY USE) ARE OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303 113	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	115 WR3B
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Immunopotentiation of Microbial Peptide Antigens

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 012100 Organic Chemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	0.0	0 0
	CURRENT 84	2.0	35

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL
NAME: Top, F H
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Div CD&I
Washington, DC 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Lowell, G H
TELEPHONE: (202) 576-3058
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Seid, R
NAME: Zollinger, W
NAME: Hall, T, Smith, L. INOC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Peptide Vaccine; (U) Adjuvants; (U) Outer Membrane Proteins; (U) Trypanosomes; (U) MDP

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Peptide vaccines are advantageous because contemporary techniques can readily be used to synthesize great quantities of pure peptides derived from many microorganisms which cause diseases of military importance. Such small peptides, however, invariably lack immunogenicity when administered without adjuvants. Most currently available adjuvants, however, are toxic. The purpose of this work unit is to develop safe and efficacious immunopotentiating methodology and agents which will enhance the immunogenicity of peptide antigens. Trypanosomes cause extensive disease among millions of people in Africa. Troops deployed in endemic areas expect high morbidity. Current treatment is insufficient or non-existent. Synthetic peptides to be used in this unit will be identical to conserved portions of the variable surface glycoprotein (VSG) of african trypanosomes.

24. (U) Peptides representing four areas of VSG which are common to many trypanosome variants will be synthesized by Peninsula Labs., Inc., according to our specifications. These peptides will be covalently linked to muramyl dipeptide (MDP) analogs, fatty acids or detoxified LPS and then used either directly or following hydrophobic complexing to meningococcal outer membrane protein vesicles (protosomes). Peptides synthesized with trypanosomal hydrophobic tails will be used alone and complexed to protosomes without lipid spacers. Control mice will be immunized with peptide alone, in Freund's adjuvant or alum, or bound to KLH. Thus, the role of covalent and hydrophobic complexing will be compared using adjuvants and peptides with significant human use potential.

25. (U) New.

* Additions to contractor upon author's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA 303 114	2. DATE OF SUMMARY: 83 10 01		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY	4. KIND OF SUMMARY: A. New	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8a. ORG'N INSTR'N: RL	8b. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT
10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61101A	3A161101A91C		00	116	WUSC	
b. SECONDARY							
c. CONTRIBUTING							

11. TITLE (Include with Security Classification Code)
(U) DNA Hybridization Identification of Leishmania in Mammals and Insect Vectors

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	0.0	00
	CURRENT 84	1.0	45

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
Washington, DC 20307
ADDRESS:

RESPONSIBLE INDIVIDUAL
NAME: Top, F H
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Div CD&I
Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)
NAME: Jackson, P
TELEPHONE: 202-576-3063
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign Intelligence Considered

POC: DA

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) DNA Hybridization; (U) Leishmania; (U) Parasite; (U) Identification

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) The objective of this work is to develop a sensitive DNA Hybridization procedure to detect and identify Leishmania parasites. Leishmaniasis is endemic in Africa, the Mid-East, Indian Subcontinent and South America posing a significant potential threat to military operations in these areas.

24 (U) DNA will be extracted from Leishmania of different species and labelled by radioactive and non-radioactive means to construct species-specific DNA probes. These probes will be hybridized to Leishmania from infected tissues or insect vectors to develop sensitive methods for detecting and identifying Leishmania. Sensitive detection methods will assist Leishmania chemotherapy procedures, drug and vaccine development and epidemiology studies.

25 (U) New.

*Available to contractors upon originator's approval.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 83

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 303 115	2. DATE OF SUMMARY* 83 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY A. New	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8. ORG'N METH'N NL	8A. SPECIFIC DATA - CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	117 WWIY
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code)*

(U) Lymphocyte Paralysis in Malaria - The Role of Cyclic Nucleotide Metabolism

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012600 Pharmacology, 012900 Physiology, 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
83 10	Cont	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	0.0	00
	CURRENT 84	3.0	40

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Walter Reed Army Institute of Research

ADDRESS:* Washington, DC 20307

RESPONSIBLE INDIVIDUAL

NAME: Top, F H

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME:* Walter Reed Army Institute of Research
Division of Medicine

ADDRESS:* Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)

NAME:* Wiesmann, W P

TELEPHONE: (202) 576-3636

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Webster, H K

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Malaria; (U) Lymphocytes; (U) Acquired Immunodeficiency; (U) Cyclic Nucleotides; (U) Adenosine Receptor

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Provide text of each with Security Classification Code.)

23.(U) 1. Define lymphocyte subset profiles in relation to the stage of infection and immune status of malaria infected individuals. 2. Define purine nucleotide pathways in normal and infected lymphocytes with emphasis on adenylate cyclase substrate availability. 3. Study lymphocyte adenylate cyclase in membrane preparations. a. Is an adenosine receptor interlocked with activation? b. What is the status of the GTP coupler. 4. Quantitate the effects of pharmacologic manipulation of cAMP content with known phosphodiesterase inhibitors and immunostimulents of lymphocyte blastogenesis. There is military relevance in this research.

24.(U) Lymphocytes obtained from malaria patients will be identified with available immunofluorescent tages. Cell sorting for purification of subsets will be performed with the Coulter fluorescent activated cell sorter available at WRAIR. Extractions for nucleotides profiles, purine pathway analysis, and cAMP content will be measured on selected subsets utilizing techniques already developed. Adenylate cyclase extraction and analysis will be performed at WRAIR. The effects of various immunostimulents and cAMP analogues will be performed in functional assays currently available at AFRIMS.

25.(U) New.

*Available to requestors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND FORMS 1498-A AND 1498-1 MAY BE USED UNTIL EXHAUSTED

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	Report Control Symbol
DA 303 116	83 10 01	RD-DRSE(AR)635

3. DATE PREV SUMRY	4. KIND OF SUMRY	5. SUMRY LEVEL	6. WORK SECURITY	7. REGRADING	8a. ORDER INSTR	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3M161101A91C	00	118 WRQA
b. CONTRIBUTING				
c. COMPUTING				

11. TITLE (Procede with Security Classification Code)

(U) Differentiation of Mosquito Sibling Species Using Recombinant DNA Probes

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	0.0	00
FISCAL YEAR CURRENT 84	0.3	28

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: AFRIMS

ADDRESS: Bangkok, Thailand

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)

NAME: ROSENBERG, R

TELEPHONE:

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: ANDRE, R

NAME: PANYIM, S

POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Medical Entomology; (U) Recombinant DNA probes; (U) Mosquito; (U) Malaria

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The technical objective is to develop a rapid, sensitive test using labeled, cloned mosquito DNA for differentiating morphologically identical malaria vector species, particularly members of the Anopheles balabacensis complex. There is a military requirement for this research.

24. (U) The following steps will be carried out sequentially: (a) Isolation and colonization of each of the four species, (b) Preparation, analysis and cloning of DNA from each species, and (c) Selection of clones specific only for one species and testing their specificity on wild material.

25. (U) New.

* Available in instructions upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND FORMS 1498A 1 NOV 65 AND 1498 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1284	83	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8b. ORGZN HIST'N	8c. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	H. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	119
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) The Biochemistry and Physiology of Erythrocyte Membrane Proteins: Role in Normal Erythrocyte Function and in Disease

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002600 Biology 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	19	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	PRECEDING	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR		3.6	207
	CURRENT	2.0	200

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
Washington, DC 20307
ADDRESS:

RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, P K
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Division of Medicine
ADDRESS: Washington, DC 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: [redacted], D C
TELEPHONE: (202) 576-3358
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME [redacted]
NAME [redacted]

21. GENERAL USE
Foreign intelligence considered

FOC: DA

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Erythrocyte; (U) Acetylcholinesterase; (U) Acetylcholine receptor; (U) Structural membrane proteins; (U) Leukocytes

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23.(U) To investigate the role of the acetylcholine receptor (AChR) in red blood cell (RBC) and leukocyte (whole blood cell, WBC) structure and function. To perform studies of the RBC membrane-bound enzyme, acetylcholinesterase (AChE), the function of which is closely linked to AChR. Stimulation of the AChR in excitable membranes, e.g., muscle and nerve, is terminated through hydrolysis of ACh by AChE. These studies are important for military chemical defense in the development of new approaches for treatment and prophylaxis against chemical nerve agents.

24.(U) Assays for measuring AChR by binding of labelled agonist, for calcium flux, and for cGMP generation. Isolation of purified RBC AChE by affinity chromatography. Reconstitution of AChE into artificial membranes (lipisomes) of varying lipid content and measurement of AChE activity with and without inhibitors, lipophilic agents, and with varying extracellular lipid environments. Evaluation of AChR on WBC at different stages of the maturation and function of these cells.

25.(U) 82 10-83 09 AChR Studies: By using non-hydrolyzable, radiolabelled cholinergic agonists and various neurotransmitter antagonists, a specific, saturable muscarinic AChR has been identified, characterized, and enumerated on human RBC and WBC (neutrophils) as well as on blood precursor cells harvested from normal bone marrow aspirates. These studies were extensions of previous studies done with animals. AChR studies have been carried out both with whole blood cells and plasma membrane preparations. Methods have been established to study the effects of ACh, ACh analogues, and ACh-AChE blockade on RBC rigidity, deformability and membrane integrity using blood viscometry and RBC osmotic fragility measurements. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82-30 Sep 83. Work unit has been terminated at end of FY83 due to loss of critical personnel from project.

*Available to contractors upon originator's approval

DD FORM 1498 — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Project 3A161101A91C: IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 119 The Biochemistry and Physiology of Erythrocyte Membrane Proteins: Role in Normal Erythrocyte Function and in Disease

Investigators LTC Daniel Wright, MC; MAJ Eric Schoomaker, MC; Dr. Lily Tang (IPA investigator)

Description

Studies of the physiology of blood cell membranes have concentrated upon the ectoenzyme, acetylchlinesterase, and the acetylcholine receptor complex present on the surfaces of circulating red and white blood cells as on neuromuscular tissues. This work has been concerned with the structural relationship of enzyme and receptor with the membrane lipoprotein structure as this relationship affects enzyme activity and their susceptibility to acetylcholinesterase inhibitors of military importance (nerve agents). This work also involves basic investigations into the structure of blood cell membranes in general with mature blood cells and blood precursor cells separated from normal human marrow.

Studies of the acetylcholine-acetylcholinesterase apparatus on human blood cells may provide a very accessible and useful model for understanding the toxic effects of nerve agents used in chemical warfare upon nervous tissues and for developing novel tactics for protecting against these effects.

Work in this area is aimed at three problems:

1. To investigate the role of the acetylcholine receptor (AChR) in red blood cell (RBC) structure and function. These studies are intended to delineate the relationship between AChR stimulation and RBC shape and deformability. This appears to involve the stimulation of the enyzme guanylcyclase resulting in an increase in cyclic guanosine monophosphate (cGMP). Cyclic GMP levels and other effects of AChR stimulation may be mediated by changes in calcium (Ca++) flux. Changes in intraerythrocyte calcium concentration are known to influence membrane shape and deformability; cGMP in other tissues has been observed to modulate the phosphorylation of certain key proteins. Both events may prove to play an important role in the function of the mature RBC as well as erythroid differentiation and/or proliferation in the bone marrow.

2. To perform studies of the RBC membrane-bound enzyme, acetylcholinesterase (AChE), the function of which is closely linked to AChR. Stimulation of the AChR in excitable membranes, e.g., muscle and nerve, is terminated through hydrolysis of ACh by AChE. Its function in the RBC remains unknown. It is an ideal source of membrane-bound, lipid-dependent enzyme for investigations of the regulation of such enzymes by changes in membrane lipids. Such studies should prove useful in our understanding of the control of enzyme activity under normal conditions. In addition, protection against complete inactivation of AChE by inhibitors such as the anti-AChE nerve agents may be afforded through alterations in the lipid microenvironment of the enzyme.

3. To investigate the mechanism by which abnormal RBC structural membrane proteins result in premature RBC destruction. Techniques developed to study the above two issues have led to methods by which dysfunctional structural protein mutations may be examined. Abnormal interactions among these proteins appear to

underline RBC shape changes and cell lysis in disorders such as hemolytic hereditary elliptocytosis.

Progress

1. Because of transfer of key personnel involved with this project, this work unit has been terminated as of the end of FY83. Work during the final year of this project has focused principally upon the characterization of cholinergic receptors (AChR) of human neutrophils and myeloid precursor cells.

Cholinergic agents have been shown to enhance the functional responses of neutrophils (e.g. chemotaxis and secretion) and to interact with these cells via specific receptors (AChR) analogous to those on neuromuscular and secretory cells. To evaluate AChR availability on human neutrophils during their maturation and function, neutrophils and neutrophil precursor cells were isolated from aspirated marrow and remove blood of volunteers and from exudates produced in skin chambers. Cells were incubated with the muscarine antagonist, quinuclidinyl-benzilate (^{3}H-QNB), at 22 °C for 20 min with and without atropine. QNB bound to cells collected on glass fiber filters, or pelleted by centrifugation through silicone oil to separate cell-bound from free radioactivity, was then measured. Specific, atropine-displaceable ^{3}H-QNB binding (SQB) by blood neutrophils was shown to be saturable with 0.8 - 1.5 x 10^5 binding sites per cell and a Kd for binding of 8-14 nm. Neutrophilic cells isolated from marrow by density gradient/sedimentation (enriched in blasts, promyelocytes and myelocytes) had 2 to 4 times more SQB sites/cell than did blood neutrophils. The immature, human promyelocytic leukemia cell line, HL-6 , also had greater receptor numbers (> 10^6/cell). In contrast, SQB by exudate neutrophils and by blood neutrophils exposed to the chemotactic peptide, f-met-leu-phe (10^{-7}M), was 30-50% less than that of control neutrophils. These studies have demonstrated that muscarinic, cholinergic receptors are acquired early in the development of myeloid cells, are particularly numerous on neutrophil precursor cells recovered from the hematopoietic marrow, and are lost or become less available for binding once neutrophils are engaged in an intlammatory response. These findings have suggested the novel concept that a cholinergic response apparatus may be particularly important to neutrophils during their development and storage in the marrow, which is a richly innervated organ.

Studies of isolated neutrophil membrane preparations have further indicated that SQB by neutrophils is associated with a separable, membrane AChR structure and that binding is not influenced by anion channel blockers.

2. In order to assess the effects of cholinergic agonist-receptor interactions upon the erythrocyte (RBC) functions of rigidity and shape change, techniques were established to measure these RBC characteristics with a disc viscometer that is adapted to a video camera system which can record the character and degree of RBC shape changes in vitro in response to shear stresses.

Future Plans

As indicated above this project has been terminated as a specific work unit. Certain derivitive projects from research conducted in this work unit will be pursued as part of other work units within the Dept. of Hematology, WRAIR.

Publication

1. Tang, L.C., Schoomaker, E.,and Weismann, W.P.: Cholinergic agonists stimulate calcium uptake and cGMP formation in human red blood cells. Science (in review), 1983.

2. Wright, D.G., Meierovics, A.I., Lucas, D.L., Schoomaker, E., and Tang, L.: Muscarinic cholinergic receptors on human neutrophils during their development and function. J. Clin. Invest. (in review), 1983.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1296	83 09 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORGN INSTR'N	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	H, Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	120 WW09
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with security classification code): (U) Identification of Virus Polypeptides in Immune Complexes in Dengue Hrmorrhagic Fever Sera

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	83 10	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE — EXPIRATION
b. NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE

	FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING	82	2.0	204
CURRENT	83	2.0	200

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER

21. GENERAL USE
Foreign intelligence considered.

ASSOCIATE INVESTIGATORS
NAME: HENCHAL, E A
NAME: BRANDT, W E POC: DA

22. KEYWORDS (Precede each with security classification code)
(U) Arbovirus; (U) Dengue Virus; (U) Antigen; (U) Immunology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security classification code.)

23 (U) Dengue Fever (DF) and dengue hemorrhagic fever (DHF) occur in epidemic form throughout tropical areas and are significant health hazards to military personnel. The pathogenesis of DHF is attributed to the formation of antigen-antibody complexes. Evaluation of dengue antigen interactions with serotype specific and crossreactive antibodies using an experimental model (mouse polyclonal and monoclonal antibodies) as well as antisera from DF, DHF, and dengue virus vaccine recipients will allow a precise understanding of the immune response to dengue antigens.

24 (U) Attempts to isolate dengue specific immune complexes using specialized solid phase methods have not been successful. The current approach consists of (1) examining the epitopic structure of major dengue antigens using monoclonal antibodies and (2) relating the immune response of DF and DHF patients as well as dengue vaccine recipients to specific antigens and antigen epitopes.

25 (U) 82 10-83 09 Monoclonal antibodies prepared to each type of dengue virus were classed as type-specific, subcomplex-specific dengue complex-specific or flavivirus group-reactive. These antibodies were used in competitive antibody binding assays to evaluate the spatial relationships of antigenic epitopes on the surface of purified dengue-2 viruses. At least 4 major epitopes were discovered. Some antibodies actually promoted the binding of others, representing a new interaction of dengue antibodies. Mouse protection assays are being used to determine which antigen epitopes should be blocked to gain maximum immune protection. This work will facilitate the development of future dengue vaccine. This research will be continued in the Institute's integrated research program. (For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82, to 30 Sep 83).

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE
* U.S. GPO: 1974-540-843/8491

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 120 Identification of Virus Polypeptides in Immune Complexes in Dengue Hemorrhagic Fever Sera

Investigators:

Principal:

CPT Erik A. Henchal, MSC
Walter E. Brandt, Ph.D.
COL William H. Bancroft, MC

Associates:

Ms. Jeanne Burrous, MS
SSG Wanda Williams
SP5 Matthew Seguin

Problems and Objectives

Soluble immune complexes have been found in the sera of dengue hemorrhagic fever (DHF) patients and are believed to play a critical role in the pathogenesis of this disease. But the immune complexes are so far unidentified. The development of a battery of mouse monoclonal antibodes to dengue viruses provides a new tool for identifying the critical viral epitopes. The objectives are to examine the epitopic structure of major dengue antigens using monoclonal antibodies and to detail the immune responses of dengue fever (DF) and DHF patients and dengue vaccine recipients to specific viral antigens and antigenic epitopes.

Progress

Monoclonal antibodies (mAB) prepared against the four dengue (DEN) virus serotypes have been used to examine the structure and biological function of eight unique epitopes on the surface of the DEN-2 virion. The specificities of the mAB and the spacial arrangement of their binding sites were determined by hemagglutination-inhibition (HI), plaque reduction neutralization (PRNT), immunofluorescence (IF), and competitive antibody binding assays. Further characterization of their biological functions included antibody-mediated enhancement of virus yields and mouse passive protection assays. Each mAB was tested against a panel of arboviruses using an indirect immunofluorescence assay. The mABs were grouped into four specificity groups: serotype-specific, DEN subcomplex-specific, DEN complex-specific, and flavivirus group-reactive. Each specificity group, however, contains mABs which

differ in their relative reactivity in the common serological assays. Competitive antibody binding assays, in which dilutions of unlabelled mABs were allowed to compete with partially purified radiolabelled mAB for antibody binding sites, suggested that four spacially distinct antigenic regions exist on the surface of the virus. Antibodies to region 1 (4 epitopes) were either a) type-specific, b) subcomplex-specific by IF, HI, and PRNT assays, or c) subcomplex specific by IF and HI assays only. Antibodies to region 2 were either a) type-specific, or b) complex-specific by IF, HI, and PRNT assays, or c) subcomplex specific by IF and HI assays. Antibodies to region 3 (1 epitope) were complex-specific by IF assay only; and antibodies to region 4 (1 epitope) were flavivirus group-reactive by IF and HI, but DEN complex specific by PRNT. Some mAB increase on "promote" the binding of other mAB in this test. For instance, antibody to regions 2 and 4 mutually promoted each other, while one DEN subcomplex mAB to region 1 promoted antibody to regions 2 and 4. The in vivo significance of this promotion phenomenon has not been determined, but the data suggests that conformational changes may occur in the antigen when highly specific antibody is bound to it. Mouse passive protection studies suggested that all of the mABs could reduce mortality. However, mAB to region 3 which lacks significant HI and PRNT titers, passively protects mice against intracerebral challenge and participates in antibody-mediated enhancement of virus yields in vitro. Other mABs required helper antibody to induce full protection. All of the mABs studied could enhance DEN-2 virus yields in U-937 (human monocyte) cells except for serotype specific antibody when a low multiplicity of infection was used.

Recommendation

This work unit has been highly productive during the past three years. It has provided dengue monoclonal antibodies which are accepted as diagnostic reagents around the world. Moreover it has done the ground work to map the surface epitopes of dengue viruses. This work has been incorporated into that of other work units.

Presentations

Henchal, E.A., Brandt, W.E., McCown, J.M., Gentry, M.K., Repik, P.B. and Padmanabhan, R.K. Recent Advances in the Molecular Biology of The Dengue Viruses. WHO International Meeting on Dengue Hemorrhagic Fever, Kuala Lumpur, Malaysia, September 1983.

Abstracts

Kalyan, N., Nohara, M., Henchal, E.A. and Padmanabhan, R.K. Structural Analysis of Dengue-2 Virus Genome. Fed. Proceedings 42: Abstract No. 1257, 1983.

Publications

1. Henchal, E.H., McCown, J.M., Seguin, M.C., Gentry, M.K. and Brandt, W.E.
   Rapid Identification of Dengue Virus Isolates by Using Monoclonal Antibodies in an Indirect Immunofluorescence Assay. Amer. J. Trop. Med. Hyg. 32: 164-169, 1983.

2. Henchal, E.A., Brandt, W.E., McCown, J.M., Gentry, M.K., Repik, P.B. and Padmanabhan, R.K. Recent Advances in the Molecular Biology of the Dengue Viruses. Proceedings of the International Conference on Dengue/Dengue Hemorrhagic Fever. University of Malaya Press (In Press), 1983.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6750	83 09 30	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORD'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	H. Prog	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	122 WW1C
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Studies of Vitamin B12 and B12 Binding Proteins for the Development of Antidotes to Acute Cyanide Poisoning

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
008800 Life Support 002600 Biology 012900 Physiology 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	83 10	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE — EXPIRATION
b. NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 82	2.5	217
CURRENT FISCAL YEAR 83	2.5	200

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: [illegible], B
NAME: [illegible] POS: PA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Vitamin B12; (U) Cobalamins; (U) Transcobalamins; (U) Hemaglobin; (U) Cyanide

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23.(U) To study the use of Vitamin B12 analogues (hydroxocobalamin, B12a, in particular) as prophylactic and therapeutic antidotes for acute cyanide poisoning. Development of new methods of protecting troops against chemical agents such as cyanide is of great importance to chemical warfare defense.

24.(U) Laboratory studies include the evaluation of different B12 analogues that have different substiution groups associated with the cobalt moiety of the molecule for binding affinity for CN. Radioisotopic and physiochemical techniques will be developed to study urinary excretion of B12 and CN, in order to follow the kinetics of CN excretion mediated by B12 in animal models of acute CN poisoning. The relative susceptibility of animals to CN toxicity will be related to blood B12 levels maintained at different levels artificially. Laboratory studies also include animal models of acute cyanide poisoning using mice, rats, and dogs that given intravenous cyanide salt with and without prior loading with B12 compounds.

25.(U) 82 10-83 09 To develop an animal model for studying the protective effects of varying blood levels of B12a against CN, pharmacokinetic studies were carried out with female foxhounds. The distribution and excretion kinetics of CN-B12 and B12a were defined with computer modeling in these animals using single bolus, IV doses: 5, 10, and 25 mg/kg. These data were then applied to develop formulae for loading and maintenance B12a doses that would maintain B12a plasma levels in the animals constant at different discrete levels. The model was adapted to study of CN^- effects with and without B12a. Protective effects of B12a against CN intoxication are being defined. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82-30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 1 MAR 68 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3A161101A91C: IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 122 Studies of Vitamin B_{12} and B_{12} Binding Proteins for the Development of Antidotes to Acute Cyanide Poisoning

Investigators Dr. Harold Williams, MS (GS-13); LTC Daniel G. Wright, LTC Charles Pamplin, MC (Div. Exp. Therapeutics, WRAIR); LTC John A Kark, MC

Description

Studies of the biology and biochemistry of Vitamin B_{12} have concentrated upon the use of B_{12} analogues as antidotes to acute cyanide poisoning. Although it has been recognized for some time that B_{12}a (hydroxocobalamin) may be a useful cyanide antidote, our work represents the first rigorous pharmacologic studies of this question. Our objective is to study the feasibility of using B_{12}a both as a therapeutic and a prophylactic measure against cyanide poisoning as may be encountered by military personnel during chemical warfare.

The use of cyanide gas (HCN) by a military adversary in the event of tactical warfare is considered to be a serious possibility. The feasibility of treating poisonings of troops in a combat zone is likely to be very difficult considering the rapidity with which toxicity occurs and the problems of transporting troops to an appropriate treatment facility. Therefore, the development of prophylactic measures that can be used when exposures are likely to occur is of considerable military importance.

Hydroxocobalamin (B_{12}a) avidly binds cyanide anion (CN) to form Vitamin B_{12} (CN-B_{12}) which is rapidly excreted by the kidneys if plasma levels of CN-B_{12} exceed the plasma protein binding capacity for cobalamins. It has been recognized for some time that B_{12}a might be a useful antidote against cyanide poisoning but rigorous pharmacologic studies of its use for this purpose have not been done. Our initial studies involved the use of mice and rats to define the capacity of B_{12}a administered intravenously to detoxify cyanide salt given to the animals intravenously or intraperitoneally. Subsequent studies with dogs have been designed to define the pharmacokinetics of very large doses of B_{12}a administered intravenously. Dogs are being used to determine the prophylactic, antidotal effects of B_{12}a maintained at different plasma concentrations against challenge with cyanide when B_{12}a is given to the animals by itself or in combination with other agents with anti-cyanide effects (e.g. sodium thiosulfate). The emphasis of these studies is to define the feasibility of using B_{12}a to increase the resistance of an individual to the toxic effects of an exposure to cyanide gas (HCN).

Progress

The three-year term of this ILIR project was completed in FY83. During this year, study of the animal model for cyanide toxicity and B12a protection, developed in female foxhounds, continued along lines previously outlined.

Dogs were given measured doses of CN in both the presence and absence of Cbl-OH. Various physiological repsonses were observed and recorded and blood and urine specimens were analyzed for total vitamin B_{12} as well as total and

free CN. The efficacy of the use of Cbl-OH as an antidote was determined by giving dogs loading and maintenance doses of this medication. We found that to achieve a blood level of 0.01 mg/ml, a loading dose of 1.08 mg/Kg and a maintenance dose of 1.7 mg/Kg/ml/min were required, when maintenance drug was given at a flow rate of 1.0 ml/min. Administration of Cbl-OH alone produced no observable adverse physiological effects on the animals tested. Dogs were given bolus injections of CN alone. These were given over 15-20 minute time intervals in increments ranging from 0.05 - 0.6 mg/Kg. Incremental increases in CN administration produced symptoms which were more acute and included a rapid rise in the blood pressure, rapid breathing (until the apparent point of lethal doses were reached) and tachycardia. It was observed that bolus injections of CN produed these effects at significantly lower total concentrations than when the same equivalent of CN was administered by constant IV infusion. Dogs were given bolus injections of CN while being constantly infused with Cbl-OH in which the blood level of Cbl-OH was maintained at 0.02 mg/ml. The analysis of data from these experiments indicates that Cbl-OH does provide some protection against CN intoxication in that we were able to give dogs CN under this condition ranging from 0.05 - 0.8 mg/Kg. We also have administered CN alone and CN in combination with Cbl-OH at a constant infusion rate. CN was administered at a rate of 0.02 mg/Kg/min and Cbl-OH at a rate of 0.6 mg/Kg/min. This concentration of Cbl-OH represented a stoichiometric amount of this medication plus a slight excess to react with CN. The maximum amount of CN given without the prophylactic was 4.8 mg/Kg and with prophylactic it was 4.6 mg/Kg. Data have thus far been obtained on a limited number of animals and the variation between dogs in their ability to tolerate different amounts of CN has been found to be large; conclusions from these studies await further study.

In related studies, we have modified a procedure for measuring CN in blood and other tissues. This modification permits one to detect significantly lower levels of CN than does the original method. In addition, the modification permits the analysis to be conducted in less than one-half the original time without a diminution in sensitivity.

Future Plans

With completion of the 3 year term of this ILIR project, research conducted under this work unit will be incoporated into other work units in the Dept. of Hematology. It is our intention to fulfill the original goals of this work unit which are to define the feasibility and theoretical value of developing a scheme of cyanide proplylaxis which includes the use of hydroxocobalamin, $B_{12}a$.

Abstract/Publication

None in FY83.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 7012	83 09 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	H. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A61101A91C	00	123 WWM7
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Test Systems for Specific Biological Effects of Chemicals

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	83 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER

c. TYPE d. AMOUNT:

e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	0.8	67
	CURRENT 83	0.8	45

19. RESPONSIBLE DOD ORGANIZATION

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NAME: JACKSON, J E

NAME POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Laboratory Models; (U) Pharmacology; (U) Biology; (U) Side-effects; (U) Mechanism of Action

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Development of laboratory models for testing selected chemical compounds for pharmacological side-effects which may limit their utilization in military medical applications, for examination of mechanisms of pharmacological activity and for studying the effects of chemical modifications on pharmacological activity.

24. (U) Laboratory models will be developed in this laboratory and utilized for detailed assessment of modes of action and deleterious effects of chemical compounds in use or under consideration for treatment of militarily important diseases. This includes identification of adverse biological mechanisms of action, relationship of response to concentration, determination of range of response within a chemical class of compounds, effect of variation of structure within the chemical class and identification of populations at risk if genetically determined metabolic defects are responsible for the adverse effects.

25. (U) 8210-8309 The radiometric micro-volume test for erythrocytic hexose monophosphate shunt (HMS) activity was applied in studies for recognition of hemolytic potential in primaquine-like candidate drugs. HMS and proteolytic responses to methylene blue and sodium nitrite were measured and the results suggested two distinct mechanisms for activation of the HMS. Nitrite treatment increased HMS activity through oxidative challenge to erythrocytic protein, whereas methylene blue activated the HMS without injurious oxidative challenge. Methylene blue-treated erythrocytes did not activate proteolysis of erythrocyte protein, whereas nitrite-treated cells actively degraded protein. Primaquine and two presumed metabolites had activity similar in characteristics to that of methylene blue. Thus, the hemolysis produced by primaquine appears not to be due to oxidative challenge. This Work Unit is being terminated by reason of expiration of its three-year period. For Technical Report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82-30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1 MAR 64 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3A161101A91C In-House Laboratory Independent Research

WORK UNIT 123 Test Systems for Specific Biological Effects of Chemicals

INVESTIGATORS:

Principal: Dr. Joan E. Jackson

PROBLEM:

Chemical compounds which are candidate drugs may have pharmacological side-effects which would limit or preclude their utilization in medical applications. The U.S. Army Drug Development Program invests considerable resources in evaluations of promising compounds being developed as drugs for military applications against infectious diseases, as radioprotectants and as agents of defense against toxic chemicals. Compounds which are discovered to have potentially useful medicinal activity early in the drug development process may have to be abandoned after considerable work and expense because toxic problems are discovered in later studies. Recognition of these limitations at an early stage would allow increased efficiency in management of the drug program. In this Work Unit, laboratory models are developed for detailed assessment of modes of action or deleterious effects of chemical compounds in use or under consideration for military drug applications. A specific area of current investigation is the hemolytic potential of primaquine and its analogues in persons with deficiency in glucose-6-phosphate dehydrogenase (G6PD). G6PD deficiency is an important military problem. It is common among people of Mediterranean and Oriental origins, and occurs in approximately 10% of American Black males. Primaquine is currently the only drug available for treatment of tissue stages of vivaz malaria.

OBJECTIVES:

The objective of research just completed was to investigate the use of a 3-hour, *in vitro*, radiorespirometric micro-test for the rapid evaluation of the potential hemolytic toxicity of new anti-malarials undergoing drug development. The hypothesis of the study was as follows: Radiorespirometric *in vitro* tests for quantitative measurement of erythrocyte hexose monophosphate shunt (HMS) activity would provide a rapid and quantitative assessment

of the oxidative drug challenge to the human erythrocyte (RBC). This hypothesis was based on previously published work indicating a direct cause-and-effect relationship between oxidative drug challenge to RBC and hemolytic drug toxicity. Unfortunately, previously published work and the above hypothesis were determined by our studies to be substantially in error, i.e., hemolytic toxicity of primaquine-related antimalarials is NOT DUE to an overwhelming drug-related oxidative challenge to the erythrocyte.

PROGRESS:

In addition to the above mentioned radiorespirometric microtest, the following tests were used to collect information on RBC physiologic response to drugs in both normal and G6PD-deficient RBC:

TEST	PHYSIOLOGIC PARAMETER MEASURED
1. Radiorespirometric	1. RBC HMS activity measured by following the catabolism of D(1-^{14}C) glucose and D(2-^{14}C) glucose (HMS recycling to $^{14}CO_2$)
2. NADPH-dependent diaphorase activity;	2. RBC diaphorase II enzyme activity measured by following the decrease in NADPH due to this enzyme activity over time;
3. Proteolytic activity of RBC	3. RBC proteolysis measured by following free tyrosine and tyramine fluorescence increase with time.

Through use of the aforementioned tests and a variety of known, non-primaquine, oxidant drugs, including sodium nitrate and phenylhydrazine and nonoxidizing, nonprimaquine drugs, methylene blue and methylene blue-like compounds, it was possible to distinguish drugs of several types:

Type I - Oxidatively Injurious Compounds:

These compounds induced a 15-25 fold elevation of HMS activity and promoted the activity of a RBC proteolytic system which is known to degrade oxidately denatured protein. These two processes appear quantitatively related. Glutathione redox reactions coupled to the HMS afford the erythrocyte primary protection against oxidative attack and are known to be nearly

inactive at maximal HMS activity levels. Proteolytic activity was not detected until HMS activity levels approached maximum. This observation substantiates the hypothesis that oxidative stress and HMS activity are in a cause-and-effect relationship. The "oxidatively injurious compounds" are nitrite and phenylhydrazine. These compounds were relatively inactive in causing diaphorase oxidation of NADPH.

Type II - Methylene Blue-like Compounds:

These drugs induced a 25-45 fold elevation of HMS activity and did so without promoting detectable levels of RBC oxidative proteolysis. Drug concentrations 3 to 8 fold in excess of those required for maximal HMS activity failed to induce RBC proteolysis. These drugs were relatively potent activators of the diaphorase-mediated oxidation of NADPH. It thus appears that this type of drug effect upon the HMS is related to diminished NADPH levels and NOT upon the glutathione defense system against oxidation. The "methylene blue-like compounds" include: methylene blue chloride, primaquine, 5,6-dihydroxy primaquine, 8-amino-5,6-dihydroxy quinoline, and 8-amino-6-hydroxy quinoline. The hydroxylated primaquine analogs are putative primaquine metabolites. These drugs clearly do NOT elevate HMS activity as a consequence of oxidative attack.

CONCLUSIONS:

1. Elevation of HMS activity can NOT be used as a criterion by which the hemolytic potential of primaquine-like candidate antimalarials may be predetermined.

2. The oxidative activity of primaquine and hydroxylated primaquine analogs in intact RBC *in vitro* is very similar to that of methylene blue. Since methylene blue is nontoxic to primaquine-sensitive individuals, it appears that the hemolytic toxicity of primaquine is unrelated to its oxidative activity in RBC.

3. Maximal levels of HMS activity appear to be required for oxidant-induced degradation of RBC proteins. Since primaquine-induced HMS activity *in vivo* has been estimated to be only 0.2 fold above baseline (Welt, et al. Ann. NY Acad. Sci.), its oxidative effects *in vivo* appear almost trivial in comparison to those required to cause cell injury *in vitro*.

FUTURE OBJECTIVES:

Not applicable; this work unit is terminated with this final report.

PRESENTATIONS:

1. Baird. JK, Decker Jackson, JE, Davidson, DE. 1982. An in vitro radiometric micro-volume procedure for rapid measurement of erythrocyte hexose monophosphate shunt activity. Paper presented at 32nd annual meeting of the American Society of Tropical Medicine and Hygiene, 7-11 November 1982, Cleveland, Ohio.

2. Baird, JK, Davidson, DE, Decker Jackson, JE. 1983. Oxidative activity of hydroxylated primaquine analogs: nontoxicity to hemolytically sensitive red blood cells in vitro. Paper presented at the annual meeting of tn American Society of Hematology, 5-11 December 1983, San Francisco, California.

PUBLICATIONS:

1. Baird, JK and Lambros, C. 1983. Effect of membrane filtration of standard antimalarials on in vitro activity against Plasmodium falciparum. Bulletin of the World Health Organization. In Press.

2. Baird, JK, Decker Jackson, JE, Davidson, DE. 1983. An in vitro micro-volume procedure for rapid masurement of erythrocyte hexose monophosphate shunt activity. Journal of Laboratory and Clinical Medicine. In Press.

3. Baird, JK. 1983. Methylene blue-mediated stimulation of the erythrocyte hexose monophosphate shunt: independence from intracellular oxidative injury. Biochemical Pharmacology. In review.

4. Baird, JK, Davidson, DE, Decker Jackson, JE. 1983. Non-toxic oxidative activity of two putative hydroxylated primaquine metabolites to glucose-6-phosphate dehydrogenase deficient erythrocytes in vitro. Manuscript in In-House Review Prior To Submission for Publication.

5. Baird, JK. 1983. Measuremeent of erythrocyte hexose monphosphate shunt activity. In: Radiochemical Methods in Parasitology (Hayunga, E. ed.) International Atomic Energy Agency Publication, Vienna Austria. In Press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 7010	83 10 01	DD DRA&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101.91C	00	124 WWGN
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Development of specific cell directed antibody-toxin conjugates

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 01 01	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	2.0	75
FISCAL YEAR CURRENT 84	2.0	90

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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RESPONSIBLE INDIVIDUAL

NAME: Russell, P K

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

Division of [illegible]

ADDRESS: Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)

NAME: DAIOFF, J C

TELEPHONE: (202) 576-3759

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: SEID, R

NAME:

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Toxins; (U) Antibodies; (U) Monoclonal; (U) Cytotoxicity

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code)

23. (U) Develop techniques for coupling toxins to monoclonal antibodies such that the toxins are internalized by and kill only cells or parasites against which the antibodies are directed. Cell directed toxins have potential for treatment of militarily important parasite and viral infections; disorders of immune regulation following trauma, exposure to radiation or chemicals; and in transplantation. An understanding of toxin entry and biochemistry is critical and relevant in designing strategies for defense against biological warfare.

24. (U) Toxins, such as ricin, following chemical modification or removal of their cell binding (B) regions will be coupled to monoclonal antibodies against cells and parasites. Intracellular toxins with no B region, such as gelonin, will also be coupled to antibody. Modification and coupling procedures will be optimized for cell entry and death.

25. (U) 82 10-83 09 A technique for purification of the A fragment of ricin using monoclonal antibodies is in the development stage. Monoclonal antibodies against ricin A chain have been made. Monoclonal anti A chain antibodies have been used for affinity purification of A chain which is an essential component for coupling. These monoclonal antibodies have potential therapeutic potential for ricin intoxification and diagnostic value for rapid ricin identification. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 124: Development of Specific Cell Directed Antibody-Toxin Conjugates

Investigators:

Principals: Samuel B. Formal, Ph.D.
COL Jerald C. Sadoff, MC

Progress

We have learned that immunotoxins constructed by coupling monoclonal antibody to Ricin A fragment have cell and animal toxicity related to contamination with B fragment. Affinity purified A fragment using polyclonal anti-A sera was therefore obtained. This A fragment had no toxicity in mice at a 5 ug/mouse dose. Mice were immunized with purified A fragment and 9 monoclonal antibodies have been found, cloned and produced as ascites fluid in mice. These monoclonals have specificity for A fragment. Several of them bind to A fragment in a Western Blot. Each of them are being evaluated for their potential in affinity purification of A fragment and in mouse protection studies. We, therefore, have developed reagents which allow us to provide absolute identification of ricin in biological samples.

Future Plans

ILIR projects run only 2 years. We will continue our investigations at a reduced level. We will investigate whether conjugates consisting of cell surface monoclonal coupled to anti A chain monoclonal which is bound to Ricin A chain can effectively deliver A chain inside the cell. We will couple Ricin A chain to monoclonal antibodies against parasite surface antigens (trypanosomes) and evaluate toxicity.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303 117	83 10 C1	DD-DR&L(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. CRED'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101 A91C	[illegible]	125 WWGD
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Isolation and Characterization of Potential Scrub Typhus Vaccine Components

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY		16. PERFORMANCE METHOD
83 10	CONT	DA		C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PRECEDING FISCAL YEAR 83	0.0	0.0
CURRENT FISCAL YEAR 84	1.0	50

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: DIV CD&I
Washington, DC 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: HEDELUND, K W

TELEPHONE: (202) 576-2146

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: RICE, R M

NAME:

21. GENERAL USE

Foreign Intelligence Considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Rickettsial Diseases; (U) Vaccine; (U) Structure - Antigenicity

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Precede individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The morbidity that occurred among U.S. troops infected with scrub typhus both in World War II and Vietnam is well recognized. At present, while we know that prior infection leads to solid homologous immunity, we do not know the nature of immunologically important components of this organism. These studies are directed at developing a vaccine to protect troops deployed throughout the Far East.

24. (U) Current technologies will be used to isolate intact scrub typhus organisms from the bulk of eucaryotic cell membranes by a variety of non-denaturing and non-destructive techniques incorporating a gentle process simultaneously coupling centrifugation and counterflow to isolate the intact rickettsiae from the host cell membranes and their degradative enzymatic components. The isolated organisms would then selectively be fractionated into its components by high pressure liquid chromatography which in turn would be evaluated for immunodominance.

25. (U) New.

*A valid No is contractor's upon contractor's approval

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 303 118	83 10 01	DD-DR&E(AR)636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. FORM SECURITY	7. REGRADING	8A. GRADE INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	126 WWL7
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Effects of Various Endorphins and their Antagonists on Regional Blood Flow in Normal Rabbits and on Rabbits in Hypovolemic Shock

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 016200 Stress physiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
83 10	CONT	DA			C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE
b. NUMBER
c. TYPE
d. KIND OF AWARD
EXPIRATION
d. AMOUNT
f. CUM. AMT.

18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	B. FUNDS (In thousands)
FISCAL YEAR	PREVIOUS 83	0.0	00
	CURRENT 84	3.0	30

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL
NAME: TOP, F H
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Division of Surgery
ADDRESS: Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)
NAME: HARMON, J W
TELEPHONE: (202) 576-3791
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: SAMPSON, J
NAME: POC: DA

21. GENERAL USE
Foreign Intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) endorphin; (U) shock; (U) rabbit; (U) radioactive microspheres; (U) blood flow

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23(U) The objective will be to follow-up the observations of Holaday and Faden of the WRAIR that endorphin antagonists such as naloxone can reverse the hypotension of hypovolemic shock in the rat. This suggests that endorphins play a role in the pathophysiology of shock. This role has been confirmed in endotoxin shock as well as in hypovolemic shock, and in dogs and pigs as well as rats. The mechanism by which they preserve blood pressure is unknown: in some situations they raise vascular resistance and in others they do not. There is military relevance in this research.

24(U) The objective will be to utilize radioactive microspheres in a rabbit system for measuring regional blood flow. This system has been developed in the Division. It will be used to evaluate the effects of various endorphins and their antagonists on regional blood flow in normal rabbits and on rabbits in hypovolemic shock. This work will benefit from collaboration with Dr. John Holaday of the Division of Neuropsychiatry, WRAIR.

25(U) New.

*Available to contractors upon originator's approval

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303 119	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPN INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. [illegible] UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	127
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Modes of Action of Antiparasitic Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
002600 Biology 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	Cont.	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	0.0	0
	CURRENT 84	0.5	50

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL
NAME: TOP, F H
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Division of Experimental Therapeutics
Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide Name if U.S. Academic Institution)
NAME: Reid, W A
TELEPHONE: (301) 427-5029
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Jackson, J E
NAME:

21. GENERAL USE
Foreign Intelligence Considered

POC:DA

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Mechanism of Action; (U) Laboratory Models; (U) Biology; (U)Pharmacology; (U)Side effects; (U)Methodology; (U) Parasitic Diseases.

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief statement paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Development of new procedures to assess efficacy of candidate antiparasitic drugs, investigation of modes of activity of drugs and development of techniques to examine host or parasite responses which may enhance, limit or preclude the development of the candidate drugs. There is military relevance in this research.

24. (U) Compounds which show enhanced as unusual activity against parasites against which they have not been tested previously or compounds which show unexpected results in unusual treatment regimens or in combination with other compounds will be studied in modifications of established laboratory models and technical procedures or specifically developed techniques. Modified or new procedures will be evaluated for incorporation into the overall program. Procedures will be developed for detailed study of modes of action of compounds on metabolic and structural processes of parasites and their hosts so that limits of application of compounds as drugs may be determined or opportunities for drug intervention may be recognized.

25. (U) New.

* Available in computer-to-computer exchange

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498-1 NOV 68 AND 1498-1 MAR 69 FOR ARMY USE ARE OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 9280	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORGN INSTRN	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	I. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	6.101A	3A161101A91C	00	128 WW09
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security C classification Code): (U) Regulation of the Human Immune Response to Dengue Virus Infection By Auto Anti-Idiotypic Antibodies

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. END OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	0.3	30
FISCAL YEAR CURRENT 84	0.3	33

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
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20. PERFORMING ORGANIZATION
NAME: AFRIMS
ADDRESS: Bangkok, Thailand
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: BURKE, D S
TELEPHONE
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME
NAME

21. GENERAL USE
Foreign Intelligence Considered

DOC: DA

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Virus; (U) Dengue fever; (U) Infectious Diseases; (U) Anti-idiotypic antibodies

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The technical objectives are: (1) to screen human hybridomas for production of naturally occurring anti-idiotypic antibodies directed against idiotypic determinants on anti-dengue immunoglobulins; (2) to produce and purify these anti-idiotypic antibodies in quantity; (3) to purify from serum the corresponding set of anti-dengue antibodies bearing these idiotypic determinants; (4) to develop immunoassays for detection and quantitation of both the set of idiotype-bearing anti-dengue antibodies and the corresponding anti-idiotype antibodies; (5) to determine the kinetics of both the idiotype bearing anti-dengue antibodies and the anti-idiotype antibodies during natural dengue infections in human, and (6) to determine if exogenously added autologous monoclonal anti-idiotype can regulate the production of idiotype bearing antibodies by in vitro cultures of peripheral blood monoclonal leukocytes from humans with acute dengue virus infection. There is a military requirement for research leading to a better understanding of the antibody response to acute dengue virus infection. These infections represent a serious hazard to troops operating in tropical areas.

24. (U) Conventional virological and immunological techniques will be utilized and modified as required.

25. (U) 82 10 - 83 09 In order to identify dengue antibody producing cells in human blood sensitive test was developed that can be completed overnight. A human myeloma cell line was obtained and the optimum conditions for growth at various rates was determined. Fusions between the myeloma cells and the antibody producing cells are in progress and should provide immortal human cell hybrids that continue to secrete antibodies. For technical report see Walter Reed Army Institute of Research Progress Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE

Project Number: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH
Title: Regulation of the Human Immune Response to Dengue Virus Infection by Auto Anti-Idiotypic Antibodies
Work Unit Number: 128

PROBLEM: The technical objectives are:

1. To screen human hybridomas for production of naturally occurring anti-idiotypic antibodies directed against idiotypic determinants on anti-dengue immunoglobulins.

2. To produce and purify these anti-idiotypic antibodies in quantity.

3. To purify from serum the corresponding set of anti-dengue antibodies bearing these idiotypic determinants.

4. To develop immunoassays for detection and quantitation of both the set of idiotype-bearing anti-dengue antibodies and the corresponding anti-idiotype antibodies.

5. To determine the kinetics of both the idiotype bearing anti-dengue antibodies and the anti-idiotype antibodies during natural dengue infections in human.

6. To determine if exogenously added autologous monoclonal anti-idiotype can regulate the production of idiotype bearing antibodies by in vitro cultures of peripheral blood monoclonal leukocytes from humans with acute dengue virus infection. There is a military requirement for research leading to a better understanding of the antibody response to acute dengue virus infection. These infections represent a serious hazard to troops operating in tropical areas.

METHOD: Conventional virological and immunological techniques will be utilized and modified as required.

PROGRESS: Satisfactory conditions for production of stable hybridomas have been determined. Four fusions of continuous UC729-6 lymphoblastoid cells with peripheral blood mononuclear cells obtained from dengue hemorrhagic fever patients have produced 35 viable clones of hybrid cells. Nineteen of these hybrid clones produced IgM and one produced IgG. Eight of the IgM producing clones are stable and have been grown in continuous culture for more than three months. Antibodies produced by all eight of these clones do not react directly with intact virions. Further characterization of these antibodies, and additional fusions, are in progress.

FUTURE OBJECTIVES: This study is yeiling interesting results and plans are to continue it for an additional year.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OG 9281	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
A. PRIMARY	61101A	3A161101A91C	00	129 WWID
B. CONTRIBUTING				
C. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Protection of Gonadal Function from Cytotoxic Therapy

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 012900 Physiology 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	1.1	25
	CURRENT 84	1.1	35

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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TELEPHONE (202) 576-3305

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: CUTTING, W A

NAME:

21. GENERAL USE

Foreign Intelligence Considered

FOC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U)Chemical Toxicity; (U)Radiation Damage; (U)Gonadal Protection; (U)Marrow Protection

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine the efficacy of hormones in protecting an organ system from exposure to toxic chemicals or radiation. If it is possible to protect reproductive organs of animals by hormonal suppression, it may also be possible to protect gonads of troops if they are exposed to chemicals of a similar nature or to radiation. The ability to protect gonads of patients undergoing chemotherapy or irradiation is also significant.

24. (U) Procedures include: small laboratory animal models exposed to radiation or chemicals; histopathologic processing of tissues; radioimmunoassay of serum hormone levels.

25. (U) 82 10 - 83 09. Experiments have been conducted using levels of radiation or cytotoxic chemical which were shown in pilot studies to cause significant gonadal damage without mortality. In female mice, treatment with a hormone prior to irradiation of the gonads had no protective effect at the levels of radiation and hormone used. In one experiment with cyclophosphamide, a majority of the animals died even though the drug dose had not caused any deaths in pilot studies. It was discovered that the mice were carrying an infection present in the Walter Reed colony. The experiment has been repeated in uninfected mice and histological analyses are in progress. Although hormonal treatment at the levels used did not prevent irradiation damage, it may prove effective against cytotoxic drugs because the mechanisms of damage may be different. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available in limited form upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3A161101A91C: IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 129 Protection of Gonadal Junction from Cytotoxic Therapy

Investigators COL William H. Crosby, MC; LTC Daniel G. Wright, MC
Mary Cutting, MS

Description

The purpose of this work unit is to study the efficacy of hormones in protecting an organ system from exposure to toxic chemicals or radiation. The concepts explored in these studies are relevant to the protection of troops who may be exposed to toxic chemicals or radiation. If it is possible to use hormones to protect the reproductive organs of animals from cytotoxic damage, it may also be possible to protect the gonads of troops exposed to cytotoxic agents. The protection of gonads of patients undergoing chemotherapy or radiation treatments is also an important problem addressed by these experiments. This work has broad relevance in that it may provide a general model to study the protection or organ systems against the actions of various toxic agents.

We have used the mouse reproductive system as a model. We conducted pilot studies to determine levels of exposure to radiation or the cytotoxic drug cyclophosphamide which cause significant damage to the gonads of male of female mice. Damage is determined by histological quantitative analyses of gonadal tissue. With the information on effective doses gained from pilot studies, experiments have been undertaken in which some animals are treated with hormones prior to irradiation or cyclophosphamide treatment. These mice are compared with those receiving no hormones to determine if the hormones protect the gonads from radiation or cyclophosphamide-induced damage.

Progress

Female mice treated with a hormone prior to irradiation of the gonads showed no protective effect of the hormone. Damage to the ovaries was comparable to that observed in mice not receiving the hormone. Studies with cyclophosphamide were initially hindered when a majority of the experimental animals died due to a bacterial infection in the Walter Reed mouse colony. The experiments have now been completed in both males and females and histological analyses are in progress. Results of these studies will be important, because the mechanisms of damage from radiation and cytotoxic drugs may be different, and the effectiveness of the hormone treatments may be determined by the way in which damage occurs.

Future Plans

During the third year of this ILIR work unit, long-term experiments will be conducted to determine whether recovery of gonadal function occurs. The assessment will bee made on the basis of both histology and fertility studies.

Abstracts/Publication

None in FY83

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300028	83 09 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	H. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	130
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Development of Anti-Parasitic Monoclonal Antibody-Toxin Conjugates

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	83 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 82	0.5	7
FISCAL YEAR CURRENT 83	0.5	15

19. RESPONSIBLE DOD ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Brown, J E

TELEPHONE: (202) 576-4235

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Gemski, P

NAME:

21. GENERAL USE

Foreign intelligence considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Monoclonal Antibody; (U) Toxin; (U) Parasite; (U) Trypanosome; (U) Immunotoxins

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The technical objective of this work unit was to construct and test "immunotoxins" consisting of enzymatic subunits from toxin proteins covalently conjugated to monoclonal antibodies specific for cell surface antigens of parasitic organisms, such as Trypanosoma rhodesiense. The goal would be eventually to provide reagents for adjunctive therapy of parasitic infections threatening military troops.

24. (U) Currently available monoclonal antibodies and toxins are utilized for experiments. The toxic plant protein ricin and its enzymatic A subunit are used. Monoclonal IgG antibody specific for parasitic cell surface antigens is derivatized with N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) to provide covalent linkage of the antibody to the toxin. The resulting bond is a disulfide linkage which can be easily cleaved intracellularly after uptake of the conjugate. The hybrid protein is purified by HPLC gel permeation techniques from the mixture of components in the conjugation reaction mixture. The resulting purified "immunotoxins" can be tested for efficacy and specificity in parasite cell culture. It was expected that numerous preparations would have to be tested since not all cell surface antigens would facilitate receptor-mediated endocytosis of a bound ligand.

25. (U) 83 10-83 06 This work unit has been terminated. Experimental protocols were standardized for synthesis of the intermediate protein products, SPDP-immunoglobulin G and SPDP-ricin, and for the final protein cojugate, IgG-S-S-Ricin. Condition were determined for the recovery of this conjugate from the reaction mixture by gel permeation HPLC. For technical report see Walter Reed Army Institute of Research Annual Program Report 1 Oct 82 - 30 Sep 83.

*A variable number of lines upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 130 Development of Anti-Parasitic Monoclonal Antibody-Toxin Conjugates

INVESTIGATORS:
Principle: CPT James E. Brown, Ph.D., MSC
Associate Peter Gemski, Ph.D.
In collaboration with: Klaus M. Esser (CDI)

PROBLEM:

New strategies which would be useful in effectively combating parasitic diseases of military interest are needed. We have given consideration to an alternative approach whereby a toxin or toxin subunit would be linked covalently to a parasite-specific monoclonal antibody thereby producing a complex which is not only toxic but also highly selective. The basic philosophy is to use a carrier molecule, the function of which is to bind selectively to the target covalently attached to a 'warhead' which enzymatically kills or modifies the target cell.

1. Preparation of Immunotoxin Conjugates.

Current therapies useful in tropical medicine appear to contain inherent difficulties. Two central problems in parasitic chemotherapeutic are (a) the lack of selectivity of present anti-parasitic drugs and (b) the emergence of drug-resistant strains. Likewise the limited success of immunologic approaches reflects (a) the complex defense mechanisms developed by parasites, (b) the lengthy period required for the body to amount a full-fledged immune response, and (c) the inherent intracellular natue of some parasites.

Interest in the preparation of cell-specific cytotoxic agents has recently increased for two reasons. First, the hybridoma cell-culture technique permits the production of large amounts of monoclonal antibodies to a large variety of cell-surface antigens. Second, the extreme toxicity of certain bacterial and plant toxins - a single molecule may kill a cell - might compensate for the small amount of complexes accumulating in a parasite cell.

Successful demonstration of this "immunotoxin" approach has already been achieved in vitro and in vivo. Several extensive reviews are now available. Application of an "immunotoxin" therapy to diseases of tropical medicine could hold great promise. At a minimum this approach should eventually provide a useful adjunct to currently employed chemo- or immuno- therapeutic regiments. Monoclonal antibodies which have been prepared at the WRAIR have been utilized for initial experiments. The toxic plant protein ricin was used since (a) the molecular mechanism is understood and (b) feasible methods exist to separate quantitatively the enzymatic subunit from the binding poly-peptide. Other well-characterized toxins which could be used are diptheria toxin, abrin, pseudomonas exotoxin A, cholera toxin and Shiga toxin. "Immunotoxin" conjugates to be

constructed first will employ monoclonal antibody specific for Trypanosoma rhodesiense, because this organism is essentially an extracellular parasite.

Experimental protocols were standardized for synthesis of the intermediate protein products, SPDP-immunoglobulin G and SPDP-ricin, and for the fenal protein conjugate, IgG-S-S-Ricin.

These procedures are outlined as follows:

1. Apply the IgC preparation (1-10 mg/ml) to a Sephadex PD-10 column containing Sephadex G-25, equilibrated with sodium phosphate buffer (0.1M, pH7.5) containing 0.1 M NaCl.

2. Dissolve N-succinemidyl-3-(2-pyridyl dethio) propiorate (SPDP, Pharmacia Fine Chemicals) in ethand. Add SPDP to the IgG solution to achieve a 10-fold molar excess of SPDP. React for 30 minutes at 23°C with gentle stirring.

3. Apply the mixture to a fresh PD-10 column containing Sephadex G-25 equilibrated with sodium acetate buffer (0.1M pH 4.5) containing 0.1M NaCl.

4. Immediately proform step 1-3 for ricin except that the derivatized ricin mixture is applied to a PD-10 colmin containing the sodium phosphate buffer.

5. Add dithiothreitol (1M) to the derivatized IgG preparation to achieve a final concentration of 50 mM. React for 20 minutes at 23°C.

6. Recover the theolated IgG by applying to a fresh PD10 column equilibrated with the sodium phosphate buffer.

7. Immediate mix the thiolated IgG and 2-pyridyl disulphide containing ricin. Allow reaction of proceed for 30 minutes at 23°C.

Procedures were established for the recovery of this conjugate from the reaction mixture by sequential chromatography on Sepharose 4B and gel permeation HPLC. The mixture is first applied to a 10 ml Sepharose 4B column equilibrated with the sodium phosphate. Since ricin binds to lactose residues of this gel, IgG conjugates and monomeric IgG are quickly separated. Unreacted ricin and IgG-ricin conjugate are recovered by elution with sodium phosphate buffer containing 0.1M lactose. Fractionation of the conjugate from ricin is performed by gel filtration HPLC on a waters HPLC system using a BioRad TSK400 column in series with a Waters Assoc I125 column. Eluction was carried out at 1 ml/min with 0.1M sodium phosphate, pH7.2. Ricin (Mr=60,000) is quantitatively separated from comjugate (Mr=210,000). Isolated conjugate is then available for physical/chemical characterization, or for testing of biological efficacy and specificity.

This work unit is termintated and merged into 124, DA 0G 7010. Therefore this is a final report.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303 120	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORGN INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	170 WWIW
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Studies of Biochemical Changes in Human Red Blood Cells Infected with Plasmodia (malaria) Organisms in vitro.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
002600 Biology 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	Cont	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	0.0	00
FISCAL YEAR CURRENT 84	2.0	94

19. RESPONSIBLE DOD ORGANIZATION
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20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: WHAUN, J M
NAME: WEBSTER, H K

21. GENERAL USE
Foreign intelligence considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Red blood cells; (U) Metabolism; (U) Malaria

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23.(U) To describe and understand biochemical differences between human host red blood cells and malaria parasites in order to define metabolic targets for the design of new, specific chemotherapy. Development of new antimalarial chemotherapy is of major current and historical military importance because of needs to station or deploy military personnel in regions of the world where malaria is endemic. Because the emergence chloroquine-resistant malaria is increasing around the world, it has become particularly important to identify alternate chemotherapy that circumvents chloroquine resistance. Our approach to this problem is to define basic differences in biochemical and metabolic pathways necessary for growth or maintenance of normal function that distinguish normal red blood cells (RBC) and RBC infected with Plasmodium falciparum, the major malaria pathogen in humans. Strategies may then be developed to exploit such differences in the obligatory metabolism of malaria parasites and the normal RBC to design malaria specific anti-metabolites.

24.(U) Laboratory studies include measurement of (1) intermediates and enzyme levels of polyamine metabolism; (2) intermediates and enzymes of purine and pyrimidine salvage and interconversion pathways; (3) enzymes mediating methylation reactions; (4) effects of selected antimetabolites on these biochemical pathways. High performance liquid chromatography and suspension tissue culture of human RBC infected with P.falciparum in vitro and radiolabeling studies with metabolic precursors are major components of technical approach.

25.(U) New.

*Available to contractors upon originator's approval

DD FORM 1 MAR 84 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 303 121	2. DATE OF SUMMARY* 83 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY A. New	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8A. DISP'N INSTR'S NL	8B. SPECIFIC DATA - CONTRACTOR ACCESS ☒ YES ☐ NO
9. LEVEL OF SUM A. WORK UNIT						

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	171 WWIX
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Development of Prophylaxis Against Acute Cyanide Poisoning: Studies with a Canine Model

12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 008800 Life Support 002600 Biology 012900 Physiology 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	CON'T	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 83	0.0	00
CURRENT FISCAL YEAR 84	2.5	95

19. RESPONSIBLE DOD ORGANIZATION
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ADDRESS:* Washington, DC 20307

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TELEPHONE: (202) 576-3358
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: WILLIAMS, H
NAME: KARK, J A POC: DA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Cobalamins; (U) Cyanide Poisoning; (U) Cyanide Prophylaxis

23. TECHNICAL OBJECTIVE.* 24. APPROACH. 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23.(U) To define effective methods to increase resistance against acute cyanide poisoning using an experimental canine model. In developing strategies for medical defense against rapidly lethal chemical agents such as hydrogen cyanide gas, which troops may encounter on the modern battlefield, chemical prophylaxis is an important consideration since combat conditions may prevent effective treatment once symptomatic intoxication has occurred. Agents with proven anti-cyanide effects in vivo which do not themselves produce incapacitating side effects, such as thiosulfate and cobalamins are the most promising candidates for cyanide-prophylaxis regimens, and so these agents are to be studied in particular.

24.(U) Laboratory studies involve a canine model in which female foxhounds are monitored for respiratory rate and effort, arterial blood pressure, heart rate, arterial pO_2, pCO_2, and pH, methemoglobin, cyanomethemoglobin, blood and urine cyanide anion levels. These measurements permit calculation of the ED50 for cyanide given to the animals intravenously or by inhalation in causing discrete cardiovascular/respiratory signs of early cyanide toxicity, and they allow for a determination of the protective effects of candidate prophylactic agents: hydroxycobalamin and/or sodium thiosulfate.

25.(U) New.

*Applicable to contractor(s) upon authorized approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M161102BS10

RESEARCH ON MILITARY DISEASE, INJURY
AND HEALTH HAZARDS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6441	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORGN INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AA	201 WUGA
b. CONTRIBUTING				
c. CONTRIBUTING	STAT 3M1[illegible]			

11. TITLE (Procode with Security Classification Code)

(U) Viral Infections of Man

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
63 08	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	3.0	430
FISCAL YEAR CURRENT 84	3.0	532

19a. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: BRANDT, W E
NAME: SCOTT, R McN POC:DA

21. GENERAL [illegible]

Foreign intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Virology; (U) Immunology; (U) Arbovirus Infections; (U) Human Volunteers

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Indent text at each new security classification. Code.)

23 (U) To define etiology of acute infectious diseas[illegible] of special hazard to military personnel, to determine and evaluate factors influencing the occurrence, distribution, severity and medical result of human virus infections, and to develop means for reducing disability due to virus diseases.
24 (U) Contemporary virological and immunological methods are applied to disease problems occurring in troops or in susceptible civilian populations in strategically important areas. New conceptual approaches and methods are developed as needed.
25 (U) 82 10-83 09. Antibody dependent infection enhancement of flaviviruses was studied with dengue and Japanese encephalitis (JE) viruses. JE virus is enhanced by IgM antibody in cerebrospinal fluid of encephalitis patients. Only LLC-MK2 cells used at the WRAIR have IgM receptors permitting the study of JE enhancement in vitro. An alternative method for studying infection enhancement of dengue viruses uses an indirect fluorescent antibody test of infected cells rather than infectivity titrations. Successful in vitro models of infection enhancement utilize cell adapted dengue virus, low multiplicity of infection and cells resistant to dengue infection in the absence of antibody. Wild strains of dengue types 2 and 3 viruses were enhanced readily while dengue type 1 was not indicating nonuniformity of enhancibility in vitro as there seems to be in vivo. Chloroquine selectively inhibits dengue viral infection via Fc receptors. No new dengue vaccine studies were started, but a MOBDES assignee demonstrated that one dengue immune volunteer developed natural killer cell activity against dengue virus. A collaborative study with NIH and Chicago scientists failed to incriminate chlamydia or Ehrlichia sp in the etiology of Acquired Immunodeficiency Syndrome. For technical report, see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 201 Viral Infections of Man

Investigators:

Principal: COL William H. Bancroft, MC
COL Robert McN. Scott, MC
Dr. Walter E. Brandt, Ph.D.

Associates: MAJ Robert R. Redfield, MC
CPT Erik A. Henchal, MSC
Dr. Robert Feighny, Ph.D.
Dr. Srisakul C. Kliks, Ph.D.
Mr. Jack McCown
Mrs. Jeanne Burrous, M.S.
SSG Carlton Brown
SGT Wanda E. Williams
Mr. Roger Jackson
SP4 Maria Ruiz
SP4 Julius Anongos

Problems and Objectives

Characterization of viruses which threaten military personnel is necessary for effective disease control. Emphasis is placed on dengue viruses, but other viral diseases are studied when necessary. Basic research on dengue viruses is directed toward evaluation of the genetic lesions causing attenuation, the enhancement of virus replication by antibody, and viral reactivity with monoclonal antibodies. Human volunteer studies are conducted to evaluate the safety and immunogenicity of candidate dengue vaccines.

Progress

1. Japanese Encephalitis virus

a. Infection Enhancement by IgM. Additional studies were carried out on IgM isolated from spinal fluids from cases of Japanese encephalitis provided by LTC D. Burke, AFRIMS. The original observation was the increased infectivity of the virus for monkey kidney cells when the virus was complexed with spinal fluid IgM. An infection-enhancement titer was defined as the highest four-fold dilution that increased the JE virus plaque count by two fold. Three of five spinal fluids from non-fatal cases had no

enhancement activity on the first hospital day, but spinal fluids taken at one week and one month exhibited infection-enhancement titers as high as 1/10,240. At about 200 days, infection-enhancement titers had fallen to 1/20 to 1/40. The corresponding serum specimens exhibited enhancement titers of up to 1/2560 on either the first hospital admission day, or at one week, or both; at one month, neutralization of JE virus by the serum samples was observed. Spinal fluids and/or sera from six fatal cases contained enhancement activity of up to 1/2560 on the day of death, which ranged from the first to the eighth hospital day.

Dilutions of antiserum against IgM was twice as effective in precipitating the antibody-virus complexes as anti-IgG, supporting the previous data that the factor in spinal fluid was indeed IgM. However, the precipitation observed with anti-IgG indicates that a) there may be a cross reaction with these reagents not previously reported, and found here because of the exquisite sensitivity of an assay system based on a single infectious unit, or, b) aggregates of IgG might be contaminating the 19S fraction of sucrose gradients containing IgM.

The previous work on the infection-enhancement of JE virus by IgM was carried out with a high passage laboratory strain of JE virus. A recent isolate of JE virus from the brain of a fatal human case was shown to be enhanced by spinal fluid IgM. In addition, virus stocks of this isolate prepared in insect (Aedes albopictus) cells, monkey kidney (LLC-MK_2) cells, and in suckling mouse brain were all enhanced by spinal fluid IgM. Enhancing IgM was found as late as 6 months post-hospital admission in the non-fatal cases, whereas the enhancing activity in serum lasted only a few weeks.

Sheep cells coated with IgM or IgG were used to probe for immunoglobulin receptors on various cell lines. IgG receptors were found only on a macrophage cell line. IgM receptors were found only on WRAIR LLC-MK_2 cells. Sublines of LLC-MK_2 cells cultured in different media in Bangkok and Hawaii did not have IgM receptors, neither did neuroblastoma or glial cell lines. We conclude that the ability to demonstrate infection enhancement by virus-specific IgM antibodies is due to a cell surface component on a subpopulation of one line of LLC-MK_2 cells that specifically binds IgM.

b. Immune response to a killed Japanese encephalitis vaccine produced in Japan. Deaths due to infection by Japanese encephalitis virus prompted the use of Japanese manufactured vaccines in field laboratories in Southeast Asia and U.S. civilians visiting China. Antibodies induced by this vaccine in humans neutralized the live parent virus from which the vaccine was made,

but neutralized very little, or not at all, the laboratory strain of prototype Nakayama JE virus used to diagnose JE infections. The vaccine-induced antibodies will be tested for their ability to neutralize current strains of JE virus from S.E. Asia. Strains of JE virus in India were found not to be neutralized by the vaccine antibodies in Indian laboratories (R. Shope, Yale, Univ., personal communication). Formulation of JE vaccines should be based on immunologic evaluation of currently circulating strains of JE virus.

2. IgG antibody enhancement of dengue-2 virus in human monocyte cell line

Previous studies showed that only monoclonal antibodies directed against two or more dengue virus serotypes (therefore cross-reactive antibodies) form a complex with the virus in such a manner as to infect cells bearing antibody (Fc) receptors. Antibodies reactive against the type-specific neutralization determinants of the virus did not produce this immunological enhancement of infection phenomenon. Thus, vaccines that induce antibodies only against the neutralization determinant may be safer if they do not prime the recipients for enhanced secondary dengue infections. These studies were carried out at a multiplicity of infection (MOI) of 0.01 to 0.005 (one infectious unit per 100 to 200 monocytes). The recent use of higher multiplicities of infection indicate that any antibodies that bind to the virus particle enhance the ability of the virus to infect monocytes or Fc-receptor bearing cells. However, a) monoclonal antibodies directed against neutralization determinants must be diluted beyond their neutralization potency before enhancement capabilities can be demonstrated, and b) the lower MOI used to distinguish cross-reactive and type-specific neutralization antibodies may be more representive of the level of infection in nature. Thus, vaccines containing only the neutralization epitope (made by recombinant DNA technology) should still be tested for their ability to induce protection and be tested for their ability to sensitize individuals for enhanced responses when challenged with heterologous dengue viruses.

3. Indirect Immunofluorescent Assay of a Dengue Infected Human Monocyte Cell Line

Previously, the determination of antibody-mediated dengue infection of mononuclear phagocytes via the Fc receptor was based on the production of larger quantities of virus than that produced by monocytes infected only via the natural viral receptor. The procedure involved titration of collected culture fluids on LLC-MK_2 cell monolayers. About one week is required for the virus in the

culture fluids to form plaques in the monolayers. This procedure is labor intensive and time consuming when large numbers of serum specimens are to be tested for antibody-dependent enhancement (ADE) activities at multiple dilutions of the specimens.

A more direct and less laborious method of detecting infected mononuclear phagocytes was developed. The indirect fluorescent antibody (FA) technique, using mouse anti-dengue hyperimmune ascitic fluid or a dengue cross-reactive monoclonal antibody (4G2) with fluorescein conjugates rabbit anti-mouse antibody, was employed to detect infected cells. Presence of FA positive cells was shown to be proportionately related to virus production, although the overall sensitivity is somewhat lower than measurement of infectivity in plaque forming units (PFU/ml); the specificity is comparable. Furthermore, in the presence of dengue antiserum with neutralizing activity, newly synthesized virus released into the medium is neutralized so that infectivity assays are of little value. FA positive cells on the other hand demonstrate the presence of dengue specific antigens intracellularly, therefore representing more truely the biological events of interest. Measurement of ADE by this technique is applicable to all 3 cell types invariably used in our experiments, namely, human U-937 and mouse P-388D_1 cell lines, and human elutriated monocytes.

4. Comparison and Evaluation of Antibody Dependent Enhancement activity measured in U-937 and P-388D_1 cells, and Human Elutriated monocytes

Factors influencing ADE of dengue virus growth are as follows:

a. Host cell culture. Virus propagated in BHK and C6/36 cell cultures are better enhanced than that propagated in suckling mouse brain.

b. Multiplicity of infection (MOI). The higher the MOI, the higher the number of FA positive cells and virus production. The test is more sensitive when higher moi up to 1.0 or 5.0 is employed. The dependence on MOI for detection of ADE activity varies depending on the type of antibodies; for example, ADE by dengue neutralizing specific 3H5 is markedly dependent on MOI, while 4G2 is marginally affected by MOI.

c. Dengue virus receptors. Human mononuclear phagocytes possess natural dengue receptors and can be infected at a marginal level. The permissiveness to dengue infection increases when these phagocytes are stimulated by adherence. The U-937 human monocyte cell line is different in that it is rarely permissive to dengue infection but occasionally a marginal infection is detected when the MOI is raised to 1.0 or more. In contrast to U-937 cells,

P-388 D_1, and human elutriated monocytes are somewhat permissive to dengue infection via the natural dengue receptor even at a low MOI of 0.1. The degree of permissiveness increases with MOI to the point where ADE may be difficult to detect when the MOI is > 1.0.

5. Antibody Dependent Enhancement of Dengue-1 Virus

Analysis of the epidemiological behavior of dengue-1 and dengue-2 in the Bangkok 1980 outbreak showed that the two serotypes had different disease patterns. Antibody dependent infection enhancement is probably less important for the development of DHF/DSS with dengue-1 than with dengue-2. To investigate the growth pattern of dengue-1 virus in mononuclear phagocytes, one dengue-1 and two dengue-2 viruses (Bangkok 1980 isolates) were used to infect U-937 cell culture in the presence and absence of monoclonal group reactive antibody (4G2) at the same MOI of 0.5. None of U-937 cells were infected by any of the three virus strains in the absence of dengue antibody. However, in the presence of 4G2 monoclonal antibody at an enhancing concentration, 50-60% and 10.7% of U-937 cells were infected by dengue-2 virus strains PUO-218 and PUO-294, respectively, while only 2.4% of U-937 cells were infected by dengue-1 strain PUO-451. Dengue-3 strain CH_5-3489 from Bangkok infected a low proportion of U-937 cells (2.2%) in the presence of flavivirus-negative human serum diluted 1:100 and the infection was enhanced 10 times in the presence of group-reactive monoclonal antibody 4G2. These results indicate the differences in capacity of dengue-1, 2 and 3 viruses to infect human mononuclear phagocytes. However, more strains of both dengue-1 and dengue-2 viruses should be tested to determine if dengue-1 can also infect human mononuclear phagocytes in the absence of dengue enhancing antibody and whether it is generally less enhanceable than dengue-2. Virus replication will also be compared in other cell system as in P-388D_1 or in human elutriated cells since one possible explanation for dengue-1 pathogenesis is that dengue-1 can infect mononuclear phagocytes effectively without depending on enhancing antibody to facilitate virus entry.

6. Effect of Chloroquine on Dengue Infection of Monocytes.

To further study the differences in the role of enhancing antibody towards development DHF/DSS by dengue-1 and dengue-2 viruses. The entry of virus via the natural dengue receptor and that via Fc receptor should be better understood. Fc receptor mediated entry is known to be associated with endocytosis. Since lysosomal vesicles are involved in endocytosis and chloroquine is known to concentrate in lysosmal vesicles, the effect of chloroquine on dengue ADE was determined. Due to their permissiveness to dengue infection at a detectable level, P-388D_1 and human elutriated monocytes were used in the study. These cells

were pre-treated with various concentrations of chloroquine which was also present throughout the culturing period. Cells were infected with dengue-2 virus strain 16681 (Bangkok isolate from a DHF case in 1968) in the presence and absence of enhancing antibody. Virus infection was measured by counting cells positive in an indirect immunofluorescent assay as well as determining PFU/ml for virus yield. In human monocytes, chloroquine at 0.01 mM inhibited infection via the Fc receptor by 85% whereas there was no inhibition (even an increase) in the number of monocytes infected via the virus receptor. Both Fc and virus routes of infection were inhibited (92% and 85% respectively) using 0.02 mM chloroquine; 0.05 mM chloroquine killed the cells. Similar results were obtained with the P-388D_1 macrophage cell line, but both virus and Fc receptor routes of infection were easier to demonstrate in the human cells. The selective inhibition by chloroquine suggests that the pathways of infection after virus entry were different between the two routes. This may be used as a tool to understand how dengue-1 and dengue-2 may depend upon two different pathways of infection to develop DHF/DSS in nature.

7. Natural Killer Cells in Dengue Infected Cells

Dr. Francis Ennis carried out experiments on natural killer (NK) cells during a two week MOBDES assignment in the department. Using continuous human mononuclear cells infected with dengue-3 and labelled with radioactive chromium; he observed increased lysis of infected cells with the addition of peripheral blood leukocytes from a vaccine recipient. In the absence of complement and antibody, cell lysis was attributed to the NK cells. Cell lysis was blocked by the addition of unlabelled dengue-3 infected U-937 cells to a greater degree than with uninfected or dengue-2 infected U-937 cells. These are the first observations of NK cell activity against dengue infected cells.

8. Live, Attenuated Dengue Vaccines

No new studies of dengue vaccines were initiated in FY83. Progress was made throu USAMRDC contractors and collaborating institutions fo prepare protocols for FY84. The first human trial of a dengue-1 (45AZ5) vaccine produced by the Salk Institute, Swiftwater, PA will be tested in 6 yellow fever immune volunteers at USAMRIID. The San Juan Laboratories, CDC, San Juan, Puerto Rico has prepared a protocol to test the dengue-2 (PR 159/S-1) vaccine in 20 people with pre-existing dengue antibody. Lot #1 of the dengue-3 (24/28) vaccine has been produced by Dr. Eckels, Dept of Biologics Research and is undergoing safety testing for IND application. A second dengue-4 vaccine is being developed by Dr. Marchette, University of Hawaii under contract.

9. Acquired Immunodeficiency Syndrome (AIDS) and Erhlichia Canis

In May 1982, Dr. Charles Kallick, Dr. Kaloo Thadhani, Dr. Miodrag Ristic, Dr Harold Kessler and Dr. Stuart Levin observed:

a. An Ehrlichia canis-like organism was isolated from the blood of 9 consecutive AIDS patients from the Chicago metropolitan area. (Ref. ICAAC Abstract #223, 1983.)

b. Immunological cross-reactivity was demonstrated between Ehrlichia canis and chlamydia trachomatis by indirect immunofluorescence. (Ref. ICAAC Abstract #632, 1983.)

These observations were extremely cantalizing, because Ehrlichia canis fulfilled the "profile" of a potential AIDS agent.

1) A zoonotic agent potentially introducible to man
2) A transmissible agent by blood and feces
3) An agent capable of producing chronic illness in mammals characterized by pancytopenia, adenopathy, and pulmonary disease.

During the months of June and July, our laboratory attempted to confirm the presence of an Ehrlichia canis-like organism in AIDS patients in several experiments performed in collaboration with NIH and the Chicago investigators. Primary isolation of an E. canis or a chlamydia-like organism was attempted using human monocyte cultures with and without antibiotics, and subsequent passage to McCoy cells using standard chlamydia technology. Direct isolation from whole blood and peripheral blood mononuclear cells was also attempted in McCoy cells. Isolation was attempted from 11 AIDS patients, 19 control patients and 1 pre-AIDS patient.

This laboratory was unable to isolate an Ehrlichia canis-like, or chlamydia-like organism from any tested AIDS or control patients. Positive and negative controls were performed for both E. canis and C. trachomatis employing direct immunofluorescence as a detection system with appropriate reproducible results. NIH was also unable to isolate an Ehrlichia canis-like or chlamydia-like organism from any tested AIDS or control patient. Experiments were also performed by the Chicago investigators in both our laboratory and laboratories at NIH. Under code the Chicago investigators identified "an agent" by their immunofluorescence techniques in 1/4 AIDS patients and 5/5 control patients.

Isolation of the candidate "AIDS agent" was finally attempted from supernatant of monocyte cultures from the original patients reported by the Chicago investigators. In one patient, this laboratory repeatedly isolated an C. trachomatis-like organism. The organism was detectable using direct immunofluorescence with a monoclonal antibody against C. trachomatis, however there was no immunological cross reactivity with polyclonal canine anti-E. canis FITC.

Additional experiments tested the reported immunofluourescent cross-reactivity between E. canis and C. trachomatis. No cross reactivity could be demonstrated by direct immunofluorescence (Table 1). Non-specific uptake of the conjugate was noted in various photographs including the control non-infected McCoy cells. However, by comparing the red block photographs with the corresponding fluorescence photographs, it was clear that these two organisms lack immunological cross-reactivity as measured by direct immunofluorescence. This laboratory was unable to confirm the observation described in either abstract.

Table 1

Direct Immunofluorescence Conjugate	Uninfected McCoy Cells	McCoy Cells infected with LGV	Dog monocytes infected with E. canis
E. canis FITC	-	-*	+
C. trachomatis FITC	-	+	-

* Cytopathic effect was noted in McCoy cells stained with anti E. canis FITC, however no immunological cross reactivity could be demonstrated by direct immunofluorescence.

Recommendation

Work should continue to identify and characterize the epitopes of dengue virus, especially those involved in viral neutralization. THis work, in conjunction with extramural contractors involved in dengue genome sequencing, provides the best chance for future dengue vaccine development through gene cloning and unravelling the mystery of dengue infection enhancement. Meanwhile continued testing of attenuated, live virus dengue vaccines is needed to better understand the immune responses to dengue infection and to learn the best way to provide polyvalent dengue immunity. Other viral and suspected viral illnesses, such as AIDS, should be studied as required by new situations.

Presentations

1. Henchal, E.A., McCown, J.M. and Gentry, M.K. Identification of Recent Dengue Viruses Having Altered Neutralization Determinants. 31st Annual Meeting of the American Society of Tropical Medicine & Hygiene, Cleveland, Ohio 11 Nov 82.

2. Brandt, W.E. Symposium on Use of Monoclonal Antibodies in Arbovirology. (Summary) 31st Annual Meeting of the American Society of Tropical Medicine & Hygiene, Cleveland, Ohio 11 Nov 82.

3. Henchal, E.A., McCown, J.M., Brandt, W.E. and Gentry, M.K. Antigenic and Biological Variation Occurring Among Dengue Type 4 Viruses. 83rd Annual Meeting of the American Society Microbiologists, New Orleans, LA 9 March 1983

Publications

1. Bancroft, W.H., Scott, R.McN., Brandt, W.E., McCown, J.M., Eckels, K.H., Hayes, D.E., Gould, D.J. and Russell, P.K. Dengue-2 Vaccine: Infection of Aedes aegypti Mosquitoes by Feeding on Viremic Recipients. Amer. J. Trop. Med. Hyg. 31: 1229-1231, 1982.

2. Repik, P.M., Dalrymple, J.M., Brandt, W.E., McCown, J.M. and Russell, P.K. RNA Fingerprinting as a Method for Distinguishing Dengue-1 Virus Strains. Amer. J. Trop. Med. Hyg. 32: 577-589, 1983.

3. Scott, R.McN., Eckels, K.H., Bancroft, W.H., Summers, P.L., McCown, J.M. Anderson, J.H. and Russell, P.K. Dengue-2 Vaccine: Dose Response in Subjects With and Without Yellow Fever Antibody. J. Infect. Dis. (In Press).

4. Eckels, K.H., Scott, R.McN., Bancroft, W.H., Brown, J., Dubois, D.R., Summers, P.L. and Russell, P.K. Human Response to Immunization with a Candidate, Live-Attenuated Dengue Type 4 (H-241) Virus Vaccine. Amer. J. Trop. Med. Hyg. (In Press).

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6. Scott, R.McN., Shelton, A.L., Eckels, K.H., Bancroft, W.H., Summers, R.J. and Russell, P.K. Human Hypersensitivity to a Sham Vaccine Prepared from Mosquito Cell Culture Fluids. (Submitted).

7. Butler, A.B., Scott, R. McN., Schydlower, M., Lampe, R.M., Schwab, J.A. and Muelenaer, A.A. The Immunoglobulin Response to Reimmunization with Rubella Vaccine. (In Press).

8. Scott, R.McN., Butler, A.B., Schydlower, M. and Rawlings, P. The Ineffectiveness of historical data in predicting measles susceptibility. Pediatrics (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AA	202 WWGB
b. CONTRIBUTING				
c. CONTRIBUTING	STOG 82/83-6.2/3			

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23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To define the epidemiology of hepatitis in military populations in order to establish methods for reducing disability from hepatitis. Emphasis is on developing and applying sensitive and specific assays for hepatitis viruses, antigens and antibodies and to determine factors important in resistance to disease.
24 (U) New methods for the isolation, identification and comparison of hepatitis viruses are under development. The immune response of patients with viral hepatitis is studied to define sensitive measures of infection and the critical factors relating to immunoprophylaxis. The epidemiology of hepatitis in military populations is described.
25 (U) 82 10-83 09. Hepatitis A virus was harvested from continuous African green monkey kidney cells (BS-C-1) and treated with formalin. Three intramuscular doses immunized 11 of 12 guinea pigs suggesting it may be feasible to produce an inactivated whole virus hepatitis A vaccine for humans. Unlike another enterovirus, poliovirus, the drug Arildone does not have antiviral activity against hepatitis A virus in vitro. A licensed hepatitis B vaccine was given to 50 volunteers to compare three 2 ug intradermal doses to three 20 ug intramuscular doses. At the time of the third dose (6 months), 21 of 23 recipients of intradermal and 22 of 23 recipients of intramuscular vaccine had antibody. Intradermal immunization may conserve vaccine and money if protection is not diminished. Collaborative studies with the Division of Preventive Medicine showed that enlisted personnel assigned to Europe and Korea and military prisoners are at increased risk of hepatitis B by virtue of location regardless of duty. IgM anti-hepatitis B core was found in 67 percent of Koreans with primary hepatocellular carcinoma as compared to only 1.6 percent of age- and sex-matched patients with other tumors. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval.

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Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 202 Mechanisms of Transmission of Hepatitis Viruses

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Problems and Objectives

The hepatitis viruses are among the most common infectious agents responsible for serious diseases among peacetime military forces today. The potential for increased transmission and epidemic spread of some forms of hepatitis, especially hepatitis A, and perhaps some types of non-A, non-B hepatitis, exists during times of mobilization with a possible resultant loss in combat effectiveness of troops. All forms of viral hepatitis may be prevented by interruption of virus transmission or passive and/or active immunoprophylaxis, although effective immunoprophylactic measures have not been fully developed. Current objectives within this work unit include the development of improved methods of specific viral diagnosis, characterization of hepatitis viruses, the study of modes of viral transmission and evaluation of means of preventing viral hepatitis.

Progress

1. Immunogenicity of Formalin-Inactivated Hepatitis A Virus Produced in Cell Culture

The ability to propagate hepatitis A virus (HAV) in cell culture has made possible the development of HAV vaccines. Following initial isolation and 10 serial passages of HM-175 strain

HAV in certified African green monkey kidney cells, virus titered 10^6 $TCID_{50}$/ml and was readily adapted to continuous green monkey kidney cells (BS-C-1). Virus was prepared in two lots from BS-C-1 cells maintained in Medium 199 without serum for 21 days. These two virus preparations (lot 1 and lot 2) contained $2.8x10^7$ and $8x10^6$ RFU/ml, respectively. Portions of both lots were treated with 1:4000 formalin at 36°C for 4 days. After 16-18 hours of formalin treatment, more than 99.9% of the virus was inactivated. Infectious virus was detected for up to 3 days, but only on blind passage. Following two IM doses (1 ml), 5 of 6 guinea pigs which received lot 1 and 5 of 5 guinea pigs given lot 2 developed anti-HAV (HAVAB radioimmunoassay). After 3 doses, all guinea pigs had antibody (GMT 1:199 and 1:101 for guinea pigs receiving formalin-inactivated HAV lots 1 and 2 respectively, compared with GMT 1:446 and 1:570 for guinea pigs immunized with the same virus lots prior to treatment with formalin). These studies thus demonstrate that it is possible to grow HAV in cell culture to titers sufficient for the production of immunogenic inactivated vaccines. Further studies on the safety, immunogenicity and protective capacity of these vaccines are in progress.

2. Serum Neutralizing Antibody Response to Hepatitis A Virus

Serum neutralizing antibody to hepatitis A virus (HAV) was measured in experimentally infected primates and naturally infected humans by means of an assay based on the autoradiographic detection of viral replication foci in vitro. Infection of primates with either PA-33 or HM-175 strains of HAV elicited antibody capable of neutralizing either strain. Sequential testing of two monkeys showed that neutralizing antibody correlated closely with antibody detected by immunoassay, developed prior to liver enzyme elevations and was associated with a substantial reduction in fecal shedding of viral antigen. In tests performed on human subjects involved in an outbreak of hepatitis A, neutralizing antibody was present 3 to 5 days prior to the onset of symptoms, and was found in both 19S and 7S immunoglobulin fractions. Immunity against HAV is probably due primarily to neutralizing antibody, and the ability to quantitate this antibody will be helpful in the evaluation of new HAV vaccines.

3. Recovery of Human Strains of Hepatitis A Virus (HAV)

Employing the procedures developed for the isolation of primate passaged HAV, five isolates have been obtained directly from man. African green monkey kidney cells (2nd passage) were more sensitive than BS-C-1 or FRhK-6 cells. In two instances, the isolates were detected in one month. Following serial passage in African green monkey kidney cells, three representative isolates were adapted to BS-C-1 cells. Antiserum to these 3 isolates have

been prepared in guinea pigs. Evaluation of these isolates for the preparation of inactivated vaccines are in progress.

4. The Effect of Arildone on the Replication of Hepatitis A Virus

Arildone (WIN 38020) inhibits poliovirus replication in cell culture, and prevents poliovirus-induced paralysis and death in mice. Because hepatitis A virus (HAV) is considered a picornavirus and shares many properties with poliovirus, we investigated the effect of arildone on HAV replication. Continuous green monkey kidney cells (BS-C-1) were infected with HM-175 HAV or poliovirus type 2. Anti-viral effect was assessed by counting foci of viral replication visualized by immune autoradiography (radioimmunofocus assay, HAV) or plaque assay (poliovirus). Arildone (3ug/ml), mixed with virus prior to infection, did not inhibit HAV radioimmunofocus formation but resulted instead in a 3.8 fold increase in radioimmunofoci relative to control cultures. Radioimmunofoci forming in the presence of arildone appeared denser and bound more 125-I anti-HAV than foci forming in the absence of drug (mean 287 cpm vs 114 cpm). Identical arildone treatment led to a 99% reduction in poliovirus plaque formation, but minimally inhibited cellular protein and RNA synthesis. Thus arildone does not inhibit HAV replication and may in fact lead to an increase in cell-associated HAV antigen, suggesting a fundamental difference between the early events in HAV and poliovirus replication.

5. Immunogenicity and Reactogenicity of Low Dose Intradermally Administered Hepatitis B Vaccine

The hepatitis B vaccine has been licensed for the prevention of Hepatitis B infections since June 1982 however, the high cost of the vaccine, concurrent with present day economic constructs have had a significant impact on the extent to which the HBV vaccination programs are implemented. A double-blinded randomized trial in 50 volunteers (comparing a three dose intramuscular injection series of 20ug of Heptavax B with a three dose intradermal injection series of 2ug of Heptavax B) was performed in FY83 in an attempt to determine if a lower dosage could safely provide adequate immunization. Preliminary results are summarized in Table 1.

Table 1. Antibody Response to HBV Vaccine;
2 ug Intradermally (ID) vs 20 ug Intramuscularly (IM)

	Anti-HBs Response					
	30 Day		60 Day		180 Day	
	ID	IM	ID	IM	ID	IM
Frequency of seroconversion P/N > 2.1*	4/25	8/25	19/25	22/25	21/23	22/23
Range of positive P/N values*	(6.1-42)	(2.3-162)	(2.1-140)	(2.5-228)	(3.6-163)	(5-277)
Median P/N of Responders	6.6	3.3	6.8	52.8	49	132
Mean P/N of Responders	15.6	25.4	22.2	66.8	49	133

* AUSAB (Abbott)

Reactogenicity has been limited to complaints of sore arm at the IM site for 24 hours in the intramuscular group (56%) and local erythema and induration at the ID site in the intradermal group (80%). Hyperpigmentation in the area of ID injection site was noted in 16% on the intradermal group. This method of administration of Hepatitis B vaccine is immunogenic with minimal reactogenicity. Additional testing will be needed to determine if a larger dose than 2 ug would be optimal, but this study demonstrates that intradermal HBV vaccination could potentially result in a significant savings of money and vaccine to the US Army.

6. Intraprison Transmission of Hepatitis B

During a recent hepatitis A outbreak at a military prison, in addition to 51 HAV cases, we identified 23 inmates with hepatitis B. All cases were IgM anti-HBc+. Other serologic markers included: HBsAg+ (6); HBsAg-, anti-HBs- (7); HBsAg-, anti-HBs+ (10). Of inmates with evidence of resolving HBV infection (70.8%), 17.6% had been imprisoned for more than 24 months. A random sample of inmates stratified for potential HBV risk factors demonstrated a HBV marker prevalence of 20.8% (HBsAg+ 4.6%; anti-HBc IgM+ 10.4%; anti-HBc+ anti-HBs+ 8.1% of which 64.3% were IgM anti-HBc+; and anti-HBc+, anti-HBs- 7.5% of which 40.0% were IgM anti-HBc+). Of all inmates, 79.2% were susceptible to HBV, 12.1% had recent or

current HBV infection, and 6.9% were immune. Of inmates with HBV markers confined for 3-12 months, 68% were IgM anti-HBc+, as compared to 42% of those confined < 3 months, and 22% of those confined 1-4 years ($p<.03$). Of inmates with HBV markers confined 3-12 months, 32% were immune, as compared to 78% of those confined 1-4 years ($p<.03$). These findings could not be explained by differences in pre-confinement risk factors. The prevalence of recent HBV disease (12.1%) and the point prevalence of clinical HBV disease (1.8%) establish the occurrence of intraprison viral transmission. A point prevalence survey revealed the highest prevalence of recent HBV infections occurred between 3-12 months in prison (Table 2). In light of these data seronegative inmates should be considered for HBV immunization.

Table 2. Prevalence of HBV Markers in Prisoners

Time in Prison (Months)	#Inmates	HBsAg+	% anti-HBc+ IgM+	% anti-HBc+ IgM-
Admission	232	2.6	5.4	4.9
<3	45	2.2	6.7	8.9
3-12	71	5.6	18.3	8.5
13-24	37	2.7	2.7	13.5
>24	20	1.0	5.0	10.0

7. Assessment of HBV Risk in Military Populations by Geographic Assignment

In collaboration with Dr. L. Gardner and MAJ W. Lednar, Division of Preventive Medicine, the incidence of hospitalizations for Hepatitis B disease were assessed in enlisted military population (number 568,404) to determine the incidence of hospitalization for HBV disease by geographic assignment and M.O.S. Results obtained from Individual Patient Data System (IPDS) data are summarized in Table 3.

Current U.S. Army Hepatitis B vaccine policy is directed at the vaccination of high risk medical personnel with frequent occupational blood exposure; however, these data clearly demonstrated the increased risk of any enlisted personnel assigned to Europe or Korea (1.47X + 2.08X respectively) as compared to high risk U.S. assigned personnel which are the certain focus point of Hepatitis B vaccination policy. Presently, this policy should be re-evaluated to determine if HBV vaccination of military personnel should be based on geographic assignment.

Table 3. HBV Hospitalization Rates Per 100,000

Occupation (Selected MOS)	Geographic Assignment CONUS	EUROPE	KOREA
1. Medical with frequent blood exposure	113	222	369
2. Combat Arms	86	171	250
3. Combat Support	75	171	238
4. Average rates for total enlisted personnel in geographic area	80	173	245

8. Low Molecular Weight IgM anti-HBc Antibody in Chronic Hepatitis B Virus Infections

IgM antibody to hepatitis B virus core antigen (IgM anti-HBc) develops during acute hepatitis B but frequently persists in chronic infections. In order to characterize persistent IgM anti-HBc better, sera from 17 patients known to have histologically proven chronic hepatitis were subjected to rate-zonal centrifugation in sucrose gradients. Low molecular weight (7-8S) and high molecular weight (19S) immunoglobulin fractions were tested for IgM anti-HBc by a sensitive antibody-capture radioimmunoassay. Three patients with acute hepatitis B served as controls. In 16 of the 17 chronic hepatitis sera peak activity was found in 7-8S fractions, although in 11 sera a minor peak was also present in the 19S fractions. In contrast, the sera from 3 controls and 1 chronic carrier had peak activity in the 19S region.

The low molecular weight of the predominant IgM anti-HBc was confirmed by gel filtration. Additionally, competitive binding experiments showed the 7-8S antibody to an anti-IgM coated solid phase was blocked more effectively by purified IgM than by purified IgG. These findings indicate that hepatitis B carriers with chronic active hepatitis have predominantly 7-8S IgM anti-HBc. This represents one of the rare instances in which defined antiviral specificity of low molecular weight IgM has been demonstrated.

9. IgM Anti-HBc in Patients with Hepatocellular Carcinoma

Primary hepatocellular carcinoma (PHC) occurs in a geographic distribution which is very similar to that of hyperendemic hepatitis B virus (HBV) infection. While all chronic HBsAg carriers can be shown to have antibody to hepatitis B core antigen, the finding of the IgM class antibody to hepatitis B core antigen (IgM anti-HBc) usually indicates the persistence of high levels of viral replication and biochemical evidence of chronic hepatitis. We investigated the frequency of serum markers of HBV infection,

including IgM anti-HBc in 110 adult Korean patients with PHC and in 63 age-and sex-matched Korean controls with other tumors. Seventy-four (67%) PHC patients had IgM anti-HBc compared with only 1 (1.6%) of the controls (P<0.001). In contrast 90 (81%) PHC patients were HBsAg-positive as well as 9 (14%) controls (P<0.001). Thus although IgM anti-HBc was found less often than HBsAg in PHC patients, it may be more specifically associated with primary hepatocellular carcinoma. IgM anti-HBc was found in more HBeAg-positive patients with PHC (91%) than in those positive for anti-HBe (74%) (p<0.04), while it did not correlate with the presence or absence of antibody to HBsAg (P=N.S.). In conclusion the presence of IgM anti-HBc in adult Korean HBsAg carriers may indicate an especially high risk for the development of primary hepatocellular carcinoma.

Recommendation

The efficacy of the inactivated HAV vaccine must be determined by direct challenge of immunized Aotus monkeys. In the meantime, the simultaneous production of one lot of vaccine by the Department of Biologics Research will speed up the vaccine development process. WRAIR should also plan to test a live attenuated HAV vaccine as they become available and seek new methods for distinguishing attenuated strains from wild HAV viruses. The diagnostic and prognostic roles of IgM anti-HBc in primary hepatocellular carcinoma is yet to be defined, as is the meaning of low molecular weight IgM anti-HBc. Work is needed to diagnose non-A, non-B hepatitis infections in military personnel.

Presentations

1. Sjogren, M., Lemon, S.M. Low Molecular Weight IgM anti-HBc antibody in chronic hepatitis B virus infections. 32nd Annual Meeting American Association for the study of Liver Diseases. Chicago, Illinois 5-8 November 1982

2. Bancroft, W.H. Hepatitis A: Prospects for Prevention. American College of Preventive Medicine, Montreal, Canada. 15 November 198[illegible]

3. Lemon, S.M., Gates, N.L., Tiniacos, C., Verduin-Zgonina, M.M., Marchwicki, R.H. and Binn, L.N. Neutralizing Antibody to Hepatitis A Virus Detected by a Radioimmunofocus Inhibition Test. 83rd Annual Meeting of the American Society Microbiologists, New Orleans, LA, 7 March 1983 (Page 297)

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7. Lednar, W.M., Redfield, R.R., Kelley, P.W., Hayne, S.T. and Miller, R.N. When is It Too Late to Investigate an Outbreak? Society of Epidemiological Research, Winnipeg, Canada. 16 June 1983

8. Gardner, L., Lednar, W., Redfield, R. and Miller, R. Hepatitis B Hospitalizations in High Risk Occupations. Society of Epidemiological Research, Winnipeg, Canada. 16 June 1983

ABSTRACTS

1. Sjogren, M., Lemon, S.M.: Low molecular weight IgM anti-HBc antibody in chronic hepatitis B Virus Infections. Hepatology 5: 690, 1982

2. Sjogren, M., Lemon, S.M., Bancroft, W.H. Anti-HBe and anti-HBs antibodies in HBsAg negative patients with acute hepatitis B. Gastroenterology 84: 1397, 1983

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OA 6443	2. DATE OF SUMMARY* 83 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 82 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8A. DISP'N INSTR'N NL	8B. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AB	203 WWG2
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	STOG 82/83-6.2/3			

11. TITLE (Precede with Security Classification Code)*

(U) Bacterial Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
58 05	CONT	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	3.0	626
	CURRENT 84	4.0	624

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NAME: Zollinger, W, Schneider, H

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Pseudomonas aeruginosa; (U) Neisseria meningitidis; (U) Gonococcus; (U) Immunology; (U) Antibiotics: (U) Infectious Diseases; (U) Bacterium

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Studies on the etiology, ecology, epidemiology, pathogenesis, physiological, immunological and diagnostic aspects of diseases of microbial origin which are current or potential problems to military forces. Current emphasis is on control of meningococcal, gonococcal and pseudomonas infections in military forces.

24. (U) Basic studies on bacterial pathogens which will elucidate mechanisms of pathogenesis and result in future development of prophylactic agents.

25. (U) 82 10-83 09 Work has continued on the development of immunoprophylactic measures against Pseudomonas aeruginosa and Escherichia coli. Monoclonal antibodies with specific binding for 11 of the 16 serotypes of Pseudomonas aeruginosa have been produced. A monoclonal which reacts with a core-determinant in the LPS of all serotypes of Pseudomonas aeruginosa and reacts with the J-5 mutants of Escherichia coli O111 has also been found. Monoclonal antibody against Pseudomonas aeruginosa toxin A and Pseudomonas aeruginosa pili have also been produced. Pseudomonas LPS vaccines have been tested and shown to be efficacious. Work on the virulence factors in bacteremia Escherichia coli has demonstrated that length or presence of O-side chain alone cannot determine sensitivity to kill in fresh serum. Immunogenicity of polypeptides was enhanced by hydrophobic complexing them to meningococcal outer membrane protein "protosomes". Two methods were developed and used to obtain new information on the human immune response to meningococcal outer membrane proteins, monoclonal antibodies specific for the major outer membrane proteins and the "western blot" method. (For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83).

*Available to contractors upon originator's approval

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY, AND HEALTH HAZARDS

Work Unit 203: Bacterial Diseases of Military Importance

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LTC George Lowell, MC
COL Jerald Sadoff, MC
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Associates: MAJ John Boslego, MC
Herman Schneider, Ph.D.
Robert Seid, Ph.D.

Progress

We have continued work on the development of immunoprophylactic measures against P. aeruginosa and E. coli. We have produced monoclonal antibodies with specific binding for 11 of the 16 serotypes of P. aeruginosa. These monoclonals bind to the O-side chain region of LPS from these organisms as demonstrated by Western blot. Several of these have been tested for protection in animals and have been efficacious in mouse intraperitoneal challenge experiments. Monoclonals that have demonstrated cross reactive determinants in P. aeruginosa LPS, both in O side chain and core regions have been found. A monoclonal which reacts with a core-determinant in the LPS of all serotypes of P. aeruginosa and reacts with the J-5 mutant of E. coli 0111 has also been found. This unique cross reactive determinant is being tested for protective efficacy and opsonic activity. This monoclonal demonstrates the existence of cross reactive determinants between E. coli J-5 and P. aeruginosa. Monoclonal antibody against P. aeruginosa toxin A and P. aeruginosa pili have also been produced. Several of the pili monoclonals show broad cross reactivity among pili from P. aeruginosa. Polyclonal rabbit antibodies against pili from strain 12.4.4 were able to block attachment of 12.4.4 organisms to damaged endotracheal rings. Purified 12-4-4 pili were also able to block attachment. The only monoclonal tests thus far for ability to block was unsuccessful. The monoclonals with cross reactivity will permit us to develop a pili typing system.

Pseudomonas LPS vaccines prepared for human use have been tested by collaborating in the chronic lung and acute pneumonia models of infection and shown to be efficacious.

Work on the virulence factors in bacteremic E. coli has demonstrated that length or presence of O-side chain alone cannot determine sensitivity to kill in fresh serum. Comparing O-12 K1 strains to O-6 K5 strains removal of the capsule in the core of O-12K1 produces a serum sensitive organism while removal of K5 from the O-61K5 strain does not. Monoclonal antibodies were prepared against core and O-side chain as well as protein determinants of the BART K1 organism. In constrast to K1 monoclonals these monoclonals were not opsonic for BART K1 implying that capsular antibody is uniquely important.

The factor which stimulates white cells which we have been characterizing was demonstrated to stimulate C3b but not Fc receptors on human WBC.

A presumed protein factor in outer membrane complex from Pseudomonas aeruginosa type 5 has been shown to confer non-specific immunity to heterologous challenge in the mouse model.

Attempts to develop a peptide conjugate system as an approach to vaccine development has proceeded. E. coli LPS was detoxified with succinic anhydride and a technique for covalently coupling this detoxified LPS to peptides was developed. The model peptide demonstration was initially utilized and later a peptide containing an important antigenic determinant in Hepatitis B virus was coupled to detoxified LPS. This conjugate is currently being tested for immunogenicity.

Immunogenicity of polypeptides was enhanced by hydrophobic complexing them to meningococcal outer membrane protein "protosomes" even in LPS-non-responding mice and even when LPS contamination was reduced to less than 1%. This indicates that the protosomes which have safely been administered to thousands of soldiers, may be used without LPS to potentiate the immunogenicity of non-related peptide antigens. Preliminary experiments using synthetic peptide antigens have been performed to support this thesis.

Influenza viruses can activate the production of heterologous anti-viral and anti-bacterial antibodies by human lymphocytes in vitro. This polyclonal activating

capability will aid in the development and analysis of immunopotentiating agents which will act as adjuvants in polypeptide vaccines.

Further development of an effective group B vaccine is dependent on identification of the specific outer membrane proteins to which human bactericidal antibodies are directed. Two methods were developed and used to obtain new information on the human immune response to meningococcal outer membrane proteins following vaccination and systemic disease. Monoclonal antibodies specific for the major outer membrane proteins (classes 1, 2, 3 and 5) of serotype 2b and 15 strains were produced and characterized with respect to specificity, and the particular outer membrane protein on which the epitope resides. Human sera obtained following vaccination (experimental group B vaccine) or natural infections were assayed for the amount of antibody with the same specificity as a given monoclonal antibody by inhibiting the binding of the monoclonal antibody to a limiting amount of antigen with serial dilutions of the sera. Both vaccination and natural infections were shown to induce antibodies to the class 1, 2, 3 and 5 major outer membrane proteins. A second approach to detecting the presence of antibodies to specific outer membrane proteins is by the "western blot" method. This procedure, which involves electrophoretic transfer of proteins to nitrocellulose paper after separation by slab gel electrophoresis in sodium dodecyl sulfate, often results in denaturation of the proteins and loss of the capacity to bind antibody. The value of this approach was enhanced by the demonstration that the antibody binding capacity of some outer membrane proteins that were denatured during electrophoresis could be partially restored by doing the electrophoretic transfer in the presence of the appropriate concentration of the zwitterionic detergent Empigen BB.

Future Plans

The specificity of human antibodies which have human complement-mediated bactericidal activity for group B meningococci will be examined and the results used to determine which outer membrane proteins are most important in a group B vaccine. Additional anti-meningococcal monoclonal antibodies will be produced to extend the number of serotyping reagents available, to further characterize the human immune response to the outer membrane proteins, and to search for common determinants which might form the basis for an improved group B vaccine.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG6763	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. SMRY INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM & WORK UNIT
82 10 01	D Change	U	U		NL	☒ YES ☐ NO	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AC	204 WRGC
b. CONTRIBUTING				
c. [redacted]	STOG 82/83-6	2/3		

11. TITLE (Procedure, with Security Classification Code)

(U) Rickettsiae - Host Interactions in Pathogenesis of Disease

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
80 10	Cont	DA			C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER

c. TYPE d. AMOUNT

e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE	PRECEDING	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	83	3.0	244
	CURRENT 84	2.0	308

19a. RESPONSIBLE DOD ORGANIZATION

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NAME: Gowda, S POC: DA

20. GENERAL USE

Foreign Intelligence Considered

21. KEYWORDS (Provide Each with Security Classification Code)

(U) Rickettsiae; (U) Biochemistry; (U) Structure - Function Relationship; (U) Structure - Antigenicity

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide Individual paragraphs identified by number, precede text with Security Classification Code.)

23. (U) Isolate and characterize subcellular fractions of rickettsiae which have potential as experimental immunogens. Locate and identify the rickettsial surface antigens that affect virulence. Evaluate in mice the immunogenic potential of rickettsial fractions. Investigate alternate techniques to facilitate the early diagnosis of rickettsial diseases and detect rickettsial antigens. These studies will aid in improving the accurate rapid diagnosis of rickettsial infections and in the development of vaccines capable of protecting troops deployed in areas endemic for rickettsial diseases.

24. (U) Isolate subcellular fractions of rickettsial organisms using low ionic strength buffer, ether extraction, and genetic recombination methodologies. Characterize potential immunogens by physiochemical and immunological methodologies. Use antibody prepared against rickettsial surface antigens to evaluate the possible role of the antigens in body fluids of infected laboratory animals. Isolate and determine the biochemical and immunologic characteristics of these antigens.

25. (U) 82 10 - 83 09 Recombinant DNA methods have been utilized with organisms representing both the scrub typhus and spotted fever groups of rickettsial organisms. Rickettsial citrate synthase has been transferred to a deficient E. coli demonstrating the technological feasibility of the approach. Clones continue to be screened for candidate rickettsial vaccine components. An enzyme linked immunosorbant assay was developed for both spotted fever groups and scrub typhus group rickettsiae. This technique is able to detect a rickettsial antigen in the urine of infected guinea pigs. For technical report see Walter Reed Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 FOR ARMY USE ARE OBSOLETE

Project 3M161102BS10 — RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 204 — Rickettsiae-Host Interactions in Pathogenesis of Disease

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1LT Srinivas Gowda, MSC,

Associates: SP4 Wilfredo A. Cardona

Problems and Objectives

The impact of scrub typhus upon American troops during World War II and Viet Nam has of course been well recognized. The development of a vaccine against this disease has been hampered by the lack of knowledge concerning the nature of the components of the whole organism that are responsible for the generation of the protective immune response that one sees for instance following a natural infection. One approach to vaccine development utilizes subunits of rickettsiae as vaccine components and has the advantage of minimizing undesired reactogenicity while tailoring an optimal protective response. Problems associated with this approach include the identification, purification and characterization of the immunodominant rickettsial components, made all the difficult if one depends upon eucaryotic cell systems to replicate the rickettsial organisms. The success of DNA recombinant techniques in other areas of medicine and industry make the use of these methods an attractive alternative for the production of potential rickettsial antigens. The DNA recombinant approach with particular regard to scrub typhus has been set up within the last three to four months. Our goals in employing the recombinant techniques would be to generate immunogens for potential vaccine use without having to rely on costly and time consuming eucaryotic cell systems.

Progress

Recombinant DNA methodologies have been utilized with organisms representatives of two major rickettsial groups, Rickettsia conorii (spotted fever group) and the Karp strain of R. tsutsugamushi (scrub typhus group). DNA was isolated by phenol extraction from purified R. conorii cells or from partially purified Karp strain rickettsiae, and was purified by centrifugation in cesium chloride-ethidium bromide gradients. The optimal conditions for digesting rickettsial DNA were established by

varying restriction enzymes and times of digestion and by analyzing the extent of R. conorii DNA fragmentation using ethidium bromide-stained agarose gels. R. conorii and Karp strain DNA were then digested with the restriction endonuoclease, Sau3A, and cloned into an E. coli K12 strain recipient using a Bam-digested pHC79 cosmid vector. Recombination was verified by analyzing the digestion patterns of selected recombinant DNAs on agarose gels, or in the case of Karp strain DNA, for complementation of a metabolic deficiency (citrate synthase activity) in a mutant E. coli recipient. Recombinant clones also were screened on antibiotic-containing media and then were tested with anti-rickettsial antibody to determine if detectable rickettsial antigens were being produced. The antibody assay utilized for this testing was chosen after trial and modification of a number of established techniques for the immunologic detection of recombinant proteins. Essentially, recombinant organisms were applied to a nitrocellulose support film, reacted with high-titered rabbit anti-rickettsial antibody, and then probed with and ^{125}I-labeled, affinity purified anti-rabbit IgG. The sensitivity of this assay has been determined to be 1-10 ng of R. conorii protein, and approximately 100 ng of Karp strain antigen. For each of these rickettsiae, approximately 1,000 recombinants have been tested immunologically; however, the number of recombinants available is virtually unlimited, and larger numbers of recombinants will have to be tested before rickettsial antigen-expressive organisms are found.

Studies aimed at analyzing and potentiating the immunogenicity of isolated rickettsial antigens have concentrated on the erythrocyte-sensitizing substance (ESS), a loosely bound, carbohydrate antigen present on the surface of most (except scrub typhus) rickettsiae. Infection of T-cell-deficient mice with typhus group, spotted fever group, and scrub typhus group rickettsiae resulted in an IgM antibody response in all but the scrub typhus-infected animals. These results pointed out a significant difference in the interactions of scrub typhus antigens and antigens of the other rickettsiae with the immune system of the murine host, and also suggested that the ESS is a T-cell-independent antigen. In experiments performed to enhance the immuno-genicity of this carbohydrate antigen, ESS from R. typhi rickettsiae was chemically coupled to tetanus toxoid (i.e., became T-cell-dependent) and injected into rabbits. The animals responded with anti-ESS antibody three weeks after immunization, and maintained significant antibody titers in the serum past the twelfth week. As least two antibody populations were produced by this immunization and they differed kinetically, in antibody class, and reactivity with different rickettsial antigens (i.e., in various serological tests).

An enzyme-linked immunosorbant assay (ELISA) was developed for both spotted fever group (R. conorii) and scrub typhus group (Karp strain) rickettsiae. Initial experiments were performed to investigate the ability to detect rickettsial antigen in the body fluids of infected animals using this ELISA. Guinea pigs inoculated with a mouse-lethal strain of R. conorii responded with clear-cut clinical signs (fever, scrotal reactions) and appeared to excrete a rickettsial-specific substance in the urine. This substance is inhibitory to the ELISA reaction of R. conorii soluble antigen and rabbit antibody against R. conorii, and thus, is likely to contain some of the surface antigens of this rickettsia. The feasibility of using other animal species (mice, monkeys) for antigen detection and characterization is being pursued.

Recommendation:

At the start recombinant DNA methodologies were worked out using the R. conorii model. In mid to late June 1983, permission to also include Rickettsia tsutsugamushi was obtained and within three months evidence of the successful transfer of rickettsial genetic materials into E. coli was obtained. Having demonstrated the technological feasibility of performing this recombinant work with Rickettsia tsutsugamushi and E. coli we will screen the resultant clones for rickettsial antigen expression. Expressed rickettsial antigens will be evaluated individually or in combination as potential vaccine candidates. Only approximately 1000 clones have been studied thus far. If at the end of 5000 clone screenings we were still unable to detect rickettsial antigens, an alternate host other than E. coli will be introduced. Along with the basic screening activity we are constantly engaged in upgrading the sensitivity of our assay procedures and maintaining a running dialogue with Dr. Kopecko and through him with the community of genetic scientists. We are ready to exploit any new alternatives that may serve improve our chances to obtain rickettsial expression.

A joint venture with Dr. Dasch's group at the Naval Medical Research Institute has been undertaken for the production of monoclonal antibodies to a variety of scrub typhus strains. These antibodies should prove mutually beneficial not only as molecular probes but in projected affinity chromatographic isolation of potential rickettsial vaccine antigens.

The development of an improved ELISA system using polyvalent or monoclonal antibodies where appropriate will continue. This technique not only bears upon our recognition of discrete rickettsial antigens produced by DNA recombinant technology but also is a key step in the identification of scrub typhus

rickettsial antigens that may or may not be found in patient urine.

Presentation

Eisemann, Christine S.: "Susceptibility of Inbred Mice to Rickettsiae of the Spotted Fever Group," Annual Meeting of the American Society for Microbiology, March 1983.

Bibliographies

1. Eisemann, C.S., M.J. Nypaver, and J.V. Osterman. 1983. Susceptibility of Inbred Mice to Rickettsiae of the Spotted Fever Group. (Accepted for publication.)

2. Jerrells, T.R, an C.S. Eisemann. 1983. Role of T-Lymphocytes in Antibody Production to Antigens of Rickettsia tsutsugamushi and other Rickettsia species. Infect. Immun. 41: 666-674.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6514	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORG'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102B510	AD	205 WWG4
b. CONTRIBUTING				
c. [redacted]	STOG 82/5[illegible]			

11. TITLE (Precede with Security Classification Code)

(U) Vector Transmission of Militarily Important Infectious Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	5.0	553
FISCAL YEAR CURRENT 84	6.0	657

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ASSOCIATE INVESTIGATORS

NAME: Schneider, I

NAME: Ward, R POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Malaria; (U) Mosquitoes; (U) Trypanosomiasis; (U) Tsetse flies; (U) Leishmaniasis; (U) Sand flies; (U) Scrub Typhus

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Develop physiological means of interrupting malaria and leishmaniasis transmission through an understanding of factors affecting parasite infectivity in vivo and in vitro. Refine models of malaria, leishmaniasis and African trypanosomiasis transmission to obtain large numbers of parasites for the study of immune mechanisms. Assess competence of closely related species as malaria and leishmaniasis vectors. Develop method of testing repellents against tsetse flies. Develop a field applicable serological test to detect anophelines infected with falciparum malaria. Realization of objectives may lead to prevention or control of malaria, leishmaniasis, trypanosomiasis and scrub typhus in military troops.

24. (U) Continue producing falciparum sporozoites needed for characterization of monoclonal antibodies. Identify factors that influence that infection process of falciparum malaria in anopheline mosquitoes. Establish colonies of phlebotomine sand flies for leishmaniasis transmission studies. Compare susceptibility of different sand fly and anopheline species to leishmanial and malarial parasites respectively. Develop the ELISA test to detect P. falciparum sporozoites in mosquitoes. Refine methods for testing repellents against tsetse flies. Produce large numbers of procyclic trypanosomes for African trypanosomiasis vaccine feasibility studies.

25 (U) 82 10 - 83 09 Improvements were made in the malaria model and 58 million P. falciparum sporozoites were produced. Monoclonal antibodies to vivax malaria sporozoites were produced and ELISAs were developed for detecting vivax and falciparum malaria sporozoites in anophelines. Colonies of 5 sand fly species were established and infection studies are underway. The tsetse colony was greatly improved and the pace of infections and vaccine feasibility studies have been accelerated. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 82 to 30 Sep 83.

*Available in machine form upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. [illegible]

PROJECT 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

WORK UNIT 205 Vector Transmission of Militarily Important Infectious Diseases

Investigators
Principal: Donald R. Roberts, LTC, MSC

Associate: Lyman W. Roberts,MAJ, MSC; Peter V. Perkins, MAJ, MSC; Robert A. Wirtz, CPT, MSC; John Werren, CPT, MSC; Ronald A. Ward, Ph.D.; Imogene Schneider, Ph.D.; Thomas R. Burkot, Ph.D.; Edgar D. Rowton, Ph.D. (NRC Fellow); David E. Hayes; Lawrence M. Macken; SSG Charles Hayes; SP5 Megan G. Dowler; SP4 Lesyle Graves; SP4 Pedro Quintero

Problems and Objectives

Malaria, trypanosomiasis and leishmaniasis are arthropod-borne diseases of great military medical importance. Consequently, the development of vaccines for these diseases is a high priority endeavor. The detection of protective antigens against the sporozoite stage of falciparum malaria is one of the most promising approaches to a malaria vaccine. Antigen variation at least in the tsetse fly transmitted forms of African trypanosomiasis and use of fly-form parasites for identifying protective antigens also offers the most promising approach to vaccine development. An effective vaccine must be active against the arthropod-borne form of parasite. Thus laboratory system also are needed for producing infected vectors to challenge experimentally immunized animals against leishmaniasis, trypanosomiasis and malaria. The value of sand fly transmitted leishmanial parasites for detecting protective antigens is currently under study. Current research objectives are to 1) develop or improve on existing laboratory transmission systems for the groups of diseases listed above, 2) develop immunochemical tests for detecting infectious parasites in natural vector populations for malaria and leishmaniasis and 3) determine factors influencing infection rates of malaria, African trypanosomiasis and leishmaniasis parasites in anopheline, tsetse and sand fly vectors, respectively.

Progress

Malaria: A system was developed for producing large numbers of falciparum malaria sporozoites by infecting Anopheles freeborni mosquitoes with cultured Plasmodium falciparum malaria parasites.

This successful effort formed the basis for creating a major sporozoite vaccine development effort within the WRAIR. Infection rates have been consistently much higher than a year ago both in percentage of mosquitoes infected and in numbers of oocysts and sporozoites per mosquito. The sporozoites are isolated first from extracted salivary glands and then from homogenized mosquito bodies placed on density gradients. Sporozoite preparations from the former are much cleaner than those from the latter but have fewer numbers. To date, 58 x 10^6 sporozoites have been isolated and frozen in aliquots ranging from 50,000 to 12 million parasites. Sporozoite yield per infected mosquito has ranged from a few hundred to 15,000.

Additional studies comparing different species of anophelines for susceptibility to infection with cultured Plasmodium falciparum gametocytes have reinforced earlier findings that An. freeborni is the most susceptible vector yet tried, based on oocyst counts. However, An. stephensi mosquitoes, although usually possessing lower oocyst numbers have, on occasion, produced considerably larger number of sporozoites on a per infected mosquito basis. Two strains of An. quadrimaculatus were also tested and found to be virtually unsusceptible to the strain of P. falciparum currently in culture.

Monoclonal antibodies against the sporozoites of the two most prevalent human malarias, Plasmodium falciparum and P. vivax, were developed. These antibodies were used to develop ELISA's for both parasites. Postive reactions were obtained only with homologous parasites when tested against sporozoites of various other species of Plasmodium. An in-depth evaluation of the falciparum double antibody ELISA indicates that the assay will detect as few as 300 sporozoites per infected mosquito. Using extract of dried infected mosquitoes as antigen, the assays were sensitive enough to detect one infected mosquito in a pool of 20 specimens. Results from laboratory evaluations indicate that the assays 1) are specific and highly sensitive, 2) can be performed with fresh or dried mosquitoes, 3) can process mosquitoes singly or in pools, 4) can utilize lyophilized antisera without loss of activity and 5) do not require the use of expensive capital equipment. These assays are currently undergoing field evaluation to determine the merit of producing kits to provide a test capability for Army field preventive medicine units.

Leishmaniasis: Research on sand flies and leishmaniasis was initiated within the last 12 months. Colonies of the following species have been established:

1. Lutzomyia longipalpis (Brazil)
2. L. anthophora (USA)
3. Phlebotomus papatasi (Israel)

4. P. martini (Kenya)
5. L. shannoni (USA)

Working colonies are in full production for the first 3 species. The laboratory facility has been renovated and a double screened holding chamber for infected specimens has been constructed. Prior to completion of this chamber infection studies were performed with L. anthophora only since it will not bite humans. This species was successfully infected with Leishmania tropica, L. donovani, L. braziliensis and L. mexicana. Infection studies are currently underway with the natural vectors of these disease agents.

African Trypanosomiasis: The tsetse colony has been greatly improved during the last year. Current production of flies (over 4000 reproducing females) exceeds requirements. A total of 21 infections have been conducted since January of this year compared to only 63 for the preceeding 6 years. The success in producing tsetse flies and producing fly-form parasites has greatly accelerated the vaccine feasibility studies for African trypanosomiasis. A new method of detecting infected flies was developed that decreases the time required for processing each fly for infection. The flies are allowed to probe on warm glass slides. At that time the infected flies extrude parasites in salivary fluids. The slides are then stained and examined for trypanosome parasites. The repellent action of DEET and Permethrin has been tested against Glossina morsitans and test results are being prepared for publication. Permethrin did not prevent the tsetses from probing before toxic effects occurred.

Recommendations for the future

The production of malaria sporozoites should be continued in support of the sporozoite vaccine development effort. Emphasis should be placed of selecting and properly managing malaria clones that produce high infection rates and large numbers of sporozoites.

Population selection studies should be conducted to develop strains of mosquitoes and parasites that produce maximum numbers of sporozoites. As part of this work studies should be performed to identify the barriers to sporozoite production.

Develop methods for culturing the exoerythrocytic stages of falciparum malaria.

Develop systems for producing large numbers of the various strains (species/subspecies) of leishmanial parasites.

Continue to improve the tsetse fly colony and increase the yield of tsetse fly-form parasites.

Formal Presentations

Roberts, D.R. U.S. Army research programs on mosquitoes and mosquito-borne diseases. An invited presentation made to the opening session of the annual meeting of the American Mosquito Control Association in Florida, 1983.

Publications

Rener, J., P.M. Graves, R. Carter, J.L. Williams and T.R. Burkot. 1983. Target antigens of transmission blocking immunity on gametes of Pasmodium falciparum. J. Exp. Med. (In press).

Williams, J.L., B. Innis, T.R. Burkot, D.E. Hayes and I. Schneider. 1983. Falciparum malaria: accidental transmission to man by mosquito after infection with culture derived gametocytes. Am. J. Trop. Med. Hyg 32: 657-659

Burkot, T.R., J.L. Williams and I. Schneider. Falciparum malaria clones grown in vitro differ in infectivity to mosquitoes. (Submitted for publication).

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Lightner, L. and L.W. Roberts. Mechanical transmission of Leishmania major by Glossina morsitans morsitans (Diptera: Glossinidae). J. Med. Ent. (In press).

Parker, A.G., M.E.C. Giglioli, S. Mussington, A.B. Knudsen, R.A. Ward and R. Aarons. 1983. Rock hole habitats of a feral population of Aedes aegypti on the island of Anguilla, West Indies. Mosq News 43: 79-81.

Roberts, L.W., Lars Karstad, Y. Varma and F.K. Onyargo. Attraction of Glossina pallidipes Austen (Diptera, Glossinidae) to four species of wild ruminants in Kenya. (In preparation).

Schneider, I. 1983. Book review: K. Maramorosch and J. Mituhashi (eds.) 1982. Invertebrate Cell Culture Applications. Quart. Rev. Biol. 58: 418.

Wirtz, R.A. 1983. Health aspects: Disease agents. In: Ecology and Management of Food-Industry Pests, J.R.R. Gorman, ed., FDA Tech. Bull. No. 4 (Accepted for publication).

Wirtz, R.A. 1983. Allergic and toxic reactions to non-stinging arthropods. Ann. Rev. Entomol. 29: 47-69 (In press).

Wirtz, R.A. 1983. Health and safety in arthropod rearing. In: Advances and Challenges in Insect Rearing, E.G. King and N.C. Leppla, eds. U.S. Dept. Agric. Tech. Bull. (In press).

Wirtz, R.A. 1983. Book review: W.F. Tordoir and E.A.H. van Heemstra (eds.) 1980. Field Worker Exposure During Pesticide Application. J. Occupational Medicine 25: 14.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6436	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N MGT'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
A. PRIMARY	61102A	3M161102 BS10	AE	206 WWGF
XXXXXXXX				
XXXXXXXX	STOG			

11. TITLE (Procede with Security Classification Code)*

(U) Microbial Genetics and Taxonomy

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
63 08	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	4.0	715
	CURRENT 84	4.0	748

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
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NAME: Kopecko, D J
NAME: Wohlhieter, J A POC: DA

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Vaccine; (U) Enteric Bacteria; (U) Antigens; (U) Virulence; (U) Salmonella; (U) Plasmids; (U) Shigella

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) To define in genetic and molecular terms gene transfer, antigenicity, and virulence of pathogenic enteric bacteria which because of their disease producing capabilities are of importance to military medicine, a major concern of which is the prevention and treatment of enteric infections in Army personnel. The goal is to modify enteric bacteria genetically to produce any desired antigenic structure and level of pathogenicity. Such strains can serve as vaccine strains or as tools to study the infectious process.

24. (U) Genetic recombination between strains of enteric bacteria and recombinant DNA techniques are used for strain construction and modification. Genetic results are extended to include the physical study of the informational macromolecules (i.e., DNA).

25. (U) 82 10 - 83 09 All Shigella sonnei strains carry a 120 Mdal plasmid needed for form I antigen (i.e., O-side chain) synthesis and for epithelial cell penetration. These form I O-side chain determinants have been subcloned on a DNA fragment of 10 Mdal. Fragments representing the entire 120 Mdal S. sonnei plasmid have been cloned and are now being examined for additional virulence traits. Potential oral vaccine strains have been constructed by gene fusion resulting in Salmonella typhi Ty2la derivatives carrying the O-antigens of S. sonnei and S. flexneri serotype 2a. Similar Ty2la derivatives to protect against S. flexneri 3 and S. dysenteriae 1 are being developed. A combination of these four oral vaccine strains should protect soldiers against typhoid fever and the predominant causes of shigellosis. Also, we are developing specific DNA probe techniques to identify rapidly under field conditions pathogenic organisms such as Leishmania, Yersinia, Salmonella, and Shigella species. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M161102BS10 RESEARCH ON MILITARY DISEASES, INJURY AND HEALTH HAZARDS

Work Unit 206: Microbial Genetics and Taxonomy

Investigators:
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Assistant: F.A. Rubin, Ph.D.
Associates: C.A. Life; N.J. Snellings, M.S.; K.F. Noon, M.S.; SP5 J.N. Coulby, B.S.; SP5 N. Calderon, B.S.

Problem

Bacterial infections of the gastrointestinal tract have always been a serious health hazard to those entering an area where modern sanitary practices and facilities are lacking. More than 50% of the military personnel involved in field operations frequently are incapacitated by enteric bacterial illnesses. These enteric organisms can produce severe stomach cramps, nausea, vomiting, intestinal ulcerations, bacteremia, dysentery and diarrhea. Such enteric bacterial infections generally occur within several days after personnel enter an area where sanitary conditions are deficient or disrupted. Effective prophylactic field measures do not exist for many of the severe enteric disease agents e.g., Shigella, Salmonella, enterotoxigenic E. coli. Since native populations do develop an immunity to the enteric organisms normally indigenous to their environment, the development and use of effective enteric vaccines should act to augment the level of natural immunity thus reducing the inherent disease level in these areas. Also, effective enteric vaccines would stimulate immunity in military troops who frequently are highly susceptible individuals. Current broad objectives within this work unit include the development of mono-and multi-valent vaccines against enteric organisms, testing vaccine efficacy in animal models, and using molecular and genetic approaches to study the mechanism of disease pathogenesis resulting in pertinent information that can lead to the development of suitable techniques for the construction of improved vaccines or for the rapid detection and treatment of pathogenic bacteria in a diseased patient. Towards this end, a variety of basic investigations into the molecular nature of bacterial gene expression and intercellular gene transfer, employing classical and new molecular genetic procedures including recombinant DNA technology, are conducted. Whole animal and cell culture systems are employed to assess bacterial virulence traits and vaccine efficacy.

Progress

Our research studies, conducted in collaboration with the Dept. Bacterial Diseases, have resulted in the conclusion that large plasmids (120-140 megadaltons in size) are necessary for the virulence of all strains of bacteria that cause dysentery (i.e., all Shigella species and certain Escherichia coli strains; see ref. 1). Specifically, we have shown that all virulent Shigella sonnei strains harbor a 120 Mdal plasmid that encodes the major protective cell surface antigen, the form I somatic antigen, of this species (2,3). Also, virulent isolates of all six serotypes of S. flexneri have been found to carry a 140 megadalton plasmid (4), as do dysenteric strains of E. coli. Animal and tissue culture assays have revealed that these large plasmids encode some virulence property that allows these virulent bacteria to invade epithelial cells, which is the first step in the disease process of dysentery. Recombinant DNA procedures have been developed to clone the plasmid-mediated and chromosomally-controlled genetic determinants of Shigella virulence. Thus far, large DNA regions representing the entire 120 Mdal and 140 megadalton (Mdal) Shigella virulence plasmids have been cloned. The genes determining synthesis of the S. sonnei form I O-antigen have been cosmid cloned and then subcloned on a defined DNA fragment of approx. 10 Mdal. These cloned genes will be used to study the mechanism of O-antigen synthesis and, potentially, to construct an improved oral vaccine against S. sonnei (see below).

As reported last year, the galactose epimeraseless S. typhi Ty21a oral vaccine strain of Germanier and co-workers (5) appears to be a potential carrier of many antigens and should be useful in constructing multi-valent oral vaccines that will be protective against many different enteric diseases. To test this hypothesis, we initially transferred the genes for S. sonnei form I somatic antigen synthesis into the S. typhi Ty21a strain (6). The derivative strain stimulates the production of specific intestinal IgA in rabbits (7) and protects mice against challenge by virulent S. typhi and S. sonnei cells (6). More recently, volunteer studies have demonstrated the safety of this oral vaccine, and preliminary immunization and challenge tests in humans showed a significant degree of protection against S. sonnei, the leading cause of shigellosis. This vaccine strain will be further analyzed and manipulated as necessary to develope a genetically stable, safe and effective oral vaccine.

Vaccines to protect against several other predominant Shigella serotypes are also being developed and tested. The chromosomal genes encoding the S. flexneri serotype 2a O-antigen have been transferred to the Ty21a S. typhi vaccine strain. The resulting

derivative expressed the S. flexneri 2a O-antigen and protected mice against challenge by this organism. Analogous vaccines to protect against S. flexneri serotype 3 and S. dysenteriae serotype 1 strains are presently being developed. These 4 oral vaccines should protect soldiers against typhoid fever and the organisms that are the predominant cause of shigellosis. Other potential enteric vaccine combinations (e.g., a vaccine to protect against cholera or traveler's diarrhea) are also being studied.

Recent preliminary studies indicate that plasmids are also important for the virulence of certain Salmonella species. Further studies are aimed at determining the precise involvement of these plasmids in Salmonella disease. Other studies underway are directed at genetically isolating, via recombinant DNA technology, the chromosomal genes that are necessary for the virulence of Shigella and Salmonella. The genes encoding the Salmonella and Citrobacter virulence (Vi) surface antigens have recently been cloned and are being analyzed in detail. Further studies have revealed that various DNA segments of these Vi antigen genes behave in DNA hybridization reactions as highly specific probes for S. typhi. These DNA probes, presently being patented, should be useful in a rapid assay to detect typhoid fever bacteria in diseased soldiers in the field.

More recently, recombinant DNA technology has been used in collaboration with workers in the Dept. of Bacterial Diseases to isolate the genes that encode the Neisseria gonorrhoeae surface attachment pili. These attachment organelles already serve as the basis for a gonorrhea vaccine. It is anticipated that these cloned genes can be used to study pili gene expression and to amplify the production of pili gene products for vaccine use. Additionally, collaborative studies with the Dept. Rickettsial Diseases are underway to clone rickettsial surface antigen genes for vaccine use. Rickettsial gene segments have now been cloned and are expressed in E. coli, but cloned protective antigen genes have not yet been detected.

DNA specific, hybridization probes have been developed for rapid detection of a number of pathogenic organisms. These precise, specific genetic probes have been used in procedures to classify pathogenic Leishmania species and to detect Leishmania parasites in infected sandflies. Identification of clinically important Yersinia, Salmonella and Shigella strains has also been possible using these procedures. Finally, these probes have been successfully employed to detect DNA in recombinant E. coli and Salmonella hybrid strains. At the present time, the probes employed are radiolabelled with ^{32}P nucleotides for detection purposes. Recently, a program has been initiated in collaboration

with the Division of Biochemistry, to investigate the use of safer, more stable methods to tag and detect probes with color reactions instead of radioisotopes.

Recommendations for the future:

1. Genetically modify the S. typhi Ty21a strain to express the cell surface antigenic determinants of S. flexneri serotype 3 and S. dysenteriae serotype 1. These new strains could be used in combination with the previously developed S. typhi-Shigella hybrid vaccine strains to produce a single multivalent vaccine that will protect against typhoid fever and the four most common causative agents of shigellosis. Also, the recently cloned form I antigen genes will be inserted into the S. typhi Ty21a strain; this derivative should have both increased stability and expression of the form I antigen and should be a better vaccine than the one initially constructed.

2. Use the Vi antigen DNA probes in the development of a rapid assay for field use to detect typhoid fever bacteria in diseased troops.

3. Inserting the genes for toxoid antigens into the S. typhi oral vaccine strain to produce a vaccine protective against typhoid fever as well as the enterotoxigenic diseases caused by Vibrio cholera and E. coli. Similar technology is contemplated to produce effective vaccines against still other enteric diseases.

4. Once cloned by recombinant DNA techniques, the Shigella plasmid-borne determinants needed for epithelial cell penetration should serve as an excellent molecular probe with which to rapidly detect dysentery bacteria in the stools of diseased soldiers /patients. Such a test would be very valuable to the military.

5. Use of genetic manipulations of Shigella and Salmonella species to dissect the steps involved in the respective disease processes, the findings of which should provide basic genetic information and new insights into methods for both prophylactic and chemotherapeutic intervention.

6. Further examination of the specific role of plasmids in the virulence of Salmonella species.

7. New genetic studies directed at creating mutations in Shigella and Salmonella which inhibit the virulence of the organism at various steps in the disease process. The purpose of these mutants is to define new steps in the pathogenic process and to map the new genetic determinants of virulence.

8. Continue collaborative efforts at cloning various surface antigenic determinants of Neisseria gonorrhoeae, E. coli, and Rickettsia for the development of vaccines.

9. Develop DNA probes that will be sensitive enough to detect a very small number of Leishmania parasites in any kind of sample, such as, a culture of promastigotes grown in a small volume of culture media, a clinical specimen prepared by blotting a filter directly onto a patient's lesion, or from a single infected sandfly.

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Formal Presentations:

(By L.S. Baron)

March 10, 1982	Cloning and characterization in *Escherichia coli* of the genes encoding reversible Vi antigen expression in *Citrobacter freundii*. 83rd Annual Meeting of the American Society of Microbiology, New Orleans, LA.
March 10, 1982	Cloning the plasmid-mediated form 1 O-antigenic determinants of *Shigella sonnei*. 83rd Annual Meeting of the American Society of Microbiology, New Orleans, LA.

(By D.J. Kopecko)

Sep 82-Jan 83	Lecturer in semester course on the Biology of Bacterial Plasmids, FAES Graduate School at NIH, Bethesda, MD.
October 13, 1982	*Shigella* virulence plasmids: application to oral vaccine construction. Microbiology Seminar, Dept. Microbiology, MCV-VA Commonwealth University, Richmond, VA.
November 13, 1982	Cloning of the determinants responsible for reversible expression of the virulence (Vi) antigen of *Citrobacter freundii*. 6th Annual Mid-Atlantic Conference on Extrachromosomal Genetic Elements, Virginia Beach, VA.
November 16, 1982	Genetics and virulence of *Shigella*. Intern. workshop on "The Clone concept in Epidemiology, Toxonomy, and Evolution of the *Enterobacteriaceae* and other bacteria". Fogarty Center, NIH, Bethesda, MD.
December 10, 1982	Bacterial plasmids, virulence, and antimicrobial resistance. Seminar, Dept. Vet. Pathology, Walter Reed Army Institute of Research, Washington, DC.
February 8, 1983	*Shigella* virulence determinants--potential vaccine approaches. Seminar, Center for Vaccine Development, University of Maryland Medical School, Baltimore, MD.

February 24, 1983	The genetics of virulence in Shigella. Dept. Microbiol., School of Pharmacy, Univ. Bonn, Bonn, West Germany.
February 28, 1983	The genetic determinants of Shigella virulence: potential anti-dysentery vaccines. International Congress on Molecular Pathogenesis. Tubingen, West Germany.
March 7, 1983	Cosmid cloning and expression in Escherichia coli of Neisseria gonorrhea pili genes. Annual Meeting of the American Society Microbiol., New Orleans, LA.
April 4, 1983	Plasmids, invited seminar, D.C. Society of Medical Technologists, Washington, DC.
April 27, 1983	Genetic studies of Shigella virulence. Infec. Dis. Grand Rds., Walter Reed Army Hospital, Washington, DC.
April 29, 1983	General Properties of Bacterial Plasmids. Molec. Genetics Seminar, Walter Reed Army Institute of Research, Washington, DC.
May 6, 1983	The involvement of plasmid encoded traits in Shigella virulence. Molec. Genetics Seminar, Walter Reed Army Institute of Research, Washington, DC.
May 9, 1983	The identification of virulence plasmids in Shigella: application to oral vaccine strain construction. Vet. Microbiology, Cornell University School of Vet. Med., Ithaca, NY.
May 27, 1983	Plasmids and Virulence in Bacteria. Naval Medical Research Institute, Bethesda, MD.
June 21, 1983	Use of Genetic Engineering in the Development of Immunogenic Peptides. Walter Reed Army Institute of Research Peptide Vaccine Workshop, Washington, DC.
June 28, 1983	Cloning and characterization in Escherichia coli of the determinants responsible for reversible expression of the Vi antigen of Citrobacter freundii. Gordon Research Conference on Biological Regulatory Mechanism, Plymouth, NH.

(By J.A. Wohlhieter)

November 17, 1982 — Plasmids associated with bacterial virulence. Department of Microbiology, University of Maryland, College Park, MD.

February 1, 1983 — Analysis of kinetoplast DNA from pathogenic Leishmania. 12th Annual UCLA Symposium on Molecular Biology of Host-Parasite Interactions, Park City, UT.

August 8, 1983 — Characterization of kinetoplast DNA from pathogenic Leishmania, Gordon Research Conference on Immunologic and Molecular Aspects of Parasitism, Plymouth, NH.

Publications:

1. Jackson, P.R., J.A. Wohlhieter and P.B. McGreevy. 1982. Restriction endonuclease analysis of kinetoplast DNA from Leishmania responsible for cutaneous disease in Panama. American Society of Tropical Medicine and Hygiene, Cleveland, OH.

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Patent Applications Pending:

Formal, S.B., L.S. Baron, and D.J. Kopecko. U.S. Patent Application #289,013, entitled "Oral Vaccine for Immunization Against Enteric Disease".

Kopecko, D.J., L.S. Baron, and F.A. Rubin. U.S. Patent Application submitted October 1983, entitled "A Method for the Rapid Detection of Typhoid Fever Bacteria".

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 0A 6445	2. DATE OF SUMMARY* 83 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 82 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8A. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AE	207 WWG3
b. CONTRIBUTING				
c. ~~CONTRIBUTING~~	STOG [illegible]			

11. TITLE (Procede with Security Classification Code)*
(U) Pathogenesis of Enteric Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
59 05	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR (BUDGET) 83	2.0	367
FISCAL YEAR (CURRENT) 84	2.0	395

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21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Diarrhea; (U) Dysentery; (U) Bacillary; (U) Salmonellosis; (U) Immunity; (U) Immunization; (U) Plasmids; (U) Genetics

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede text of each with Security Classification Code.)

23. (U) The pathogenesis of bacterial infections of the gastrointestinal tract is being studied to establish factors and mechanisms by which disease is provoked. Through an elucidation of such elements, procedures for prevention and control of diarrheal diseases can be devised. Diarrhea is a significant problem in military personnel operating overseas.

24. (U) The genetic control of O-antigen specificity of enteric pathogens is being studied since such cell envelope components are of importance in disease and its prevention through vaccination. Interactions of bacterial pathogens and epithelial cells, especially mechanisms of penetration are investigated. Attentuated living vaccines are developed.

25. (U) 82 10 - 83 09 Extrachromosomal transcription in Shigella flexneri has been studied in avirulent mutants with transposon insertions in the virulence-associated plasmid. A quantitative plaque assay has been developed for study of shigella invasion in vitro. Over 200 hybridoma cell lines producing monoclonal antibody against Shigella antigens have been developed. In addition, five anti-Vi monoclonal antibodies which recognize various domains of this Salmonella-Citrobacter antigen have been produced. The genes for expression of the S. dysenteriae I somatic antigen have been identified and mobilized into an avirulent Escherichia coli K12 strain. (For technical report see Walter Reed Army Institute of Research Progress Report, 1 Oct 82-30 Sep 83).

*Available to contractors upon originator's approval

DD FORM 1 MAR 86 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND FORMS 1498A 1 NOV 85 AND 1498-1 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY, AND HEALTH HAZARDS

Work Unit 207: Pathogenesis of Enteric Diseases

Investigators:

Principal: Thomas L. Hale, Ph.D.

Associates: Edwin V. Oaks, Ph.D.
Samuel B. Formal, Ph.D.

Problem

Diarrheal disease has been a component of military campaigns since biblical times. In recent history these diseases play an important role in the British defeat at Gallipoli and caused significant illness in American troops in North Africa and the South Pacific in WWII, in Korea, in Lebanon and in Viet Nam. The pathogenesis of bacterial infections of the intestinal tract is studied using techniques of biochemistry, genetics, molecular biology, physiology and pathology to establish the factors and mechanisms which are involved in the disease process. The current objectives of this work are to understand the interaction of enteric pathogens with intestinal epithelial cells and to develop vaccines to prevent disease.

Progress

The invasive phenotype in *S. flexneri* is associated with the presence of a 140 Mdal plasmid. Plasmid expression was studied in anucleate minicells, and fifteen polypeptides were identified as virulence plasmid transcripts. Noninvasive mutants were made by insertion of the Tn*5* antibiotic resistance transposon into the 140 Mdal plasmid, and it was found that various combinations of six polypeptides were no longer transcribed in minicells. Similar changes in plasmid expression were observed in shigellae grown at 30°C, a temperature which causes a reversible loss of the invasive phenotype. Radioiodination of intact, viable shigellae labeled three of the six polypeptides associated with the invasive phenotypes. This indicates that these three polypeptides are exposed on the bacterial surface. The plasmid-coded polypeptides thus far identified are probably important antigens to be included in a shigella vaccine.

The complete *Shigella dysenteriae* 1 somatic antigen has been transferred to a *E. coli* K-12 strain which now enhances opportunities to construct a vaccine against this serotype.

Over 200 hybridoma cell lines have been developed which produce antibody reactive with *S. flexneri* 5 lysates in an enzyme-linked immunoassay. The antibodies produced by these cultures recognize at least 5 different shigella protein antigens, three of which are exposed on the organism's surface. The antigen specificity of the monoclonal antibodies was determined by immune precipitation of radiolabeled shigella polypeptides or by immunoblotting procedures. These monoclonal antibodies will aid in the identification of shigella antigens involved in invasion.

A new, sensitive measure of shigella invasiveness has been developed for *in vitro* experiments. This system measures the ability of virulent shigella to form plaques in HeLa-cell of L-cell monolayers. The plaques are a result of intracellular multiplication and intercellular spread with resultant cytopathic effects on the host cells. Initial studies have indicated that plaque formation is correlated completely with the presence of the 140 megadalton virulence plasmid. The shigella-plaque assay will allow sensitive quantitative investigations on shigella invasiveness such as the capacity of antibody to neutralize plaque formation.

Using hybridoma technology, five different mouse cell lines have been produced which secrete antibody recognizing the Vi antigen of *Citrobacter intermedius*. To determine if more than one epitope on the citrobacter Vi antigen was recognized by the 5 monoclonal antibodies, competitive binding assays were used in an enzyme-linked immunoassay. In this system unlabeled monoclonal antibodies were used to inhibit the binding of horseradish peroxidase-conjugated monoclonal antibodies. Two antibodies (52B4 and 56B2) blocked the binding of all other monoclones, while another monoclone (55F12) was inhibited by all 5 Vi monoclones. These results indicate that several different antigenic determinants exist on the Vi antigen. In addition, mouse ascitic fluids from four of the hybridomas protected ICR mice from a lethal challenge of *Salmonella typhi* (TY2). These monoclonal antibodies may provide useful diagnostic reagents for the detection of Vi antigen in urine or serum and they will also provide a tool for evaluating the antigenicity of chemically modified Vi antigen.

Future studies

1. Plasmid-coded polypeptides from virulent and avirulent S. flexneri will be analyzed in a two-dimensional electrophoresis system which will give data on both isoelectric point and molecular weight of these components.

2. Plasmid-coded polypeptides will be isolated by preparative isoelectric focusing and fractions enriched in individual polypeptides will be tested as protective antigens. The plasmid-coded polypeptides isolated from preparation IEF gels will also be used to immunize mice for the production of monoclonal antibodies. The monoclonal antibodies will be useful in the rapid identification of the invasive phenotype and will provide specific antibody necessary to study the biological function of the plasmid-coded polypeptides.

3. The plaque assay will be evaluated for the capacity to isolate genetically constructed (by classical genetic or recombinant DNA procedures) shigella strains which are invasive. In addition, to study the temperature sensitive aspects of invasion, the plaque assay will be used to isolate mutants capable of forming plaques at 30°C (wild-type shigella do not form plaques at 30°C). The plasmid-coded polypeptides of these mutants will be compared to temperature-sensitive strains.

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Abstracts

1. Kopecko, D.J., P.J. Sansonetti, L.S. Baron and S.B. Formal. 1981. Invasive Bacterial Pathogens of the Intestine: Shigella Virulence Plasmids and Potential Vaccine Approaches. Molecular Biol., Path. and Ecology of Bacterial Plasmids, Santo Domingo, Dominican Republic S28.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6435	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORG'N HIST'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AF	208 WWG8
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	STOG 82/83-6	2/3		

11. TITLE (Procede with Security Classification Code)

(U) Immunity in Protozoan Diseases.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	5.0	574
FISCAL YEAR CURRENT 84	7.0	613

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Haynes, J D

NAME:

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Antigens; (U) Protozoa; (U) Immunity; (U) Tropical Medicine; (U) Malaria

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) To conduct immunological studies of protozoan diseases with emphasis on malaria, to produce P. falciparum malaria antigens by in vitro techniques for immunoassay and immunochemical analysis. These studies will aid in the development of a vaccine to protect soldiers stationed in many areas of the world against a major military disease.
24 (U) The approach used in these studies is to study, in both animal models and through the use of in vitro techniques, the response elicited by the immune system, to determine the role of cellular and molecular mediators in these processes, and to design experimental immunogens which will provide the basis for future vaccine development programs.
25 (U) 82 10-83 09 We stabilized labile malaria merozoite (blood stage) antigens and then used a monoclonal antibody to remove the immunodominant antigen, thus allowing the successful immunization of mice and the production of monoclonal hybridoma antibodies to several additional merozoite-surface antigens. These are now being produced in quantity for further studies. Genomic DNA expression libraries have been made in E. coli and are being screened with antibodies to sporozoite and merozoite surface antigens. A number of immunochemical and molecular genetic approaches to a vaccine are underway. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 1 MAR 88 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 83 AND 1498-1 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3M161102BS10 RESEARCH ON MILITARY DISEASES, INJURY AND HEALTH HAZARDS

Work Unit 208 Immunity in Protozoan Diseases

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	SP4 Lisandro Reyes

Problems and Objectives:

Malaria has caused many lost man years in every major military conflict. Protecting soldiers in the field by administering one or more immunizing malaria vaccine doses before deployment would be logistically much simpler than currently available methods of controlling mosquito populations or administering antimalarial drugs weekly in the field. We are investigating immune responses to malaria antigens and are attempting to produce candidate vaccine antigens by genetic engineering.

The major objectives are (1) to identify the best parasite antigens to further evaluate as vaccine candidates, (2) to determine how much antigenic variation there is, and (3) to produce the antigens through genetic engineering in bacteria in sufficient quantity and purity, so that then (4) they can be tested for their ability to induce protective immunity in experimental animals.

Progress:

Asexual blood stages:

Major progress has been made in producing hybridomas which secrete monoclonal antibodies (MAbs) to parasite surface antigens likely to be involved in protective immunity. These were produced

by immunizing mice with antigens stabilized by techniques we developed last year using immune sera and Streptomyces-derived protease inhibitors. The MAbs include (a) several which react with 75 and 85 kd antigens which appear on the merozoite surface after processing from a heavier precursor antigen in the schizont, (b) one which seems to react with activated merozoites attached to erythrocytes and with early ring-infected erythrocytes, and (c) one which reacts with the schizont-infected erythrocyte surface. These are being examined immunochemically and ultrastructurally, and evaluated for their ability to inhibit parasite growth.

Using another MAb which we had produced earlier against a major 195 kd schizont glycoprotein antigen, we demonstrated in collaboration with a group at NIH that contrary to previous reports, this antigen does not appear on the schizont-infected erythrocyte surface (Howard ref.)

Work on strain-specific immunity has yielded little progress this year, in part because of a mix-up in parasite strains which has now been corrected. One paper from previous work was accepted for publication (Vernes ref.), and we are now renewing our efforts.

The recombinant DNA lab is now in operation. We have produced genomic DNA expression libraries of P. falciparum which are being screened with monoclonal antibodies to parasite antigens. We have also begun to utilize techniques of parasite mRNA translation, copying, and insertion into Okayama-Berg type cDNA cloning and expression vectors.

We demonstrated that tumor-necrosis factor (TNF) or a TNF-like factor in serum can kill P. falciparum inside erythrocytes (Haidaris ref.). We also demonstrated several oxygen-dependent mechanisms of parasite killing.

We collaborated with the group working on the sporozoite surface antigen. This included developing methods for an ELISA which detects sporozoite-infected mosquitos and electrophoresis and detection of small amounts of the sporozoite antigen using western blotting.

In late September three scientists joined us. Dr. Jeffrey Chulay returned from Nairobi where he worked on leishmaniasis and malaria. He will lead the immunobiology effort, concentrating on in vitro growth inhibitition and strain-specificity. Dr. Terence Hadley came from NIH where he studied malaria proteases,

erythrocyte receptors, and surface antigens. He will continue immunochemical studies of a P. falciparum merozite surface antigen that cross-reacts with an inhibitory MAb against a P. knowlesi merozoite antigen. Dr. Daniel Camus is a visiting professor from the University of Lille, France, where he most recently investigated alterations in blood antigens during malaria infections. He will be comparing parasite antigens found in culture superntants with protease-labile cell-associated antigens.

We are now hiring a much needed supervisor-investigator for the culture and immunobiology lab. We are beginning to use computers to better handle much of our scientific data manipulation and inventory control.

Recommendations:

Continue with present research plans. We need to (1) recruit another recombinant DNA scientist; (2) further explore the possibilities for coordinating in-house efforts with other groups and companies through collaborations and contracts; (3) recruit an immunochemistry lab assistant to replace Ms. Pratt, who left to attend medical school; (4) expand our staff of research assistants in order to make better use of the newly arrived professional staff; (5) renovate some of the labs and add two well equipped chemistry labs with chemical fume hoods; (6) acquire several microcomputers for stand-alone use, and for use as VAX terminals, so that they are accessible to all investigators for data entry and manipulation; (7) prepare for large budget increases to support testing of vaccine candidate antigens as they are produced in the next year or so.

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Howard, R.J., Lyon, J.A., Diggs, C.L., Haynes, J.D., Leech, J.H., Barnwell, J.W., Aley, S.B., Aikawa, M., and Miller, L.H. 1983. Localization of the major Plasmodium falciparum glycoprotein on the surface of mature intraerythrocytic trophozoites and schizonts. Molec. Biochem. Parasitol. in press.

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Presentations:

Diggs, C.L. September 1983. Research towards vaccination against malaria: an update. Second International Conference on Malaria and Babesiosis. Annecy, France. (Invited talk).

Publications:

Chulay, J.D., Haynes, J.D., Diggs, C.L. 1983 Plasmodium falciparum: Assessment of in vitro growth by ^{3}H hypoxanthine incorporation. Exp. Parasitol. 55:138-146.

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Howard, R.J., Lyon, J.A., Diggs, C.L., Haynes, J.D., Leech, J.H., Barnwell, J.W., Aley, S.B., Aikawa, M., and Miller, L.H. 1983. Localization of the major Plasmodium falciparum glycoprotein on the surface of mature intraerythrocytic trophozoites and schizonts. Molec. Biochem. Parasitol. in press.

Vernes, A., Haynes, J.D., Tapchaisri, P., Williams, J.L., Dutoit, E., and Diggs, C.L. 1983. Plasmodium falciparum strain-specific human antibody inhibits merozoite invasion of erythrocytes. Am. J. Trop. Med. Hyg. in press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	83 10 01	DD-DR&E-AR(6)6

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISEN INSTR	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		XX	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY				
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Biochemical Research on Military Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR (PREVIOUS) 83	3.0	787
FISCAL YEAR (CURRENT) 84	3.0	652

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Brown, J E

NAME:

21. GENERAL USE

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Antigen; (U) DNA; (U) Plasmid; (U) Hybridoma; (U) Immunoglobulin; (U) Toxin

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective is to conduct studies on biochemical molecular and cellular processes related to bacterial, parasitic and viral diseases of importance to the military. Molecules which may be by-products of the disease state, of the invading organism and of the immune system of the host are being identified and characterized. Types of molecules of interest include toxins, antigens, nucleic acids, enzymes, immunoglobulins; diagnostic tests and prophylaxis for military diseases are the envisioned products of this work.

24. (U) The approach includes the disciplines of biochemistry, microbiology, immunology and cell biology. Macromolecules are purified and characterized. Studies of virulence potential will be performed using cell-free enzyme assays, immunochemical assays and cell culture and animal toxicity assays. Hybridoma technology to prepare monoclonal antibodies to components of pathogens is employed. Recombinant DNA technology is being applied to study of virulence.

25. (U) Hybridomas which produce monoclonal antibodies to Shiga toxin have been selected and the antibodies have been isolated and characterized. Salmonella typhimurium hybrids which express Shigella flexneri somatic LPS antigens have been isolated and characterized. The glycolipid cell surface receptor for Shiga toxin has been isolated and identified as a globoside containing a glcd-1-4 gal disaccharide. Cytotoxin from C. difficile has been purified; found to inhibit protein synthesis and cause K + loss from cells. Via use of DNA molecular homology technology, new species of Vibrio associated with human disease have been defined. Shiga toxin does not cause hydrolysis of 28sRNA and does not prevent initiation of protein synthesis. For technical reports, see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sept 83.

*Available [illegible] when [illegible] approved

DD FORM 1498

PROJECT: 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

WORK UNIT: 210 Biochemical Research on Military Diseases

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In collaboration with: MAJ C. R. Bartz, VC (DVM); J. Brendle (DVM); D. Brenner, Ph.D. (CDC, Atlanta, GA); H. Collins (DCD & I); R. V. Lewis, Ph.D. (University of Wyoming); T. Obrig, Ph.D. (Albany, NY); A. D. O'Brien M.D. (National Bacteriological Lab, Stockholm, Sweden).

DESCRIPTION:

The mission is to design and execute research programs that provide fundamental biochemical and molecular definitions of diseases relevant to the military. Factors associated with disease processes such as virulence determinants and toxins of organisms, biochemical and metabolic mechanisms of pathogens, and products of host responses to disease are being studied through the use of physiocochemical, biochemical, microbiolo ical and immunological concepts and techniques. Such information provides a rational basis for immunological and chemotherapeutic protection against disease and the development of accurate diagnostic procedures.

A. Studies of Shigella and Their Toxins.

1. Release of Shiga Toxin.

The release of Shiga toxin from Shigella dysenteria 1 by polymyxin B was investigated. The amount of Shiga toxin released from exponential cultures of S. dysenteriae 1 strain 3818-0 was dependent on both polymyxin concentration and time of incubation. Cell lysis was demonstrated following the exposure of a cell suspension to polymyxin B by a decrease in turbidity of the suspension. An immunoblot characterization of Shiga toxin released by polymyxin demonstrated that it is electrophoretically similar to purified Shiga toxin and to Shiga toxin present in crude bacterial lysates of S. dysenteriae 1 cells.

2. Isolation and Characterization of Monoclonal Antibodies to Shiga Toxin.

Hybridoma cell lines which produce monoclonal antibodies to Shiga toxin from Shigella dysenteriae were prepared. The monoclonal antibodies

lere all of the immunoglobulin G1 isotype and differed in their ability to neutralize cytotoxicity and to bind to Shiga toxin in a solid-phase radioimmunoassay. When used for immunoblot analysis, these antibodies were able to identify specifically both nicked and unnicked Shiga toxin in crude lysates of S. dysenteriae.

3. On the mechanism of Action of Shiga Toxin in the Inhibition of Eukaryotic Protein Synthesis.

We have observed that Shiga toxin appears to affect eukaryotic protein synthesis by inhibition of the aminoacyl tRNA binding step of peptide elongation in a manner similar to that of α-sarcin. Since the mechanism of α-sarcin involves hydrolysis of 28S rRNA in 60S ribosomal subunits, the action of Shiga toxin on 28S rRNA was examined to determine if its mechanism of inhibition was similar to that of α-sarcin. Toxin purified to homogeneity from Shigella dysenteriae 1, strain 3818 0, was preincubated with 5 ug/ml TPCK-trypsin followed by 19 ng/ml phenylmethylsulfonyl fluoride, 2.5 mg/ml urea and 10mM dithiothreitol. Toxin-inactivation of rabbit reticulocyte ribosomes was carried out by addition of toxin to either lysate or crude ribosomal pellet at a molar ratio of toxin to 80S ribosome of 1:10. [^{3}H]leucine incorporation into acid-insoluble material in lysate preparations was completely inhibited by this amount of toxin. Ribosomal RNA was extracted from control or toxin-treated ribosomes and analyzed by polyacrylamide-agarose gel electrophoresis. Although fragments derived from 28S rRNA were observed from α-sarcin-treated ribosomes, no hydrolysis of 28S or 18S rRNA was detected from Shiga toxin-treated ribosomes. Thus, it appears that Shiga toxin inactivates ribosomes in a manner different than α-sarcin.

4. Shiga Toxin Does Not Affect Initiation of Protein Synthesis.

Shiga toxin, the protein toxin of Shigella dysenteriae 1 has been shown to inhibit eukaryotic protein synthesis catalytically. We have now examined toxin action for its effect on initiation of protein synthesis. Toxin, purified to homogeneity from S. dysenteriae 1, strain 38180, was activated with TPCK-trypsin followed by phenylmethylsulfonyl fluoride, urea and dithiothreitol. A Shiga toxin concentration of 1 ug/ml which completely inhibits protein synthesis in reticulocyte lysate was utilized. In the presence of ribosomal salt wash fraction, formation of the ternary initiation complex [^{35}S]Met • $tRNA_f$-eIF 2-GTP was not affected by the toxin. Further, when sucrose gradient-isolated ribosomes were added, codon-directed binding of [^{35}S]Met • $tRNA_f$ to the 40S ribosome was not inhibited by toxin. Toxin effects on protein synthesis initiation in complete reticulocyte lysates were monitored in the presence [^{35}S]Met • $tRNA_f$. Accumulation of the initiation complex [^{35}S]Met •$tRNA_f$-40S ribosomal subunit-mRNA, promoted in response to NaF and β, γ-methylene GTP, was not inhibited in the presence of Shiga toxin. Finally, [^{35}S] •tRNA-80S ribosome-mRNA complex formed in Shiga toxin-treated lysates was fully capable of reacting with puromycin to yield [^{35}S] methionylpuromycin. We conclude that Shiga toxin inhibits protein synthesis without affecting the initiation process.

5. Identification of the Receptor Glycolipid for the Toxin of Shigella dysenteriae.

Necrosis of the epithelium of the colon is part of the pathogenesis of bacillary dysentery after infection with Shigella dysenteriae. The necrosis is due to an invasion of the bacterium into the epithelium and to production of a toxin which appears to inhibit protein synthesis. The purified toxin has a molecular weight of 70,000 and is composed of one heavy and 4-5 light subunits similar to the case for cholera toxin. The toxin has recently been purified on a milligram scale allowing more detailed studies on its structure and mechanism of action. In the present work the binding of 125-I-labelled toxin to glycolipids was studied using a modification of a technique for antibody-binding to a thin-layer chromatogram (5) and also ordinary microtiter plates. The results from analysis of total glycolipid extracts from many tissues and quantitative studies of pure glycolipids show a binding specificity for Gal α1 →4Gal in terminal or internal position with highest affinity for the blood group P_1 antigen. The toxin is more restrictive in its binding then uropathogenic E.coli, which have been proposed to have an identical disaccharide receptor and which tolerate any extension of the saccharide chain.

The role of the toxin in the pathogenesis of dysentery in human colon is unclear. Inhibition of protein synthesis through binding to the 60S ribosomal subunit appears to be the basic toxin effect and the different expressions in different animal species and tissues may in part be explained by selection at the receptor level. A number of cell lines have been analyzed for presence of receptor. Vero cells were highly reactive and shown to contain receptor type glycolipids. Epithelial cells of human small intestine seem to lack receptor type glycolipids, correlating with an absence of toxin effect in this organ. However, rabbit small intestine contains globotriaosylceramide of the more hydroxylated type typical of epithelial origin which may explain electrolyte fluid secretion of ileal loops when exposed to toxin. Similarly, the presence of slow-moving globotriaosylceramide of human meconium may suggest receptors on the colon epithelium.

B. Studies of Clostridial Toxins.

1. Differential Cytotoxic Effects of Toxin A and Toxin B Isolated from Clostridium difficile.

Clostridium difficile, the causative agent of antibiotic-associated colitis, produces two protein cytotoxins, designated A and B. We have examined the partially purified toxins isolated from culture supernatant fluids for their effect on HeLa cells. The toxins were purified and separated by hollow fiber ultrafiltration, Phenyl-Sepharose CL-4B chromatography, and DEAE-Sepharose CL-6B chromatography. Analysis after polyacrylamide gel electrophoresis using Coomassie blue staining and elution of cytotoxicity demonstrated complete separation of toxin A and toxin B. Cytotoxic activity was quantitated by absorbance measurement of crystal violet-stained HeLa cell monolayers after overnight incubation.

Both preparations were adjusted to equivalent cytotoxic activity. Subconfluent cell monolayers were incubated with serial dilutions of each toxin for periods from 5 min to 24 hr. Incorporation of [^{14}C]-leucine into acid-precipitable material was inhibited at 3 hr by toxin A, whereas toxin B did not inhibit even at 4 hr. Intracellular K^+ levels decreased within 120 min after treatment with toxin A. An effect by toxin B was not detected even at 4 hr. As expected from the cytotoxicity assay, [^{14}C]-leucine incorporation and K^+ retention after 24 hr incubation were decreased by each toxin in proportion to the cytotoxic dose.

C. Studies of Intergerneric Hybrids.

1. Isolation of Salmonella typhimurium Hybrids Which Express Shigella fexneri Somatic Antigens.

The genes controlling synthesis and expression of S. flexneri group- and type- specific antigens were transferred to S. typhimurium LT7 recipients by conjugation with a S. flexneri Hfr donor. Previous studies have established that the S. flexneri 3,4 group antigen gene(s) are positioned near the his operon whereas the type-specific 2 antigen gene(s) are linked to the pro locus. S. flexneri 2a Hfr 256 was crossed with recipient S. typhimurium LT7 his 404 pro 51 and PRO^+ transconjugants were recovered at a frequency of about 10^{-6} per donor. Such PRO^+ hybrids were then remated with the same Hfr donor, selection being made for inheritance of the donor his chromosomal region. Genetic analysis revealed that some of these S. typhimurium PRO^+ HIS^+ hybrids had also received genes controlling S. flexneri O antigens. Antisera prepared by immunizing rabbits with such hybrids had high agglutinin titers against O antigens of both genera. In addition to expressing their native 4,12 somatic antigen, these hybrids readily expressed both the group 3,4 and type 2 antigens of S. flexneri.

D. Nucleotide Sequence Relatedness Among Enterobactereaceae

1. Classification of Group DF-2: A Cause of Septicemia Following Dog Bite.

Twelve strains of an unclassified gram-negative bacterium, designated group DF-2, and three related strains, were studied phenotypically and genetically. DNAs from the DF-2 strains (isolated from human blood specimens and frequently associated with dog bites) were >80% related (hydroxyapatite method 60°C or 75°C). The other three strains (two isolated from hand wounds caused by dog bites and one from a dog's mouth were 68%-75% related at 75°C. DNA relatedness between the groups was 13%-21%. Three species of Capnocytophaga were <8% related to both groups. The G+C content of DF-2 strains (optical thermal denaturation) was 36% and, of the second group, 34%. Major characteristics of DF-2 include the following: acid but no gas from D-glucose, lactose, and maltose; positive reactions from oxidase, catalase, arginine dihydrolase, gliding motility, and ONPG; growth enhanced by serum and by incubation in a candle jar atmosphere; negative reactions for nitrate reduction, indole, and growth on MacConkey agar. Two strains of the second DNA relatedness group had the above characteristics except that acid was formed from sucrose. The

third strain was also sucrose-negative but was unique by being markedly adherent to the surface of blood agar. The DNA data indicate the 12 DF-2 strains are a single species, and the additional three strains constitute a second species. Both species are distinct from Capnocytophaga. There are, however, similarities between the two unclassified species and Capnocytophaga, i.e., gliding motility, enhancement of growth in a candle jar atmosphere, cellular morphology, and G+C content, that indicate they could be considered new species in the genus Capnocytophaga. This would require the inclusion of oxidase- and catalase-positive strains in the genus.

2. DNA Relatedness Among Vibrionaceae, with Emphasis on the Vibrio Species Associated with Human Infection.

Relatedness, as assayed by DNA hybridization (hydroxyapatite method at 60°C and 75°C) was determined among type and reference strains that represented almost all species of Vibrio. Strains from species of known or potential clinical significance were characterized phenotypically and by source of isolation, as well as by DNA relatedness. The results confirmed and extended indications of extreme genetic heterogeneity within the genus Vibrio previously observed by Baumann and others. DNA relatedness fell to less than 10% among Vibrio species. Heterogeneity was not confined to a single group as less than 10% relatedness was seen among environmental strains as well as among clinically significant strains. Phenotypic and DNA relatedness (polyphasic) studies of clinical strains indicated the existence of three new pathogenic species, Vibrio mimicus, Vibrio hollisae, and Vibrio damsela, and confirmed the existence within Vibrio fluvialis of a fourth new, potentially pathogenic species. We have now shown that the gas-producing (aerogenic) strains that had been included in V. fluvialis are a separate, as yet unnamed, species. Biochemically atypical strains of Vibrio cholerae (lysine-negative, salicin-positive, or luminescent), Vibrio parahaemolyticus (urea-positive), and V. vulnificus (sucrose-positive) were genetically typical members of their respective species (above 70% DNA relatedness). The only Vibrio species (of 26 tested) that were 40% or more interrelated were V. cholerae-V. mimicus; V. fluvialis - aerogenic, unnamed species; and V. parahaemolyticus-V. alginolyticus-V. campbelli-V. harveyi. At some point it may be desirable to propose a classification for the genus Vibrio that will reflect phenotypic and genetic species groups. One approach to this problem is discussed.

E. Studies of Lipoproteins.

1. Quantitation of Apoliproteins from High Density Lipoprotein by Size Exclusion High Performance Liquid Chromatography.

A method for quantitation of apoliproteins by size exclusion HPLC has been developed that does not employ chemical denaturants such as urea, guanididium chloride or SDS. Plasma was obtained from fasted human donors, and HDL was isolated by sequential ultracentrifugation. Apo HDL was prepared by the method of Shore and Shore [Biochemistry 8, 4510-4516 (1969)]. HPLC was performed on TSK-125 and TSK-250 columns (BioRad Laboratories) connected in series and eluted with 0.1M phosphate buffer

(pH 7.2) at 1 ml/min. The columns were calibrated with molecular weight standards including Apo A-I, A-II, and C-III (Calbiochem-Behring) monitored at 280nm. When 20 to 250 µg of apo HDL was chromatographed, protein recoveries were approximately 85%. The column eluants were screened for reactivity against antisera to apo A peptides (Calbiochem-Behring) and identity of peaks was confirmed by SDS/urea- PAGE followed by immunoblot analysis. When Apo A-I was isolated by HPLC and interacted with unilamellar egg yolk lecithin vesicles, it was effective in the disruption of the vesicles to form smaller apo A-I stabilized particles. Thus, a method for isolation and quantitation of biologically active apo HDL molecules has been developed using size exclusion HPLC. Supported by U.S. Army Health Services Command.

F. Studies of Campylobacter

Isolation and Characterization of Campylobacter jejuni from Selected Vertebrates.

Characterization of Campylobacter jejuni isolates by antibiotic sensitivity and plasmid profile from a survey of 1,087 animals (38 species) was performed to study diarrhetic epizootics within captive animal groups. Fecal material was taken during routine bacterial surveys or during disease outbreaks in colonies. Isolation was performed at 43 C with subculturing at 37 C. Standard disc antibiotic sensitivities were performed using high concentration antibiotic discs and MIC's were performed using Gibco Sensititre plates. Bacterial plasmids were isolated using NaOH-SDS lysing and EtOH/Tris-Acetate extraction methods. C. jejuni was isolated from 8.5% (92) of the animals cultured. Antibiotic sensitivity patterns confirm previous literature reports. Of the 43 isolates tested,76% had at least one plasmid and 28% had three plasmids. The results substantiate current therapeutic regimen and suggest the need for examination of specimens for other etiologic agents when C. jejuni is present in a clinical sample as all Campylobacter positive cultures were associated with animals confirmed for other viral or bacteria pathogens or that had recently suffered shipping stress.

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1. Griffin, D. E. and P. Gemski. 1983. Release of Shiga Toxin from Shigella dysenteriae 1 by polymyxin B. Inf. and Immun. 40: 425-428.

2. Griffin, D. E., M. K. Gentry, and J. E. Brown. 1983. Isolation and Characterization of Monoclonal Antibodies to Shiga Toxin Infect. Immun. 41: 430-433.

3. O'Brien, A. D., T. A. Lively, M. E. Chen, S. W. Rothman, and S. B. Formal. 1983. Escherichia coli 0157:H7 strains associated with haemorrhagic colitis in the United States produce a Shigella dysenteriae 1 (Shiga)-like toxin. Lancet. 1:702.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SECURITY	6. WORK SECURITY	7. REGRADING	8. ORGANIZATION	8a. SPECIFIC DATA FOR CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D.Change	U	U	NL		☒ YES ☐ NO	A. WORK UNIT

10. NO CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BD	122 Malaria
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Biochemistry of Parasitic Dru...

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry (U)100 Mic...

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMING METHOD
78 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE	EXPIRATION	b. PERIOD ESTIMATE	c. PROFESSIONAL MAN YRS	d. FUNDS (In Thousands)
b. NUMBER		FY 83	3.0	290
c. TYPE	a. AMOUNT	YEAR TO DATE		
d. KIND OF AWARD	FUNDING AMT	CUMULATIVE		382

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ASSOCIATE INVESTIGATORS

NAME: Owens, R

NAME: Tait, N

F.2: DA

22. KEYWORDS (Provide each with Security Classification Code)

(U) Drug Carriers; (U) Antibody; (U) Parasites; (U) Endotoxin; (U) Arachidonic acid

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code)

23. (U) The objective is to investigate biochemical aspects that influence lipids and membranes of parasitic diseases. The goal is to develop drug carriers that will deliver conventional antimicrobial drugs or serve as vehicles for vaccines. The main emphasis is to examine liposomes as drug carriers or as carriers of adjuvants and proteins for immunization. Adjuvants to be examined include lipid A from endotoxin and other lipidic or lipid-conjugated materials. The role of the arachidonic acid cascade in relation to the infectious process is to be examined, as well as how these compounds may influence the effectiveness of drug delivery. There is considerable military relevance in this research because liposomes are projected for use as drug carriers for treatment of leishmaniasis, and virus diseases (Rift Valley Fever), and possibly for delivery of protein antigens in a malaria vaccine.

24. (U) An attempt will be made to utilize liposomes as carriers of prostaglandins and leukotrienes and to examine the effects of [illegible] delivery on phospholipid metabolism in macrophages. Incorporation of protein antigens into liposomes and investigation of the immune response generated in animals will be performed.

25. (U) 82 10 - 83 09 Liposome-encapsulated antimonial drug was successfully used for treatment of visceral leishmaniasis in the dog. As in rodents, the liposome-encapsulated drug was more than 200 times more effective than the unencapsulated drug. New hypothesis was developed based on electron microscopic evidence that the liposomes are taken up by lysosomes of the host cell and subsequently by lysosomes of the parasite. Liposomes were successfully utilized as carriers of ribavirin for treatment of experimental Rift Valley Fever infections in rodents. The liposomal drug was much more effective than the unencapsulated drug. Liposomes also were successfully used for enhanced oral immunization against an enterotoxin. For technical report see WRAIR Annual Progress Report 1 Oct 82 - 30 Sept 83.

*Available to contractors upon originator's approval

DD FORM 1498 [illegible]

The mouse immune sera against the 140,000 m.w. protein on the merozoite surface did not immunoprecipitate a 140,000 m.w. protein from metabolically labeled schixonts. Instead, the major protein immunoprecipitated had a m.w. of 144,000. By analogy to the 250,000 m.w. protein and its cleavage products, we propose that the 140,000 m.w. protein on the merozoite surface is a cleavage product of the higher m.w. protein.

5. Selective Cytotoxicity of Tumor Cells Induced by Liposomes Containing Plant Phosphatidylinositol.

Liposomes containing highly purified phosphatidylinositol (PI) from plant origin selectively killed tumor cells from 8 out of 9 cultured cell lines, but did not kill 4 types of normal cells. Other phospholipids, including PI or phosphatidylserine from animal origin, synthetic phosphatidic acid, phosphatidylglycerol, or phosphatidylcholine, were not cytotoxic. Cholesterol enrichment of cells, shown by other investigators to inhibit tumor development, was slightly cytotoxic in this system, but the toxic effect of cholesterol was minor compared to the massive cytotoxicity induced by plant PI.

6. Enhancement of Specific Mucosal Antibody Responses by Locally Administered Adjuvants.

CP-20961, is a small lipoidal amine that is known to act as an interferon inducer and also to have adjuvant activity when given parenterally. The second is lipid A from Gram negative bacteria, which is a potent adjuvant and B cell mitogen. In addition, we have studied the use of liposomes as vehicles for effective delivery of mucosally applied adjuvants and/or antigens. Liposomes were either of the multilamellar or unilamellar type, as indicated. When used as carriers for lipid adjuvants, such as CP-20961 or lipid A, these materials were incorporated directly into the phospholipid bilayer of the liposomal membrane.

7. Interactions of Lipid A and Liposome-Associated Lipid A with Limulus pllyphemus Amoebacytes.

Lipid A or lipid A fractions and liposomes containing lipid A were tested for the ability to gel Limulus amoebocyte lysates and for effects on intact Limulus amoebocytes. Liposomes having a relatively low concentration of lipid A did not produce coagulation of lysate and were designated as Limulus-negative, but liposomes having a high concentration of lipid A were Limulus-positive. Limulus-negative liposomes had no effect on intact amoebocytes. Limulus-positive liposomes caused a striking transformation in the appearance of amoebocytes in that the cells sent out long filamentous extensions that formed a tangled network of processes between cells. The filamentous projections were similar to those that have been previously observed in the presence of gram-negative bacteria. We conclude that amoebocytes have the ability to recognize Limulus-positive liposomes, but the lack of activation of Limulus lysate or the absence of amoebocyte recognition does not prove the absence of liposomal lipid A. We also found that individual lipid A fractions were

are discussed which may account for the greatly enhanced effectiveness of liposomal chemotherapy for experimental visceral leishmaniasis.

3. Monoclonal Antibodies to a 140,000 m.w. Protein on Plasmodium Knowlesi Merozoites Inhibit Their Invasion of Rhesus Erythrocytes.

Merozoites are the invasive stage of the malaria parasite which are released from infected erythrocytes to invade other rythrocytes. Antibody to surface antigens on merozoites may prevent invasion by agglutinating merozoites as they are released from infected erythrocytes or by blocking receptors before contact of merozoites with the host erythrocyte. Monoclonal antibodies were produced to a 140,000 m.w. protein on the merozoite surface. The protein was synthesized by the mature intra-rythrocytic parasite, the schizont, as a 143,000 m.w. protein and had a m.w. of 140,000 on the surface of free merozoites. The monoclonal antibodies were shown to bind to the surface of merozoites by immune electron microscopy. Ascitic fluid containing four of 11 anti-140,000 monoclonal antibodies partially blocked invasion of erythrocytes by merozoites released from schizont-infected cells. The low invasion rate was always associated with a high frequency of multiply infected erythrocytes (two or more rings per erythrocyte). Monoclonal antibodies purified by $(NH_4)_2SO_4$ precipitation and DEAE column fractionation also blocked invasion and caused multiple invasion of individual erythrocytes. The monoclonal antibodies, incubated with free merozoites, did not block invasion, indicating that the antibodies did not bind to merozoite receptors for erythrocytes. We propose that the reduced rate of invasion and the multiple invasion of erythrocytes, the characteristic of these monoclonal antibodies, was caused by weak agglutination of merozoites as they were released from infected erythrocytes.

4. The Malaria Merozoite Surface: A 140,000 M.W. Protein Antigenically Unrelated to Other Surface Components on Plasmodium Knowles Merozoites.

We previously identified three proteins on the surface of merozoites (140,000, 105,000 and 75,000 m.w.). To determine if 140,000 m.w. protein was related to other surface proteins, we immunized mice with liposomes containing merozoite proteins from the 140,000 m.w. region of the polyacrylamide gel. The immune sera reacted with the surface of viable merozoites and acetone-fixed schizonts by immunofluorescence. The sera immunoprecipitated only the 140,000 m.w. protein from surface-labeled merozoites. We demonstrated that monoclonal antibody 13C11 immunoprecipitated a 250,000 m.w. protein from metabolically labeled schizonts and bound to the merozoite surface. This monoclonal antibody immunoprecipitated the 75,000 and lower m.w. proteins from surface-labeled merozoites but did not bring down the 140,000 m.w. protein. Because the mouse immune sera did not immunoprecipitate the 250,000 m.w. protein from metabolically labeled schizonts or proteins other than the 140,000 m.w. protein from surface-labeled merozoites, we conclude that the 140,000 m.w. protein is unrealted to other merozoite surface antigens identified to date.

PROJECT: 3M161102BS10 BASIC RESEARCH ON MILITARY DISEASES

WORK UNIT: 211 Biochemistry of Parasitic Drugs

INVESTIGATORS:

Principal:	Carl R. Alving, M.D., COL, MC
Associates:	Roberta L. Richards (Owens), Ph.D., DAC; Nabila M. Wassef, Ph.D., DAC; Marti Jett, Ph.D., DAC.
Assistants:	SP4 Ramon Pacheco; SP4 Pearl Burke

DESCRIPTION:

The major goal was to develop liposomes as drug carriers in treatment of parasitic diseases. The influence of liposomes on the immune system was also examined because of our discovery that antibodies against liposomes could be generated under certain conditions. The possible feasibility of using liposomes as vehicles for vaccines was examined.

1. Binding of Choleragen and Anti-Ganglioside Antibodies to Gangliosides Incorporated Into Preformed Liposomes.

Exogenously added gangliosides were taken up and incorporated into liposomes just as they are incorporated into cells. Ganglioside G_{M1} was rapidly taken up by liposomes containing dimyristoyl- or dipalmitoyl-phosphatidylcholine, cholesterol and dicetyl phosphate. When incubated with a wide range of G_{M1} concentrations for 18 hr, the liposomes incorporated about 10% of the added ganglioside. The rate of G_{M1} uptake by preformed liposomes was both time- and temperature-dependent. The liposomes also incorporated other gangliosides to a similar extent. The G_{M1} taken up by preformed liposomes was predominantly located on the outer surface of the liposomes and did not appear to be internalized into the inner half of the lipid bilayer. Liposomes containing G_{M1} added after liposome formation bound as many anti-G_{M1} antibodies and as much choleragen as liposomes having G_{M1} added during the formation of the lipid bilayers. Thus, preformed liposomes sensitized by incubation with G_{M1} are a good model system for studying the interactions of antibodies and toxins with membrane-associated gangliosides.

2. Liposomal Chemotherapy in Visceral Leishmaniasis: An Ultrastructural Study of An Intracellular Pathway.

The intracellular fate of liposomes administered intracardially was examined in the liver and spleen of hamsters experimentally infected with Leishmania donovani. Separate groups of animals were treated with liposomes containing either an antileishmanial agent, a colloidal gold marker or saline. Ultrastructural examinations of lysosomal interactions with the parasitophorous vacuole and with phagocytized liposomes were made. Lysosomes readily fused with the parasitophorous vacuoles but appeared to have little effect on the parasite, possibly due to the production of enzyme inhibitors. Liposomes rapidly became localized in lysosomes subsequent to endocytosis by macrophages. Morphologic evidence suggested that secondary lysosomes containing liposomal residues then fused with the parasitophorous vacuole. Aspects of one possible pathway

heterogeneous in their ability to gel lysate. Of eight fractions tested, one (fraction 1) had no detectable activity above the background, and the seven others had activity that ranged from 10-fold to 10,000-fold above the background. The heterogeneity of lipid A fractions detected in assays with amoebocyte lysate was consistent with the finding of heterogeneity in other functional assays of lipid A fractions.

8. Effects of Negatively Charged Lipids on Phagocytosis of Liposomes Opsonized by Complement.

Ingestion of liposomes opsonized by specific antibody plus complement was investigated in vitro. Although the antibodies alone (IgM) did not have an opsonizing effect, in the presence of such antibodies uptake and ingestion of liposomes by mouse peritoneal macrophages was enhanced 5- to 10-fold by addition of complement. Phagocytosis of complement-opsonized liposomes was strongly dependent on the charge of the liposomal lipids. The presence of a negatively charged (i.e., acidic) lipid profoundly suppressed the uptake of the liposomes. Each of three acidic liposomal lipids, phosphatidylserine, phosphatidylinositol and dicetylphosphate, suppressed liposome uptake. We conclude that opsonization of liposomes with complement greatly stimulates ingestion of liposomes by murine macrophages. However, most of the opsonic enhancement conferred by complement can be prevented by the presence of negatively charged membrane lipids.

9. Antibodies Reactive with Liposomal Phospholipids are Produced During Experimental Trypanosoma Rhodesiense Infections in Rabbits.

Antibodies against phosopholipids appeared spontaneously during the course of experimental Trypanosoma rhodeniense infections in rabbits. These antibodies were observed in rabbits infected either with a lethal strain or with a strain newly discovered to give a spontaneous self-cure. Serum antibodies reacting with liposomes containing dimyristoyl phosphatidylcholine (DMPC), phosphatidylinositol (PI), phosphatidylinositol phosphate (PIP), or cardiolipin were detected at 3 to 4 wk by complement-mediated release of trapped marker from liposomes. Antibodies were also detected against a trypanosomal lipid fraction (TrF2) that contained PI as a major constituent. The antibody activities against DMPC, PI, or TrF2 all reacted (or cross-reacted) with DMPC, and were removed from the serum by adsorbing with liposomes containing DMPC as the only phospholipid. Phosphocholine inhibited the antibodies reactive with liposomes containing either DMPC or DMPC and pI as phospholipids. Antibodies against PIP, however, reacted only with liposomes containing PIP and were not removed by adsorbing with liposomes lacking PIP. We conclude that antiphospholipid antibodies appear during the course of trypanosomal infections that either undergo apparent self-cure or are lethal, and at least two anti-phospholipid antibody specificities can be detected.

10. Localization of Urinary Lactosylceramide in Cytoplasmic Vesicles of Renal Tubular Cells in Homozygous Familial Hypercholester-

An average 15-fold increase in lactosylceramide (LacCer) in the sediment of receptor-negative, familial hypercholesterolemic (FH) homoxygotes has been reported [Chatterjee, S., Sekerke, C. S. and Kwiterovich, P.O., Jr. (1982) J. Lipid Res. 23, 513-522]. We report here the abnormal urinary exretion of significant numbers of renal tubular cells in eight FH homozygoes. The mean activity of -glutamyltransferase, a marker for renal tubular cells, was twice as high in urinary sediment of FH homozygoes as in normals. Membrane-enclosed cytoplasmic vesicles that stained strongly positive with a fluorescein-labeled antibody against LacCer were found in the renal tubular cells of all homozygoes except two who had undergone a protacaval shunt. These two had normal urinary levels of LacCer, and the cytoplasmic vesicles were vacuolated. In the other six, most of the fluorescent antibody label was intracellular and perinuclear. The cytoplasmic vesicles stained strongly with polychromatic Papanicolaou stain, periodic acid/Schiff reagent, and oil red O. Electron microscopy revealed perinuclear membrane-enclosed lipid and free lipid droplets. When two FH homoxygotes, who excreted increased LacCer, underwent plasma exchange, the cytoplasmic vesicles became empty, and the urinary LacCer level decreased into the normal range. We conclude that the increased urinary excretion of LacCer in FH homozygotes occures in renal tubular cells and that the intracellular location of LacCer is within cytoplasmic vesicles. The presence of LacCer within these vesicles can be modulated by treatment with plasma exchange.

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ABSTRACTS

1. Wassef, N. M., Roerdink, F., Richardson, Earl C. and Alving, C. R. Phagocytosis of complement-opsonized liposomes by murine macrophages: effects of negatively charged lipids. Fed. Proc. 42(7) 1826 (1983) (Abs. No. 402).

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MEETINGS AND SYMPOSIA

COL Alving and Dr. Mattsby-Baltzer were co-chairperson and co-organizers of a Symposium on "Molecular Concepts of Lipid A", held at the Walter Reed Army Institute of Research, Washington, DC 4-6 April 1983.

COL Alving was a faculty member, NATO Advanced Study Institute on "Receptor-Mediated Targeting of Drugs" held at Cape Sounion Beach, Greece, 20 June - 1 July 1983.

COL Alving was an invited speaker, Symposium on "Use of Liposomes in Medicine", in honor of Prof. Brenda Ryman on the occasion of her retirement, held in London on 22 September 1983.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6471	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CG	212 WWI6
b. CONTRIBUTING				
c. CONTRIBUTING	STOC 82/83-6 2/2			

11. TITLE (Precede with Security Classification Code)

(U) Physiology of Systemic Effects of Blast Overpressure

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

017100 Weapons Effects 002300 Biochemistry 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 03	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	3.0	100
FISCAL YEAR CURRENT 84	6.0	211

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: HOYT, R F

NAME: YOUNG, A J POC:DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Blast overpressure; (U) Pulmonary biochemistry; (U) Pulmonary Receptors; (U) Impulse Noise; (U) Biophysical Modeling

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To define the physiologic effects of blast overpressure and to determine the limits of human safety for exposure to impulse noise. To develop a mathematical model of the thoraco-abdominal response to blast waves. There is military relevance.

24. (U) Approach uses biochemical assays and physiologic tests bfore and after blast and impact injury. Blood is analyzed for enzymes, elastin related products and protein changes detected by 2 dimensional gel electrophoresis. Pulmonary tissue is examined histologically. A finite element model of the sheep and human torso will be developed. Engineering materiel properties of lung parenchyma are to be determined.

25. (U) 82 10 - 83 09 The search for a biochemical marker of blast injury now focuses on Angiotensin I Converting Enyzme (ACE), related moieties and two-dimensional gel electrophoresis as a screening tool for examination of blood. The pharmacokinetics of intravenous desmosine have been studied in sheep. A detailed 2 dimensional finite element model (FEM) of a crossection of the sheep at the seventh vertebra was developed. This FEM predicts wave propogation in lung parenchyma as the cause of changes in intrathoracic pressure. Measurements of elastic and bulk moduli and the speed of sound have been made in rabbit, cat, and goat lungs at various trans pulmonary pressures. For technical report, see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M161102B S10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 212: Physiology of Systemic Effects of Blast Overpressure

Principal Investigator: Yancy Y Phillips, M.D., MAJ, MC

Associate Investigators: James J. Jaeger, Ph.D., MAJ, MSC
Robert F. Hoyt, Jr., D.V.M., M.S., MAJ, VC
Andrew J. Young, Ph.D., CPT, MSC
Kenneth T. Dodd, Ph.D., GS-12
Thomas G. Mundie, Ph.D., CPT, MSC
Pritam S. Verma, Ph.D., CPT, MSC

Problem Statement and Objectives

Certain Army weapon systems, some currently in use, others still in development, produce levels of blast overpressure which exceed the limits defined in MIL STD 1474B. The research objective of the Department of Respiratory Research is to define the risk of non-auditory injury to crew members from the blast overpressure produced by these weapon systems. To obtain a general understanding of the interaction of blast waves with crewmembers, mathematical models are being developed using the inherent biomechanical properties of the human structure. Validation of the model's predictions will be made at frequent intervals to avoid costly development along non-productive paths. Biochemical indicators of non-auditory blast injury are also being sought in order to increase the probability of detecting subclinical injury and to reduce the complexity and invasiveness of current techniques for assessing injury.

Progress And Accomplishments In FY83

a. Contract milestones in the second year of a three year mathematical modeling contract for the characterization of the thoraco-abdominal response to blast waves with the JAYCOR Corporation, San Diego, CA include:

1. The anatomical elements and appropriate material properties were identified for the two and three-dimensional finite element model (FEM) of sheep-blast wave interaction. The two-dimensional FEM of the thorax predicts wave phenomena in lung parenchyma and correlates well with observed data for intrathoracic pressures (ITP) measured in the esophagus of sheep when exposed to blast.

2. The near field mathematical model of blast wave loading of a human torso was completed by JAYCOR and validated by actual blast exposure of a torso model at the Lovelace test facility in Albuquerque, NM.

3. Trial runs of a large element three-dimensional FEM demonstrated reasonable agreement with the Lovelace spring/dashpot lumped parameter model. Both, however, were found to differ from actual ITP pressure traces.

4. The mechanical properties of lung parenchyma during impulsive loading are being determined by the Bioengineering Department of the University of California at San Diego, acting as a sub-contractor to JAYCOR. To date, incremental elasticity, bulk modulus and wave speed measurements have been made as a function of lung inflation.

b. The pharmacokinetics of desmosine, a degradation product of elastin, were determined in sheep as a first step in evaluating the utility of desmosine as a biochemical marker of blast injury to the lung.

c. A one-year contract was awarded to Electronucleonics for the study of serum from blast-injured animals by two dimensional gel electrophoresis

Recommendations and Objectives for FY84

The major research efforts under this work unit for FY84 will be:

a. Continued efforts to assess biochemical markers of injury. The advantage of such noninvasive measures is clear in both animal and human work. Results from a recent uncontrolled field study of sheep exposed to lung injury threshold levels of blast overpressure show angiotension converting enzyme (ACE) levels are substantially elevated following impulse loading. A new study planned for the first quarter FY84 will attempt to further define this potential marker. The study will require single shots to groups of six animals. Groups will have graded levels of injury-approximately LD_{20}, LD_1 threshold, and sub-threshold. In addition, there will be handling and exercise controls. Blood will be drawn by percutaneous intravenous stick prior to and for up to 2 hours after blast exposure. The collected specimens will be analyzed for angiotension converting enzyme for desmosine for two-dimensional gel electrophorectic analysis and for routine chemistries.

b. Continued development and validation of a mathematical model of blast-thorax interaction in animals. Included in this area will be both the validation of wave phenomena in lung parencyhma by endobronchially placed catheters and the validation of the two-dimensional FEM structure by measurement of thoracic deformation with static loading. Intrathoracic pressure traces from actual blast exposure will be compared to model output exercised by similar blast waves.

c. Contract work will continue on material properties of the lung. Effects of temperature, specimen age, elapsed time from death, transpulmonary pressure difference, and normalized lung volume will be studied for bulk and elastic moduli. Study will begin on damage mechanism of lung tissue.

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Young, A. J., Evans, W. J., Sharp, R. L., Costill, D. L., and Maher J. T.: Intramuscular pH with excercise at high altitude. Presented at the 67th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Ill., April, 1983.

Jaeger, J., Young, A. J., Phillips, Y. Y and Hoyt, R. F., Jr: Lung inflation effects on blast lung injury. Presented at the fourth annual meeting of RSG-6 (NATO Panel VIII, Effects of Impulse Noise), WRAIR, Washington , D.C., May 1983.

Jaeger, J., Young, A. J., Phillips, Y. Y and Hoyt, R. F., Jr.,: Low level human blast exposures. Presented at the fourth annual meeting of RSG-6 (NATO Panel VIII, Effects of Impulse Noise), WRAIR, Washington, D. C. May 1983

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Phillips, Y. Y: Dry gas eucapnic hyperventilation: a simplified technique for bronchoprovocation. Presented at the thirty-fifth annual Carl W. Tempel Symposium on Pulmonary Disease and Allergy-Immunology, Fitzsimons Army Medical Center, Aurora, Colo., January 1983.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OC 451	2. DATE OF SUMMARY: 83 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 82 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. ORDER NUMBER: NL	9. SPECIFIC DATA - CONTRACTOR ACCESS: ☒ YES ☐ NO

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CD	213 WW.JA
b. CONTRIBUTING				
XXXXXXX	JTCG [illegible]			

11. TITLE (Precede with Security Classification Code):
(U) Biological Modulation of Military Performance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
012900 Physiology 016200 Stress Physiology 013400 Psychology 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING: 83	7.0	688
FISCAL YEAR CURRENT: 84	7.0	725

19. RESPONSIBLE DOD ORGANIZATION
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RESPONSIBLE INDIVIDUAL
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21. GENERAL USE
Foreign Intelligence Considered

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Division of Neuropsychiatry
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Elsmore, T F
TELEPHONE: (202) 576-2483
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Hursh, S R
NAME: Wylie, R M

POC: DA

22. KEYWORDS (Precede each with Security Classification Code): (U) Neuropsychiatry; (U) Physiology; (U) Performance; (U) Neurophysiology; (U) Neuroanatomy; (U) Stress

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Investigations will seek to describe the means by which the nervous system effects bodily responses to stress and injury, and to discern those combinations of physiologic parameters which collectively define the optimal conditions for effective military performance.

24. (U) Animal models of performance will be created using the techniques of operant and respondent conditioning and the role of internal factors in performance variability assessed by neurophysiologic recording of intracellular and extracellular bioelectric potentials; the descriptive and experimental neuroanatomical techniques of light and electron microscopy and histochemistry; stimulation or lesioning of discrete brain areas; and experimental modifications of hormonal status by ablation and/or administration of exogenous hormones or other drugs.

25. (U) 82 10 - 83 09 Major findings: For use as baselines in studying the impact of stressors upon military performance, two methods for the study of time perception in rats and a technique for the investigation of short-term memory in mice have been developed. Studies on behavioral and physiological variables affecting short-term memory in monkeys are continuing. In studies on the neural mechanisms controlling limb movement in primates, three different procedures have been developed for studying motor tracking of a visual stimulus in monkeys. Three normal animals and one animal wit.. a deafferented forelimb are being trained on these procedures. Studies on the neural control of respiration concluded that at least two mechanisms exist for the control of termination of both inspiration and expiration, and that baroreceptor input had no consistent effect upon phrenic nerve control of inspiration and expiration. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

Project: 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 213: Biological Modulation of Military Performance

Investigators:

Principal:	Elsmore, T.F. Ph.D.
Associate:	Hursh, MAJ, S.R.; Campbell, LTC, C.B.G.; Raslear, CPT, T.G.; Leu, CPT, J.R.; Petras, J.M., Ph.D.; Wylie, R.M., Ph.D.

Objectives:

The objectives of this project include the definition of the means by which the nervous system mediates bodily responses to stress and injury, and to discern those combinations of physiologic parameters which collectively define the optimal conditions for effective military performance. A major thrust of research in this work unit is the development of animal behavior models that more closely approximate realistic conditions outside of the laboratory. Techniques and methods are drawn from a broad spectrum of neuroscience disciplines including psychology, neurophysiology, neuroanatomy, neuropharmacology, and chronobiology.

Progress:

One major thrust of this work unit is the development of animal models of human performance that are clearly relevant to military situations. Behavioral research with animals has traditionally emphasized short experimental sessions under highly controlled conditions, and ignored the larger context in which the animals exist. We have recently been applying economic concepts in an attempt to broaden the framework of our laboratory behavior research, and therefore to increase the validity of our animal models. In nearly all situations, as the price of a commodity is increased, consumption of it decreases. The slope of this decrease is called "elasticity of demand," and indicates how sensitive consumption is to price changes. The level of the demand curve is simply the amount of demand for the commodity. Economists have proposed a number of environmental variables that alter these two parameters but little controlled experimental work exists to verify these proposals. One of the most obvious variables to alter the demand curve is the availability of substitutes. Work this year has focused on providing various alternative substances to rats that are working for food and determining if the type of substitute alters demand elasticity, amount of demand, or both. Initial efforts used the non-nutritive sweetener aspartame, which was found to be unstable in our experimental situation. These experiments are now being repeated with saccharin. Generality of findings between species is critical in evaluating the validity of animal models of behavior. Two studies demonstrated that increasing the economic variable of meal cost produced results in

wild-caught rats and domestic chicks that were quite similar to those that had previously been reported in standard laboratory rats. That is, increasing meal cost led to increased meal size and decreased meal frequency.

Perception is a process fundamental to most complex human tasks. Time perception in particular, is critical for tasks requiring sensory/motor coordination. Two methods have been developed for studying time perception in rats, one in which the animals are trained to respond to the absolute duration of visual or auditory stimuli, and one which derives a subjective midpoint between two extreme stimuli. Both techniques are currently in use in the evaluation of long-term effects of exposure to organophosphorous compounds (See WU 164, DAOG 8600, FY83 WRAIR Annual Report.

Short-term memory is being studied in both mice and non-human primates. In mice, a radial-arm maze procedure has successfully been used to obtain measures of both long-term and short-term memory. Studies on short-term memory processes in monkeys are continuing.

Another major thrust of this work unit is the study of the physiological bases of behavior, particularly the neural mechanisms underlying behavior. The neural basis of memory has been the topic of a series of collaborative studies with NIH. The tentative conclusion from this work is that the hippocampus is primarily responsible for spatial associations. An ongoing project is investigating memory for auditory stimuli, and cross-modality memory for sounds and spatial locations.

A continuing series of studies is investigating the neural control of movement. Previous work in our Department has demonstrated that monkeys that have been deprived of sensory input from a limb are capable of performance on a weight-lifting task quite comparable to that of normal animals. In an effort to place greater demands upon the animal's motor control system, three different visual tracking tasks, one requiring the animal to point his forearm at a stationary spot of light, one that moves the spot of light from place to place in a step-wise fashion, and one which moves it in an smooth analog fashion have been developed. To date three normal animals have been trained on all three of these these tasks, and one deafferented monkey has been been trained to point at a stationary spot. No conclusions are available from this study as yet.

Two studies investigated the neural control of respiration in cats using neurophysiological techniques. In one the effects of stimulating muscle afferents on respiration were studied. It was concluded that at least two distinct central mechanisms must exist for the control of termination of both inspiration and expiration. A second study evaluated the effects of constant baroreceptor input on neural inspiratory drive at different levels of central chemoreceptor drive. It was concluded from these studies that baroreceptor input had no consistent effect on phrenic nerve activity or

upon relationships between phrenic activity and either inspiratory or expiratory time.

Future objectives:

Applicability and utility of economic concepts in the analysis of behavior will continue to be investigated, including a simulation of the "Laffer curve." Short-term memory models of animal behavior will continue to be evaluated. Studies on the neural bases of movement, respiration, and memory will continue.

Presentations

Bachevalier, J., Parkinson, J. K., Aggleton, J. P., and Mishkin, M. Severe recognition impairment after combined but not separate transection of the fornix and the amygdalofugal pathways. Society for Neurosciences November 1982. (Abstract: Society for Neurosciences Abstracts, 1982, 8,23.)

Gill-Kumar, P. and Campbell, C.B.G. Effects of baroreceptors on the respiratory centre complex. FASEB, 1983. (Abstract: Federation Proceedings, 1983, 42, 1253.)

Hursh, S.R. Supply, Demand, and Response Rate. Invited paper presented at the First European Meeting on the Experimental Analysis of Behavior, Liege, Belgium, 1983.

Hursh, S.R. Economics. Invited paper presented at the West Virginia Conference on the Future Directions in the Experimental Analysis of Behavior, 1983.

Parkinson, J. K. and Mishkin, M. A selective mnemonic role for the hippocampus in monkeys: memory for the location of objects. Society for Neurosciences November, 1982. (Abstract: Society for Neurosciences Abstracts, 1982, 8,23.).

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Campbell, C.B.G. Behaviorism and natural selection The Behavioral and Brain Sciences, in press.

Kaufman, L.W. and Collier, G. Cost and meal patterns in wild-caught rats. Physiology and Behavior, 1983, 30, 445-449.

Kaufman, L.W. and Collier, G. Meal-taking by domestic chicks (Gallus gallus). Animal Behavior, 1983, 31, 397-403.

Hursh, S.R. Maximization and reinforcement theory compared. The Behavioral and Brain Sciences, 1983, 6, 324-326.

Hursh, S.R. and Bauman, R.A. The behavioral analysis of demand. In Advances in Behavioral Economics, Vol. 1., Eds: Howard Rachlin and Leonard Green, Norwood, N.J., Ablex Publishing Corporation, in press.

Parkinson, J.K. and Medin, D.L. Emerging attributes in monkey short term memory. Journal of Experimental Psychology: Animal Behavior Processes, 1983, 9, 31-40.

Raslear, T.G. A test of the Pfanzagel bisection model in rats. Journal of Experimental Psychology: Animal Behavior Processes, 1983, 9, 49-62.

Raslear, T.G., Pierrel-Sorrentino, R., and Rudnick, F. Loudness scaling and masking in rats. Behavioral Neuroscience, 1983, 97, 392-398.

Raslear, R.G. and Keck, L. Comment on Foster and White's "Ethnic Identity and Perceived Distance between Ethnic Categories." Human Organization, 1983, 42, 91-92.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION: DA OG 6755	2. DATE OF SUMMARY: 83 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CE	214 WWJE
b. CONTRIBUTING				
c. CONTRIBUTING	STO: 82/83-6.2/2			

11. TITLE (Precede with Security Classification Code)

(U) Millimeter Wave Biophysics and Bionazards

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

014100 Radiobiol 012900 Physiol 014000 Rad Chem 017000 Wave Prop

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
30 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING: 83	2.0	89
FISCAL YEAR CURRENT: 84	2.0	328

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Dept of Microwave Research
Div of Neuropsychiatry

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PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)

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TELEPHONE: (202) 576-3615

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: JACOBI, J H

NAME: HUNT, E L POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Biophysics; (U) Millimeter Wave; (U) Bioeffects; (U) Permittivity

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with DoD Security Classification Code)

23. (U) The objectives of the millimeter wave bioeffects program are to (1) establish a technology base in millimeter wave instrumentation as needed for biophysical research in this region of the electromagnetic spectrum, (2) to develop millimeter wave exposure systems for use with biological specimens under conditions of both continuous wave and high peak power operations, and (3) to explore biological hazards with special interest in the eye. The military relevance in this research derives from millimeter wave radars

24. (U) The millimeter wave instrumentation system will consist of a millimeter wave phase locked synthesizer for the range 40-60 GHz. This will serve as the source for a six-port network analyzer that will provide network analysis based description of biological dielectrics in vitro. The continuous wave exposure system will consist of a 35 GHz, 1 kilowatt klystron amplifier, a 10 watt traveling wave tube driver and a 100 milliwatt Gunn diode oscillator. The pulse transmitter will consist of a 35 GHz traveling wave tube amplifier of 30 kilowatts peak power and 3 kilowatts average power. The antenna will consist of a WR 28 feed to an elliptical reflector. The biological hazard studies will emphasize two features: (1) the direct heating action of millimeter waves on the cornea of the eye and (2) the production of thermoacoustic expansion in cornea, lens and retina.

25. (U) 82 10 - 83 09 Development of the 35 GHz pulsed and CW transmitters continued. At the present time, both are ca. 90 - 95% complete. Site preparation is fully completed. Preliminary millimeter wave (37 GHz) measurements of emissivity in vitro indicate a value near 0.90. Spectral scanning equipment development has progressed into the system integration phase. The 40 - 60 GHz six-port network analyzer and digital synthesizer subsystems are completed. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD, FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 214: Millimeter Wave Biophysics and Biohazards

Investigators.
Principal: LTC(P) Lawrence E. Larsen, MD
Associate: John H. Jacobi, M.S.; Charles N. Rafferty, Ph.D.

Introduction

The millimeter wave program began as an unfunded requirement in 1979/1980. It was funded in FY 1981 at which time a MCA for new laboratory space was completed on time and on budget. The program consists of a biophysical segment which is still in the hardware development phase; and a biohazards segment which has only recently emerged from its hardware development phase. Also a new biophysicl chemistry program has just just completed the equipping of a laboratory located in Forest Glen.

Dielectric Relaxation

This program is designed to develop the first biosystem dielectric data base in the 40 to 60 GHz range. The hardware developments needed to support this goal consist of a 40 to 60 GHz digital synthesizer of previously unobtainable accuracy, a 40 to 60 GHz six port network analyzer, and a system integration step to include automatic process control as well as error correction. These steps have recently been completed; and an additional requirement has been added to accommodate dielectric measurements under transient conditions.

New physical theories have also been developed in the context of this program. This theoretical thrust has been directed to the issue of dielectric properties under transient conditions. All existing theory is limited to steady-state conditions. The new transient theory depends upon cooperative effects at the molecular level. This effect produces energy deposition in the dielectric that is not accountable in normal Debye theory.

Biophysical Chemisty

This program is designed to investigate conformational state changes in micromolecules consequent to exposure in dual beam systems which use RF power as either an effector or as a sensor in combination with optical beams. The laboratory needed to accomplish these objectives is located in space formerly occupied by neurochemisty at Forest Glen. The equipment

needed for this laboratory has been acquired over the last year. Protocols have been developed along with a program plan which we expect to implement in the next fiscal year.

Millimeter Wave Biohazards

This program has developed the first high power pulsed millimeter wave exposure system for biomedical use in the free world. The program goal is to explore the limitations of safety standard based on continuous wave exposure and whole body averaged dosimetry. It consists of a 30 kilowatt pulsed transmitter, a 100 dB anechoic chamber tested from 18 to 100 GHz, a polarization twist reflector antenna, a staring infra-red radiometer, and closed circuit TV for subject monitoring. The frequency of operation is 35 GHz.

The site of greatest hazard to high power millimeter wave exposure is thought to be the cornea. The cornea of anesthetized rabbit subjects were examimed in a pilot study.

Although the data is a very preliminary, it does appear that pulsed millimeter wave exposures produce anterior chamber signs at much lower power densities than similar studies in the same experimental animal with continuous wave exposures at 2450 MHz performed in this department about ten years ago. These studies are presently in the stage of replication. However, progress is slow due to the staff shortages mentioned in the microwave portion of the Annual Report.

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2. T.C. Guo and W.W. Guo, A Transient-State Theory of Dielectric Relaxation and the Curie-VonSchweidler Law", J. Physics C: Solid State Physics, 10:1955-1960, 1982.

3. T.C. Guo and W.W. Guo, "An After Field Effect on the Distribution of Relaxation Times and a Transient-State Theory of Dielectric Relaxation", IEEE Proc Conf Conduction and Breakdown in Solid Dielectrics, Pub. #82-837361, p 448, 1983.

4. T.C. Guo, W.W. Guo and L.E. Larsen, "Microwave Tomography for Biomedical Application, Intl J Infrared and Millimeter Waves, in press, 1983.

5. N.A. Dencher, C.N. Rafferty, and W. Sperling, "Photochemistry and Isomer Determination of 13-cis and Trans Bacteriorhodopsin", In Methods in Enzymology, Vol 88, Biomembranes, Part I, Visual Pigments and Purple Membranes, II, (L. Packer, Editor), Academic Press, New York, pp 168-174, 1982.

DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORDER NUMBER	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	61102A	3M161102BS1C	CD	215 WWJ8
CONTRIBUTING				
XXXXXXX	STOG 82 -6			

TITLE (Provide with Security Classification Code)

(U) Mechanism of Response to Stress

SCIENTIFIC AND TECHNOLOGICAL AREAS

2900 Physiology 002300 Biochemistry 013400 Psychology

START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	C. In-House

CONTRACT/GRANT

DATES/EFFECTIVE: EXPIRATION:

NUMBER:

TYPE: 4. AMOUNT:

KIND OF AWARD: f. CUM. AMT.

17. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	1.0	379
FISCAL YEAR CURRENT 84	3.0	377

RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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GENERAL USE

Foreign Intelligence Considered

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

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NAME: Meyerhoff, J L

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Kant, G J Lynch, T

NAME: Walczak, D POC: DA

KEYWORDS (Provide EACH with Security Classification Code) (U) Stress; (U) Neurochemistry; (U) Neurotransmitters; (U) Cyclic nucleotides; (U) Neuropeptides; (U) Kindling; (U) Post-traumatic epilepsy

TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Provide text of each with security Classification Code.)

3. (U) To examine neurochemical mechanisms in adaptation to stress and continuous performance. To study neurochemical mechanisms in development of post-traumatic epilepsy, which occurs in 40% of soldiers suffering head wounds, despite anti-convulsant drugs. To provide database for interpretation of military clinical and field studies, and recommendations for prevention and/or treatment in soldiers.

4. (U) Analysis of neurochemical regulation of hormonal response during adaptation to stress. Repeated electrical stimulation of the brain ("kindling") has been selected as the best animal model of post-traumatic epilepsy, because of similarities in time-course. Studies entail brain lesion and stimulation; measurement of neurotransmitters, neuropeptides, cyclic nucleotides and phosphorylation in specific brain regions.

5. (U) 82 10 - 83 09. Increasing intensities of footshock induced proportionally greater increases of plasma corticosterone, prolactin and pituitary cyclic AMP levels in rats. Hippocampal lesions enhanced the pituitary cyclic AMP response to acute stress without affecting habituation to repeated stress. Adrenalectomy totally abolished the stress induced increase in pituitary cyclic AMP, without affecting the increase in plasma prolactin. Footshock decreased hippocampal corticosteroid binding by 50%, while adrenalectomy doubled binding capacity. Thyrotropin releasing Hormone levels in pyriform cortex of the rat were moderately increased after partial seizures and were increased six fold after fully kindled seizures. For Technical Report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

Available to contractors upon originator's approval

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 215 Mechanisms of Response to Military Stress

Investigators

Principal: Meyerhoff, J.L., M.D.

Associate: Kant, G.J., Ph.D.; Walczak, D.D., Ph.D., Cpt., MSC; Mougey, E.H., M.S.; Collins, D.R., B.S.; Pennington, L.L., B.S.

Objectives:

Studies are conducted to evaluate neurochemical mechanisms of response to stress, brain injury and other factors which produce psychiatric incapacitation or brain syndromes pertinent to military medicine. Included are (1), studies of CNS regulation of neuroendocrine function in acute and repeated exposure to stressors, as well as in adaptation to chronic stress; (2), effects of stress or injury on CNS neurotransmitter turnover, neuropeptide chemistry and receptor function, as well as studies of neurochemical system interactions. These fundamental studies are intended to provide a database for interpretation of clinical psychoendocrine studies of stress in soldiers, and for understanding the mechanisms by which traumatic factors cause decrements in CNS function and performance.

Studies are conducted to explore possible neurochemical mechanisms in the development of post-traumatic epilepsy. More than 50% of soldiers receiving penetrating missile injuries of the brain will subsequently develop post-traumatic epilepsy. Despite the development of anticonvulsant drugs and improvements in aseptic surgical technique, the incidence of post-traumatic epilepsy has not decreased since World War I. Understanding the biochemical mechanisms active during the latent period between the injury and the observation of clinical seizures is essential to developing rational preventive measures which might be initiated immediately post-injury. Based on our neurochemical findings, appropriate novel pharmacological interventions will be explored.

Progress:

The effect of hypothalamic releasing factors on pituitary function may be mediated via cyclic AMP acting as a second messenger, translating responses to individual releasing factors into changes in regulation of pituitary hormone synthesis and release. In a series of experiments, we have investigated biochemical responses to graded levels of environmental stress. Increasing intensities of footshock (0.0 - 3.2 mA) produced increasing levels of several hormonal indices of stress in rats. The maximal elevations observed in plasma hormones were 5-fold for beta endorphin, 14-

fold for prolactin, and 5-fold for corticosterone. At the highest intensity of footshock, plasma levels of beta-lipotrophic hormone (the precursor of beta-endorphin) were increased 25-fold, and 17-fold increases were seen in pituitary levels of cyclic AMP. Generally, there was little or no pituitary cyclic AMP or hormonal response observed at the lowest current intensities, a proportional response over the medium intensities and a maximal plateau response at the highest intensities. Corticosterone, however, appeared to be a more sensitive responder to mild current intensities than the other indices measured but was less sensitive to differences between higher levels of current. There was a positive correlation among all biochemical indices with an observer rated behavioral index of stress response.

In a study comparing a variety of environmental stressors in rats, forced running, immobilization and footshock all increased levels of pituitary cyclic AMP as compared to controls. Pretreatment with atropine, an antidote for nerve agent poisoning, resulted in greater pituitary cyclic AMP responses to immobilization or footshock, as compared to saline-pretreated controls. The prolactin response to footshock was also increased in atropine-pretreated rats. In a follow-up experiment, atropine was also shown to increase responsiveness to pain in rats. These data suggest that atropine potentiates neurochemical and neuroendocrine responses to stressors at least partly due to lowering of pain threshold by atropine.

Hippocampal lesions appeared to potentiate the stress response much like atropine. Rats with hippocampal lesions had greater pituitary cyclic AMP, plasma corticosterone and plasma prolactin responses to stress than sham-operated controls. Rats subjected to repeated stress showed a diminished cyclic AMP response compared to the response to acute stress, thus demonstrating habituation. No differences in habituation were observed between hippocampal and sham-operated groups. These data complement reports of behavioral hyperreactivity to acute stress in hippocampal-lesioned animals, but suggest that they are able to habituate to repeated stressful stimuli.

Hormones released by acute stress are believed to alter the hormonal responses to subsequent stressors. An inhibitory feedback effect of the adrenal glucocorticoid, corticosterone, may be mediated through its binding to glucocorticoid receptors abundant on the hippocampus. We have begun a series of experiments designed to characterize the effects of stress-induced release of corticosterone on hippocampal glucocorticoid receptors. Adrenalectomy doubled the corticosteroid binding capacity of the hippocampal cytosol as compared to normal animals. Footshock markedly decreased the binding capacity of normal rats (by 50%), but did not affect glucocorticoid binding in adrenalectomized rats. The shock-induced decrease in hippocampal glucocorticoid binding capacity appeared to be dependent upon shock intensity and session length.

Because of the numerous studies demonstrating effects of adrenal factors on the hypothalamo-pituitary axis, we examined the effect of bilateral adrenalectomy on the stress-induced increase in pituitary cyclic AMP. We found that adrenalectomy totally abolished the cyclic AMP response to stress, without affecting the stress-induced increase in plasma prolactin. Our data suggest that either adrenal cortical or adrenal medullary hormones are essential to the pituitary cyclic AMP response. Moreover, it appears that the cyclic AMP response can be dissociated from the release of pituitary prolactin, ACTH and beta-endorphin.

In our project directed at post-traumatic epilepsy, we have succeeded in employing the "kindling" technique for producing epileptiform seizures. Kindling consists of repeated, intermittent low intensity electrical stimulation of the amygdala. This results in progressive changes in both electrographic and behavioral responses over several weeks, and culminates in a generalized seizure in response to an electrical stimulus which initially had produced no behavioral effect. Kindling seems a particularly good model of post-traumatic epilepsy because it permit biochemical study of seizure-prone brain tissue without requiring the use of seizure-inducing drugs. Moreover, the latent period seen in the kindling phenomenon is similar to the delay of seizure onset seen in post-traumatic epilepsy. Thyrotropin Releasing Hormone (TRH) is released from the hypothalamus to regulate the release of thyroid stimulating hormone from the pituitary. TRH is also found in many brain regions outside the hypothalamus where it is viewed as a neuromodulator, affecting the function of neurotransmitter systems without having a direct post-synaptic effect. Because high levels of TRH and its receptors are found in brain regions associated with seizure activity, we have studied the effect of kindled seizures on brain regional TRH. We found that TRH in fully kindled (stage 5) rats was elevated 6-fold in pyriform cortex and 2-fold in both the amygdala and hippocampus. Additional studies with partially kindled (stage 2) rats suggest that the degree of TRH elevation may be related to the stage of kindled seizure elicited.

Future Objectives:

We plan to continue to study the mechanism of the pituitary cyclic AMP and other neuroendocrine and neuropeptide responses to stress. The interactions of neurotransmitters and hormones in modulating stress responses will be analysed using specific pharmacological blockers. The contribution of the adrenal gland will be further analysed by endocrine manipulations such as, adrenal medullectomy, to specifically eliminate circulating epinephrine, or the use of drugs such as dexamethasone or metyrapone to mimic or block the feedback effects of corticosterone. Further physiologic studies (i.e. amygdalar or septal lesions, etc.) will be initiated to understand limbic system regulation of pituitary responses. We shall extend the acute stress studies to include consideration of the chronic stress of continuous performance requirements. We will also continue to

study the interaction between stress responses and pretreatment with pharmacological agents such as carbamate cholinesterase inhibitors, as well as with drugs known to enhance performance. We plan to assay brain noradrenergic receptors in stressed animals, and to study the effects of "stress hormones" on release of neurotransmitters. We plan to extend studies of sex differences in responses to acute and chronic stress.

We plan to continue studies of neurochemical mechanism of development of seizure disorders following trauma, extending neuropeptide studies to include neurotensin, somatostatin, enkephalin, cholecystokinin, vasoactive intestinal peptide and motilin. We are planning further studies of novel pharmacologic interventions to prevent development of seizures following brain injury. Further studies are under way to determine if the effects of kindling on TRH levels are persistent, or of limited duration.

Presentations:

1. Belenky, G.L., Gelinas-Sorrell, D., Kenner, J.R., and Holaday, J.W. Delta antagonist modifies the post-ictal effects of electroconvulsive shock (ECS). Paper presented at the International Narcotics Research Conference, June 1983.

Publications:

1. Kant, G.J., Meyerhoff, J.L., Bunnell, B.N. and Lenox, R.H. Cyclic AMP and cyclic GMP response to stress in brain and pituitary: stress elevates pituitary cyclic AMP. Pharmacology, Biochemistry and Behavior 17, 1067-1072 (1982).

2. Kubek, M.J., Meyerhoff, J.L., and Sattin, A. Alterations of CNS thyrotropin-releasing hormone (TRH) following convulsive (ECS) and subconvulsive (SCS) electroshock in the rat. Neuroscience Abstracts 8, 982 (1982).

3. Kant, J.G., Meyerhoff, J.L., Bunnell, B.N., Mougey, E.H., Collins, D.R., Pennington, L.L., Kenion, C.C., Driver, G.C., Gamble, W.L., Wormley, C.B., Landman-Roberts, L., and Eggleston, T. Effect of stress on levels of pituitary cyclic AMP and plasma hormones. Neuroscience Abstracts 8, 425 (1982).

4. Meyerhoff, J.L., Bates, V.E. and Kubek, M.J. Increases in brain thyrotropin-releasing hormone (TRH) following kindled seizures. Neuroscience Abstracts 8, 457 (1982).

5. Bunnell, B.N., Meyerhoff, J.L., Collins, D.R., Mougey, E.H, Pennington, L.L., and Lenox, R.H. Pituitary cyclic AMP in rats: effects of presenting or withholding an appetitive stimulus. Neuroscience Abstracts 8,459 (1982).

6. Kant, G.J., Bates, V.E., Lenox, R.H., and Meyerhoff, J.L. Effects of acute and chronic desmethyl imipramine on levels of cyclic AMP in vivo. Biochemical Pharmacology 32, 732-735 (1983).

7. Belenky, G.L., Gelinas-Sorrell, D., Kenner, J.R., and Holaday, J.W. Delta antagonist modifies the post-ictal effects of electroconvulsive shock (ECS). Life Sciences (in press).

8. Kant, G.J., Bunnell, B.N., Mougey, E.H., Pennington, L.L. and Meyerhoff, J.L. Effects of repeated stress on pituitary cyclic AMP and plasma prolactin, corticosterone and growth hormone in male rats. Pharmacol. Biochem Behav 18, 967-971 (1983).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6473	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORGN'S INSTR'N	9a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	61102A	3M161102BS10	CD	216 WU/5
CONTRIBUTING				
CONTRIBUTING	(STDG)			

11. TITLE (Procede with Security Classification Code)

) Military Stress: Non-invasive Monitoring of Health Performance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

6200 Stress Physiology 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
10	CONT	DA	C. In-House

17. CONTRACT/GRANT

DATES/EFFECTIVE	EXPIRATION
NUMBER	
TYPE	AMOUNT
KIND OF AWARD	CUM. AMT.

18. RESOURCES ESTIMATE

	PRECEDING/CURRENT	A. PROFESSIONAL MAN YRS	B. FUNDS (In Thousands)
FISCAL YEAR	83	4.0	432
	84	4.0	455

19a. RESPONSIBLE DOD ORGANIZATION

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19b. RESPONSIBLE INDIVIDUAL

T , F H JR

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20. PERFORMING ORGANIZATION

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Division of Neuropsychiatry
Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide Name if U.S. Academic Institution)

Genser, S G
(301) 427-5521

ASSOCIATE INVESTIGATORS

Thorne, D
Sing, H C POC:DA

21. GENERAL USE

reign intelligence considered.

22. KEYWORDS (Provide Each with Security Classification Code) (U) Electrophysiology; (U) Psychophysiology;) Psychophysics; (U) Stress; (U) Performance; (U) Human Volunteer

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide Individual Paragraphs Identified by Number. Precede Text of Each with Security Classification Code.)

. (U) Objective is the development of non-invasive human psychophysiological nitoring technology in support of field studies of stress in military environments.

. (U) Approach is to exploit advances in signal acquisition and processing chnologies to enlarge the scope of psychophysiological measurements that can be made der field conditions. Techniques are validated in the laboratory prior to deployment in ntrolled field trials.

. (U) 82 10 - 83 09 This work provides the technology base for Work Unit 043, litary Stress: Circadian and Ultradian Factors (Accession Number DA OC 6457).

Development of a field deployable Actigraph system has progressed to the release of RFQ and first Source Selection Board review for its contracted design. Arrangements ve been completed with NASA to acquire their technology for improving our present system r recording human core temperatures using "pill" telemetry. The revision of the rformance Assessment Battery (PAB), the major portions of the user's manual and utility ograms to aid subject training, data analysis and test parameter modification have now en completed. The revised battery has been sent to Ft. Rucker for field use in forger-83 and in ongoing experiments at AFIP. Findings from our sustained performance udies led to the development of an efficient and robust new performance index called hroughput". RFQs have been released for the development of a Field Deployable rformance Assessment Battery (FPAB), a Neuropsychological Test System (NPTS), a obtrusive Ocular Monitoring System (UOMS) and feature extraction procedures utilizing ttern recognition and signal processing techniques. A data base has been formed mposed of the PAB, Mood Activation Scale, Lexical Decision and Vigilance Discrimination sks. Subsequent analyses will focus on the effects of fatigue on cognitive, affective d physiologic functions. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

DD FORM 1498

Project 3M161102BS10 RESEARCH ON DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 216: Military Stress: Non-invasive Monitoring of Health and Performance

Investigators:

Principal: LTC(P) Sander G. Genser, MC

Associates: LTC Daniel P. Redmond, MC;
MAJ Steven Taube, MC;
David Thorne, Ph.D.;
Stanley Hall, B.A.;
Helen Sing, M.S.

Problems and Objectives

This work unit provides the supportive technology base for Work Unit 043, Military Stress: Circadian and Ultradian Factors (Accession Number DA OC 6457), which is designed to address the central psychophysiologic problems of modern combat stress through laboratory and field studies. The technical goals are the exploitation, refinement and application of rapidly improving technologies applicable to physiologic data acquisition and performance assessment. Laboratory studies emphasizing both innovation in instrumentation and data processing techniques are coupled with field studies in military and appropriate civilian environments. The objective is to minimize the intrusion of research into and consequent interference with military operations that are the subject of study.

Progress

For continued development of a wrist-worn Actigraph system, it was essential to first design linear analog wrist motion recording methodology based on highly sensitive and accurate transducers. Fifteen such systems were fabricated and deployed in a variety of studies in-house at the University of Arkansas, and at Ft. Hood. Their dependability was demonstrated and with refinements they will become the standard for in-house use. Through these studies we have now determined the basic orientation, frequency, band width and amplitude characteristics of human wrist movement in natural environments during wakefulness and sleep and have identified common causes of artifact and noise. These observations, plus comparisons of the analog transducer with competing ones, have permitted the estimation of the technical requirements for an adequate digital memory based system and have progressed to the release of an RFQ and first Source Selection Board review for

the contracted design of a field deployable Actigraph system.

To correct drawbacks and improve our present system for recording human core temperatures using telemetered "pill" signals, we have completed arrangements with NASA to acquire their technical refinements in miniaturization and signal emission quality. Once acquired, these units and six analog Actigraphs will be shared with our Canadian collaborators under the WRAIR/DCIEM Memorandum of Understanding for sustained operations/sleep deprivation investigations.

The revision of the Performance Assessment Battery (PAB), the major portions of the user's manual and utility programs to aid subject-training, data analysis and test parameter modification have now been completed. Copies have been requested by and supplied to individual laboratories in the Navy, Air Force, and NASA. We have sent the revised battery to Ft. Rucker for field use in project Reforger-83, and have continued collaborative support of its use in ongoing experiments at AFIP.

Findings from our sustained performance studies led to the development of a new performance index (throughput) that is simple, applicable to widely different tasks, unaffected by details of task implementation, consistent with speed-accuracy trade-off phenomena, and both more sensitive and less variable than traditional reaction time or accuracy measures. Throughput declined approximately 25% per day awake when circadian variation was removed. (See Thorne, Genser, Sing and Hegge, 1983).

The importance of determining the full impact of various combat stressors and their interactions on the performance capacity of the soldier has resulted in RFQs for a variety of hardware and software systems. The Field Deployable Performance Assessment Battery (FPAB) is a microprocessor system suitable for field deployment and will be utilized to collect cognitive and psychomotor data, subject histories, read out physiological monitoring systems, administer psychometric scales and neuropsychological instruments, run military task simulations and communicate directly with mainframe computers such as VAX. The Neuropsychological Test System (NPTS) will be used for both screening and quantitative assessment of neuropsychological function over a full range of sensory, perceptual, and cognitive abilities. An Unobtrusive Ocular Monitoring System (UOMS) has been proposed which would be employed in conjunction with PAB

(or FPAB) in an effort to map out more clearly the effects of specified stressors on both ocular and cerebral components of cognitive performance. New data analytic strategies have been proposed which would subject heart rate, interbeat interval, and motor activity to feature extraction procedures utilizing pattern recognition and signal processing techniques. Statistical properties of the extracted features will be described and software will be developed for the most promising features.

A data base composed of the Performance Assessment Battery, Mood-Activiation Scale, Lexical Decision and Vigilance Discrimination Tasks (see 1982 annual reports - Non-invasive Monitoring and Circadian and Ultradian Factors for descriptions of PAB and the tasks mentioned above) has been formed. Analysis of the data contained therein will focus on the effects of fatigue on cognitive, affective, and physiologic functions.

Future Objectives

Acquisition of the field deployable Actigraph system is expected by Summer 1984, when application to a variety of sleep loss, sustained operations and CBW discipline studies will begin. Continued use of the analog systems is expected in Toronto and Little Rock to provide data on sleep and fatigue physiology for Pattern Recognition development, both in-house and in collaboration with George Washington University.

Future plans for PAB are to complete the remaining portions of the user's manual, to develop a resident analysis program for the Mood-Activation Scale, to begin compiling normative data on the existing tasks, and to begin developing tests of critical skills not assessed by the present battery.

The contract efforts mentioned above are currently in the acquisition process.

Presentations and Publications

1. Thorne, D., Genser, S., Sing, H., and Hegge, F. Plumbing human performance limits during 72 hours of high task load. Proceedings of the 24th Defense Research Group Seminar; 1983 May 2-4; Defense and Civil Institute of Environmental Medicine, Downsview, Ontario; Vol 1.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OB 6448	83 10 01	DD-DR&E(AR)636

TE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORGN HISTRY	9A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

3./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
MMARY	61102A	3M161102BS10	AG	217 WWMA
NTRIBUTING				
XXXXXXX	STOG	[illegible]-1.2/3		

TLE (Precede with Security Classification Code)

U) Basic Studies in Infectious Diseases

IENTIFIC AND TECHNOLOGICAL AREAS

002100 Organic Chemistry, 002600 Biology, 012600 Pharmacology

ART DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
07	Cont	DA	C. In-House

NTRACT/GRANT

TES/EFFECTIVE: EXPIRATION:

MBER:

PE: 4. AMOUNT:

ID OF AWARD: f. CUM. AMT.

17. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	7.0	289
	CURRENT 84	3.0	492

SPONSORING DOD ORGANIZATION

Walter Reed Army Institute of Research

ESS: Washington, DC 20307

20. PERFORMING ORGANIZATION

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SPONSORING INDIVIDUAL

TOP, F H JR

PHONE: (202) 576-3551

PRINCIPAL INVESTIGATOR (Provide SEAN if U.S. Academic institution)

NAME: CANFIELD, C J

TELEPHONE: (301) 427-5411

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

GENERAL USE

eign Intelligence Considered

POC: DA

KEYWORDS (Precede EACH with Security Classification Code) (U)Malaria;(U)Schistosomiasis;(U)Leishmaniasis;(U)
panosomiasis;(U)Parasitology;(U)Medicinal Chemistry;(U)Pharmacology;(U)Toxicology

TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief individual paragraphs identified by number. Precede text of each with Security Classification Code.)

(U)To investigate basic chemical, biological, and pharmacological aspects in drug de-
opment for use against parasitic diseases of military importance.
(U)Chemicals are synthesized, characterized, and analyzed for study, culture systems
animals models of parasitic diseases are developed and used, drug delivery systems
animal models are developed, all to study the efficacy and toxicology of potential
gs. New diagnostic methods are investigated. 25. (U) 82 10 -
09 The effects of mefloquine.HCl on atrial and ventricular automaticity were evaluated
pentobarbial-anesthetized dogs with complete AV block. Mefloquine produced little
nge in atrial and ventricular automaticity alone or in combination with primaquine, but
entiated the depression in atrial automaticity produced by the beta adrenolytic agent,
pranolol. These data suggest that mefloquine and primaquien can be coadministered with
adverse effects while concomitant mefloquine therapy with propranolol requires care-
patient monitoring and possibly dose reduction. In the canine model, several chemical
physical characteristics of bile were identified which will ultimately serve as refe-
nce indices for this model's use in establishment of the enterohepatic circulation
racteristics of candidate antiparasitic drugs. Preliminary results have shown a small
consistent inverse relationship between sodium taurocholate induced choleresis and
cosity. A surgical procedure for long-term implantation of a catheter into the hepatic
tal vein has been devised which ensures this vessel's patency and provides the oppor-
ity for studying the liver's handling of small quantities of test drug. Over twenty
no acids were screened for their in vitro utilization requirements by P. falciparum.
tures with oleic acid, cis vaccenic acid and linoleic acid sustained best growth for
r one month and were studied in various concentrations, both singly and combined.
g sensitivity assay studies were performed for hospitalized and clinical phase II drug
elopment patients. For technical report see WRAIR Annual Progress Report, 1Oct82-30Sep
83

to contractors upon originator's approval

FORM 1498 1 MAR 88 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3M161102BS10 Research Hazards on Military Disease, Injury and Health Hazards

WORK UNIT 217 Basic Studies in Infectious Diseases

INVESTIGATORS:

Principal: MAJ D. Korte
Dr. H. Lowensohn
Mrs. G.P. Willet

Associate: SP6 N. Wright

PROBLEM AND OBJECTIVES:

The development of effective antiparasitic and infectious disease agents is dependent upon the simultaneous development of pharmacologic, toxicologic and chemical analysis technology and model systems for evaluating efficacy and host responses. The objective of this work unit is to design and implement such methodologies for the evaluation of identified candidate compounds. Specifically, studies were designed to evaluate the interactions of drugs known to possess cardiac antiarrhythmic effects, enterohepatic effects and *in vitro* fatty acid requirements for malaria cultivation.

PROGRESS:

The effects of the candidate antimalarial, mefloquine.HCl, on atrial and ventricular automaticity were evaluated in pentobarbial-anesthetized dogs with complete AV block. Mefloquine produced little change in atrial and ventricular automaticity alone or in combination with the antimalarial, primaquine, but potentiated the depression in atrial automaticity produced by the beta adrenolytic agent, propranolol. These data suggest that mefloquine and primaquine can be coadministered without adverse effects while concommitant mefloquine therapy with propranolol requires careful patient monitoring and possibly dose reduction.

Using the canine model previously developed in this laboratory, several chemical and physical characteristics of bile were identified which will ultimately serve as reference indices for this model's use in establishment of the enterohepatic circulation characteristics of candidate antiparasitic drugs.

Contrary to published information, no correlation was found between bile flow rate and varying strengths of the choleretic agent, sodium taurocholate. Intravenous administration produced a uniform bile flow for 8 hours in the anesthetized dog. Bile flows varied from dog-to-dog, but ranged from 2 ml/hour to 12 ml/hour. Bile osmolarity consistently amounted to slightly less than that observed for plasma (92 ± .05% SD) and, in 3 dogs, bile pH remained relatively stable. Preliminary results have shown a small but consistent inverse relationship between sodium taurocholate induced choleresis and viscosity. Having attained a stable model, quinine was infused into one dog; quantitative recovery techniques from blood plasma appear adequate, yet difficulty was experienced with quantitative separations of quinine from bile. Bile interfering substances have caused analytical problems but these difficulties were overcome by separating interference from quinine with a column clean-up procedure. A surgical procedure for long-term implantation of a catheter into the hepatic portal vein has been devised which ensures this vessel's patency and provides the opportunity for studying the liver's handling of small quantities of test drug.

Continuous in vitro cultivation of the malaria parasite P. falciparum was performed in a plasma-free medium supplemented with fatty-acids and fatty-acid free albumin. Over twenty fatty acids were screened at different concentrations. Cultures with oleic acid, cis vaccenic acid and linoleic acid sustained best growth for a period of over a month and were maintained at various concentrations, single and combined. This work was completed and is in press (see publications). This laboratory was also responsible for performing the malaria in vitro drug sensitivity assay studies for patients of the clinical phase II drug development program and patients from WRAMC. Several such studies were conducted.

FUTURE OBJECTIVES

Studies will be initiated to establish tolerable i.v. doses of atropine sulfate with respect to hemodynamic and myocardial metabolic responses, temporal relationships between peak plasma levels of atropine and physiological testing and the time span required between atropine tests to preclude tachyphylaxis. After solving the above questons we will commence studies to ascertain the effects of intravenous atropine upon myocardial performance under the same protocol. Current bile flow studies to establish

initial hepatic kinetics of quine and WR6026 will be continued after perfecting a quantitative assay for thse test substances in bile. A chronic canine model will be utilized to study the effects of infusion of test antiparasitic drugs into the hepatic portal vein in order to evaluate the initial distribution kinetics.

Studies will be continued to identify growth requirements in long term cultivation of P. falciparum malaria using lipids, fatty acids and amino acids in pursuiit of the development of a completely defined medium.

PUBLICATIONS:

Willet, G.P. and C.J. Canfield. 1993. Plasmodium falciparum: Continuous Cultivation of Erythrocyte Stages in Plasma Free Culture Medium. Experimental Parasitology (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6449	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORD'R NUMB'R	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
A. PRIMARY	61102A	3M161102BS10	AF	218 WWG7
B. CONTRIBUTING				
C. XXXXXXX	Stog 62163A.2/3			

11. TITLE (Precede with Security Classification Code)

(U) Immunological Mechanisms in Microbial Infections.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 003400 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 08	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	2.0	381
FISCAL YEAR CURRENT 84	4.0	415

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)
NAME: Hockmeyer, W T
TELEPHONE: (202) 576-3544
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Gore, R
NAME: Williams, J POC:DA

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Immunity; (U) Antibodies; (U) Antigens; (U) Protozoa; (U) Immunoassays; (U) Animal Model; (U) Leishmania

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede start of each with Security Classification Code.)

23 (U) The objective is to find suitable antigens that can be used to develop vaccines for parasitic diseases that pose a significant threat to military operations in endemic areas.

24 (U) The approaches used for these studies involve development of an in vitro C' mediated assay to measure cidal activity of polyconal immune serum against promastigotes and amastigotes of L. donovani, to use various techniques to produce anti-sporozoite P. falciparum') mab's recognizing Pf67.

25 (U) 82 10-83 09 An in vitro C' mediated killing assay was developed which demonstrated that human immune serum (antibody) killed both promastigotes and amastigotes of L. donovani; the former by the classical and the later by the alternate pathway. Furthermore in vitro observations correlate with in vivo effects. Surface reactive Mab's against L. donovani have been developed and are being screened in the C' mediated assay. Four Mab's show significant cytopathic effect. Surface proteins recognized by these mab's are being characterized by SDS-PAGE Western Blot Techniques. It is anticipated that recombinant DNA methods will allow production of these proteins for immunogeneity and immunization trials. Twenty anti-P. falciparum sporozoite mab's were developed and characterized. SDS-PAGE Western Blot techniques were developed to demonstrate that they recognize Pf67. Recombinant DNA technology is being utilized to prepare genomic and DNA libraries. A pool of 5 mab's is being used to screen the libraries for clones containing DNA coding for Pf67. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

Reverse to continue upon originator's approval

DD FORM 1498 (1 MAR 68) PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3M161102BS10 RESEARCH ON MILITARY DISEASES, INJURY AND HEALTH HAZARDS

Work Unit: 218 Immunological Mechanisms in Microbial Infections

Investigators:

Principals: LTC Wayne T. Hockmeyer, MSC
MAJ W. Ripley Ballou, MC
CPT Jackie Williams, MSC

Associates: CPT Robert Crawford, MSC
Mr. Rufus Gore
Mr. Joseph S. Williams
Mr. William H. Hildreth
SP4 David Soos

Problem and Objectives:

Leishmaniasis vaccine development is hampered by the apparent lack of protection associated with antibody and thus the inability to use polyclonal immune sera or monoclonal antibodies to identify relevant protective antigens. Since most of this evidence was derived from studies conducted in laboratory mice, it was important to determine whether immune sera from patients cured of visceral leishmaniasis was protective either *in vitro* or *in vivo*.

Malaria remains are of the Army's most important infectious disease problems. Development of a protective vaccine against *Plasmodium falciparum* malaria is a major goal to augment traditional chemoprophylactic approaches.

Progress:

Immune sera was collected from sixty-eight Kenyan patients cured of visceral leishmaniasis. Five to seven day old cultured *L. donovani* promastigotes were washed and adjusted to 2×10^7 organisms/ml in RPMI medium. Fresh normal human serum is diluted 1/20 in RPMI to provide a source of exogenous complement

(C'). Human immune serum was heat inactivated for 30 min at 56 C^{o} and variously diluted in RPMI to provide a source of antibody (Δ I). Promastigote suspensions (0.25ml) were incubated for 30 min at 37C^{o} with 0.125 ml 1/20 C' and 0.125 ml ΔI or with medium alone. The promastigote suspensions were washed by centrifugation and resuspended in 0.3 ml RPMI. Twenty µl of the washed promastigote suspension was mixed with 20 µl of a fluorescein diacetate-ethidium bromide solution (FDA-EB) and examined by incident fluorescent microscopy. Viable organisms transport FDA intracellularly, enzymatically cleave diacetate and fluoresce green. Dead organisms cannot exclude EB and fluoresce red. The ratio of dead to alive promastigotes among 100-200 organisms is determined in triplicate samples. Cytopathic activity (CPA) is calculated as follows:

$$\frac{\% \text{ killed control} - \% \text{ killed treated}}{\% \text{ killed control}} \times 100$$

Media treated controls do not differ from ΔI 1/40 treated controls. C' (1/30) controls typically show 10% CPA. However 1/80 C' plus 1/40 Δ I results in a 40 - 60% CPA at 30 min. Dose response experiments demonstrate significant loss of CPA at Δ I dilutions above 1/400. Evidence that this activity is mediated by classical complement pathways is confirmed by loss of CPA if fresh C-2 deficient or fresh Mg-EGTA chelated serum is used as a source of complement. In similar studies, amastigotes of L. donovani are also killed by Δ I plus C' though killing is mediated by the alternate rather than classical pathways.

Surface reactive monoclonal antibodies are currently being screened in the C' mediated killing assay. Of 4 screened to date, three demonstrated significant CPA, 1 as high as 40%. The intent is to use these mab's to identify and characterize immunochemically the relevant protective surface reactive antigens, to clone the gene(s) coding for expression of these antigen(s) and finally, to produce the gene product for immunization studies.

During the past year more than 20 mab's were produced against P. falciparum sporozoites. These mab's produced high IFA titers and strong CSP reactions. Five of these mab's were analyzed by SDS-PAGE electrophoresis and Western Blot and react with Pf 67 and Pf 58, an antigen which has been demonstrated to be protective. An intensive effort is underway to clone the gene coding for PF 67 by making both genomic and cDNA libraries.

Publications:

1. Hockmeyer, Wayne T., David Walters, Rufus W. Gore, Joseph S. Williams, Anne H. Fortier, Carol A. Nacy. 1983. Intracellular Distruction of Leishmania donovani and Leishmania tropica amastigotes by Activated Macrophages: Dissociation of these microbicidal Effector Activities In vitro. J. Immunol. (submitted).

2. Hoover, David L., Melvin Berger, Carol A. Nacy, Wayne T. Hockmeyer, Monte M. Meltzer. 1983. Killing of Leishmania tropica amastigotes of normal human serum. J. Immunol (In Press).

3. Nacy, C.A., W.T. Hockmeyer, W.R. Benjamin, J. J. Farrar, S.J. James, M.S. Meltzer. 1983. Activation of macrophages for microbicidal and tumoricidal effector functions by soluble factors from EL-4, a continuous T cell line. Inf. Imm 40: 820-824.

4 Hockmeyer, W.T., B.T. Wellde, L.L. Sabwa, D.H. Smith, P.H. Rees, P.A. Kager. 1983. I. Complement Fixation Test for Kala Azar using Homologous Parasite Antigen. Ann. Trop. Med. Parasit. (In press).

5. Smith, D.H., B.T. Wellde, L.L. Sabwa, W.T. Hockmeyer. 1983. II. Complement Fixation Test for Visceral Leishmaniasis using Parasite Antigen. Results in an Endemic Area. Ann. Trop. Med. Parasital (In Press).

6. Pappas, M.G., Cannon, L.T., W.T. Hockmeyer. 1983. Evaluation of Complement Fixation Procedures for the Diagnosis of Visceral Leishmaniasis. Ann Trop Med. Parasit (In Press).

7. Jackson, Peter R., J.A. Wohlheiter, Perry Sayles, Carter L. Diggs, Wayne T. Hockmeyer. 1983. Restriction Endonuclease Analysis of Leishmania kinetoplast DNA characterizes parasites responsible for visceral and cutaneous disease. Am. Soc. Trop. Med. Hyg. (In Press).

8. Pappas, Michael G., Ruta Hajkowski, Wayne T. Hockmeyer. 1983. Dot Enzyme-Linked Immunosorbent Assay (DOI-ELISA): A Micro-Technique for the Rapid Diagnosis of Visceral Leishmaniasis. J. Immunol. Meth. (In Press).

9. Pappas, Michael G., Patrick B. McGreevy, Ruta Hajkowski, Larry D. Hendricks, Charles N. Oster, Wayne T. Hockmeyer 1983. Evaluation of Promastigote and Amastigotes Antigens In the Indirect Fluorescent Antibody Test for American Cutaneous Leishmaniasis. Am. Soc. Trop. Med. Hyg. (In Press).

10. Williams, J.L., Innis, B.T., Burkot, T.R., Hayes, D.E. and Schneider, I. 1983. Falciparum malaria: Accidental transmission to man by mosquitoes after infection with culture-derived gametocytes. Am. J. Trop. Med. Hyg. 32: 657-659.

11. Pavia, C.S., Diggs, C.L., and Williams, J.L. 1983. The use of metrizamide for isopyknic separation and enrichment of Plasmodium falciparum schizonts from continuous culture. Am. J. Trop. Med. Hyg. 32: 675-681.

12. Rener, J. Graves, P.M., Carter, R., Williams, J.L., and Burkot, T.R. 1983. Target antigens of transmission-blocking immunity on gametes of Plasmodium falciparum. J. Exp. Med. 158: 976.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OA 6464	2. DATE OF SUMMARY: 83 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 82 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. ORDER NUMBER: NL	9. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BD	220 WW1h
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procedure, and Security Classification Code): (U) Pathogenesis of Renal Disease of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 012900 Physiology, 003500 Clinical Medicine, 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
54 09	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: — EXPIRATION:
b. NUMBER:
c. TYPE: — d. AMOUNT:
e. KIND OF AWARD: — f. CUM. AMT.:

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	17.0	895
	CURRENT 84	13.0	652

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21. GENERAL USE: Foreign Intelligence Considered — POC: DA

22. KEYWORDS (Provide Each with Security Classification Code): (U) Renal Failure; (U) Renal Hemodynamics; (U) Heat Stress; (U) Shock; (U) Fluid and Solute Hemeostasis; (U) Dialysis; (U) Kidney Function

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief but self-contained paragraphs identified by number. Precede text of each with Security Classification Code.)

23.(U) To investigate methods for maintaining fluid, electrolyte and hemodynamic homeostasis in response to disease, injury and environmental stress of military significance such as shock, infectious disease, heat stress, gastrointestinal disorders and nephrotoxic drugs in order to provide rational basis for prevention and treatment of renal failure.

24.(U) Clearance methods, isolated tubule perfusion, membrane transport, intracellular microelectrodes, tissue culture, enzyme kinetics, chromatography and isotope dilution.

25.(U) Vasoactive factors have been studied in several models of acute renal failure including hemmorhagic hypotension in the dog, hemmorhagic and myoglobin infusion in the rat and gentimicin nephrotoxicity in the rat. Marked changes in bradykinin, kallikrein and catecholamine activity occur but do not correlate well with the degree of renal failure seen. Prostaglandin inhibition is associated with an increase in rate of development and severity of ARF. The latter two models provide more highly reproducible animal ARF in which to evaluate therapy. Studies in two other models, renal artery clamp and dye-induced ARF in the rat have been initiated to examine role of pre-existing electrolyte imbalance on genesis of ARF and to look for suitable enzymatic markers of ARF. Studies have been initiated in therapy of established acute renal failure by use of metabolic agents. A tubule suspension preparation has been used to demonstrate maintance of tissue ATP levels in anoxia by use of purine precursors. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

* Available for distribution upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498 1 MAR 66 FOR ARMY USE ARE OBSOLETE

Project: 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 220 Pathogenesis of Renal Disease of Military Importance

Investigators:

Principal:	LTC John P. Johnson, MC
Associates:	COL Donald E. Butkus, MC
	LTC William P. Wiesmann
	MAJ James L. Atkins
	Mr. Richard S. Fisher, Ph.D.
	LTC Cristobal G. Duarte, MC
	Mr. John A. Gagnon
	Mr. James S. McNeil

Problems and Objectives

Acute renal failure in combat casualties is the major problem addressed by this Department. Renal failure in this setting is associated with a persistant high mortality despite two decades of advances in therapy directed at controlling consequences of ARF (1). The incidence of acute renal failure has decreased in severley injured in the Vietnam conflict as compared to the Korean conflict due to more rapid triage and volume replacement on the battlefield. The impact of established renal failure on survival remained unchanged in spite of more vigorous dialysis and nutritional support measures. It is also apparent that the pattern of ARF developing in combat casualties may be changing with a greater incidence late in treatment courses due to complications of sepsis and antibiotic toxicity (2). Early insights into therapy were developed from studies of vasomotor events in the initiation phase ARF. This data suggested that establishment of a solute diuresis with osmotically active agents which reduce cell swelling due to anoxia might ameliorate the course of ARF. Indeed, early volume replacement and reduced shock duration as practiced in Vietnam, did result in a decreased frequency of ARF (2) but has not resulted in a change in our approach to established renal failure. For this reason, the Department has chosen to develop combat casuality analogous, animal models of establish ARF with which to examine therapeutic interventions. The underlying approach will be a metabolic intervention design to preserve damaged tissue and hasten renal regeneration. Based on studies

derived from RBC survival, ischemic damage to CNS, myocardium, and kidney our approach will be to augment anaerobic ATP synthesis in an attempt to retain or reclaim organ function.

Progress

Considerable evidence of vasomotor changes during the first twelve hours following the initial insult in ischemic or toxic renal failure have suggested that pretreatment or very early intervention designed to maintain solute diuresis or decrease afferent artoriolar resistance may modify the course of ARF. In particular, inhibition of the vasodiluting prostaglandin system has been shown to accentrate the course of a wide variety of experimental AFR models (see below). Recently, however, emphasis has shifted to examining the role of cell death in established renal failure and the importance of imparied cellular metabolism in the establishment of acute renal failure. While the course of ARF may be influenced in its initiation, it is likely that most AFR in combat casualties will be recognized during its maintainance phase. The department has therefore initiated studies to develop reliable markers of acute renal failure to correlate with metabolic cell damage at the earliest and latest reversible stages. This will be examined by looking for enzyme markers or products of purine degradation in ischemic (clamped) kidneys and in perfusate from isolated tubules perfused under anoxic conditions. Additionally we will continue to develop a variety of models of acute renal failure in large and small animals in which to attempt metabolic intervention at varying stages of developing or established acute renal failure.

Studies in Ischemic and Combined Toxic-Ischemic Renal Failure

In a previous study on the interaction of vasoconstrictor and vasodilator hormones released systemically and intrarenally in response to the combined insult of hemorrhagic hypotension and suprarenal aortic constriction, we proposed to maximize renal injury without causing the acute death of the animal. Partial constriction of the aorta at the level of the right renal artery, in unilaterally nephrectomized animals, would selectively reduce renal blood flow without severely impairing flow to the gut.

In nine anesthetized dogs severe renal ischemia of three hours duration was obtained by the combined insults of sublethal hemorrhage (30 ml/kg) and partial aortic constriction in acutely uninephrectomized dogs. Mild transient azotemia occured in all animals in the first two post-ischemia days. Four animals demonstrated persistant azotemia through day 7 (P_{cr} 3.1 - 20

mg%). The mean GFR and RPF values for the 9 animals, however, was not significantly reduced below control 7 days after insult.

In an effort to impose a greater renal insult the amount of blood withdrawn was increased to 35 ml/kg and 40 ml/kg in 2 animals. One animal (40 ml/kg) died the day following the experiment from a necrotic gut. The second animal (35 ml/kg) was sacrificed 7 days post-insult with GFR and RPF exceeding control values.

Utilizing the same model a 30-60 min period of hypoxia ($\bar{x}$ PO_2 = 57 mm/Hg) was imposed during the period of H.H. and SRAC in 3 dogs. By day 7 post-ischemia GFR and RPF had returned to control on above in all 3 animals. Since hypoxia did not appear to enhance the renal insult it was discontinued and the period of HH and SRAC extended to 4 hours in 3 additional animals. All three died within 24 hours of insult with bloody stools. It was apparent that constriction of the aorta, although distal to the cranial mesenteric artery, could contribute to the ischemic gut by impairing flow to the caudal mesenteric artery which primarily supplies the colon.

It was concluded that this approach, which involved rather extensive surgery, would not lead to the development of a highly reproducible model of ARF. For this reason further study of this model was discontinued, and attempts were initiated to develop a simpler model by combining hemorrhage and a toxic insult in the awake, non-nephrectomized animal. Initial studies on feasibility were carried out in the rat.

Post-traumatic renal insufficiency associated with myoglobinuria is a common complication of traumatic injuries. Because of the relevance of this syndrome to the military a study has been undertaken to reproduce myoglobinuric acute renal failure in the rat.

While it is generally acknowledged that multiple factors are involved in the development of myoglobinuria ARF, many of the experimental studies designed to develop this syndrome have simply involved the administration of the heme pigments, (primarily hemoglobin) to otherwise unstressed animals in which diet or water intake was altered. In no instance, to our knowledge, has myoglobin been infused in combination with an episode of hemorrhagic hypotension.

It is well established that prostaglandins exert strong direct vasomotor effects yet their role under conditions of stress is not well defined. Ischemia tends to cause a release of vasodilating

prostaglandins in the kidney, therefore, an increase in the renal release of prostaglandins, secondary to hemorrhage or trauma could theoretically provide protection by opposing renal vasoconstriction. Inhibition of renal prostaglandin synthesis, on the other hand, might be expected to potentiate the renal ischemic response. Therefore, the present study on acute renal failure, the renal effects of the intraarterial infusion of myoglobin and inhibition of prostaglandin synthesis during hemorrhage in the rat, was undertaken.

Water, but not food, was withheld for 48 hrs prior to the experimental procedure. Lyophylized equine myoglobin (0.5-0.7 g/kg body wgt) was administered intraarterially in a volume of 0.5 ml normal saline over a period of 15 min. In the prostaglandin - inhibited animals indomethacin (5 mg/kg body wgt) was slowly administered via the arterial cannula 30 min prior to hemorrhage and/or myoglobin administration. Hemorrhage consisted of the withdrawal of 25% of the estimated blood volume (blood vol. of rat = 65 ml/kg body wgt) over a period of twenty minutes. To prolong the period of hypovolemia and prevent a sudden increase in the excretion of myoglobin secondary to an increase in arterial blood pressure and urine flow, the shed blood was not reinfused. To further delay the excretion of the heme pigment water was withheld for 24 hrs after the insult. Control animals were handled and prepared in the same manner as the experimental animals but were not hemorrhaged nor administered indomethacin or heme pigment.

The plasma concentrations of creatinine (P_{cr}) and urea nitrogen (BUN), at the time of sacrifice (48 hrs post-insult) were used as indices of renal insufficiency. The appearance of each kidney was noted and then removed, weighed, sectioned and placed in 10% buffered formalin until processed for histologic examination.

The mean P_{cr} and BUN, on the day of sacrifice, of eleven control animals (group I) was 0.62 $\pm$ 0.17 mg% and 22.0 $\pm$ 7.3 mg%, respectively and the mean PK^+ was 4.62 $\pm$ 0.58 mEq/L. Withdrawal of 25% of the estimated blood volume in 3 rats (group II) was not significantly increased above control; P_{cr} was 0.80 $\pm$ 0.12 mg% and BUN was 17.0 $\pm$ 2.7 mg% while PK^+ was 3.87 $\pm$ 0.10 mEq/L. Administration of myoglobin (group III) in 9 rats resulted in a moderate but not significant increase in P_{cr} to 1.56 $\pm$ 1.7 mg% and BUN to 53.4 $\pm$ 54.6 mg% ($p > 0.05$); PK^+ increased, but not significantly to 4.49 $\pm$ 0.79 mEq/L. When hemorrhage preceded the administration of myoglobin in 8 animals (group IV) P_{cr} (3.06 $\pm$ 4.0 mg%: $p > 0.05$) and BUN (93.8 $\pm$ 82.4 mg%: $p < 0.025$) nearly doubled the mean values observed when myoglobin alone was administered. However, since P_{cr} was below control or only moderately increased in 5 of the 8 animals the increase was not significant. Although

the increase in BUN was significant it was only moderately elevated in 4 of the 8 animals (31-49 mg%). PK^+ also increased to 5.63 $\pm$ 1.04 mEq/L ($p > 0.05$) in this group.

In group V, in which myoglobin was administered after inhibiting prostaglandin synthesis on release, P_{cr} and BUN increased in all 13 animals to 5.70 $\pm$ 3.95 mg% ($p < 0.001$) and 149.7 $\pm$ 81 mg% ($p < 0.001$), respectively. PK^+ was 5.75 $\pm$ 1.97 mEq/L ($p > 0.05$).

The administration of myoglobin after prostaglandin inhibition and hemorrhage (4 animals) resulted in only moderate and insignificant increases in P_{cr} (2.47 $\pm$ 1.76 mg%) and BUN (85.9 $\pm$ 64 mg). PK^+ (4.17 $\pm$ 0.61 mEq/L) was also not significantly increased.

Preliminary result support the generally held notion that a funtional vasomotor disturbance within the kidney may be, in part, responsible for the induction of acute renal failure in this as in other models.

Because an acid concentrated urine appears to be important in the genesis of ARF in this model and appears to be typical of clinical syndromes in which AFR occurs, a separate pilot study has been initiated to examine the role of luminal pH and toxicity on the response to graded clamp hypoperfusion.

Studies in Non-Ischemic Models of Acute Renal Failure

The Department has completed a study of Gentimicin-Induced Nephrotoxicity (12) which provides an easily reproducible and well characterized toxic model. In addition, studies in cis-platinum nephrotoxicity (12) have demonstrated the value of sulfydryl reactive agents in ameliorating the nephrotoxicity of this commonly used anti-neoplastic agent. The agents do not seem to work primarly through chelation as urinary levels of the cis-platinum were unchanged. Tissues levels may help determine whether uptake is altered or the agents are acting directly at the membrane level. A model of dye-induced renal failure is being developed and studied in the rat in an attempt to delineate the role of pre-existing electrolyte imbalance on susceptibility to this form of toxic ARF. Studies are ongoing in varying states of volume depletion and K^+ imbalance. This may be of value in terms of defining conditions for dye studies which will minimize the occurance of this form of ARF.

Metabolic and Functional Studies of Anoxic Epithelial Cell and Tubules

In line with the Departments goal of defining and influencing cellular metabolic consequences of renal injury, a major effort has been undertaken to establish precise conditions for monitoring response of individual cells or tubules to anoxia. An improved cortical tubule suspension dervied from pathogen free rats has been developed which demonstrate sustained O_2 uptake in-vitro and has been employed in preliminary studies of anoxia. Tubules were incubated in-vitro under aerobic and anerobic conditions in control buffer solutions or solutions supplemented with adenine, adenosine or calcium ionophore, and tissue ATP levels were measured after 1 hour by high performance liquid chromatography. Under aerobic conditions neither adenine or adenosine (both precursors of anerobic ATP synthesis) had any effect on ATP elvels while the Ca^{++} ionophore markedly reduced ATP levels. This finding is consistent with observations in nor-epinephrine induced renal failure that tissue Ca^{++} levels correlate inversely with oxidative phosphorylation potential. Under anerobic conditions, control and Ca^{++} ionophore preparations had reduced ATP levels and adenosine and adenine returned these to near control aerobic ATP levels.

Considerable developmental efforts have been expanded to acquire the capabilities to measure O_2 consumption and simultaneously monitor phosphorylation potential in tubule suspensions and cultured cells. The department has also developed the capability to monitor intracellular potentials and cell volume simultaneously as a measure of functional response to anoxia. These efforts have been undertaken with a view to projected studies in FY 84.

Basic Scientific Research

Collaborative efforts of individuals within the Department with others outside Department or Division have resulted in several basic scientific observations of note. Studies have been carried out in the area of purine metabolism in malaria (14), cell volume regulation and pathways of ionic permeability (6), and basic alterations in phospholipid composition of membranes in response to hormonal and metabolic stimulation (20). These lines of research relate directly to proposed mission-related research in the kidneys response to toxic and metabolic injury.

Future Plan and Recommendations

The Department plans to undertake a series of studies of the efforts of nephrotoxins and anoxia on metabolism and cell volume

regulation in cultured cells and tubule suspensions along with a search for markers of cell injury or death in isolated cells and tubules. We will then study the ability of precursors of anerobic glycolysis and ATP synthesis to reverse or block these efforts in-vitro. Agents which show promise in-vitro will then be employed in-vivo in animal models of acute renal failure in an attempt to ameliorate or reverse ARF.

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2. Butkus, D.E.: Post-Traumatic Acute Renal Failure in Combat Casualties: A Historical Review. Military Medicine, In Press.

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7. Johnson, J.P., McCauley, C.R. and Copley, J.B.: The Quality of Life of Hemodialysis and Transport Patients. Kid. Int. 22:286-291, 1982.

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9. Moore, J. Gagnon, J, Verma, P.S., Sander, G.G. and Butkus, D.E. Plasma Kinin Levels in Acute Renovascular Hypertension in Dogs. Renal Physiology, In Press.

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14. Wiesmann, W.P, Webster, H.K. Daddona, P.: Adenosine Deaminase in the Malaria Infected Red Cell. Proc. 6th International Conference on the Red Blood Cell. In Press.

15. Wiesmann, W.P. and Hsu, H.: Hypo and Hyperurecemia. Chapter 14 in Handbook of Clinical Nephrology. H. Preuss ed. John Wiley Sons New York. In Press.

Abstracts and Presentations

16. Atkins, J.L. and Burg, M.B.: Secretion and Absorption of Bicarbonate by Rat Collecting Ducts. AFCR National Meeting 1983.

17. Fisher, R.S. and Spring, K.R.: Ionic Dependence of Epithelial Cell Volume Regulation. Federation Proceedings, 42(4):987, 1983.

18. McNeil, J.S., Jackson, B.D., Taylor, O., Allen, A.Y. and Butkus, D.E.: Amelioration of Cis-Platinum Nephrotoxicity (American Physiological Scoeity 34 Annual Fall Meeting, 169:22, 1983.

19. Wiesmann, W.P., Webster, H.K. and Daddona, P.: Culture of Human Malaria Parasite in Adenosine Deaminase Deficient Erythrocytes.

20. Wiesmann, W.P., Chiang, P.K. and Johnson, J.P.: Aldosterone Stimulated Methylations in Cultured Toad Urinary Bladder Epithelial Cells. Presented at National Meeting of American Federal Clinical Research, Clin. Res. 31:445A, 1983.

21. Johnson, J.P. and Jones. D.C.: Hormonal Regulation of Na+-K+ ATPase in Cultured Epithelial Cells. 16th American Society of Nephrology, 1983.

22. Fraser, B., Buko, A., Moore, J. and Johnson, J.P.: Similarity of Peptides from the Serum of Uremic and Volume Expanded Man. 16th American Society of Nephrology, 1983.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OC 6466	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GRADE/GRADE	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AI	222 WWP2
b. CONTRIBUTING				
c. XXXXXXXX	STOG 82/83 6.2/3			

11. TITLE (Precede with Security Classification Code)

(U) Histopathologic Manifestation of Military Diseases and Injuries

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
63 08	Continue	DA	C. In House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	1.0	295
FISCAL YEAR CURRENT 84	1.0	303

19a. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

FINA

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Gut lamina propria; (U) IgA plasma cells; (U) Peyer's patches; (U) Migration and differentiation

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief individual paragraphs identified by number, provide end of each with security classification code)

23(U) The gut lamina propria houses a variety of lymphoid cells which consist of IgA plasma cells, B cells and T cells. The IgA plasma cells produce antibodies which play the major role in humoral immunity. The functions of the B and T cells remain obscure. Previously, we studied the morphology and function of the B and T cells. This objective was undertaken to study the ontogenic relationship of the B cells to the IgA plasma cells. There is military relevance in this research.

24(U) The B cells of the gut lamina propria were passively transferred between Ig allotype congenic mice (BALB/c and CB-20). Migration and lodging of the B cells and their capability to repopulate the IgA plasma cells in the recipients gut lamina propria were studied by flourescent antibody microscopy and radioactivity counting for 125IUDR and 51 Cr labeled cells.

82 10 - 83 09

25(U) The B cells of the gut lamina propria are capable of repopulating the IgA plasma cells in the gut lamina propria. The repopulating cells are heterogeneous in differentiation stages and migration properties; some are blasts and differentiating IgA bearing cells while the others are resting B cells. The former cells migrate quickly to and lodge in the gut lamina propria. The latter are recirculating cells migrating through the gut lamina propria. The factors controlling the lodging and recirculation remain to be solved. For technical report see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 85

Project 3M161102B510 RESEARCH ON MILITARY DISEASES, INJURY, AND HEALTH HAZARDS

Work Unit 222 Histopathologic Manifestation of Military Diseases and Injuries

Investigators:
Principal: Jeenan Tseng, Ph.D.
Assistants: SP5 Mildred Lopez
SP4 Michael Williams

Description:

The humoral immunity of the intestine is mainly affected by IgA antibodies which are produced by the plasma cells in the gut lamina propria. The IgA plasma cells are derived from precursor cells in Peyer's patches which are in separated anatomical sites from gut lamina propria. A migration, from Peyer's patches to the gut lamina propria appear to be the source of these IgA precursor cells. However, the migration route and differentiation events occurring in the migration are still obscure.

Previously, we developed a lymphocyte transfer system between two inbreed mouse strains congenic in immunoglobulin (Ig) allotypes. Using the allotypes as the markers, we knew that the IgA plasma cell precursors in Peyer's patches migrate first to spleen where they stay for 5 days and then to the gut lamina propria where they differentiate into plasma cells. Recently we also characterized the lymphoid population in the gut lamina propria and found that B cell population also have potential to differentiate into IgA plasma cells. Research effort was thus concentrated using the congenic cell transfer system and techniques of isolating cells from the gut lamina propria to study the migration and repopulation of B cells from the gut lamina propria.

Progress:

I. Repopulation Potential

The repopulation potential of lymphoid cells from gut lamina propria was studied by passive transfer of gut lamina propria lymphoid cells between BALB/c and C8-20 mice (these two mouse strains are congenic in Ig allotypes). Although the lymphoid cells of the gut lamina propria are substantially less efficient than lymphoid cells of Peyer's patches (PP), they were able to repopulate the IgA plasma cells in the gut lamina propria. The repopulation began at day 1, peaked at days 13-15, and declined gradually thereafter. When large numbers of lymphoid cells were transferred, an additional peak at day 3 was seen. The early appearing IgA plasma

cells were not seen in the recipients of PP cells which were mainly small resting lymphocytes. Thus, the gut lamina propria lymphoid cells responsible for the repopulation are heterogeneous in differentiation stages and migration properties. Further studies using splenectomized mice and ^{125}IUDR and ^{51} CR-labeled cells revealed that the early (day 3) repopulating cells were mainly IgA blasts and some well differentiated IgA precursor cells and the late repopulating cells were mainly small resting, IgA precursor cells which came from Peyer's patches and remained as resting or unchanged cells after the migration.

II. Tissue Distribution

Tissue distribution of lymphoid cells of gut lamina propria after transfer was also examined. The blast cells preferentially lodged in gut lamina propria, while resting cells lodged in the spleen. Although a substantial number of blasts also lodged in the spleen shortly after transfer, they migrated to the gut lamina propria within 3-5 days. The small resting lymphocytes lodging in the spleen began to migrate to the gut lamina at days 6-7 and differentiated there into IgA plasma cells. Some IgA cells and blasts could be seen in mesenteric lymph nodes 13-15 days after transfer, suggesting that some B Cells of the gut lamina propria recirculate through the gut lamina propria and differentiate during the recirculation.

III. Conclusion

The lymphoid cells of the gut lamina propria responsible for repopulating IgA plasma cells in the gut lamina propria are heterogeneous in differentiation stages and migration properties. These heterogeneities suggest that IgA plasma cell precursors of Peyer's patches differentiate asynchronously during their migration to the gut lamina propria. Some IgA precursors may differentiate completely while others remain undifferentiated after arrival in the gut lamina propria. The former cells become resident plasma cells. The latter migrate through the gut lamina and recirculate, these cells may differentiate and lodge in the same manner as the differentiating IGA precursors seen in the first journey.

Publications:

1. Tseng, J., Repopulation of IgA plasma cells in the gut lamina propria with lymphoid cells isolated from gut lamina propria (accepted. Eur. J. Immunol.).

2. Tseng, J., Migration and differentiation of Peyer's patches IgA precursor cells uring repopulation of IgA plasma cells in the gut lamina propria 5th International Congress of Immunology, Kyoto, Japan. August 21-28, 1983 (Abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6937	83 10 01	DD-DR&E(AR)636

3 DATE PREV SUM'RY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8A ORDER NUMBER	8B SPECIFIC DATA - CONTRACTOR ACCESS	9 LEVEL OF SUM
82 10 01	D. Change	U	U		NI	X YES ☐ NO	A. WORK UNIT

10 NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AI	223 WW05
b. SECONDARY				
c. ADDITIONAL				

11 TITLE (Procede with Security Classification Code)

(U) Pathologic Manifestations of Zoonotic Diseases of Military Importance

12 SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

13 START DATE	14 ESTIMATED COMPLETION DATE	15 FUNDING AGENCY	16 PERFORMANCE METHOD
74 02	Continuous	DA	C. In House

17 CONTRACT/GRANT

a. DATE/EFFECTIVE — EXPIRATION

b. NUMBER

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM AMT

18 RESOURCES ESTIMATE

	PREVIOUS/CURRENT	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	83	8.0	368
	84	8.0	354

19 RESPONSIBLE DOD ORGANIZATION

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Washington, D.C. 20307

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d. GENERAL USE
FYNA

20 PERFORMING ORGANIZATION

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21 KEYWORDS (Provide ESCM with Security Classification Code)

(U) Pathogenesis; (U) Animal model; (U) Trypanosomiasis; (U) Leishmaniasis, (U) Morphologic Pathology

22 TECHNICAL OBJECTIVE, 23 APPROACH, 24 PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23(U) To study and define the pathology and pathogenesis of experimantal trypanosomiasis and leishmaniasis and the effects of other infectious, toxic, and environmental bio-hazards in a variety of animal hosts. Initiate and provide pathologic studies needed to prevent/control diseases and conditions that impact on quality assurance of the WRAIR-reared and purchased laboratory animals. Provide diagnostic pathology for animals acquiring natural diseases and deaths during quarantine or colonization at the WRAIR. Provide clinical pathology and histopathology support to the WRAIR and other eligible government agencies. All projects are generated from approved protocols and are related to military medical problems.

24(U) Studies utilized conventional gross and histopathology, clinical pathology, histochemistry, immunohistochemistry, and electron microscopy techniques.

25(U) 8210-83-09 The sequence of events involved in the cytodynamics of repair of airway epithelial damage was resolved, resulting in a new concept in this phenomenon. The pathogenetic features of experimental Shigella entertoxin-induced diarrhea were characterized, leading to a better understanding of the basis of disease with this organism. The reliability and predictability of central nervous lesions in the C57BL/Jb mouse experimental model of trypanosoma rhodesiense infection were demonstrated. Results from a study of the tissue effects of blast injury induced by fuel air explosives suggested that the porcine species is superior to the ovine species for deriving more effective diagnostic and therapeutic modalities. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

* Continue in continuation sheet, if necessary

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NOV 85 AND 1498, 1 MAR 80 FROM ARMY JSEP ARE OBSOLETE

Project: 3M161102BS10 RESEARCH ON MILITARY DISEASES, INJURY AND HEALTH HAZARDS.

Work Unit 223 Pathologic Manifestations of Zoonotic Diseases of Military Importance.

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Description:

To diagnose, define, investigate and compare known and potential diseases common to man and animal, particularly those of military significance. To devise and evaluate means for precise diagnosis, control and/or prevention of inflammation and tissue injury induced by these diseases. To develop new animal models for the study of human diseases. A major effort has been directed toward defining the pathogenesis and fundamental mechanistic events operative at the cellular and subcellular levels during the induction of tissue injury. Studies have applied methods of microscopic pathology, histopathology, clinical pathology, ultrastructural pathology, histochemistry, and immunohistochemistry.

Progress:

Progress in original and collaborative studies is presented below:

I. Studies on Tracheal and Bronchial Injury.

A. Results obtained in conjunction with ongoing investigations of the potential effects of free field artillery blasts on operator personnel have revealed new concepts in the cytodynamics of respiratory epithelial repair. Epithelial regeneration is a basic response to injury and precise definition of this process is fundamental to our understanding of development processes, to maintenance of the adult state, and to many pathological processes including mechanical, toxicological and infectious respiratory injury. An understanding of the proliferative capabilities of epithelial cells, appreciation of the wide spectrum of phenotypic modulations and pathways of differentiation, and determination of the origins of nascent cells during the regenerative process, have widespread significance and implication in pathology.

All stages of regeneration in hamster tracheal epithelium were studied following a denuding mechanical injury. At 1 h all the cells had sloughed from the wound site leaving a bare and sometimes disrupted basal lamina. Viable cells at the wound margins rapidly changed shape, flattened and migrated to cover the denuded lesion by 12 h. In addition, epithelial cells that remained viable demonstrated sublethal changes that included the rapid discharge of mucous granules from secretory cells, internalization of cilia by ciliated cells and evidence of heterophagy in both cell types.

By 24 h a wave of epithelial cell divisions occurred, primarily by secretory cells. This produced a multilayered epidermoid metaplasia that was best developed at 48 h. The metaplastic epithelium was largely composed of cells with both secretory (mucous granules) and epidermoid (tonofilament bundles and numerous desmosomes) characteristics. The peroxidase-anti-peroxidase (PAP) method demonstrated a few keratin-positive cells in the wound as early as 12 h post-wounding and keratin was demonstrated in more cells by 24 h. All cells in the metaplastic wound epithelium were keratin-positive by 48 h.

Following 48 h some of the most superficial keratinized cells sloughed from the epithelium and the keratin content of the remaining cells began to decline. At 72 h pre-ciliated and pre-secretory cells were seen in the wound. Pre-ciliated cells were characterized by an abundant electronlucent cytoplasm, large pale nucleus, filiform apical microvilli and evidence of ciliogenesis, similar to that seen during fetal development. Pre-ciliated cells often contained apical mucous granules, apparently carried over from the parent secretory cells. With the appearance of these columnar cells the normal mucociliary morphology was restored in small wounds by 120 h, but some persistent epidermoid metaplasia remained in the large wounds through 168 h post wounding.

These data provide further evidence for the important role of secretory cells in the histogenesis of epidermoid metaplasia and the regeneration of normal morphology following injury. The knowledge gained from these studies provides a firmer basis for the understanding of the histogenesis of other lesions observed in the tracheobronchial epithelium.

B. In order to learn more about the respective roles played by basal cells and mucous cells in maintenance of tracheal mucociliary epithelium, cell kinetics and epithelial cell morphology were characterized over a 7-day period, during which dietary vitamin A was restored to previously deprived hamsters. Hamsters were reared from birth to 35 days of age on vitamin A-replete or deficient diets. Deprived hamsters were made replete by 5 mg

vitamin A-acetate orally, plus a vitamin A-replete diet. Colchicine and ^{3}HTdR were given 6 hours before death. The numbers of basal cells, mucous cells, preciliated cells and ciliated cells, and mitotic rates (MR) and labeling indices (LI) of basal cells and mucous cells, were quantified in glycol methacrylate sections stained with PAS-lead hematoxylin.

Vitamin A-deprivation inhibited replication of basal cells and mucous cells in tracheal epithelium which showed minimal morphologic change. The proportion of basal cells was increased and proportions of mucous, preciliated and ciliated cells were decreased. Following restoration of vitamin A to the diet, basal cell MR remained below control level throughout the experimental period, but mucous cell MR started to rise on day 2-replete, and on day 3-replete and thereafter mucous cell MR was within control range. Basal cell and mucous cell LI's showed similar trends. Preciliated cells were reduced or absent in vitamin A-deprived epithelium. Their number had risen by day 3-replete and thereafter they were generated within control range. These cells matured into ciliated cells. By day 4-replete, the proportion of basal cells had decreased markedly and proportions of mucous cells, and of preciliated plus ciliated cells had increased, so that at this time cellular proportionswere within or near control values. This trend continued so that by day 7-replete, nearly normal mucociliary epithelium was restored. The results show that vitamin A-levels modulate replication rates of basal cells and mucous cells and indicate that mitotic division of mucous cells is prerequisite for genesis of preciliated cells and new mucous cells and for restoration of the mucociliary epithelium following deprivation of vitamin A in the diet. The effects of vitamin A-deprivation on the tracheal epithelium were studied in 35-day old hamsters that had been raised since birth on a vitamin A-deficient diet. Colchicine and ^{3}HTdR were given 6 hours before death and the proliferative activities of basal cells and mucous cells were quantitied separately by ^{3}HTdR labeling indices and mitotic rates.

Vitamin A-deprivation inhibited replication of basal cells and mucous cells in tracheal epithelium which showed minimal morphologic change. The mitotic rates and labeling indices were reduced 3 to 4-fold in basal cells and 14-fold in mucous cells (analyzed as percent of total number of each cell type) compared with controls. Thus, replication of mucous cells was more inhibited by lack of vitamin A, than replication of basal cells. The disparate hypoplasia of basal cells and mucous cells in epithelium showing minimal change, resulted in a relative increase in the proportion of basal cells and a relative decrease in the proportion of mucous cells, which could be erroneously interpreted as "basal cell hyperplasia".

Proportions of preciliated and ciliated cells were also decreased compared to controls. At foci of stratification and epidermoid metaplasia, cell replication rates were increased over controls and more than 70% of all mitotic activity was associated with "non-basal" cells. Genesis of these lesions was coincident with cell death and cell loss. The histogenesis of stratification and epidermoid metaplasia was characterized. Morphological evidence indicated that these lesions were closely related histogenetically and were composed, for the most part, of altered mucous cells which expressed dual phenotypes i.e. keratinization and mucous synthesis.

II. Studies on Shigella dysenteriae I enterotoxin on the morphology of the rabbit ileum.

Collaborative study with the Dept. Bacterial Diseases, Division of Communicable Disease and Immunology. The effects of crude and purified Shigella dysenteriae I enterotoxin at different doses on the morphology of the intestinal mucosa in the rabbit ileal loop models are being studied. Light microscopy and histochemistry studies are ongoing and transmission and scanning electron microscopy studies have been initiated. Present findings indicate a dose dependent response of the ileal mucosa to both crude and purified enterotoxin preparations. Initial results suggest that direct cytotoxic damage to the absorptive epithelial cells covering the intestinal villus is the first event in the pathogenesis of the enterotoxins action. This results in sloughing of the absorptive epithelium, areas of microulceration, reduction of the villous length (villous atrophy) and thus a reduction of the absorptive surface area. In contrast to the villous epithelium, the undifferentiated crypt epithelium becomes extremely hyperplastic and this cell population rapidly expands to repair and rebuild the atrophic villi. The initial lesions of villous atrophy and crypt hyperplasia suggest a functional deficit leading to malabsorption. However, since the crypt enterocytes have secretory as well as proliferative capabilities, it appears likely that the overall mechanism of diarrhea and fluid production is the result of a net secretion from the hyperplastic crypts overcoming the absorptive ability of the atrophic villi. Ultrastructural studies confirm the above observations. The villus epithelium is more rapidly lost from the villus and contain sublethal changes as early as two hours post-exposure to the enterotoxin. The main subcellular change observed is autophagocytosis with digestion of cell organelles within cytoplasmic vacuoles. This suggests an ultrastructural organelle or organelles as the target of the enterotoxin. Studies are underway to identify the target(s) and describe the ultrastructural sequence of all damage.

III. Studies on the Effects of Shigella dysenteriae I-like enterotoxin produced by Escherichia coli.

Collaborative study with Dept. Bacterial Diseases, Division of Communicable Disease and Immunology. Work has been initiated to study the effects of a shiga-like toxin isolated and purified from an enteropathogenic Escherichia coli on the small intestinial epithelium in the rabbit ileal loop model.

Light and Electron microscopy studies ar ongoing and initial observations indicate that the morphological effects of this enterotoxin are identical to those of Shigella dysenteriae I enterotoxin. Direct cytotoxic damage to the absorptive villus epithelium with accelerated cell loss and subcellular changes including autophagocytosis have been observed; along with crypt epithelial hyperplasia. These observations suggest the toxin acts by a mechanism similar to the Shiga toxin.

IV. Pathology in C57BL/Jb Mice Infected with Trypanosoma rhodesiense.

Collaborative study with the Department of Immunology, WRAIR. There is a need for a practical laboratory animal model for the study of chronic trypanosomiasis. The infection of Trypanosoma rhodesiense in laboratory rats and mice generally leads to an acute fatal disease without the development of the chronic disease or cerebral lesions so much a feature of the disease in man. In recent years there have been some reports of chronic trpanosomiasis with cerebral lesions in mice infected with T. equiperdum and T. brucei. However, a rodent model for the study of chronic trypanosomiasis caused by T. rhodesiense is still lacking. Initial studies in our laboratories indicated that a chronic infection of trypanosomiasis with significant cerebral lesions could be produced in C57BL/Jb mice infected with a human strain of T. rhodesiense. These findings led to a more detailed study. One hundred and nineteen (119) mice were inoculated intraperitoneally (IP) with 10^3 T. rhodesiense strain ZVH 18A9. The mice were sequentially killed every two weeks PI with the last surviving infected mice killed at 147 days PI. The infected mice became anemic, hypoglycemic, hypergammaglobulinemic (with IgM greatly increased) and hypoalbuminemic. Immunofluorescence studies of the kidneys suggested an immune complex glomerulonephritis. Lesions histologically similar to those described in chronic trpanosomiasis in man were found in the brain, spleen, lymph nodes, liver, heart, kidney, pancreas, and epididymis. Data on parasitology, immunology and pathology are being compiled. An additional forty (40) mice have been studied by light and electron microscopy to determine additional details of the morphology of the changes in the kidneys and brain. Changes in the kidney, particularly the glomerulus do not persist and evidence of chronic

immune complex glomerulonephritis were not observed in chronically infected mice. However, progressive changes were observed in the brains of C57BL/Jb mice chronically infected with T. rhodesiense. The changes are most evident in the choroid plexus and periventricular cerebral cortex. Extensive inflammatory cell infiltrates were seen in the brains of all chronically infected mice. The ultrastructural details of these changes are presently being documented.

The morphological, hematological, serological and immunological results plus the duration of the infection indicates that the C57BL/Jb mouse infected with this strain of T. rhodesiense makes an excellent model for the study of chronic cerebral trypanosomiasis.

V. The Use of the German Shepherd Dog as an Experimental Model for Visceral Leishmaniasis.

Collaborative study with the Department of Parasitic Diseases, WRAIR. Visceral leishmaniasis of man and dogs is a disease that is widely distributed geographically. It is endemic in many areas and extensive epidemics can occur with mortality reaching 98% in untreated cases. There is an increasing awareness of the risk of exposure to infection in military units operating in many parts of the world. Treatment with the currently available drugs is prolonged and by no means entirely safe or uniformly successful. While the hamster model has been used successfully for screening of new antileishmanial compounds, additional nonrodent models should be developed. Experimental infection in the dog (Beagles and Mongrels) has either been equivocal or incompletely evaluated. It was the objective of this preliminary study to determine if the German Shepherd dog might prove to be an animal model that would develop a uniform infection which when characterized clinically and pathologically would be similar to the infection in man.

In this preliminary study six young German Shepherd dogs were used as experimental animals-three were infected with 1.7×10^8/kg of Leishmania chagasi and three were infected with 2.8×10^8/kg of Leishmania donovani. All dogs became infected and remained infected throughout the study. This was substantiated by periodic cultures of bone marrow aspirates. Infected animals did not show the weight gain expected for dogs of comparable size and age. Several dogs exhibited splenomegaly and lymphadenopathy by day 41 post infection. The three dogs infected with L. donovani also developed dermatitis associated with demodectic mange. Funduscopic examinations were done periodically and were unremarkable. Evaluation of the clinical pathology data revealed a mild to moderate anemia, elevated sedimentation rate, elevated total protein, hypergammaglobulinemia,

and hypoalbuminemia. Whole blood tryptophan levels were decreased. The histopathology of the lymph nodes and spleens was characterized by follicular hyperplasia, plasmacytosis, and proliferation of macrophages in paracortical areas and medullary cords of lymph nodes and proliferation of macrophages in red and white pulp of the spleens. Clusters of parasitized macrophages were present in other organs, including liver, tonsils, bone marrow, intestine, and lung. The clinicopathologic findings are consistent with those observed in human visceral leishmaniasis. Two manuscripts have been accepted for publication.

VI. Pathologic and Clinicopathologic Evaluation of the Owl Monkey (Aotus trivirgatus) as a Model of Hepatitis A.

Collaborative study with the Department of Virology, WRAIR. Hepatitis A (HAV), hepatitis B and non-A, non-B hepatitis viruses are not readily propagated in vitro and have very limited non-human animal hosts. Laboratory animals are needed for virus production, infectivity detection and assay, studies of virus transmission, pathogenesis of disease and immune responses. In 1979 and 1980, Aotus trivirgatus at WRAIR and newly captured monkeys in Panama were found to be seropositive for HAV. These findings provide suggestive evidence that Aotus monkeys may be susceptible to infection with HAV.

The objective of this initial experimental was to confirm the susceptibility of Aotus monkeys to HAV and to record the clinical, viral, pathological, and serological response to experimental infection. Six seronegative, colony-bred monkeys were inoculated intravenously with a fecal suspension of PA33 strain hepatitis A virus, recovered previously from a naturally infected Aotus in Panama. Six to 17 days after inoculation, viral antigen was shed in the feces of all monkeys, and four to eight days later, serum aminotransferase activities became significantly elevated in each. Liver biopsies obtained 16 to 24 days after inoculation demonstrated mild to moderate histopathologic changes including portal region. Antibody to virus developed in each monkey by 28 days after inoculation.

Six additional monkeys were inoculated i.v. with a fecal suspension from an infected human (HM-175). Findings similar to those seen in monkeys infected with the PA-33 strain were observed, although the inoculation period preceding aminotransferase elevations was somewhat longer (25 to 39 days). These data confirm the susceptibility of Aotus to hepatitis A virus and indicated that infection of this primate provides a useful animal model of human hepatitis A. The details of the results of these experiments are presented in two manuscripts.

VII. Toxicity of Formycin B in Hamsters.

Collaborative study with the Department of Parasitology, WRAIR. The treatment of choice for leishmaniasis is pentavalent antimony; antimony failures are treated with pentamidine or with Amphotericin B. Lack of knowledge of the mechanism of action of antimony, and of pentamidine, has not permitted development of derivatives of these drugs as new antileishmanial agents. Rather, the antileishmanial efficacy of experimental agents such as purine analogues are being investigated. Formycin B, an inosine analogue, has proven to be an attractive antileishmanial agent. Formycin B is 100 times more active than antimony or allopurinol in vitro and a dose of 100 MKD IM for 3 days eliminates 90% of L. donovani from hamsters (G=0.46). At least 3 purine synthetic enzymes are inhibited by this drug. Formycin B is therefore an attractive experimental agent because it is active in vitro and in vivo, and its mechanisms of action have been significantly elucidated.

Four hamsters were administered Formycin B in 1 ml H_2O P.O. each day for 4 days at the ED_{90} and at 4 times the ED_{90}. Four control hamsters were administered 1 ml H_2O P.O. for 4 days. On the 4th day, the hamsters were anesthetized and exsanguinated. Hamster blood was analyzed to include a complete blood count, liver function tests (SGOT, SGPT, total bilirubin), kidney function tests (BUN, creatinine, phosphate, calcium, total protein, albumin), electrolytes (Na, K, Cl), glucose, alkaline phosphatase, CPK, and uric acid. Because of the possible effect of Formycin B on mucleic acid synthesis in rapidly dividing cells, histopathology of the hemolyphatic system (thymus, spleen, bone marrow, lymph node) and liver was performed. The broad range of laboratory and pathologic studies were done because, except for an anecdotal report of leukopenia in dogs, the side effects of Formycin B are unknown.

Mild cytoplasmic vacuolization of hepatocytes was observed in 3 of 4 control animals, in 3 low-dose Formycin B animals and in 3 high-dose Formycin B animals. Livers of the fourth low-dose Formycin B and high-dose Formycin B animals exhibited moderate vacuolization. There was no demonstrable necrosis of parenchymal cells or evidence of cholestasis. There were no lesions noted in the spleen, lymph nodes and thymus of Formycin B treated animals. The myeloid/erythroid ratio showed normal maturation. Analysis of the CBC revealed an 11% increase in hemoglobin in the Formycin B treated animals. Alkaline phosphatase levels were higher in both treated groups than in the controls. Total bilirubin levels were significantly elevated in both treated groups to a level approximately twice that of controls. The levels of glucose, BUN, albumin, globulin, total protein, cholesterol and triglycerides were significantly decreased in the high-dose Formycin B group.

In addition there was a dose related weight loss. The in vivo efficacy and mild acute toxicity of this compound in hamsters are sufficiently encouraging to suggest further evaluation of oral administration of Formycin B be performed. The results of this study have been published in the journal of Experimental Parasitology.

VIII. Tumorigenesis of N-Butyl Cyanoacrylate in Rats.

Collaborative study with the Division of Surgery. N-butyl cyanoacrylate, a tissue adhesive, was injected subcutaneously in approximately 160 rats to determine the tumorigenesis of this agent. Treatment groups included those injected with either 0.4 ml or 0.1 ml of the cyanoacrylate. Control rats were injected with either 0.4 ml or 0.1 ml normal saline. The rats were injected on 3-4 June 1982 for a 2 year chronic study. To date, 94 rats have either been killed or died. Twenty three (23) of these rats had been injected with 0.4 ml of cyanoacrylate and, of this group, six (6) had malignant fibrous histiocytomas involving the subcutaneous injection site on the neck. Thirty (30) received 0.1 ml of cyanoacrylate and, of these, five (5) had malignant fibrous histiocytomas of the subcutaneous injection site on the neck. The tumors were limited to the subcutaneous tissue in all cases. The remaining 41 rats received normal saline and did not develop tumors. Most rats died from either bacterial or mycoplasmal pneumonia. The study is ongoing.

IX Assessment of the Passage of Candidate Antitrypanosomal Drugs Through the Blood-Brain Barrier of Inbred Mouse and Rabbit Models.

Collaborative study with the Department of Experimental Therapeutics. Eight groups of mice (C57BL/C) consisting of five mice per group were inoculated with 1×10^5 trypanosomes. Six groups received antitrypanosomal drugs and two groups received water or propylene glycol. The treatments were as follows:

GROUP(S)	TREATMENT
1 and 2	Suramin
3 and 4	Pentamidine
5 and 6	Melarsoprol
7	Water
8	Propylene Glycol

Treatment groups were evaluated by quantifying inflammatory infiltrates within the brain. Suramin was effective as an antitrypanosomal drug as evidenced by the lack of significant histological lesions noted in the brains of mice receiving this treatment. Pentamidine was more protective than melarsoprol, but

less protective than suramin based on the number of brains containing inflammatory cellular infiltrates. The mice treated with water or propylene glycol were not protected. This project was completed to the satisfaction of the principal investigator.

X. Acute Renal Failure: The renal effects of the intravenous administration of myoglobin and theinhibition of prostaglandin synthesis during hemorrhagic hypotension in the rat.

Collaborative study with the Department of Nephrology, WRAIR. Post traumatic renal insufficiency associated with myoglobinuria is a common complication of traumatic injuries. Such injuries expected in the military might include gunshot or shrapnel wounds, crushing injuries, exertional rhabdomyolysis, hyperthermia and misuse of tourniquets. Although many factors have been proposed as the primary factor responsible for renal injury, renal ischemia, heme pigments (myoglobin, hemoglobin), acid urine, and prostaglandin inhibition may contribute to renal failure.

The objective of this experiment is to develop a reproducible myoglobinuric model of acute renal failure in moderately dehydrated, aciduric, and prostaglandin-inhibited rats by the intravenous infusion of purified equine myglobin during a period of hemorrhagic hypotension. The test animals consist of 300-350 gram (body weight), male, Sprague-Dawley rats which are divided in 7 groups, one of which represents a control group. The other groups consist of a hemorrhage group, hemorrhage plus hyoglobin, hemorrhage plus prostaglandin inhibitor (PGI), hemorrhage plus myoglobin and PGI, myoglobin alone and PGI alone. This study is currently in progress and sections prepared from each kidney will be examined by light microscopy.

XI. Care of Blast-Injured Casualties.

Collaborative study with the Division of Surgery. Compilation of data from a pilot study performed in late FY 82 exploring porcine-ovine interspecies pathomorphologic blast response parameters has been completed. Results suggest a greater operational suitability for the porcine as a model of blast-induced gastrointestinal injury to select and refine both diagnostic and therapeutic modolities. The successive program phase, developing appropriate methodology to monitor serum biochemical parameters, followed by field testing after blast exposure, is in final developmental stages. A film, summarizing the potential threat of primary blast injury and medical research efforts up to the present, has been produced in collaboration with the Division of Medical Audiovisual Service.

XII. Assessment of Local Tolerance to Parenteral Administration of Various Salts of the Candidate Antitrypanosomal Compound (WR-163, 577) in Calves.

Collaborative study with the Department of Parasitology, WRAIR. WR-163, 577 is a bisquinalone with antitrypansomal activity which has been shown to protect mice against Trypanosoma rhodesiense challenge for at least 10 months after one subcutaneous injection. In clinical trials in man, local reactions developed at the sites of injection of the dihydrochloride salt of the compound and the trials were stopped. In mouse studies, of numerous salts of the compound assessed for local and systemic toxicity, the dihydrochloride, acetate, and nitrate salts were found to be the least toxic. In this study designed by the Department of Parasitology, the dihydrochloride, acetate, and nitrate salts were injected subcutaneously and intramuscularly in calves at a dosage of 25 and 50 mg/kg body weight to assess the local and possibly systemic toxicity. Injection sites were observed daily for approximately 30 days post injection. The calves were then killed and the injection sites were dissected, examined, and measured. In subcutaneous sites the salt residues were indistinguishable from each other and were easily recognized as flattened, irregular islands and cords of bright yellow, dry, crumbly, and flaky material.

The areas involved were approximately 10-20 cm in diameter and involved the subcutaneous fascial planes. There appeared to be minimal absorption of the material and the quantity found generally corresponded to the dosage injected. There was minimal connective tissue response. Moderate edema surrounded the nitrate salt residue.

Intramuscular injection sites contained identical material in fascial planes dissecting between muscle bundles. Little tissue reaction was noted. Because of the apparently minimal absorption of the compound injected intramuscularly, there will be significant loss of muscle tissue from condemnation at slaughter. This route of administration, therefore, appears less desirable then subcutaneous injection.

Additional calves were injected subcutaneously with 100 mg/kg body weight of each salt, observed for 30 days, and killed. A large sterile abscess containing approximately 400 ml of serosanguinous fluid was found around the nitrate salt. The acetate and dihydrochloride salts were surrounded by smaller abscesses with approximately 40 ml of fluid each of which contained Staphylococcus aureus.

The histologic appearance of all injection sites at all dose levels were essentially the same. Granulomas, granulomatous

inflammation, necrosis, fibrosis and aggregates of lymphoid tissue were present to varying degrees. The only possibly significant difference in the reactions appears to be with the subcutaneous method of injection. In this instance the compound is not as well contained by the host response as in the intramuscular injection sites. In reference to severity the reactions ranged from mild to severe depending more on the variability of the sections at any one injection site than on any differences between the compounds, dosages or location of injection.

Major microscopic findings in other organs included necrotizing hepatitis, the cause of which could not be readily determined. Additional studies would be required to determine whether necrotizing hepatitis was related to the administration of the experimental compounds.

XIII. Study of Gastric Emptying by use of 99mTc-Tagged Chicken Liver as a Marker of Solid Food in Patients with Refl., Esophagitis.

Collaborative study with the Gastroenterolcgy Service, WRAMC. This study is designed to identify patients with distinct patterns of gastroesophageal reflux (GER) who have delayed gastric emptying of solid food contributing to their symptomatology.

Patients with GER are carefully categorized with GER before the gastric emptying study by routine tests for GER, including 24 hour pH monitoring. Previous data suggests that four different groups of patients can be distinguished within the reflux population on the basis of dyspepsia, pyrosis, and endoscoptic esophagitis. Only 2/33 patients studied to date have not been clearly classified into one of four groups by this combination of studies.

The gastric emptying study employs 99mTc-tagged chicken livers which are incorporated into a meal and used as a marker to quantitate solid food emptying by the stomach. Tagged chicken livers are prepared by injecting 0.5 mcu of the 99mTc sulfur-colloid into the wing vein of a live chicken. After 30 minutes, the chicken is sacrificed by cervical dislocation. The liver and chicken carcass are thoroughly examined by a staff veterinary pathologist for evidence of disease. The liver is removed, washed, and cut into small pieces (about 1 cm) which are then mixed with a commercially available preparation of beef stew (7½ oz. Dinty Moore).

After an overnight fast, patients and normal volunteers ingest the meal along with 150cc of water. The patient is then placed under the counter and the stomach imaged. Counts are taken every 15 minutes until T 1/2 for gastric emptying is

reached. This is determined by computer analysis of the area of interest, and the 99mTc half-life. This study is ongoing.

XIV. Experimental Visceral Leishmaniasis in Non Human Primates: Pathologic and Serologic Characterization of Aotus trivarigatus infected with L. donovani Promastigotes.

Collaborative study with the Department of Immunology, WPAIR. The development of a suitable vaccine for the prevention of visceral leishmaniasis (kala Azar) would be greatly aided by the availability of a primate model of the disease. A model of visceral leishmaniasis using the Owl Monkey (Aotus trivarigatus) has been recently developed. Intravenous infection with L. donovani amastigotes (32.5×10^6 organisms/kg) resulted in severe clinical illness with heavy parasite burdens in the liver, spleen, and bone marrow at 8-10 weeks.

This protocol utilizes this model to determine whether intravenous infection with the 25 strain L. donovani (sudan strain) promastigotes results in a similar illness. The promastigote form of the parasite is required for infection in a model vaccine system because: 1. This is the stage which is introduced into the host by infected sandflies and is the stage against which one would ideally direct an immune response. 2. Viable promastigotes can be more precisely quantitated than amastigotes thus enhancing the reproducibility of the infectious inoculum. 3. More importantly, BALB/c mice immunized with irradiated L. tropica promastigotes, but not amastigotes, have responded with specific anti-leishmanial antibody isotypes (IgG1, 2a, 3) which confered protection from visceral disease following a subsequent intravenous promastigote challenge. These data suggest that relevant protective antigens are recognized on the surface of promastigotes and can be used to immunize and protect mice. Confirmatory studies using an L. donovani murine immunization model are underway in our laboratory. This work must then be repeated in non human primates.

The study is being performed in two phases. During phase 1, the preinfection period, the animals are studied to determine baseline values for the parameters to be observed, and to exclude intercurrent illness which may affect the outcome of the study. During phase 2, the infection period, the animals are followed prospectively, with all procedures and studies being performed on both study and control animals. The study is currently in phase 1. Hematologic and serologic data are being obtained and evaluated prior to proceeding to phase 2.

XV. Treatment of L. tropica Infected C57Bl/6 Mice with Liposomes-Encapsulated Lymphokines.

Collaborative study with the Department of Immunology, WRAIR. This laboratory has previously shown that lymphokines enhance the killing and/or decrease intracellular replication of L. tropica amastigotes within macrophages in vitro. The objective of this study is to develop an in vivo mouse model to evaluate the efficacy of lymphokines encapsulated in liposomes for the treatment of Leishmania tropica infections. With this goal in mind this study is being conducted to determine the optimal concentration of L. tropica amastigotes to inject into C57Bl/6 mice for the development of systemic disease and metastatic cutaneous lesions.

Forty-five C57Bl/6 male mice, 6-8 wk old, are divided into three groups of 15 mice. Each group will be injected, i.v., with either 10^5, 10^6, or 10^7 amastigotes of L. tropica. At 1, 3, 6, 9 and 12-16 weeks post-injection three (3) mice from each group are sacrified and tissues collected for further studies. Throughout the experiment each animal is necropsied by personnel in the Department of Comparative Pathology, WRAIR, to determine gross pathological lesions, with emphasis on the identification of matastatic lesions. Only gross pathological lesions are examined microscopically. Microscopic evaluation of tissues from selected animals showed a granulomatous inflammatory reaction in a variety of organs. This is an ongoing study.

XVI. Neurovirulence Testing of Argentinian Hemorrhagic Fever (Junin) Vaccine.

The Division of Pathology served as the lead organization in collaboration with investigators from the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in the determination of neurovirulence of a candidate vaccine against Junin. The following materials were inoculated bilaterally into the thalami (0.5 ml each side) and the lumbar enlargement of the spinal cord (0.2 ml total) of 45 young rhesus monkeys: control culture fluids (n=5); secondary vaccine seed (n=10); clarified bulk vaccine (n=10); virulent Ledesma strain (n=5); XJ clone 3 (n=5); and strain XJ 13 (n=10). The Ledesma virulent wild strain was clearly the most neurovirulent of the strains tested, in terms of proportion (incidence) of tissues affected and in severity of lesions, suggesting a widespread viral infection of central nervous system (CNS) tissues. In descending order, neurovirulence of the other viral strains was as follows: XJ clone 3 > strain XJ 13 > secondary seed > clarified bulk vaccine. Both secondary seed and clarified bulk vaccine resulted in low levels of incidence of neurovirulence lesions in the CNS, the majority of which were minimal in severity. Based on these results, it was concluded that the attenuation of Junin virus had produced a vaccine virus strain that was significantly less neurovirulent than the wild strain as indicated by the limited

evidence of neurovirulence under the vigorous conditions of this test. Therefore, these neurovirulence test results were incorporated into an Investigational New Drug proposal which was forwarded to the Bureau of Biologics, Food and Drug Administration as part of the approval process for use of Junin vaccine in humans.

XVII. Leishmaniasis Tissue Response Studies.

Collaborative study with the Department of Immunology, WRAIR. Studies were conducted in order to characterize the tissue response(s) in various tissues from different strains of mice following inoculation with Leishmania. The prototypical response to Leishmania was pyogranulomations regardless of tissue (liver, spleen, footpad) or strain of mouse inoculated. The number of organisms indentifiable in the inflammatory lesions varied, usually in direct proportion to the severity of the inflammatory response. These results served as the basis for selection of strains of mice for more definitive pathogenetic and immunological studies of leishmaniasis.

XVIII. Autotransfusion Studies in Dogs.

In collaborative study with the Division of Surgery, the efficacy and safety of a simple, field transportable autotransfusion device have been tested in dogs. Following induction of hemothorax by transection of the internal mammary artery, a chest tube was used to collect blood into a blood bag containing anticoagulant. This blood was then transfused back into the dog from which it had been collected and the animals evaluated for evidence of harmful effects. A principal concern had been to determine whether clotting occurred in the collected blood, resulting in microthrombosis following autotransfusion. There was no histopathological evidence of microthrombosis in any of the tissues from dogs which were autotransfused.

XIX. Effects of Ketamine Hydrochloride on the Hemogram, Serum Enzyme Activity and other Biological Parameters in Cynomolgus Monkeys (Macaca fascicularis).

Collaborative study with the Department of Animal Resources, Division of Veterinary Medicine, WRAIR. Ketamine hydrochloride is a dissociative anesthetic routinely used to immobilize non human primates. Properties of ketamine hydrochloride include rapid induction of anesthetic effects, analgesia and short duration of action. Given the extensive use of ketamine hydrochloride in non human primates, it is important to determine the effects, if any, on various biological parameters including hemogram and various serum enzyme activities. This pilot project was undertaken in an attempt to define these effects, if any, in the adult cynomolgus monkey (Macaca fascicularis).

Eight adult cynomolgus monkeys were bred using physical restraint after having not been under the influence of ketamine anesthesia for at least 6-8 weeks. Routine complete blood counts, extensive serum enzyme and electrolyte analysis and isoenzyme analysis of CPK and LDH by electrophoresis were performed on all animals. One week later the same animals were bled at 5 and 15 minutes after the intramuscular injection of 50 mg ketamine hydrochloride. All previous blood and serum parameters were repeated. One week after the IM method, the same monkeys were given ketamine hydrochloride (.5 mg/kg body weight) intravenously and were again bled at 5 and 15 minutes post-injection with all blood and serum parameters measured. A subsequent experiment was conducted using seven of the eight monkeys to determine if physical restraint in itself had any effect on the various parameters. While being physically restrained, each animal was bled at 0, 5 and 15 minutes. Again, samples were analyzed as before.

Numerous significant alteration were detected in the hemogram, serum enzyme determinations, serum electrolytes and isoenzyme analysis. Ketamine given intramuscularly caused a significant decrease in red blood cell count, hematocrit and hemoglobin concentration whereas ketamine given intravenously did not. Total leukocyte count decreased with both modes of ketamine administration. Ketamine also increased creatine phosphokinase, lactate dehydrogenase, sodium and total bilirubin while decreases were detected in alkaline phosphatase, aspartate aminotransferase, and total protein. Additionally, CPK isoenzyme BB was found in serum of normal adult cynomolgus monkeys, a finding not previously reported. The significance of these findings can have far-reaching implications regarding interpretation of data from experiments where ketamine hydrochloride is commonly used for handling this laboratory animal.

The results of this pilot project have given rise to a formal protocol currently being drafted whereby a modified crisscross pattern of monkey groups will be utilized in an effort to verify our previous findings.

XX. Quality Assurance Pathology Evaluation for Extramural Contracts.

The Division of Pathology continued to serve as a source of pathology expertise for the Institute. Most notably, quality assurance pathology services were provided to the Division of Experimental Therapeutics in support of the toxicology testing of promising antiprotozoan drugs. With only minor exceptions the quality of pathology provided under extramural contracts by commercial laboratories was determined to be excellent. The toxic effects of compound WR 228258 were found to be reversible

in 17 week long studies conducted under one of these extramural contracts.

XXI. Clinical Pathology Laboratory Support, Histopathology Laboratory Support and Necropsy Support.

The clinical pathology laboratory handled approximately 6,897 requests for hematology and 36,735 determinations for serum biochemistry during the reporting period. The histopathology laboratory processed approximately 8,197 paraffin blocks and 13,067 microslides. A new process of plastic embedding was also initiated during this reporting period giving rise to 277 blocks and 565 microslides. A total of 1,395 necropsies were performed by the necropsy laboratory. These three laboratories support research protocols at WRAIR and its overseas laboratories and other government agencies as well as providing diagnostic support for the Institute's laboratory animal facilities.

A storage area was established in Room B097, Bldg. 40, WRAIR, in compliance with the provisions of the Good Laboratory Practices Act. This area serves as a secure repository for pathology materials including wet tissues, paraffin blocks, tissue slides, protocols, pathology reports and final reports. Access to this area is controlled and accountability of material stored is emphasized.

Publications:

1. Keenan, K.P., Wilson, T.S., McDowell, E.M. Regeneration of Hamster Tracheal Epithelium after Mechanical Injury: Histochemical, Immunocytochemical and Ultrastructural Studies. Virchows Arch (Cell Pathol) 1983, 43: 213-240.

2. McDowell, E.M., Keenan, K.P., Huang, M. Effects of Vitamin A-Deprivation on Hamster Epithelium: A Quantitative Morphologic Study. Virchows Arch (Cell Pathol) 1983, in press.

3. McDowell, E.M., Keenan, K.P., Huang, M. Restoration of Mucociliary Tracheal Epithelium Following Deprivation of Vitamin A: A Quantitative Morphologic Study. Virchows Arch (Cell Pathol) 1983, in press.

4. Keenan, C.M., Hendricks, L.D., Lightner, L., Webster, H.K., and Johnson, A.J.: Visceral Leishmaniasis in the German Shepherd Dog. I. Infection, Clinical Disease, and Clinical Pathology. Veterinary Pathology. 1984, in press.

5. Keenan, C.M., Hendricks, L.D., Lightner, L., and Johnson, A.J.: Visceral Leishmaniasis in the German Shepherd Dog. II. Pathology. Veterinary Pathology. 1984, in press.

6. LeDuc, J.W., Lemon, S.M., Keenan, C.M., Graham, R.R., Marchwiccki, R.H., and Binn, L.N.: Experimental Infection of the New World Owl Monkey (Aotus trivirgatus) with Hepatitis A Virus. Infection and Immunity, 40(2): 766-772, 1983.

7. Keenan, C.M., Lemon, S.M. LeDuc, J.W., McNamee, G.A., and Binn, L.N.: Pathology of Hepatitis A in the Owl Monkey (Aotus trivirgatus). American Journal of Pathology. 1983, in press.

8. Berman, J.D., Keenan, C.M., Lamb, S., Hanson, W.L., Waits, V.B.: Leishmania donovani Infected Hamsters: Efficacy and Toxicity of Formycin B After Oral Administration. 1983, in press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 06 6751	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORDER INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CG	224 WWP4
b. CONTRIBUTING				
c. XXXXXXXX				

11. TITLE (Include Title Security Classification Code)

(U) Functional and Structural Bases of Blast-Related Tissue Injuries

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 017100 Weapons Effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	Continuous	DA	C. In House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: b. EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	2.0	76
FISCAL YEAR CURRENT 84	2.0	78

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS
NAME: Clifford, C B
NAME: Sharpnack, D D

21. GENERAL USE

FINA

POC: DA

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Functional correlation; (U) Exposure factors; (U)Blast overpressure; (U) Vascular permeability; (U) Vascular ultrastructural; (U) Pathogenesis

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide Individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23(U) To determine the finite structural and functional bases of the pathologic changes classically associated with blast-related injury to various tissues, especially in the respiratory and gastrointestinal systems. Correlate structural and functional pathologic changes with various levels and amounts of blast exposure, emphasizing dose ranges which are near the environmental exposure associated with crew operator positions of large field artillery weapons. Study effects of repeated blast over time periods covering up to 14 days to determine cumulative effects and resolution dynamics. Map the relative sensitivities of airways and vessels in the respiratory system. Compare the tissues throughout the body. Determine the effects of blast injury on other parameters e.g., susceptibility to infectious diseases of military importance.

24(U) Conventional morphologic techniques including light and electron microscopy will be used. Other procedures will involve use of substances such as carbon particles, ferritin and horseradish peroxidase to determine vascular permeability and clearance functions. Small laboratory rodents, especially rats and guinea pigs will be the predominant laboratory animals used.

25(U) 82-10 - 83-09 Threshold levels of repeated blast were determined for airway epithelial damage in rats. Furthermore, the cellular bases of airway epithelial damage were partially defined and resolved. The relationship of airway epithelial damage and pulmonary parenchymal injury was established. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project: 3M161102BS10 RESEARCH ON MILITARY DISEASES, INJURY AND HEALTH HAZARDS

Work Unit 224 Functional and Structural Bases of Blast Related Tissue Injuries

Investigators:
Principal: LTC James B. Moe, DVM, Ph.D.
CPT Charles B. Clifford, DVM
CPT Douglas D. Sharpnack, DVM, MS

Description:

As new weapons systems are developed, it is imperative that consideration be given to the potential effects that these may have on the health and performance of the crews operating these systems. Use of mammals exposed to blast overpressure generated by weapons or other blast-generating devices provides a means of estimating the susceptibility of mammalian tissues to blast overpressures at levels approximating those received by operators of weapons systems. More detailed study of tissues so exposed helps to resolve the biological bases of blast-related injuries. Additionally, the various pathologic structural and functional techniques are useful in determining the complex interaction between blast overpressure and other factors in the modern combat environment. Resolution at the cellular and subcellular level of the biodynamic events leading to tissue injury is essential to an accurate approach to the prevention and treatment of blast-related injury.

Progress:

To determine the finite structural and functional bases of the pathologic changes caused by blast-related injury in the various tissues. Of special interest are injuries which result from exposures similar to those received by artillery weapons crews in the field environment. Structural and functional changes are correlated with various amounts of blast overpressure, emphasizing dose ranges which are near the environmental exposure associated with crew operator positions of large field artillery weapons. Effects of repeated blasts over time periods covering up to 14 days are studied to determine cumulative damage and resolution dynamics. The relative sensitivities of airways and blood vessels in the respiratory system are mapped. The fragility of the pulmonary vascular bed is compared with that of blood vessels in other organs and tissues of the body. Other functional parameters are investigated.

Conventional morphologic techniques, including light and

electron microscopy, as well as special procedures which determine vascular permeability, mucociliary clearance and other functional parameters, are used. Complex procedures designed to determine the effects of blast overpressure exposure on susceptibility to infectious agents will be adapted as the studies progress.

As non-auditory lesions in rats due to repeated exposure to blast overpressure had not been well described at the light or scanning electron microscopic level, an experiment was conducted to examine respiratory injury. Rats were exposed to 20 repetitions of blast overpressure varying in intensity from 22.5 psi, an anticipated LD_{30}, to 1 psi. Although no mortality was produced, mild respiratory lesions were observed in the 22.5 psi and 16 psi groups. These consisted of multifocal pulmonary hemorrhage observed by light microscopy, and tracheal epithelial injury observed by scanning electron microscopy.

In order to maximize these lesions to characterize them more fully an additional project was performed exposing rats to 20 repetitions of blast overpressure exposure at 25 psi. Although pulmonary hemorrhage was still relatively mild, there was extensive loss of tracheal and bronchial lining cells. Incipient regeneration was evident at 24 hours post exposure. The potential of single or repeated episodes of blast-induced tracheo-bronchial injury to result in permanent morphologic change, increased susceptibility to lower respiratory infection or other secondary complications requires further study. Additionally, experiments are underway to validate these findings in a larger mammalian species, the sheep.

Collaborative studies with the Department of Clinical Physiology, Division of Medicine have involved consultation and documentation of pathologic aspects of chronic tracheostomy studies, lymphatic duct cannulation studies, isolated gut loop experiments and early development of the waterjet impactor as a tool for blast overpressure research. Preliminary findings have suggested that each of these approaches will have considerable utility and value in the more finite proposed study of blast biology in the future.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6767	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. SMRY MATER	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BC	225 WWL3
b. CONTRIBUTING				
c. [illegible]	STOG 82 [illegible] 12/4			

11. TITLE (Procede with Security Classification Code)

(U) Pathophysiology of Blast Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Stress Physiology 017000 Weapons Effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
80 10	CONT	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	PRECEDING/CURRENT	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	83	1.0	209
	84	1.0	288

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

FINA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Blast injury; (U) Tissue markers; (U) Serum markers; (U) CPK; (U) LDH; (U) Isoenzymes

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number, precede text of each with Security Classification Code.)

23 (U) Recent work from this laboratory has shown that serum enzyme systems (particularly CPK) change with bowel infarction. In order to assess properly the changes in various enzyme systems in the peripheral serum subsequent to blast injury, more must be known concerning each enzyme's distribution in the various parts of the GI tract. If a difference in enzyme distribution could be detected, then earlier stages of injury may be detected by assaying the changes in the isoenzymes in the peripheral serum after blast injury. There is military relevance in this research.

24 (U) Our program of serum analyses is being integrated into the program currently being conducted in conjunction with the Department of Clinical Physiology of the Division of Medicine, WRAIR. The enzyme levels in the gastrointestinal tissues and serum of laboratory animals is being assayed.

25 (U) 82 10 - 82 09 A comparison was made between serum levels of Alkaline Phosphatase and Creatine Phosphokinase (CPK) and their isoenzymes as markers of injury to the small and large bowel. CPK was found to be a more sensitive and specific marking in laboratory animal experiments. A new laboratory blast device is being built which will allow testing of these markers after blast injury under controlled circumstances. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 81 (FOR ARMY USE) ARE OBSOLETE.

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 225 Pathophysiology of Blast Injury

Investigator:
Principal: LTC Geoffrey M. Graeber, MC

Background and Objectives:

Exposure to certain levels of blast has been shown to cause injuries to specific organs depending on the amplitude and the duration of the blast impulse. Organs such as the stomach, colon and lung are particularly susceptible to injury from exposure to blast. Our work has been directed at delineating potential serum markers which could be used to assess the extent and severity of injury to specific organs caused by blast exposure. Hopefully, such markers could be used to better evaluate and treat soldiers who had been exposed to blast waves in a combat situation. Work which we have previously published has shown that severe injury to either the small bowel or the colon is associated with elevations of peripheral serum creatine phosphokinase and its isoenzymes.[1-3] Analysis of lactic dehydrogenase in animals has shown that there are only minimal changes in this isoenzyme system in the peripheral serum after severe injury to the bowel.[3] More recently published work from our laboratory has shown that infarction of as little as fifty centimeters of small bowel will cause changes in the peripheral serum isoenzymes of creatine phosphokinase.[4] Further work which is awaiting publication has shown that the isoenzymes of creatine phosphokinase and lactic dehydrogenase may have similar changes in the serum of patients who have had severe bowel injury.[5] Other reports which are awaiting publication show that creatine phosphokinase and all three of its isoenzymes are distributed throughout the gastrointestinal tract.[6] Moreover, bowel has rather characteristic distributions of this isoenzyme system which are different from those found in some other tissues in the body.[6]

Our work during the current year has looked at the isoenzymes of creatine phosphokinase and lactic dehydrogenase in chest wall muscles and in the heart chambers.[7] The distribution of these isoenzymes in the skeletal muscles of the chest wall and the heart had potential interest since these organs could also be injured, theoretically, in blast exposure. The deposition of such isoenzymes in the serum after blast exposure could possibly confuse the interpretation of isoenzymes which had been deposited in the serum from injury to the bowel. Interestingly, there are characteristic isoenzyme patterns for both the skeletal muscle and

the different portions of the myocardium which could at least, theoretically, allow differentiation from GI tract injury. We have also studied alkaline phosphatase as a potential serum marker of injury to the bowel.[8]

Progress:

Progress this year has centered on the better definition of creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) in skeletal muscle and in cardiac muscle.[7] The isoenzyme distribution of creatime phosphokinase in cardiac muscle suggests that there is virtually no CPK-BB present in the heart. This is particularly important since CPK-BB seems to be indicative of mesenteric injury.[1-3]

Further studies which we have carried out this year have shown that the isoenzyme system of alkaline phosphatase, although a potentially attractive marker of severe injury to the gastrointestinal tract, is not as good a marker as creatine phosphokinase.[8] With severe bowel infarction, peripheral serum creatine phosphokinase rose to a higher level and more rapidly that did alkaline phosphatase. The serum isoenzyme distribution of creatine phosphokinase also changed in that the CPK-MB and CPK-BB isoenzymes went up as well as the CPK-MM isoenzyme. In alkaline phosphatase, the fraction which is found to be in the beta region and which was felt to be characteristic of the intestinal alkaline phosphatase, did not appear in appreciable quantities in the serum in the first twelve hours after injury. Moreover, there was virtually no detectable intestinal alkaline phosphatase in the serum of laboratory animals which had a major mesenteric injury twelve hours subsequent to the injury. For these reasons, serum alkaline phosphatase was felt not to be as good a marker of intestinal injury as creatine phosphokinase.

Further studies on tissue distribution of alkaline phosphatase have shown that there is overlap of the different isoenzymes found in different organs. Hence, alkaline phosphatase is not as good a marker as CPK which has distinct and physically separable isoenzymes. Other experimental work conducted this year has shown that the dog was a more adequate model for studying CPK and LDH isoenzymes than was the sheep. Preliminary studies are being conducted to determine if the swine is as good a model as the dog for the study of CPK and LDH isoenzymes after injury. Very preliminary results suggest that the swine will be a model comparable to the dog for studying these enzymes in relation to injuries which may be suffered by humans.

Recommendations For The Future:

Our plans for the future are to better define the swine model with respect to the isoenzymes we wish to study. We also plan to take the swine out to the field and expose it to different levels of blast. After exposing it to different levels of blast we will analyze the serum for the common isoenzymes and proteins assayed on routine examinations. Some enzyme systems, such as those of CPK and LDH will have more extensive examination including electrophoresis. We feel that these have particular promise for future endeavors in measuring intestinal tract injury after blast exposure.

A new development which bears further investigation and further consideration is the marketing of a rapid electrophoretic process for defining isoenzymes. The time has now been cut in half and the equipment has been reduced substantially for the new assay. This will allow more rapid determination of serum isoenzymes in a field situation and will allow more expeditious processing of the samples since less equipment is required. Initial evaluation and testing of this new enzyme assay will be slated for the coming fiscal year.

PROJECT 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 225 Pathophysiology of Blast Injury

Literature Cited:

References:

1. Graeber, G.M., Cafferty, P.J., Reardon, M.J., et al.: Elevations of Serum Creatine Phosphokinase (CPK) in Experimental Mesenteric Infarction. Surg Forum 31:148-150, 1980.

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6. Graeber, G.M., Cafferty, P.J., Wolf, R.E., Harmon, J.W.: The Distribution of Creatine Phosphokinase in the Gastrointestinal Tract. Submitted for publication.

7. Graeber, G.M., Cafferty, P.J., Wolf, R.E., Cohen, D.J., Zajtchuk, R.: Concentrations of Creatime Kinase and Lactic Dehydrogenase in the muscles encountered during median stemotomy and the Wall of the Cardiac Chambers. Surg Forum 34: 337-339, 1983.

8. Graeber, G.M., Wolf, R.E., Harmon, J.W.: Serum Creatine Kinase and alkaline phosphatase in experimental small bowel infarction. J. Surg. Res. - Submitted.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6768	83 10 01	DD DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORGN INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BC	226
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Pathophysiologic Studies of Blast Injury to the Gastrointestinal Tract

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

060500 Stress Physiology 008800 Life Support

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
83 10	CONT	DA			C. In-House

17. CONTRACT/GRANT

a. DATE(S)/EFFECTIVE — EXPIRATION

b. NUMBER

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR	83	2.0	211
CURRENT FISCAL YEAR	84	3.0	369

19a. RESPONSIBLE DOD ORGANIZATION

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20. GENERAL USE

NA

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NAME BASS, B

NAME SCHWEITZER, E POC: DA

21. KEYWORDS (Provide EACH with Security Classification Code) (U) Blast injury; (U) Gastrointestinal hemorrhage; (U) Gastrointestinal perforation; (U) Combat Casualty Management

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Our technical objective will be to study the pathophysiology of the development of mucosal and serosal hemorrhage, which is known to be a consequence of blast injury and which may be an important aspect of combat casualty management in future conflicts. Low level exposure to blast injury is experienced by troops firing weapons. Much higher levels of blast injury is experienced by troops in the vicinity of an explosion, or in a tank which is struck by a projectile. Extremely high levels of blast overpressure would be experienced by troops in the field of a Fuel Air Explosion (FAE) Mine Neutralization System.

23. (U) Observations of the gastrointestinal results of blast injuries over a range of pressures, durations and frequencies will be made in sheep. Sequential laparotomies will be carried out on sheep to observe the natural history of the lesions observed. Gross and microscopic observations will be made. The general physiologic status of the animals will be assessed during these studies by measurements of pulse, respiration rate and white blood cell count.

24. (U) 82 10 - 83 09 A report reviewing world experience on gastrointestinal blast injuries was published in the journal of Military Medicine. A study to evaluate the possible usefulness of Xenon to diagnose hematomas of the bowel was begun in collaboration with Dr. Gregory Bulkley at Johns Hopkins University. Initial experiments in rats demonstrate some potential for this approach. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

25. AVAILABILITY (Unclassified only) [illegible]

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. [illegible]

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 226 Pathophysiologic studies of blast injury to the gastrointestinal tract

Investigators:
Principal: John W. Harmon, LTC, MC
Co-Investigators: Eugene J. Schweitzer, CPT, MC
Amiron Cohen, CPT, MC

Background and Objectives:

With blast overpressure injury, it is common to see gastrointestinal tract injury. The lesions observed acutely range from petechia in mild form progressing to large hematomas in the submucosa and in the most severe cases including perforation of the bowel.[1-6] The hematomas occur most commonly in the stomach and the proximal colon. They also occasionally are seen in the small bowel and retroperitoneum. The natural history of these lesions is not known. A knowledge of the natural history of the lesions is of importance for those who will be managing Blast Injury Casualties. If the lesions resolve over time, they are not a significant problem. If, however, they progress to perforation, they are a very major problem. At this time surgeons do not have guidelines for managing non-perforating bowel lesions of blast overpressure.

Progress:

During this fiscal year, we carried out two projects in the area of gastrointestinal effects of blast injury.

First we collaborated with Dr. Gregory Bulkley of Johns Hopkins University, Department of Surgery, to evaluate the potential of the Xenon washout technique for diagnosing the intramural hematomas that result from blast injury. Dr. Amiron Cohen, a resident on elective from WRAMC, was the lead investigator in this intensive evaluation which lasted three months. A final analysis of the studies performed is being prepared and will be available for next year's annual report. At this time, it can be said that Xenon washout has the potential to diagnose any intra-abdominal hematoma. Xenon is an inert, lipid soluble, radioactive gas. After it is injected into the peritoneal cavity, it is removed from tissue with a washout curve that depends on blood flow. Xenon was injected into the peritoneal cavities of rats and when a hematoma was present in the peritoneal cavity, radioactivity lingered in the area of the hematoma.

This method is expensive and requires sophisticated equipment. It also lacks precision. So at this stage, it clearly is not suitable for field use or even for use in major hospitals. However, there is no alternative way to diagnose intra-abdominal hematomas, and this approach does have potential.

The other project in this work unit is the building of a laboratory blasting device. This device is currently being built at WRAIR. It will have the potential to inflict air blast injury on small animals. The pathophysiology of blast injury as well as the effects of various treatment regimens could be nicely evaluated if this system works. It is patterned on a similar device that was built at Porton Downs in England, but which has only had initial evaluation there. We expect to have the laboratory blasting device operational in fiscal 1984.

Recommendations for the future:

The data from the Xenon project needs to be fully evaluated and reported in the scientific literature. Without significant basic improvements in the technology of this approach, it is not clear that further evaluation of it by our Division would be valuable. We will continue to monitor this area for new developments which would be of use to the United States Army Medical Corps.

We expect to be able to carry out meaningful experiments relating to the pathophysiology and treatment of blast injury using our laboratory air blast device in the near term future.

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 226 Pathophysiologic studies of blast injury to the gastrointestinal tract

Publication:

Harmon, JW and Haluszka, M. Care of Blast-Injured Casualties with Gastrointestinal Injuries. Military Medicine 148:586-588, 1983. (Appendix A)

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6761	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'Y	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'S INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	6110'A	3M161102BS10	CD	228 WW17
b. [illegible]				
c. [illegible]	STOC			

11. (U) Regulatory Mechanisms and Pathophysiology of Hematopoiesis Application to Military Hematology

12. FIELD AND TECHNOLOGICAL AREAS
005400 Life Support 002600 Biology 003500 Clinical Medicine 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATE EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PREVIOUS FISCAL YEAR 83	5.0	123
CURRENT FISCAL YEAR 84	5.0	184

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ASSOCIATE INVESTIGATORS
NAME: CROSBY, W H SALVADO, A J
NAME: MEAGHER, R POC: DA

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Leukocytes; (U) Bone Marrow; (U) Hematopoiesis; (U) Marrow Failure; (U) Erythrocytes

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief individual paragraphs identified by number, precede text of each with Security Classification Code.)

23. (U) To define the hematologic pathophysiology of bone marrow toxicity from certain families of chemical agents, drugs, radiation, and acute infection; to identify modalities that may protect against hematopoietic stem cell injury; to study basic mechanisms involved in the regulation of hematopoiesis, including iron absorption and to define and purify hematopoietic regulatory mediators. A basic understanding of the regulation of hematopoiesis is very important to the military because of numerous marrow toxic conditions (radiation, drugs, infections, chemicals) to which military personnel may be exposed during their duties.

24.(U) Experimental procedures include biochemical and cell culture techniques, animal models, and the isolation of normal human bone marrow cells. Studies also involve electron microscopic analysis of the ultrastructure of bone marrow tissue during its morphogenesis.

25.(U) 82 10 - 83 09 Studies of the long-term in vitro culture of normal human marrow have defined optional serum requirements for maintenance of human hematopietic stem cells in vitro; a microtized version of the "Dexter" long-term marrow culture system has been developed using leighton tubes in which marrow cells grow on a sterile, removable plastic slides which permits experimental modification of the surface to which marrow stroma and hematopoietic cells adhere. Using this system, marrow stromal cells separated by density gradient techniques have been characterized; nutrient requirements for in vitro hematopoiesis have also been investgated. Studies of erythropoietin purification from human urine and from a murine cell line have been carried out. Studies of iron absorption in mice and humans with and without iron loading have continued. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

* Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 66 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M161102BS10: RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 228 Regulatory Mechanisms and Pathophysiology of Hematopoiesis Application to Military Hematology

Investigators LTC Daniel Wright, MC; LTC August Salvado, MC; COL William Crosby, MC; Ms. Mary Cutting, MS (Fellow, GWU); Dr. Richard Meagher (IPA Investigator)

Description

Blood cells constitute a complex organ of which normal function requires continuous self-renewal of blood precursor cells within the bone marrow. The demands of blood cell renewal (hematopoiesis) are enormous, and for this reason hematopoiesis is particularly sensitive to the toxic effects of chemicals, drugs, radiation, and acute infections which interfere with cell division or differentiation. The objectives of this work unit are to study basic mechanisms involved in the regulation of hematopoiesis using tissue culture of stem cells and committed hematopoietic precursor cells from human, mouse, and rabbit marrrow, using leukemic cell lines, and using allogenic transplantation of bone marrow tissue in animals.

Specific studies are designed to study the effects of mediators derived from mature leukocytes and inflammatory fluids upon hematopoiesis, to study the biochemistry and physiologic effects of erythropoietin upon stem cell maturation, and to study basic mechanisms by which iron absorption is regulated and by which iron is utilized by hematopoietic tissues.

Progress

1. Studies of long-term in vitro culture of normal human bone marrow

A. Marrow stromal cells:

The adherent layer of "stromal" cells which forms on the bottom of the culture flasks during the first 2-3 weeks of bone marrow culture is critical for supporting the survival and proliferation of hematopoietic stem cells. A technique for separation of bone marrow cells on density gradients composed of PVP coated silica (Percoll) has been utilized to identify different cell types which comprise the adherent monolayer. Morphologic differences have been defined by light microscopy of adherent cells from different portions of the density gradients. At least 3-4 cell types are distinguishable by such techniques. We have begun to investigate the ability of "stromal" cells with different bouyant densities to support hematopoietic stem cells added back to the cultures. Cells capable of supporting stem cell growth are being further characterized by electron microscopy and studied for surface determinants utilizing a flourescence activated cell sorter. A microtized culture system has been developed using Leighton tubes in which cultured marrow cells grow on removable plastic slides. This technique is especially adaptable for study of the adherent stromal cell layer which supports hematopoietic stem cell self-renewal.

B. To better investigate the production of factors by the stromal

elements in long term cultures which are essential to the survival and function of hematopoietic stem cells, studies have been begun to completely define the nutrient requirements of the marrow cell culture system. Any factors produced by the stromal cells are operative over short distances and likely to be in such small quantities as to be virtually undetectable in a system flooded with a broad spectrum of proteins found in serum. Therefore, development of serum free nutrient media has been initiated. Studies are underway to fractionate fetal calf serum and horse serum by ultracentrifugation with density adjustment. The goal is to support the formation of a stromal layer and the ensueing hematopoiesis in the presence of culture medium supplemented with a well characterized serum fraction and other purified proteins and/or hormones. A particular effort has been directed at distinguishing lipid and non-lipid requirements of in vitro hematopoiesis.

2. Studies of erythropoietin (Ep)

Studies have been continued on the purification of Ep to high specific activity by methods producing both high yields and significant resolution. Thus far, the hormone has been purified over 500 fold from human urine by a combination of reverse phase high performance liquid chromatography (HPLC), affinity chromatography on wheat germ agglutinin, and size exlcusion HPLC. Although three techniques are rapid and produce better yields than most previous separations for this substance, problems remain. In particular, reverse phase chromatography necessitates that large volumes of ethanol be removed before further processing. Along with this, lectin affinity chromatography produces difficulties with loss of small quantities of protein by nonspecific binding. Newer studies to eliminate macro affinity chromatography and to utilize primarily various forms of HPLC including adsorption on hydroxyapatite have been begun. Along with this, a different and potentially much more reliable source of starting material has been identified in the form of a murine cell line which can secrete significant quantities of EP into the growth medium.

3. Studies of the regulation of iron absorption

Efforts have continued to develop an easy, non-toxic, nonradioactive test of iron adsorption from small, orally administered amounts of iron. Using this test we intend to define indicators of mild iron deficiency sufficiently sensitive and accurate that it may be used in epidemiologic studies of iron nutrition. Our basic approach has been to describe iron absorption with a low dose iron tolerance test (ITT). Instead of using pharmacologic doses (50-250 mg), we have employed 5 or 10 or 20 mg, approximating the amount of iron in a meal or a mild dietary supplement. Our subjects have been normal blood-bank donors some of whom have mild anemia and some have no anemia but partially depleted iron stores. Small doses of iron were found to cause significant increases in the plasma iron concentration of the mildly iron deficient volunteers. The changes in normally iron replete subjects on the other hand are insignificant. In the iron deficient donors the larger the dose, the greater the increase in plasma iron concentration. Heme iron (as blood) causes a smaller and delayed increase in plasma iron. The same is true of reduced iron (as carbonyl iron). The delays may be a function of digestion in the case of

heme and of dissolving in the case of metallic iron. We have learned that a large dose of iron (60 mg) on day one may interfere with the absorption of a smaller dose (20 mg) on day two suggesting that large doses result, to some extent, in a refactory period of iron absorption by the duodenum. We have also ascertained that ascorbic acid increases the rise of plasma iron and carbonate inhibits it. The implications for efficacy of vitamin-mineral preparations are a subject for further investigation.

Future Plans

Studies of the regultion of hematopoiesis using in vitro systems for the long-term culture of normal human bone marrow, and of hematopoietic growth factors, and of the regulation of iron absorption will continue in FY84 along lines of work begun in the last 2 years. Particular attention will be given to in vitro nutrient variables that influence terminal differentiation of neutrophils in long-term marrow culture, to defining the characteristics of adherent marrow stromal cells that support in vitro hematopoiesis, and to characterizing the refractory period of iron absorption provoked by small doses of oral iron in vivo.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6450	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. HANDLING INSTRS	9a. SPECIFIC DATA-CONTRACTOR ACCESS	9b. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AJ	229 WW13
b. CONTRIBUTING				
XXXXXXXXXX	STOG 82/83-6.2/1			

11. TITLE (Procede with Security Classification Code):

(U) Military Hematology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

008800 Life Support 002600 Biology 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
58 05	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	4.0	80
FISCAL YEAR CURRENT 84	4.0	539

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code): (U) Coagulation; (U) Hematopoiesis; (U) Blood; (U) Marrow Failure; (U) Erythrocytes; (U) Leukocytes

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23.(U) To define the hematologic Pathophysiology of trauma, infections, shock, marrow toxic drugs or radiation as related to diseases of military importance; to identify modelities to restore hemostasis, to augment host defense systems against infection; The importance of this basic research to the military is wide ranging and is applicable to both health maintenance of military personnel exposed to unusual enviornmental, toxic and infectious hazards but also to the treatment of militarily relevant disease.

24.(U) Experimental procedures include biochemical, immunologic, and cell culture methods; in vitro cell-free and membrane-dependent systems; large and small laboratory animal models; and studies of human subjects.

25.(U) 82 10 - 83 09 a) Studies were carried out investigating the interactions of negatively charged phospholipids, factor VIIIc and factors of the prothrombinase complex. These studies led to the development of a dilute-phospholipid APTT assay useful in detecting auto-antibodies with anticoagulant effects. b) Studies of plasminogen activator (PA) production by the human leukemic cell line, HL-60, have defined a model for studying inducible synthesis and release of PA. c) Methods were developed to assay a critical enzyme of intermediary purine metabolism, IMP dehydrogenase. Use of this assay in study of hematopoietic precursor cells has further defined a relationship between this enzymes activity and terminal differentiation of myeloid cells. d) A new human myeloid leukemia cell line has been isolated, RDFD-2, which may be induced to differentiate in vitro. e) A model of rapidly occurring essential fatty and dificiency (EFAD) was developed in monkeys to study the effects of EFAD on host-defense functions of neutrophils. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

* Classify as necessary upon originator's approval

PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A, 1 NOV 68

Project 3M161102BS10: RESEARCH ON MILITARY DISEASE, INJURY AND AND HEALTH HAZARDS

Work Unit 229 Military Hematology

Investigators LTC Daniel G. Wright, MC; LTC Barbara Alving, MC; Dr. Diane Lucas, GS-12; LTC John Kark, MC; Mr. Charles Barr, GS-12; COL William Crosby, MC; LTC August Salvado, MC; Dr. Leo Lui, GS-12; Dr. Jan Palmblad (NRC Senior Fellow); MAJ Robert Knight, MC (WRAMC); Dr. Richard Meagher (IPA Investigator from Johns Hopkins)

Description

Two distinct research areas have been explored under this work unit in FY83.

1. Studies of coagulation and plasma proteins

Blood coagulation factors, blood platelets, and plasma proteins (e.g. the kinin-kallikrein, fibrolytic and complement systems) are critical for the development and outcome of acute responses to traumatic, thermal and infectious injury. Studies are directed at changes in clotting and plasma proteins and in platelets during trauma, stress, and infection that lead to clinically significant abnormalities of hemostasis. Studies are also directed at understanding the therapeutic potential of intravenously administered immunoglobulins that have military relevance.

2. Studies of blood phagocytes

Phagocytic blood leukocytes are critical to host defense against bacterial and fungal infections and to the development and outcome of inflammatory responses. Studies of human neutrophil and monocyte function have concentrated upon understanding the secretion of soluble mediators by these cells that influence the immunoresponsive functions of macrophages and lymphocytes and affect connective tissue disorganization and repair. Studies have also been directed at understanding factors that regulate the production of neutrophils in the bone marrow and that influence the distribution, utilization, and function of these phagocytes in peripheral tissues.

Progress

1. Studies of coagulation and plasma proteins

A. Production of plasminogen activator by leukemic leukocytes

Studies of the synthesis and release of plasminogen activator (PA) by the human leukemic cell line, HL-60,

have been completed. Previous studies have shown that neoplastic cells from patients with acute myeloblastic leukemia (AML) release PA when incubated in vitro. This pro-coagulant factor may contribute to the occurrence of disseminated intravascular coagulation which is a serious complication of AML and also occurs is other disease conditions, e.g. disseminated infections, massive trauma. The HL-60 cell line provided us with an opportunity to study in detail the synthesis and release of PA by a homogeneous population of myeloid cells. The time course and quantity of PA release by HL-60 cells was studied under normal culture conditions and in the presence of various inducing agents. PA in cell culture supernatants was determined in the presence and absence of plasminogen with [^{3}H] TAME or S-2251 as the plasmin substrate. PA was detected in cell cultures by 3 days and this activity increased during subsequent culture. Production of PA was stimulated 3 fold by tetradecanoyl phorbol acetate. The PA generated was characterized by SDS gel electrophoresis techniques by which gels were placed on plasminogen-fibrin-agar indicator plates. Molecular weight of this PA was similar to that of urokinase (54 Kd) and was neutralized by urokinase-specific antibodies.

Techniques developed in these studies will be applied to the study of PA and tissue factor (a potent activator of Factor VII) by human monocytes infected with Dengue virus and intracellular bacteria such as leishmania, and also by cultured endothelial cells.

B. Development of a dilute phospholipid APTT assay

The activated partial thromboplastin time (APTT) requires negatively charged phospholipids (PL) which interact with Factor VIIIc and factors of the prothrobinase complex. So-called lupus anticoagulants, which are auto-antibodies that occur in certain disease states directed against PL, are often initially detected by their noncorrectable prolongation of the APTT. Because initial studies with negatively-charged liposomes showed that dilution of PL in the APTT system increased the sensitivity of lupus anticoagulant detection, we developed a dilute PL-APTT assay. In study of a series of plasma samples which demonstrated noncorrectable prolongation of the APTT, this dilute PL-APTT assay proved to be a sensitive technique for verifying the presence of lupus anticoagulants.

C. Other clinical coagulation studies

Studies were begun to characterize the abnormal fibrinogen that may occur in association with renal cell carcinoma or with hepatic dysfunction. Studies of a rare kindred with hereditary warfarin resistance were completed

which demonstrated that abnormal hepatic transport of the anticoagulant drug, warfarin, or altered binding of the drug to proteins involved in vitamin K metabolism caused the drug resistance, not altered absorption or clearance of the drug. Studies of a series of patients receiving IV heparin have been carried out to correlate heparin levels with routine coagulation tests and with levels of anti-thrombin III, a potent inhibitor of thrombin and Factor Xa, in order to refine techniques for monitoring heparin therapy and for evaluating heparin kinetics.

2. Studies of blood phagocytes

A. The regulation of myeloid cell maturation

In our previous studies with the human promyelocytic leukemic cell line, HL-60, we defined changes in purine metabolism that consistently occur with induced differentiation of these immature myeloid cells. When HL-60 cells are exposed to compounds that induce maturation, biosynthesis of guanylates from the central intermediate, IMP, is reduced and intracellular guanosine nucleotide (NTD) pools shrink. Furthermore, our studies suggested that these metabolic changes involve the down-regulation of the enzyme IMP dehydrogenase (IMPD), leading us to discover that specific inhibitors of IMPD, such as mycophenolic acid and 2-β-D-ribofuranosylthiazole-4-carboxamide (RTC), are potent inducers of HL-60 cell maturation. Because of these prior observations, we have investigated IMPD in induced and uninduced HL-60 cells in greater detail. IMPD activity in cell extracts was measured directly with a tritium release assay which uses anion exchange columns to separate ^3H liberated during conversion of IMP to XMP. Uninduced HL-60 cells had high levels of IMPD activity (2.4 ± .06 nmoles IMP metabolized/mg cell protein extracted/hr) compared with purified human blood neutrophils (0.45 ± .08) and monocytes (0.65 ± .36). When HL-60 cells were exposed to retinoic acid (10^{-6}M), dimethylformamide ($6x10^{-2}$M), or RTC (10^{-6}M), IMPD levels decreased by up to 65%, 80%, and 88% respectively within 3 to 12 hrs. of culture. Markedly decreased levels of IMPD activity persisted thereafter and preceded functional maturation of the cells (phagocytosis and NBT reduction) by at least 24 hrs. These findings have lent further support to our concept that the activity of IMPD may be critical to the regulation of cellular maturation, and are consistent with prior observations that high levels of this enzyme are associated with neoplastic transformation.

B. Isolation of a new, human myeloid leukemia cell line, RDFD-2

A continuously maintained cell line, RDFD-2, was successfully isolated by tissue culture of peripheral blood leukemic blood cells from a 56 y.o. man with acute myelogenous leukemic (FAB class M1). The cells were established in tissue culture by innoculation into RPMI 1640 media supplemented with 20% fetal bovine serum (FBS), insulin, transferrin, selenium, and 10% PHA stimulated human lymphocyte conditioned media (LCM). During 6 wks of culture passage, the LCM was gradually withdrawn and the FBS concentration reduced. Once established in stable maintenance culture, these cells demonstrated a limiting but appreciable capacity to undergo induced, functional maturation along myeloid lines when exposed to inducers such as retinoic acid and dimethylformamide. Depending on induction condtions, 15-50% of cells developed the differentiated myeloid functions of phagocytosis and NBT reduction. When the RDFD-2 cells were cloned by limited dilution in standard maintenance media, 6 derivitive lines were isolated that showed no response to inducing agents, while 4 other clones expressed different but stable degrees of induced maturation (14-92% maturation by 6 days' induction). The inducible clones were found to have shorter doubling times (27-35 hr) than did non-inducible clones (46-52 hr). These cell line derivitives of the new human myeloid line, RDFD-2, should prove useful in understanding cellular mechanisms that regulate terminal differentiation in myeloid cells.

C. Studies of neutrophil function in essential fatty acid deficiency

A model of essential fatty acid deficiency (EFAD) has been developed in monkeys in order to study its effects upon the host defense functions of neutrophils. Essential fatty acid, linoleic acid, is required in the diet of all mammals, including humans, in order to form the normal 20-carbon derivitive of linoleic acid, arachidonate. Arachidonic acid is incorporated into the phospholipid constituents of cell membranes and appears to be very important in propogating receptor mediated signals that stimulate cells to carry out their specific, normal functions. Arachidonate metabolism appears to be particularly important in the stimulus-response coupling of neutrophils, which are the principal blood phagocytes. Linoleic acid deficiency was produced in monkeys by total intravenous alimentation using solutions that delivered carbohydrate calories, and vitamins but no fat. Monkeys receiving fat-free IV nutrition were compared with control animals that received the same preparations but with fat emulsions (intra-lipid) added. These experimental conditions are analogous to those of

post-surgical or post-trauma patients who must receive all their nutrition intravenously. In monkeys not given lipid, biochemical evidence for essential fatty acid deficiency was clearly evident by one week and progressed during the subsequent 2 weeks of three week experimental periods. While marked decreases in plasma lipid and leukocyte membrane lipid linoleic acid levels occurred first, by 2 1/2 weeks there was also a significant decrease in leukocyte membrane arachidonate levels. Several different monkey species were evaluated in these studies before finding that the African Green monkey was the best for study by being most able to tolerate the experimental model, and by having neutrophils with separation characteristics most similar to humans. Study of functional changes in the neutrophils from EFAD is still in progress.

Future Plans

Studies of coagulation and plasma proteins and of blood phagocytes will continue in FY84 along lines of work carried out in the past three years. These studies will continue to include investigations in to the character and function of glycoproteins of human neutrophil secondary granules (discussed in previous animal reports under this work unit) on inflammatory cell function and on the regulation of granulopoiesis.

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82 10 01	D. Change	U	U		NL	YES ☒ NO ☐	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AH	230 WWH5
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Biological Roles of Surface Membrane Components: Parasite Model Systems

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE
b. NUMBER
c. TYPE
d. KIND OF AWARD
EXPIRATION
e. AMOUNT
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 83	2.5	369
CURRENT FISCAL YEAR 84	2.5	459

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21. GENERAL USE

FINA

ASSOCIATE INVESTIGATORS
NAME: Hansen, B I
NAME: Geller, R

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Surface Antigens; (U) Gene Cloning; (U) Transport Receptors; (U) 2-D Gel Analysis

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective of this work unit is to investigate the biochemistry and molecular biology of cell surface membranes as they relate to processes of antigenicity, nourishment, differentiation and multiplication. Membranes will be studied in parasites with a view to elucidating surface-associated processes that will afford the development of immunoprophylactic and/or chemotherapeutic protection of military personnel against tropical diseases of military importance.
24. (U) Surface antigens are studied by application of recombinant DNA, gene cloning, restriction analysis and probe hybridization techniques. Radiolabeled ligands are employed to determine transport processes and surface receptors. 2-D gel electrophoresis is used to characterize surface antigens of isolated membranes.
25. (U) 82 10 - 83 09 Adenosine binding sites of leishmanial promastigotes have been characterized by use of radiolabeled ligands cyclohexyladenosine and phenyl-isopropyladenosine. Double-stranded cDNA of variant-specific trypanosomes has been cloned into suitable host/vector systems. Nuclear DNA has been isolated and restricted for cloning into a cosmid vector. Antiserum to CT Wellcome strain flagellar pocket antigens has been used to demonstrate that genes coding for the antigens are conserved in other variant-specific trypanosomes. Whole cell and surface components of Leishmania Spp. have been analyzed by 2-D gel electrophoresis. Protein patterns for each species are distinctive and contain serveral protein spots that appear to be species specific. These latter findings are being correlated with translation products precipitated with human antisera and analyzed by polyacrylamide gel electrophoresis. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon requestor's approval

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3M161102BS10 BASIC RESEARCH ON MILITATY DISEASES

WORK UNIT: 230 Biological Roles of Surface Membrane Components: Parasite Model Systems

INVESTIGATORS:
Principal: John G. Olenick, Ph.D.
Associate: Ruth Geller, Ph.D.; Brian D. Hansen, Ph.D.
Assistant: SP5 Roberto Ayala-Medina; SP4 Lynn Decker; John A. Kintzios, M.S.; SP5 Jose Perez-Arbelo; CPT Ruthann Smejkal

The objective of this work unit is to investigate the biochemistry and molecular biology of cell surface membranes as they relate to processes of antigenicity, survival, nourishment, multiplication and differentiation. Membranes are studied in parasitic protozoa with a view to revealing and elucidating surface-associated processes that will afford the development of immunoprophylactic and/or chemotherapeutic protection of military personnel against leishmaniasis, trypanosomiasis or other tropical diseases of military importance. The following investigations were conducted:

1. Adenosine receptor binding in promastigotes of Leishmania Spp.
2. Fluorochromatic detection of viable intra- and extra-cellular leishmania.
3. Cloning of genes for Trypanosoma rhodesiense variant-specific glycoproteins.
4. Cloning of genes for Trypanosoma rhodesiense flagellar pocket antigens.
5. 2-D gel analysis of whole cell and surface components of Leishmania Spp.
6. Cloning of genes for surface components of Leishmania Spp.

1. Adenosine Receptor Binding in Promastigotes of Leishmania Spp.

Since Leishmania Spp. lack the ability to synthesize purines de novo, the host organism supplies a primary source of the purine ring, the nucleoside adenosine. Following our study showing drug (Thiosemicarbazone H) inhibition of adenosine transport and adenosine ligand binding to the cell surface membrane, a further study was conducted to characterize the nature of adenosine binding to the surface membrane. Initial experiments measured the total, specific and nonspecific binding of ^{3}H-cyclohexyl-adenosine (CHA) to the promastigote surface over increasing incubation periods from 15 seconds to 120 minutes. Interestingly, specific binding equilibrated in approximately 30 seconds, suggesting high affinity binding of adenosine to the receptor. This equilibration of ligand binding continued through 15 minutes followed by a significant linear increase through 120 minutes. This apparent increase of ligand bound may be due to internalization and metabolism of ^{3}H-CHA. Therefore, all CHA receptor studies were conducted for 2 minute incubation periods. Similar results

were obtained when using an additional adenosine agonist, ^{3}H-phenyl-isopropyladenosine. Dissociation constants for the specific binding of radiolabeled ligand tested were determined. Studies are currently underway utilizing isolated leishmanial membranes.

2. Fluorochromatic Detection of Viable Intracellular and Extracellular Leishmania.

Viability assays are essential for parasite drug development and immunological research. Although metabolic, staining and replication procedures have been used to test parasite viability, numerous problems have arisen. Therefore, a color epifluorescence microscope procedure was developed utilizing fluorescein diacetate (FDA) and ethidium bromide (EB). Esterases within living cells convert nonfluorescent FDA to fluorescein, inducing a striking yellow-green fluorescence. Dead cells, lacking esterase activity, yield no fluorescence when exposed to FDA although EB is rapidly accumulated. Complexes of EB and nucleic acids cause dead cells to fluoresce red. The present study demonstrated that both promastigotes and intracellular amastigotes (within murine macrophages) will fluoresce red if dead and green if live. Dead cells used as controls were killed via formalin fixation. Growth curves of formalin-killed promastigotes were plotted to confirm cell death. Formalin-fixed extracellular amastigotes were confirmed dead by their inability to transform to the promastigote form.

3. Cloning of Genes for Trypanosoma rhodesiense Variant-Specific Glycoproteins.

Utilizing recombinant DNA technology, this project seeks to elucidate the mechanism of antigenic variation in the African salivarian trypanosome, Trypanosoma rhodesiense. It has previously been shown that this variability resides in the protein moiety of the variant-specific surface glycoprotein (VSG). Antisera have been raised in rabbits to purified VSGs from a number of trypanosoma isolates expressing different VSGs (VATs). We have used such antisera to detect messenger RNA populations coding for specific VSGs by translation in a rabbit reticulocyte lysate system followed by immunoprecipitation, polyacrylamide gel electrophoresis and autoradiography. Some of these populations have been further fractionated according to size in methylmercurichydroxide-containing agarose gels. Messenger RNAs were than used to prime cDNA synthesis using AMV-reverse transcriptase and DNA polymerase I (Klenow fragment). Resulting double-stranded cDNAs were cloned into suitable host/vector systems after treatment with S_1 nuclease and terminal deoxynucleotidyl transferase. Transformants are currently under investigation to identify those containing VSG sequences. In parallel, nuclear DNA has been isolated from T. rhodesiense variants and restricted for cloning into a cosmid vector. Both approaches are necessary to fully characterize the loci coding for the VSGs and their controlling elements.

4. Cloning of genes for Trypanosoma rhodesiense Flagellar Pocket Antigens.

Although the VSG is the major suface component of T. rhodesiense, there is another much smaller group of proteins localized in the flagellar pocket (flagellar pocket antigens- FPA). To test whether these remain invariant from one VAT to another, an antiserum raised in rabbits by injection of purified FPA from CT Wellcome strain, was obtained from Dr. John McLaughlin of the University of Miami. Messenger RNA from two variants currently in use in the laboratory was used to prime a cell-free trans-lation system (rabbit reticulocyte), the translation reacted with the FPA-specific antiserum and the resulting complexes analyzed by polyacrylamide gel electrophoresis and autoradiography. Each variant contained sequences which, when translated, coded for identical proteins as recognized by the heterologous FPA antiserum. We are currently examining the accessibility of the flagellar pocket antigens to antibody recognition in situ. Pending the outcome of these accessibility studies, messenger RNAs coding for these antigens will be cloned into suitable vectors. Sequences from cDNA, as well as genomic banks, will be cloned into suitable sequencing and expression vectors to study the structure of these antigens and the feasi-bility of using these antigens for immunoproophylaxis.

5. 2-D Gel Analysis of Whole Cell and Surface Components of Leishmania Spp.

This study is designed to identify relevant surface antigens on Leishmania Spp. with the view of developing immunoprophylactic protection of military personnel against these parasitic protozoa and/or a rapid, simple and reliable method of identifying and discriminating among various species. Accordingly, antigens which are common to the various species, as well as unique to a single species, are of interest. Three phylo-genetically separate species, L. donovani donovani, L. braziliensis panamensis and L. mexicana mexicana, were chosen for study. Whole cell preparations of promastigotes were made and subjected to isoelectric focusing in the first dimension and SDS-polyacrylamide gel electrophoresis on slab gels in the second. The slab gels of each species were stained for protein using silver stain and compared with those of other species. Differences in the protein patterns obtained were quite evident and reproducible. Cell membranes from each species were also isolated and subjected to two-dimensional polyacrylamide gel electrophoresis. The protein patterns obtained from each species were distinctive, containing several protein spots that appeared to be species specific. In collabo-ration with Ms. Mary Kay Gentry, Department of Biological Chemistry, mice have been immunized with whole cell preparations of Leishmania Spp. to begin the production of monoclonal antibodies.

6. Cloning of Genes for Surface Components of Leishmania Spp

For all species of Leishmania thus far studied, there appear to be surface components which are species specific and components which appear common to all. To determine whether these differences and similarities reside in the protein or in the sugar moieties of these surface components, translation products of nonglycosylating lysates (rabbit reticulocyte) primed with messenger RNA from Leishmania Spp. studied above

observed with both homologous and heterologous antisera. Work is currently underway to define these similarities and differences and to correlate these findings with the 2-D gel patterns previously obtained.

PROJECTED STUDIES

Studies on the biochemistry and molecular biology of surface membrane-associated phenomena in parasitic protozoa will continue. Specific aims include:

1. Continuation of studies on adenosine receptor binding and the effect of antileishmanial compounds on the binding.

2. Continuation of studies on the molecular basis of antigenic variation in trypanosomes to include the isolation of cDNA and genomic clones containing sequences coding for VSGs and for FPA and the sequencing of isolated clones to determine the organization of genes and controlling elements.

3. Continuation of studies on 2-D gel analysis of Leishmania Spp. to include additional species in order to determine interspecific similarities and differences providing groundwork for the development of immunoprophylaxis and rapid assay protocols. Polyclonal and monoclonal antibodies will continue to be generated as probes for studying proteins and translation products of Leishmania Spp.

4. Determination of the 2-D gel protein patterns of Leishmania Spp. amastigotes and axenic amastigotes and comparison with the protein patterns of promastigotes. This will provide informaiton as to whether the more easily obtained axenic amastigotes are a valid model for studying the membrane composition of amastigotes.

PUBLICATIONS

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3. Olenick, J. G., and P. E. Lorenz. 1982. Float device for density gradient fractionation. Unted States Patent No. 4,346,608.

ABSTRACTS AND PRESENTATIONS

1. Hansen, D. B., J. Perez-Arbelo and P. K. Chiang. 1982. Antileishmanial activity of thiosemicarbazone H: Inhibition of adenosine receptor binding. The American Physiologist 25, p 293.

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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORDER NUMBER	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CH	231 WWIC
b. CONTRIBUTING				
c. COMPATIBLE	[illegible]			

11. TITLE (Include with Security Classification Code) (U) Studies of Military Personnel with Sickle Cell Trait (SCT)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
002600 Biology 012400 Personnel Selection and Maintenance

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 01	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PREVIOUS 83	2.5	109
	CURRENT 84	4.0	239

19. RESPONSIBLE DOD ORGANIZATION
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NAME: KARK, J A
NAME:
POC: DA

22. KEYWORDS (Provide KACs with Security Classification Code)
(U) Sickle Cell; (U) Hypoxia; (U) Thalassemias; (U) Hemolysis

23. TECHNICAL OBJECTIVE. 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code.)

23.(U) To determine medical risks to soldiers with sickle cell trait (SCT) who may be assigned to special military roles, such as Army aviation, which require performance during hypoxia. To perform studies of changes produced in their red cells by sickling to determine whether a laboratory test can be devised capable of predicting the relative susceptibility of SCT soldiers to complications of sickling as revealed in altitude chamber studies. These studies are important to Army aviation, because of its unique requirement to carry out missions in hypoxic environments, and because of a lack of data which define the incidence and degree of medical risk to individuals with SCT.

24.(U) A prospective, controlled study of the physiologic effects of hypoxia in an altitude chamber upon SCT aviator candidates and controls, using exposure similar to mission conditions and identical to aviation training. Special procedures will include analysis of hemoglobins, accurate diagnosis of thalassemias, E.M. studies of red cells to identify subtle levels of sickle changes, use of autologous 51-Cr-RBC transfusion to measure hemolysis and splenic sequestration. SCT cells from studied individuals will be examined before and after in vitro sickling for changes in size and density distribution, filterability, 51-Cr-RBC adherence to endothelium, hemoglobin binding by cell membranes, cation and anion permeability, polymerization of Hb S and levels of ATP, 2-3-DPG by NMR studies of intact red cells.

25.(U) 82-10 - 83 09 a) Seven of 12 pairs of study subjects have completed hypobaric chamber studies, and preliminary data has been acquired concerning altitude related changes in in-vivo sickling, pulmonary blood flow, arterial SaO_2, splenic sequestration of ^{51}Cr-labeled RBC, plasma factor VIII levels, RBC cell density and size, urinary concentrating capacity. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sept 83.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

Project 3M161102BS10: RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 231 Studies of Military Personnel with Sickle Cell Trait (SCT)

Investigators LTC John A. Kark, MC; LTC Daniel G. Wright, MC
MAJ James Canik, MC (AFIP); LTC David Posey, MC (AFIP)

Description

Individuals with sickle cell trait (SCT) may now enter previously restricted MOS which involve exposure to hypoxia and other stresses that can cause intravascular sickling in people with SCT and have been infrequently reported to cause medical complications. The frequency and severity of complications in SCT individuals going through usual Army aviation, high altitude parachute, and deep sea diving training and missions is unknown. It is possible that previous infrequent reports of such complications reflected a small percentage of individuals more susceptible to such complications than the usual person with SCT. It is important to be able to identify such a subgroup of SCT individuals at greater risk. Better understanding of events leading to deformed sickled cells and vascular obstruction by sickled cells is needed in order to develop definitive subclinical tests for the risk involved in hypoxia alone or with other stresses.

Army missions involve a unique requirement for performance in specialized military environments which cause hypoxia, at least equivalent to an altitude of 14,000 to 15,000 feet, at times combined with heat exposure, dehydration, and exertional stress. These requirements are evident for Army aviation, high altitude, high altitude parachute corps, and deep sea diving. Entry of individuals with SCT (7-10% of American Blacks) into these duties poses the immediate problem of determining the medical risks resulting from the potential for intravascular sickling, and the impact of such risks on the missions. Required information does not exist to specify appropriate medical regulations. Laboratory tests which could be used to identify the level of risk for given environmental conditions are needed.

Hypoxic exposures in an High Altitude Chamber will be induced in graded levels, to reproduce the aviation training protocol in a prospective, controlled study of the susceptibility of SCT aviator candidates to subclinical and/or clinical complications of hypoxia. Later, these physiologic studies will examine exercise, heat and dehydration, and combination of these stresses with hypoxia, as needed to reproduce mission conditions. Special techniques will include ultrastructural studies of reversible and irreversible sickled cells, studies of survival and splenic sequestration of autologous 51-Cr-RBC, and special studies of renal and pulmonary physiology. Well studied SCT individuals and controls will be stratified by level of SCT-complications, and will serve as a source for intensive study of red cell characteristics which might predict susceptibility to vascular complications of sickling. NMR will be used to measure extent to Hb S polymerization and ATP levels of intact RBC. Changes in RBC density distribution, using a Percoll gradient, RBC size distribution, using a Coulter channellyzer, hemoglobin binding by RBC membranes, cation and anion permeability, filterability, and adherence of RBC to endothelial cultures in response to controlled hypoxia in tonometers will be correlated with the clinical stratification.

Progress

1. Study of altitude chamber training for individuals with Sickle Cell Trait (SCT).

A preliminary study of ear oximetry in normal volunteers exposed to various degrees of altitude - simulated hypoxia in a hypobaric chamber was completed and has defined normal values for this measurement. Substantial progress was made in completing the protocol "A Study of Altitude Chamber Training for Individuals with Sickle Cell Trait". The original plan was to study 10 pairs of subjects, and seven pairs have been studied in FY 1983. This collaborative effort, administered by LTC Kark, has involved 3 Depts at WRAIR, a Div. at AFIP, and five Divisions or Departments at WRAMC. Analysis of all data obtained on these study subjects is incomplete. Nonetheless, preliminary data analysis indicates that (1) in vivo sickling in SCT individuals is trivial at ground level (less than 0.2%), but increases to a maximum of 2-5% at altitudes of 14,000 to 18,000 feet following about 15 min. of hypoxia. More severe hypoxia for shorter time periods at 20,22,and 25 x 10^3 ft. produces declining sickle cell counts to about 0.2% for the shortest flight (5 min at 25,000 ft.), indicating that time of exposure to hypoxia above 14,000 is the most important determinant for the accumulation of sickle cells and any increased risk of vascular obstruction associated with sickling. As expected SaO_2 values an altitude were lower for HbAS subjects (4 mm Hg), but such differences are unlikely to be physiologically important. The only significant change in orgain function related to hypoxia and HbAS was the occurrence of diffuse small patches of non-perfused areas in the lung in scans made post-hypoxia. This alteration in perfusion pattern does not necessarily imply any pathologic change, and, indeed, individuals who demonstrated this change all had normal Stage I incremental exercise tests and other pulmonary function tests. Data for RBC survival and splenic sequestration of RBCs (using 51-Cr-label) have not been fully analyzed to date, although no gross abnormalities have been evident in SCT subjects on inspection of individual data Plasma values for Factor VIII activity, a coagulant protein released by endothelial cells, appears to increase for all individuals exposed to hypoxia, with greater increments at intermediate altitudes for those with HbAS who had lung perfusion changes. Differences in cell density and size profiles and rates of in vitro sickling were noted for participants with HbAS, but have not yet been correlated with clinical data. All individuals with HbAS had reduced maximum urinary concentration, but this remained stable with hypoxic exposure and hematuria was not induced by altitude exposures.

2. Study of sudden unexpected deaths among recruits during basic combat training.

An epidemiologic survey of sudden unexpected death (SUD) of recruits with and without sickle cell trait (SCT) in basic combat training (BCT) was continued in collaboration with AFIP (principal investigator, David Posey, LTC). All but 4 local bases have been visited, and increased differences in the population corrected death rate for recruits in all military forces for 1977-1981 for SCT recruits versus non-tested recruits has continued to be documented. This important observation requires a review of spleen pathology and completion of the survey of local pathology reports before final results can be summerized and conclusions reached. Nonetheless, results at present

indicated that there is a 20 to 40 fold increase in corrected rate of SUD for SCT recruits during BCT compared with non-tested recruits (e.g. those not known to have SCT).

3. Basic stdies on sickle hemoglobin

(1) Studies have been completed which define the ability of pyridoxal phosphate to enter Hb S containing red cells more rapidly than normal red cells, to react with HbS and to inhibit sickling by a mechanism different from pyridoxal (previously described by our laboratory). This agent has been shown to improve oxygen-transport in red cells, in contrast with most protein-modifying antisickling agents. This principal intracellular form of vitamin B_6 has been found to have very low toxicity in animal studies, as well as in its clinical use in humans. (2) Initial methods are being worked out in a human study "Anion permeability of sickle cell trait red cells" (WRAMC #9021-82) and an animal study "Survival of pyridoxal-hemoglobin in the circulation" (WRAIR primate protocol #014-81) for the potential use of pyridoxal phosphate as an anti-sickling agent. (3) A case study of rare Hb H disease in a Black Army man has led to the "Identification of a rare alpha-thalassemia-1 deletion". Also, a case study of an aviator with a rare Hb variant combined with Hb S has led to the recognition that such a combination of variant Hb S is equivalent to sickle cell trait for purposes of determining his participation in aviation.

4. Consultations

Consultation has been provided on the risks of aviation for individuals with sickle cell trait for the Aeromedical Consultation Advisory Panel, at Ft. Rucker, Al. Consultation concerning the medical risks for military members with SCT has also been provided to TSG, to the Hematology Consultant to TSG, to the Preventive Medicine brand, OTSG, and to USAMRDC. In addition, consultation has been provided for the drawing up of a ten year plan for research and management of medical problems related to sickle cell trait in the US Army, at the request of TSG and MEDCOM.

Future Plans

1. Completion of studies outlined above. 2. Initiation of studies of operational flight hypoxia for sickle cell trait aviators compared with controls. 3. Field study of NATO altitude training for SCT aviator candidates/aviators. 4. Prospective study of SUD in BCT and in other military training: possible relation to non-fatal admissions with rhabdomyolysis. 5. Survey of biophysical techniques which might be applied to a practical test to identify individuals at risk for complications related to in vivo sickling from HbAS.

Abstracts

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2. Kark, J.A. and Tarassoff, P.G. Pyridoxal phosphate as an antisickling agent in vitro. Proceedings of the N.I.H. Workshop on Development of Therapeutic Agents for Sickle Cell Disease. p. 18, May, 1983. Presented May 17, 1983.

3. Embury, S.H., Monroy, G.C., and Kark, J.A.: Identification of the rare α -thalassemia-1 deletion in the Black population. Blood (in press), 1983.

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1.* Kark, J.A., Bongiovanni, R., Hicks, C.U., Tarassoff, P.G., Hannah, J.S., Yoshida, G.Y.: Modification of intracellular hemoglobin with pyridoxal and pyridoxal 5'-phosphate. Blood Cells 8: 299-314, 1982.

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6.* Howe, E.G., Kark, J.A., Wright, D.G. Studying sickle cell trait in healthy Army recruits: should the research be done? Clin Res 31: 119-125, 1983.

7.* Kark, J.A., Tarassoff, P.G., Bongiovanni, R. Pyridoxal phosphate as an antisickling agent in vitro. J Clin Invest 71: 1224-1229, 1983.

8. Kark, J.A., Haut, M.J., Schecter, G.P., McQuilken, C.T., Duffy, T.P., and Vigersky, R.A. The biochemical response to vitamin B_6 in sideroblastic anemia (RSA). I. Pyridoxal 5'-phosphate concentrations in plasma and erythrocytes. (in review), 1983.

9. Kark, J.A., Haut, M.J., Schechter, G.P., Hicks, C.U., Duffy, T.P., and Vigersky, R.A. The biochemical response to vitamin B_6 in refractory sideroblastic anemia (RSA). II. Pyridoxal 5'-phosphate metabolism by erythrocytes. (in review), 1983.

* Listed as "in press", FY82 annual report.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300402	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DOWN/MISTR'N	9A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AI	235 WWP3
b. [illegible]				
c. [illegible]				

11. TITLE (Procede with Security Classification Code)

(U) Ultrastructural Study and Definition of Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	Continuous	DA	C. In House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	2.0	250
	CURRENT 84	3.0	251

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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20. GENERAL USE

FOUO

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Hase, T

NAME: Sharpnack, D D

21. KEYWORDS (Procede each with Security Classification Code)

(U) Host-Parasite Relationship; (U) Pathogenesis; (U) Ultrastructural Damage; (U) Repair; (U) Toxin; (U) Trauma

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Procede each paragraph identified by number. Provide end of each with Security Classification Code.)

23(U) To study and define the ultrastructural bases of the pathogenesis of various injuries and diseases of military importance. Of particular interest are the structure and function of organs participating in oral immunity production, the nature of surface proteins of protozoans as related to vaccine development, injury to the gastrointestinal system by Shigella toxin, and repair of tracheal injuries.

24(U) Conventional ultrastructural techniques, including transmission and scanning electron microscopy, as well as enzyme histochemistry, immunochemistry, negative staining, and autoradiography.

25(U) 82 10 - 83 09 Morphologic characteristics of gut immune organs were studied to help understand functional considerations which effect mucosal immunity. A technique to process small numbers of Trypanosomes for electron microscopy has been devised and will be used to help localize surface antigens. Surface characteristics of tracheal epithelial repair were characterized by scanning electron microscopy and will aid in evaluation of tracheal damage caused by blast overpressure. A new theory of rickettsial life cycle has been forwarded, based on morphologic studies. For technical report see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83

(Available to contributing user offices only)

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, MAR 69 FOR ARMY USE ARE OBSOLETE

Project 3M161102BS10 RESEARCH ON MILITARY DISEASES, INJURY, AND HEALTH HAZARDS

Work Unit 235 Ultrastructural Study and Definition of Diseases of Military Importance

Investigators:
Principal: Wallace B. Baze, MAJ, VC
Kevin P. Keenan, MAJ, VC
Tatsuo Hase, MD
Douglas D. Sharpnack, CPT, VC
Assistants: Eugene F. Bernard
Edward A. Asafoadejei

Description:

The primary purpose of this work unit is to define histopathologic manifestations of experimentally induced injury or disease which present current or potential problems to military personnel. Of particular interest are those aspects of injury or disease which are best studied at the ultrastructural level. A multidisciplinary approach, including conventional histology, histo-and cytochemistry, autoradiography, immunocytochemistry, and scanning and transmission electron microscopy, is employed.

This work unit also serves as a central electron microscopy facility for the WRAIR, offering services to include processing of tissues, electron microscope use (scanning and transmission), and photomicrograph processing. Various special techniques, such as negative staining and immunocytochemistry, are also available.

Progress:

I. Studies on Mucosal Immunity

The surface of gut associated lymphoid tissue (GALT) is exposed to a wide variety of antigens and microbes. The surfaces of various rabbit GALT to better understand their roles in mucosal immunity were studied. Appendix (APP), ileal Peyer's patch (PP), sacculus rotundus (SR), and the cecal lymphatic patch (LP) were obtained from adult rabbits and processed for scanning electron microscopy. All of the GALT examined consisted of both lymphatic domes and some form of intervening villi or mucosal ridges. Each tissue was studied for villus structure and degree of exposure of lymphatic domes to the gut lumen. The APP had a broad, mushroom shaped mucosal covering which arose from a narrow stalk between the domes. The luminal surface appeared as a flat sheet with regularly spaced holes through which the dome apices could be seen. The apices of the APP domes were approximately

the height of the mucosal covering. The PP had domes interspersed between cylindrical villi. The villi were 250-300um above the level of the dome apex, but did not prevent visualization of the domes from the luminal surface. The SR had a mucosal covering which was indistinguishable from the mucosa of the cecum. Low mucosal ridges encircled openings through which the domes, which were 100-150um below the level of these openings, were only occasionally visible.

The serosal surface of the SR was continuous with a small area of cecum here referred to as the cecal LP. A 1mm wide strip of villi and mucosal ridges at the ileocecal junction divided the mucosal surface of the LP and SR. The LP had single mucosal ridges separating the lymphatic domes. The apices of the domes were equal to or greater than the height of the intervening ridges. Of the GALT examined, the domes of the SR were most shielded by mucosal structures while those of the adjacent cecal LP were most exposed to the lumen. The functional significance of these differences has yet to be determined.

II. Immunocytochemical Studies on Trypanosoma rhodesiense

Ultrastructural studies of Trypanosomes, especially the metacyclic form, require the ability to process very low numbers of cells for electron microscopy. A technique which utilizes 96-well microtite plates as a mold for embedding has been devised. The flat bottoms of the wells are coated with poly-L-lysine to aid the adherence of organisms. A centrifuge bucket for the entire plate is used, making possible simultaneous concentration of the cells in the entire 96-well plate. The embedding process is carried out directly in the wells, providing a single layer of embedded cells at the bottom which can then be thin sectioned for immunocytochemistry and transmission electron microscopy. These samples are now being used to label surface antigens with gold, ferritin, and horseradish peroxidase.

III. Studies on Tracheal Injury

This work unit has been investigating the cytodynamics of respiratory epithelial repair after mechanical injury in conjunction with ongoing WRAIR investigations on the effects of free field artillery blasts on operator personnel. Previous reports have described and quantified, at the light and transmission electron microscopic levels, the morphologic and kinetic events that occur during regeneration following mechanical wounding of the hamster trachea. Studies have now been completed which define the surface characteristics of this repair process with the scanning electron microscope. Epithelial sliding, squamous metaplasia, and ciliogenesis have been documented and are in agreement with the previous histologic studies. The results will aid interpretation

of field studies of blast overpressure-induced tracheal injury and repair being conducted at the Lovelace Inhalation Toxicology Research Institute, Albuquerque, N.M.

IV. Studies on the Life Cycle of Rickettsia tsutsugamushi in the Mammalian Cell

Rickettsial infections exist in certain regions of the world as endemic diseases. Therefore a massive outbreak of a rickettsial disease in a particular region is possible in a military operation when large numbers of unimmunized people enter the region under a stressful circumstance; this happened in Napoleon's army in Russia with epidemic typhus, and in allied and Japanese forces in the Southeast Asia with Scrub typhus.

Members of the genus Rickettsiae are conceived as being obligatory intracellular bacteria; however, little is known about their life cycle within host cells.

Recently, we have accumulated evidence which suggests that organisms of R. tsutsugamushi multiply in tissue culture in a cytoplasmic matrix of host cells, somewhat similar to viruses. [1, 2] Accordingly, further investigations are in progress, emphasizing the following aspects: (a) to isolate and define the particles which carry and transmit rickettsial genomes among host cells in tissue culture, since R. tsutsugumushi are not often viewed in binary fission. (b) to investigate the applicability of the novel multiplication pattern of R. tsutsugamushi in tissue culture to the infection of mammalian hosts. The purpose of the investigations is (a) to clarify the pathogenesis of scrub typhus in the mammalian host area (b) to formulate better preventive and therapeutic measures for the disease.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2532	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORG'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AF	236 WWGS
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	STOG 82/83-6 12/3			

11. TITLE: (U) Immune Mechanisms in Leishmaniasis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	4.0	279
	CURRENT 84	4.0	312

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
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ASSOCIATE INVESTIGATORS
NAME: Nacy, C A
NAME: POC: DA

21. GENERAL USE
Foreign Intelligence Considered

22. KEYWORDS (Provide Each with Security Classification Code)
(U) Immunity; (U) Leishmaniasis; (U) Tropical Medicine; (U) Macrophages

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) The objective is the elucidation of the mechanisms responsible for host destruction of leishmania during active disease or secondary challenge of immunized animals. This information will have a direct bearing on the feasibility of immunization against the disease and will provide methods for assessing immunity. Leishmaniasis extends throughout the tropics on every continent except Australia and is a threat to military operations. U.S. troops are contracting disease during training operations in Panama.

24 (U) The approach will be to identify genetically controlled host defense mechanisms that correlate with resistance to infection with Leishmania tropica in experimental animals.

25 (U) 82 10 - 83 09 We identified several mouse strains with macrophage defects in lymphokine activation for intracellular destruction of the parasite (BALB/c, C57L/J, NZW/N, P/J). These defects correlated with in vivo susceptibility to L. tropica infection: of 20 mouse strains infected with amastigotes, only 4 strains (BALB/c, C57L/J, NZW/N, P/J) failed to resolve their cutaneous lesions in 12 wks. F1, F2, and back-cross analysis of macrophage function in P/J (susceptible) by C3H/HeN (resistant) mice suggests that control of intracellular killing activity is by a single autosomal dominant gene; .. tropica infection in these mice is also controlled by a single autosomal dominant gene. We are now assessing the correlation between these two genes. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

Available to contractors upon originator's approval

DD, FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M161102BS10 RESEARCH ON MILITARY DISEASES, INJURY AND HEALTH HAZARDS

Work Unit 236 Immune Mechanisms in Leishmaniasis

Investigators:

Principals: Carol A. Nacy, Ph.D.
LTC Wayne T. Hockmeyer, MSC

Associates: MAJ David S. Finbloom, MC
MAJ W. Ripley Ballou, MC
Mrs. Anne H. Fortier

Problems and Objective:

The Leishmania are obligate intracellular protozoan parasites that replicate only within macrophages in mammalian hosts. The successful resolution of cutaneous lesions or systemic disease relies ultimately on the intracellular destruction of parasites by infected macrophages. The major interest of our laboratory is the documentation of parasite interactions with resident and inflammatory macrophages, and the analysis of changes induced in these interactions following nonspecific activation of macrophages by soluble lymphocyte products (lymphokines).

Progress:

To analyze parasite-host cell interactions in vitro, we developed a model assay with Leishmania tropica amastigotes and resident peritoneal macrophages of C3H/HeN mice. The parasite infects and replicates in these cells: infected macrophages maintained as nonadherent peritoneal cell cultures support 6 to 10-fold increases in the number of intracellular amastigotes over 72 hr (1). Addition of lymphokines to these cultures dramatically alters parasite-macrophage interactions, and lympokine-treated macrophages develop two potent antileishmanial activities (2).

Expression of the two microbicidal activities of lymphokine-activated macrophages is under separate genetic control. We identified several mouse strains with macrophage defects in either lymphokine-induced resistance to infection (A/J, C3H/HeJ, C57BL/10SnCR) or intracellular killing (BALB/c, C57L/J, NZW/N, P/J. One

of these defects, intracellular killing, correlates with in vivo susceptibility to L. tropica infection: of 20 mouse strains infected with L. tropica amastigotes, only 4 strains (BALB/c, C57L/J, NZW/N, P/J) fail to resolve lesions in 12 wks (3,4). In susceptible mice, the L. tropica infected footpad becomes necrotic, visceral metastases occur, and the mice die of systemic disease. F_1, F_2, and backcross analysis of macrophage function in P/J (susceptible) by C3H/HeN (resistant) mice suggests that control of macrophage intracellular killing activity is by a single autosomal dominant gene (5). Resistance to L. tropica infection in these mice also appears to be controlled by a single autosomal dominant gene: if regulation of macrophage activation and resistance to L. tropica infection is controlled by the same gene, we will have identified a major host defense mechanism that can be amplified by immunologic intervention.

That genetic control of L. tropica infections is more complex than previously thought has been underscored by recent studies we performed in collaboration with Dr. M. Potter (6). Using several different inbred mice, F_1 hybrids, and a congenic strain developed by Dr. Potter with the Lsh^r gene from DBA/2 mice (L. tropica resistant) on a BALB/c (L. tropica susceptible) background, we have been able to identify three different genes that regulate resistance to L. tropica infection. One of the genes controls resolution of the cutaneous lesion: the other two genes regulate events that influence systemic disease. None of these genes is the Lsh gene that confers resistance in mice to systemic L. donovani. By appropriate mating of resistant and susceptible mice, we should be able to isolate each of these genes and identify additional immunological factors that determine successful resolution of disease. These animals will be invaluable tools for analysis of host defense mechanisms that develop during different stages of disease, as well as assessment of candidate vaccine and immunotherapeutic regimens.

Recommendations:

Problems associated with the control of leishmaniasis that will be investigated are: 1) evaluation of host-parasite interactions in vitro and modulation of these interactions with soluble products of immune lymphocytes. 2) evaluation of nonspecific and specifically sensitized lymphocyte products for immunoprophylactic/therapeutic and diagnostic potenial. 3) development of in vitro methods for assessing immunity, and 4) evaluation of nonspecific immunopotentiating agents for control of leishmanial infection.

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2. Nacy, C.A., M.S. Meltzer, E.J. Leonard, and D.J. Wyler. J. Immunol. 127:2381-2386 (1981).

3. Nacy, C.A., A.H. Fortier, M.G. Pappas, and R.R. Henry. Cell. Immunol. (in press, 1983).

4. Ballou, W.R., J. Moe, and C.A. Nacy. Presented at 1982 Ann. Meeting Trop. Med. Hyg., manuscript in preparation.

5. Fortier, A.H., M.S. Meltzer, and C.A. Nacy. Fed. Proc. 42 (1983).

6. Nacy, C.A., A.H. Fortier, J.S. Wax, and M. Potter. Fed. Proc. 42 (1983).

Formal Presentations:

1. Ballou, W.R., A.H. Fortier, J. Moe, and C.A. Nacy. Defective macrophage activation for intracellular killing in C57L/J mice: Characterization of in vivo responses to Leishmania tropica. Presented at 1982 Annual Meeting of the Tropical Medicine and Hygiene Society, Cleveland, OH, Nov 1982.

2. Hoover, D.L., C.A. Nacy, and R.W. Gore. Use of frozen amastigotes of Leishmania tropica to assay microbicidal activity of macrophages from C3H/HeN mice. Presented at 1982 Annual Meeting of the Tropical Medicine and Hygiene Society, Cleveland, OH, Nov 1982.

3. Nacy, C.A., "Cell-mediated immune responses and killing of leishmania within macrophages" in the Symposium on Host Responses to Protozoan Parasites: Developments and Prospects. Annual Meeting of the Tropical Medicine and Hygiene Society. Cleveland, OH, Nov 1982.

4. Nacy, C.A.: "Inductive signals leading to macrophage activation" in the minisymposium, Macrophages as Effector Cells. Annual Meeting of the Federation of American Societies for Experimental Biology., Chicago, IL, April 1983.

5. Nacy, C.A. "Monocyte and lymphocyte function in immunity to parasitic infections" in the Symposium on Regulatory Mechanisms Governing Resistance to Parasitic Infections at the Canadian Federation of Biological Sciences, Ottawa, Canada, June 1983.

6. Nacy, C.A. "Activation and suppression of macrophage intracellular destruction of Leishmania tropica amastigotes". Presented at the Gordon Conference on Biochemistry and Immunology of Parasites, Plymouth, New Hampshire, August 1983.

7. Nacy, C.A. "Lymphokine signals that regulate transient cytotoxic responses of macrophages against intracellular and extracellular parasites". Presented at the Gordon Conference on Biochemistry and Immunology of Parasites, Plymouth, New Hampshire, August 1983.

8. Fortier, A.H. "Genetic approach to analysis of macrophage function in vivo". Symposium presented at Litton Bionetics, on Genetic Influences on Macrophage Function, Rockville, MD, September 1983.

9. Nacy, C.A. "Genetic defects in macrophage function facilitate analysis of steps involved in macrophage activation to kill tumor cells and parasites". Symposium presented at Litton Bionetics on Genetic Influences on Macrophage Function, Rockville, MD, September 1983.

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Published Papers:

1. Nacy, C. A., E. J. Leonard and M. S. Meltzer. 1982. Role of activated macrophages in resistance to rickettsial infections. In Phagocytosis Past and Future, M. Karnovsky and L. Bolis, Eds., Academic Press, New York, p. 475-504. (October).

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6. Nacy, C. A., M. S. Meltzer, E. J. Leonard, M. M. Stevenson, and E. Skamene. 1982. Activation of macrophages for killing of rickettsiae: analysis of macrophage effector function after rickettsial inoculation of inbred mouse strains In: Host Defenses to Intracellular Pathogens, T. K. Eisenstein, H. Friedman, P. Actor, Eds., Plenum Press, New York, pp. 441-459 (December).

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20. C. N. Oster and C. A. Nacy. 1984. Macrophage activation to kill Leishmania tropica; Kinetics of macrophage response to lymphokines that induce microbicidal activities against Leishmania tropica amastigotes. J. Immun. (In press).

21. Hoover, D. L. and C. A. Nacy. 1984. Macrophage activation to kill Leishmania tropica amastgotes: Defective intracellular killing of amastigotes by macrophages elicited with sterile inflammatory agents. J. Immunol. (In press).

22. Nacy, C. A. and M. S. Meltzer. 1984. Macrophages in resistance to rickettsial infection: protection against lethal Rickettsia tsutsugamushi infections by treatment of mice with macrophage activating agents. J. Reticuloendothel. Soc. (In press).

23. Hoover, D. L., M. Berger, C. A. Nacy, W. T. Hockmeyer, and M. S. Meltzer. 1984. Killing of Leishmania tropica amastigotes by factors in normal human serum. J. Immunol. (In press).

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PROJECT 3M161102BS11

MEDICAL CHEMICAL DEFENSE SCIENCE BASE/MECHANISMS
OF ACTION OF CHEMICAL AGENTS AND ANTIDOTES

1. DATE PREV SUMRY	2. KIND OF SUMMARY	3. SUMMARY SCTY	4. WORK SECURITY	5. REGRADING	6a. DISP'N INSTR'N	6b. SPECIFIC DATA - CONTRACTOR ACCESS	7. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS11	EB	219 WWH4
b. CONTRIBUTING	61102A	3M161102BS10		
c. (CONTRIBUTING)				

11. TITLE (Enter title in unclassified letters. Classified title follows in parentheses.)

(U) Biochemical Aspects of Medical Defense Against Chemical Agents

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 002600 Biology 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE
b. NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM AMT
EXPIRATION

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR 83	7.0	497
FISCAL YEAR 84	7.0	531

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21. GENERAL USE

FINA

ASSOCIATE INVESTIGATORS
NAME: Brown, M D
NAME: Olenick, J G Wolfe, A D POC: DA

22. KEYWORDS (Provide each with security classification code)

(U) Organophosphates; (U) Antidotes; (U) Acetylcholinesterase; (U) Active Sites

23. (U) The objectives of this work unit are: (1) to provide the U.S. Army Command with a safe and effective prophylactic/therapeutic nerve agent antidote which will be efficacious against a variety of chemical agents, (2) to determine the pharmacokinetics, distribution, transport, metabolism, and enzymology of both chemical and antidotal agents; (3) to investigate metabolic and degradation products of chemical and antidotal agents including their identification, synthesis, and quantitation, (4) to determine biochemical systems and components which interact with either chemical and/or therapeutic agents, and (5) to develop more specific and simple methodologies for conducting the prescribed studies.

24. (U) A multicomponent anticholinergic antidote has been formulated and tested for its stability over an extended time period. High performance liquid chromatography (HPLC) methodology has been developed for separation of the oxime HI-6 from all of its degradative by-products. Kinetic studies showing reaction rates as functions of pH and temperature were performed to develop the basis for this new multicomponent nerve agent antidote. Enzymatic studies were performed showing the influence of aprophen on enzymes, and the effect of enzymes on aprophen. Unknown metabolic or enzymatic products are being analyzed to establish metabolic pathways.

(U) The stability of HI-6, was determined in various aqueous solutions, and in solutions containing selected organic and mineral acids used to modify pH. The metabolic fate of (^{14}C) aprophen was determined. More than 24 metabolites were detected in various organs of rats and guinea pigs. Three new acetylcholinesterases were purified; monoclonal antibodies to selected enzymes were developed; tryptic digests of organophosphate labelled enzymes were analyzed by HPLC. Aprophen was found to be hydrolyzed by esterases, including human butyrylcholinesterase (BuChE). For technical report see WRAIR Annual Progress Report 1 Oct. 82 - 30 Sept 83.

DD FORM 1498

PROJECT: 3M161102BS11 MEDICAL CHEMICAL DEFENSE SCIENCE BASE/MECHANISMS OF ACTION OF CHEMICAL AGENTS AND ANTIDOTES

WORK UNIT: 219 Biochemical Aspects of Medical Defense Against Chemical Agents

INVESTIGATORS:

Principal: Bhupendra P. Doctor, Ph.D.

Associates: Nesbitt D. Brown, M.S.; John G. Olenick, Ph.D.; Alan D. Wolfe, Ph.D.

Assistants: M. Judith Gemski, B.S.; R. Richard Gray, M.S.; SP4 Bobby Holmes; Leo Kazyak, B.S.; Rush, Robert, Ph.D.; SP4 Margaret G. Stermer-Cox; SP4 Jeff S. Verdier; John S. Rolston, Ph.D.

DESCRIPTION:

The objective of this work unit is to conduct multifaceted biochemical research on chemical agents in order to provide the military with a safe and effective prophylactic/therapeutic formulation against chemical agents. These include: the identification of the metabolites and degradation products of chemical agents and antidotes; the determination of the pharmacokinetics, distribution, transport and metabolism of chemical and antidotal agents; the investigation of the effects of chemical agents and antidotes on enzyme catalysis, specifically on active sites of acetyl- and butyrylcholines-tereases. The following investigations were conducted:

1. Prolonged Stability of HI-6 in An Aqueous Solution at Ambient Temperatures and Modified pH Conditions.

2. Pharmacokinetics and Distribution of Aprophen in Animal Models.

3. Aprophen Interactions with Selected Enzymes.

4. Purification of Selected Esterases and Cholinesterases.

5. Structural Analysis of Esterases and Cholinesterases.

A. Prolonged Stability of HI-6 in An Aqueous Solution at Ambient Temperatures and Modified pH Conditions.

Previous work has shown HI-6 or 1-(2-Hydroxy iminoethyl-pyridinium)-1-(4-carboxyamido-pyridinium) dimethyl ether to be highly unstable in various pH's and temperature gradients. As such, the enhancing qualities of this bispyridinium oxime to act as an effective reactivator against acetyl-cholinesterases poisioned by an organophosphate depends entirely on this compound to maintain its chemical integrity. From recent studies it has been shown that many substituted derivatives of HI-6 were obtained as breakdown products in both acid and alkaline solutions when pH's were greater than 5 and temperature exceeded 25°C. Utilizing data obtained from these studies, the multicomponent anticholinergic formulation has been prepared and shown to be extremely stable over a prolonged period of

time. A citrate buffered solution containing atropine sulfate, HI-6 and aprophen produced no degradative products when stored at ambient room temperatures and pH values of 4. Biweekly analysis of this mixed formulation, over a 46 week period, produced a 98% stability factor for each of the three neat compounds present within the solution. The stability studies of this bispyridinium oxime are currently being evaluated with other organic and mineral acids in hopes of determining the chemical factors involved in this mechanism.

B. Pharmacokinetics and Distribution of Aprophen in Animal Models.

The metabolic fate of aprophen has been investigated in experimental animals, specifically rats and guinea pigs. Data obtained from the pharmacokinetic and distribution studies showed that many metabolites were present in the various tissue and urine specimens analyzed. The ^{14}C labelled metabolic products formed exhibited both lipophilic and lipophobic charac-teristics. Major identified metabolites in all samples analyzed showed diphenylpropionic acid and diethylaminoethanol to be in predominance. At the same time, a series of other compounds were noted in the different extracts prepared from the various organs. In most cases, more than 10 radioactive metabolites were observed in rat brain, lung, liver, and urine samples. In kidney extracts, 24 major and minor peaks representing ^{14}C labelled analogues were observed. While metabolism of aprophen occurs rapidly in both rats and guinea pigs, usually one half the administered dose, data from these studies showed that radioactive aprophen metabolites were being excreted in the urine 43 days post administration. Final studies are being planned to identify the major metabolic products collected from each organ extract. Chemical identification will be accomplished by mass spectrometry.

C. Enzyme-Aprophen Interactions:

Benzilates are noted muscarinic receptor blockers, but hydrolysis by mammalian organ homogenates and by sera suggests they are also enzyme substrates. In order to identify enzymes potentially involved in benzilate catabolism, or influenced by benzilates, we studied the interaction between a model benzilate, aprophen, and selected, highly purified serine hydrolases. Aprophen preferentially inhibited human and horse butyrylcholinesterases (BuChE) in comparison with eel acetylcholinesterase (AChE). In addition, BuChE slowly hydrolyzed aprophen. Kinetic analysis showed aprophen inhibition of BuChE was mixed in mechanism with an apparent $K_i = 1.39 \times 10^{-7}$ M; aprophen hydrolysis occurred with a $K_m = 1.34$ uM. Other enzymes which hydrolyzed aprophen included a monomeric and a dimeric carboxylesterase (CE) from rabbit liver, and a horse serum CE. Eel AChE failed to hydrolyze the drug. All enzymes which hydrolyzed aprophen appeared to do so at similar rates when hydrolysis was calculated upon the number of active centers per enzyme.

A parallelism between BuChE inhibitors and muscarinic receptor blockers has been noted. To test this hypothesis further, 16 analogs of the alcoholic portion of quinuclidine benzilate were compared with respect to their BuChE inhibitory potency, and their ability to displace QNB from

muscarinic acetylcholine receptors. Both tests yielded the same six most effective compounds, although the order of effectiveness differed within the group of six. Thus a general parallel may exist, reflective of considerable binding site homology, but specific structural homology cannot be inferred.

D. Enzyme Purification:

An acetylcholinesterase from fetal calf serum has been purified approximately 18,000 fold to near homogeneity. It is hoped to use this enzyme as a model mammalian AChE. Purification procedures included procainamide affinity gel chromatography, DEAE anion exchange chromatography, and sepharose 4B gel chromatography. The molecular weight appeared to be 360,000 daltons. Enzyme specificity was acetylcholine > propionylcholine > butyrylcholine. Substrate inhibition occurred, atropine relieved this inhibition, and diisopropylphosphofluoridate (DFP) inhibited the enzyme. Monoclonal antibodies raised against DFP-torpedo AChE cross reacted with DFP treated fetal calf serum AChE to a limited extent, indicating both homology and difference between the DFP treated enzymes.

A soluble AChE from rabbit serum has been extensively purified. Purification steps included ammonium sulfate (27.5-60%) precipitation, phenyltrimethylammonium iodide affinity gel chromatography, and DEAE anion exchange chromatography. The partially purified enzyme was completely resolved from contaminating serine hydrolases, i.e., BuChE and CE. Substrate kinetics appeared similar to other AChEs; the apparent K_m for acetylthiocholine was 0.064 mM, and for phenylthioacetate, 0.68 mM. Aliphatic substrate specificity was acetylthiocholine > propionylthiocholine > butyrylthiocholine, indicating an acetyl (C2) specificity. The bimolecular inhibition constant, K_i for DFP was $1.4 \times 10^5\ M^{-1}L^{-1}$, agrees favorably with the reported value of $2.3 \times 10^5\ M^{-1}L^{-1}$ for eel AchE. The enzyme appears unusual among AChEs in that it dissociates into active dimers at pH 4. These dimers retain nearly identical kinetics compared to the enzyme. Substrate inhibition characteristic of AChEs also occurs.

A ChE from Pseudomonas aeruginosa has been purified to electrophoretic homogeneiety. Purification procedures included ammonium sulfate precipitation, DEAE anion exchange chromatography, and red A agarose gel chromatography. A molecular weight of 31,000 was derived from sodium dodecyl sulfate acrylamide gel electrophoresis. The enzyme has a dual specificity for acetyl- and proprionyl choline.

All cholinesterases tested were retained by Red A agarose, suggesting that this gel has a specificity for serine hydrolases. However, neither substrates nor inhibitors released the enzymes from the gel, thereby limiting the specificity.

E. Enzyme Structure.

Aspects of the structure of butyrylcholinesterase, and a

carboxylesterase have been investigated. Gel chromatography of pure, untreated BuChE yields a single product with a molecular weight of approximately 340,000 daltons. Upon incubation with sodium dodecyl sulfate (SDS), and subsequent electrophoresis, the purified enzyme splits into two bands, consistent with 20-25% monomer, and 80% dimer substituents. If the enzyme is incubated with SDS and a reducing agent, identical bands appear but the proportion of monomer to dimer is reversed. Current hypothesis holds that BuChE is composed of a dimer of dimers. Monomers are thought to be associated via disulfide bonds to form the dimer, whereas the dimers are thought to be held by noncovalent bonds to form the intact enzyme. This putative structure is inconsistent with electro-phoretic patterns in that the monomer should not result from SDS treatment, and the dimer should not persist through SDS-reduction treatment. However, similar electrophoretic peptide banding patterns are obtained upon BuChE monomer and dimer incubation with Staphylccoccus aureus protease V-8, leading to the conclusion that all bands may be derived from the same polypeptide. Thus current results may be consistent with theory if it is assumed that the extra monomer and dimer bands occur as the result of proteolytic cleavage which excises disulfide bond containing peptides.

Monoclonal antibodies to human BuChE have been raised. Approximately 30 positive clones have been detected, and the structure of BuChE is being analyzed.

The microheterogeneiety of a horse serum carboxylesterase (EC 3.1.1.1) has been studied. Purification of a horse serum carboxylesterase resulted in a product with a molecular weight of 70,200 daltons by sephadex gel chroma-tography, and of 126,000 daltons by paraoxon titration. This discrepancy was the subject of an investigation which revealed that the purified enzyme contained a catalytically active fraction which could bind DFP, and an inactive fraction which did not bind DFP. The inactive fraction also contained only 10% of the carbohydrate, whereas it constituted 40% of the protein by weight. Tryptic digestion followed by HPLC peptide analysis showed each electrophoretically obtained band to contain similar peptides, thereby proving the identity of the two bands. The absence of activity was not traced to any specific molecular change, and the molecular basis for the activity loss remains speculative.

F. Studies on the Molecular Aspects of the Biosynthesis and Disposition of Acetylcholinesterase.

Acetylcholinesterase is known to exist in two classes of molecular forms: elongated species which contain several catalytic subunits disulfide-linked to a collagen-like tail unit, and globular species which are primarily found as simple dimers or tetramers of the catalytic subunit. The elongated species are localized in the basal lamina of the synapse while the globular forms are sufficiently hydrophobic to expect that they associate readily with a variety of plasma membranes. Since both forms of enzyme in Torpedo are present in near-equal quantities, the opportunity exists to study the biosynthesis and disposition of each form in a tissue where the detailed structure of the gene product can be

examined. Towards this end, we have begun to characterize catalytic subunits from each form to ascertain whether the divergence in molecular species is the result of encoding by separate acetylcholinesterase genes or post-rranslational differences giving rise to assembled species of varying complexity.

Initial peptide characterization shows that the catalytic subunits contain considerable homology and the active site peptides appear identical. Candidates for peptides comprising the peripheral site have been identified with an azido analog of propidium. Both forms have similar carbohydrate compositions but at least two noncarbohydrate-containing peptides appear unique in each enzyme form. We have prepared monoclonal antibodies to each form. While most of the clones exhibited similar cross-reactivity, one shows at least an 80-fold selectivity for the catalytic subunit of the globular form. Thus, structural studies and immunologic reactivity suggest that the forms arise either as separate gene products or as distinct RNA transcripts. The biosynthesis of acetylcholinesterase has been examined using isolated m-RNA fractions and an in vitro translation system. Analysis of the nascent protein in relation to the structure of the molecular forms of acetylcholinesterase should provide fundamental information on the regulation of expression of the various molecular species of the enzyme.

Projected Studies

1. Experimental studies in animals and cultured cell lines which will show the effects of nerve agent poisoning in major biochemical pathways. Emphasis will focus on the neurotransmiters, such as acetylcholine, serotonin, epinephrine, norepinephrine and gamma amino butyric acid (GAbA).

2. Pharmacokinetic studies in model animals using HI-6 and aprophen which will show the mode of action of these drug in various receptors.

3. Continuation of the HI-6 study using various cells lines, which will produce some information on the metabolism of HI-6.

4. Purification of selected cholinesterases and esterases.

5. Characterization of selected cholinesterases and esterases.

6. Mapping of enzymes by monoclonal antibodies and other agents and techniques.

7. Delineation of a relationship between butyrylcholinesterase and the muscarinic acetylcholine receptor.

Publications

1. Baker, L., and Wolfe, A. D. Preferential butyrylcholinesterase inhibitors. 1983. Fed. Proc., P. 2112.

2. Chiang, P. K., Gordon, P. K., Brown, N. D., DeClercq, E. and Montgomery, J. A., A Novel Antiviral Agent that Interferes with Polyamine Biosynthesis. 1983. A.C.S. Meetings (Seattle, WA) Abstract.

3. Emery, C. E., Stancato, F. A., Brown, R. E., Prichard, D. A., and Wolfe,A. D. Arylmethanol and thiosemicarbazone influence on plasmodial macromolecular synthesis and cell stability. 1983. Life Sciences, 33 1285-1294.

4. Ralston, J. S., Main, A. R., and Gemski, J. The Stability of interchain disulfide bonds in serum butyrylcholinesterase. 1983. Fed. Proc. P. 2043.

5. Rush, R. S., Ralston, J. S., and Main, A. R. Physical characterization of a horse serum carboxylesterase (EC 3.1.1.1). 1983. Fed Proc., P. 2046.

6. Whaun, J. M., Brown, N. D., and Webster H. K., studies of Host-Parasite Interaction: Identify Biochemical Pathways for Chemotherapy Targets. 1983. U.C.L.A. Symposia Abstract.

7. Whaun, J. M., Brown, N. D., and Chiang, P. K., Antimalarial Activity of Methylation Inhibitors 36th Annual Meeting of the Society of Protozoologist. (New York) 1983 Abstract.

8. Taylor, P., Lee, S. Camp, S., Amitai, G., and Doctor, B. P., Studies on the Molecular Aspects of the Biosynthesis and Disposition of Acetylcholinesterase 1983. 2nd International Cholinesterases Meeting, Bled. Yugoslavia Abstract.

9. Doctor, B. P. and Taylor, P., Antigenic and Strucutal Differences in the Catalytic Subunits of the Molecular Forms of Acetylcholinesterase. 1983. 2nd Internation Cholinesterases Meeting, Bled. Yugoslavia Abstract.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6753	83 10 01	DD-DR&E(AR)636

3. PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. OBSN INSTR'N	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. PROGRAM ELEMENT CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	61102A	3M161102BS11	EA	221 WWJ4
XXXXXX STOG	61102A	3M161102BS10		

11. TITLE (Precede with Security Classification Code)

Neural Mechanisms of Chemical Defense-Related Compounds

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

300 Biochemistry 012600 Pharmacology 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

ES/N/EFFECTIVE:	EXPIRATION:
BER:	
E:	a. AMOUNT
OF AWARD:	f. CUM. AMT.

18. RESOURCES ESTIMATE

	PRECEDING/CURRENT	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	83	2.0	430
	84	2.0	387

19a. RESPONSIBLE DOD ORGANIZATION

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NAME: Meyerhoff, J L

POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Chemical Defense; (U) Chemical Interactions; ensory-Motor Processing; (U) Experimental Neuropathology

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

(U) Investigations are directed at understanding the effects on nervous system function of ical defense-related compounds, extrinsic and intrinsic. These studies have both direct and ect military relevance.

(U) Animal experiments are based on anatomic methods for locating critical sites of /antidote action; on pharmacologic and biochemical methods for elucidating interactions een agents/antidotes and the body's chemistry; and on physiologic methods for studying the ts of agents/antidotes on neural signal processing.

(U) 82 10 - 83 09 Macaque monkey brain tissue material from animals surviving ure to soman was processed according to the experimental silver impregnation methods and ard neuropathologic tissue stains. No unequivocal degenerating neuron axonal material was . Animals experiencing seizures were noted to have multiple bilateral brain infarcts. As :ts should produce degeneration on their own, technical factors may be responsible for the e to demonstrate it. Ethylcholine aziridinium (AF64A) injected bilaterally into the lateral icles of rats produced impairments in behavior on memory tests. Depletion of acetylcholine hippocampus and corpus striatum, and nonspecific damage to the fimbria-fornix. Injection F64A into the substantia nigra of rats produced damage at the injection site and a 50% tion of striatal dopamine. It is unlikely that this putative specific cholinergic neurotoxin such a role. Collaborative work on CSF changes in cat survivors of soman exposure were ued and new work on the respiratory centers of amphibians, a group of animals resistant to olinesterase agents, was begun. For technical report see Walter Reed Army Institute of rch Annual Report, 1 Oct 82 - 30 Sep 83.

No corrections upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project: 3M161102BS11 MEDICAL CHEMICAL DEFENSE SCIENCE BASE/MECHANISMS OF ACTION OF CHEMICAL AGENTS AND ANTIDOTES

Work Unit: 221 Neural Mechanisms of Chemical Defense-Related Compounds

Investigators:
Principal: C.B.G. Campbell, M.D., Ph.D., LTC, MC
Associate: C.F. Tyner, M.D., COL, MC; J.L. Meyerhoff, M.D.; J.M. Petras, Ph.D.; G.J. Kant, Ph.D.; W. Mobley, M.D., Ph.D., MAJ, MC

Objectives:

The overall objectives of this task are the protection of military personnel from the lethal effects of chemical agents, elimination or reduction of impaired performance, and return to duty of those individuals exposed to sublethal doses of these agents. Emphasis has been placed on determining the sites of action of chemical defense-related compounds, the primary and secondary effects of these agents and their antidotes on nervous tissue, and exploring the possibility of exploiting the intrinsic defense mechanisms of the body as protection against these agents.

Progress:

Last year it was shown that cats which survived exposure to soman developed patterns of neuronal degeneration in the brain, as well as bilaterally symmetrical infarcts in one case. Similar studies of macaque monkeys utilizing doses of 3.6 to 6.4 mg/kg were in a preliminary stage. During this year the monkey material has been studied with the light microscope. Only two animals which had survived soman-induced grand mal seizures showed evidence of brain pathology. This took the form of

multiple bilateral infarcts in various brain regions. No evidence of nerve cell axon degeneration was found. This was also true in areas affected by the infarcts where it would be expected. This suggests that technical factors might be responsible for the failure to demonstrate axonal degeneration. Even if brain damage in nonhuman primates and man results from vascular insufficiency secondary to seizures, this could be a significant problem for soldiers exposed to soman.

Two studies were conducted on ethylcholine aziridinium (AF64A), a putative specific cholinergic neurotoxin. In the first, AF64A was injected bilaterally in the lateral ventricles of rats. Animals were found to be impaired on tests of reference memory applied to place tasks, but not to cue tasks. Such patterns of behavioral change are known to result from damage to the fimbria-fornix. Neurochemical measurements in a separate group of animals showed depletion of acetylcholine in the hippocampus and corpus striatum, but no depletion of norepinephrine or dopamine. Histological examination demonstrated extensive damage to the fimbria-fornix. The behavioral and neurochemical results are probably due to non-specific lesion effects rather than a specific toxic effect on cholinergic systems. In the other study, AF64A was unilaterally injected into the substantia nigra of rats. Two weeks later a 50% depletion of striatal dopamine was found, as well as extensive damage at the injection site. AF64A is apparently capable of damaging dopaminergic neurons.

Recent work has shown that nerve growth factor (NGF) may have a trophic effect on the cholinergic system. Our interest centers on those cholinergic pathways which interconnect the cerebral cortex and the basal forebrain. A project to study the role of NGF in the cholinergic system and its possible implications for defense against anticholinesterase nerve agents has been initiated. Necessary equipment is on order and NGF has been synthesized. Collaborative work on cerebrospinal fluid changes in animal survivors following exposure to soman have continued. Experiments designed to identify the respiratory centers in amphibians have been initiated. Amphibians are more resistant to anticholinesterase agents than mammals, and it is hoped that these animals can be used as a model in which anticholinesterase effects on respiratory neurons can be studied.

Recommendations for Future Work:

Contingent upon the availability of local facilities for exposure of experimental animals to nerve agents, further studies on the neuropathologic effects of these agents are planned. We would like to repeat the exposure of macaques to soman; examine the effects of sarin in rats; and study the course of development of the changes observed in rats after exposure to soman. Experiments described above as being underway will be continued.

Presentations

Petras, J.M. Brain pathology induced by organophosphate poisoning with the nerve agent soman (submitted for presentation at the Army Science Conference).

Publications

Jarrard, L., Levey, R., and Kant, G.J. Behavioral and neurochemical effects of intraventricular AF64A administration in rats (submitted).

Petras, J.M. Brain pathology in rats induced by organophosphate poisoning with the nerve agent soman (in preparation).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OH 0383	83 10 01	DD-DR&E(AR)636

PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GMP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
10 01	D. Change	U	U		NL	YES ☒ NO ☐	A. WORK UNIT

CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	61102A	3M161102BS11	EB	227 WWIF
CONTRIBUTING	61102A	3M161102BS10		
PROGRAM (Incl. Security Classification Code)				

Chronic systemic Effects of Organophosphate Esters

SCIENTIFIC AND TECHNOLOGICAL AREAS

2600 Pharmacology 012900 Physiology

START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
05	CONT	DA	C. In-House

CONTRACT/GRANT		17. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
a. EFFECTIVE DATE	b. EXPIRATION DATE			
c. NUMBER	d. AMOUNT	PRECEDING FISCAL YEAR 83	1.5	95
e. TYPE OF AWARD	f. CUM. AMT.	CURRENT FISCAL YEAR 84	2.5	205

18. RESPONSIBLE DOD ORGANIZATION

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b. NAME: FEIN, H

22. EAL USE

reign Intelligence Considered

23. KEYWORDS (Provide EACH with Security Classification Code)

(U) Cardiac receptors;
) Organophosphate esters; (U) Anticholinesterase; (U) Cardiac biochemistry

24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Provide brief individual paragraphs identified by number. Provide end of each with security Classification Code.)

. (U) To determine the chronic systemic effects of low dose single or repeated posure of organophosphate esters. These studies will examine the effect of such ents and other cholinergic drugs on myocardial function at a molecular level. A cond objective is the examination of cholinergic stimulation on pituitary peptide rmone synthesis and secretion. There is military relevance in this research.

. (U) A small animal model will be used to produce sublethal injury using single d multiple dose exposure regimens. Cardiac toxicity will be examined by measuring rdiac enzymes involved in contractility and myocardial receptors. Pituitary udies will employ cell culture and a dispersed cell superfusion system.

. (U) A computer software program for data and graphics analysis has been mpleted. An assay for liver thyroid hormone receptors has been developed. In tro studies show inhibition of binding with physostigmine and atropine, but only high concentrations. The lung receptor system is partially completed, after ich the myocardial receptor assay will be established. A single dose (LD_{20}) DFP udy demonstrated a depression of weight gain at two weeks, an increase in rgan/body weight ratio for kidney and testes, with no change in liver or heart. At weeks, DFP rats were heavier than controls, but all four organ/body weight ratios re depressed. There were no consistent hormonal changes except a low serum T_3 at o weeks. A laboratory has been established to perform the cell culture and perfusion studies. For technical report see Walter Reed Army Institute of search Annual Progress Report, 1 Oct 82 - 30 Sep 83.

Refer to contractors upon enlistment's group.

FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

Project: 3M161102BS11 MEDICAL CHEMICAL DEFENSE SCIENCE BASE/MECHANISMS OF ACTION OF CHEMICAL AGENTS AND ANTIDOTES

Work Unit 227 Chronic Systemic Effects of Organophosphate Esters

Investigators:
Principal: Robert C. Smallridge, LTC, MC
Associate: Nancy E. Whorton, GS-11
Henry Fein, MAJ, MC
SP4 Irene Gist

This work unit was established to study the chronic systemic effects of organophosphate esters in a rodent model and on selected organ systems in vitro. The acetylcholinesterase to be used is diisopropylfluorophosphate (DFP). Studies are being performed in three areas.

1. Cardiac Studies: Acute poisoning with organophosphorus compounds can reduce stroke volume and cardiac output, and prolong the circulation time in man (1); large doses of DFP in rats may produce cardiac failure and death (2). DFP also decreases heart rate and contractility in the isolated rat heart, suggesting a direct toxic effect independent of cholinesterase inhibition (2). The mechanism of the deleterious effects of DFP on myocardial contractility is not clear, although an effect on ATPase may be involved as this agent may inactivate ATPase in other tissues (3,4). It is recognized that acute exposure to acetylcholinesterases may produce long-term injury in the central nervous system (CNS). Whether such damage may occur outside the CNS is unknown although one case of chronic congestive cardiomyopathy has been described (5).

Studies are focusing on the potential toxic effects of these agents on, and cholinergic regulation of, selected myocardial enzymes and receptors. The initial enzyme under study is 5'-monodeiodinase, an enzyme studied extensively in this laboratory (6) and which has recently been identified in the heart (7). Elucidation of the degradation pathway utilizing this enzyme was completed (8) during FY83. This required establishment of an HPLC system for confirmation, which consumed a considerable amount of time. The initial receptor for study will be the myocardial nuclear thyroid hormone receptor. In descending order of difficulty in methodologic development, heart > lung > liver. Liver binding studies have been performed and give excellent reproducible results. In vitro exposures to physostigmine and atropine sulfate inhibit binding in a dose dependent manner, but only at high concentrations ($> 10^{-3}$M). This may be a nonspecific ionic strength effect and requires further study. The lung receptor binding assay is partially characterized, but development was delayed considerably due to the aforementioned enzyme studies. Cardiac receptor studies will proceed after the lung

binding assay is established. In vivo toxicity studies will of necessity be delayed for some time.

2. Pulmonary studies: Central respiratory depression is the usual cause of death after acute exposure to organophosphate esters. The chronic effects of these agents on pulmonary function are unknown. Thyroid hormone receptors in the lung have been identified and partially characterized in our laboratory. Since this hormone influences the production of certain lung phospholipids and surfactant, this receptor assay will be more completely developed. It will then be applied to animal toxicity studies.

3. Hormone/metabolic studies: The anterior pituitary gland has cholinergic receptors, and several of its hormones are under cholinergic control (9-12). Serum levels of pituitary peptide hormones will be investigated after in vivo DFP exposure. A more critical examination of pituitary function will be performed by establishing pituitary cell cultures and a pituitary superfusion system (13) and studying the effect of cholinergic agents on the biosynthesis and secretion of these hormones.

A second hormone system to be studied is the renin-angiotensin axis, since the system is a major regulator of blood pressure. Specifically, angiotensin-converting enzyme is being studied. Preliminary studies in our laboratory have demonstrated cholinergic inhibition of this enzyme in vitro with physostgmine, and reversal of this inhibition with atropine. This enzyme has also been shown to be depressed in two human studies after acute surgical stress and after caloric restriction. Since both these conditions will be common in chemical defense casualties, knowledge of their effects on ACE and on blood pressure is extremely useful in our program for elucidating the cholinergic control mechanisms on this enzyme.

One in vivo DFP study has been performed. In this study, rats were administered an LD_{20} dose of DFP, and survivors (and their controls) sacrificed at either two, six, or 52 weeks. At two weeks there was a significant decrease in body weight and in the weights of liver, heart, and kidney, but not testes. By six weeks, body weight normalized, but organ weights remained low, and at 52 weeks the DFP rats were heavier than controls, with organ weights now being normal. When the organ to body weight ratios were calculated, they showed a rise in kidney and testes at two weeks, but a decrease in all four organs tested at 52 weeks. The early weight changes in heart, kidney, and testes were confirmed in a followup study. This latter study also employed an additional control group pair fed to match the food intake of the DFP treated rats. Similar changes were observed in both the DFP and pair fed groups, suggesting that the early weight changes were due to a generalized nutritional deficit and were not specific for DFP.

Future research plans include: (1) completion of thyroid hormone receptor assays in lung and heart, and the 5'-deiodinase enzyme assay in heart so that *in vivo* toxicity studies can be done; (2) development of pituitary cell culture and superfusion systems to explore the cholinergic regulation of anterior pituitary hormones; and (3) extension of *in vitro* observations of chilinergic/anticholinergic effects on angiotensin-converting enzyme activity to *in vivo* studies.

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1. Smallridge, RC, and NE Whorton, 3'-Monoiodothyronine metabolism occurs by deiodination in rat liver homogenates: Documentation by high pressure liquid chromatography. Presented at the 65th Annual Meeting of the Endocrine Society, San Antonio, TX, June 8-10, 1983 (Abstr #742).

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4. Smallridge, RC, and NE Whorton, 3'-Monoiodothyronine degradation in rat liver hemogenate: Enzyme characteristics and documentation of deiodination by high pressure liquid chromatography (submitted).

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13. Baker, JR, JR., YG Lukes, RC Smallridge, M Berger, and KD Burman, Partial characterization and clinical correlation of circulating human immunoglobulins directed gainst thyrotrophin binding sites in guinea pig fat cell membranes. Development of a direct enzyme immunoassay, J Clin Invest (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | DA OH 0169 | 83 10 01 | REPORT CONTROL SYMBOL DD-DR&E(AR)636

1. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORG'N INSTR'N	9a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

2. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS11	EC	232 WWGT
b. CONTRIBUTING	61102A	3M161102BS10		
c. CONTRIBUTING	STOC	[illegible]		

3. TITLE (Precede with Security Classification Code)

(U) Immunochemistry of Nerve Agents

4. SCIENTIFIC AND TECHNOLOGICAL AREAS

016800 Toxicology 012600 Pharmacology

5. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 04	CONT	DA	C. In-House

7. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

17. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	1.0	98
FISCAL YEAR CURRENT 84	1.0	149

8. RESPONSIBLE DOD ORGANIZATION

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9. GENERAL USE

Foreign intelligence considered

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NAME: Kaufman, B M

NAME: Seid, R, Jr

POC: DA

12. KEYWORDS (Precede EACH with Security Classification Code)

(U) Immunochemistry; (U) Soman; (U) Monoclonal Antibodies; (U) Nerve Agents

13. TECHNICAL OBJECTIVE, 14. APPROACH, 15. PROGRESS (Precede each with Security Classification Code.)

23. (U) Development of passive and active immunization for protection against nerve agents in humans. Nerve agents represent a serious threat to combat military personnel.

24. (U) Nerve agents such as Soman and Soman analogues will be convalently coupled to protein carriers such as tetanus toxoid, and adjuvants such as gram negative bacterial membrane proteins, detoxified gram-negative lipopolysaccharides. These vaccines will be tested in mice for potency and used for vaccination of mice to produce monoclonal antibodies. Human monoclonal antibodies of high affinity will be produced by in-vitro vaccination of human lymphoid cells followed by fusion to long term human myeloma lines. Human monoclonal antibodies will be tested for potency. Tissue culture techniques for large scale production of human monoclonal antibody for human use will be explored. Recombinant DNA approaches to large scale production of antibody for human use will be explored.

25. (U) 82 10-83 09 Utilizing chloro-GD protein conjugates 16 hybridomas have been found which produce monoclonal antibodies reactive with Soman. These hybridomas have been doubly cloned and ascites fluid produced. A synthetic Soman analogue replacing the Florine with a Methoxy group has been designed, synthesized and successfully coupled to protein conjugates utilizing an inert spacer at the Pinacolyl alcohol end of the molecule. (For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83).

* Available to contractors upon originator's approval

PROJECT 3M161102BS11 MEDICAL CHEMICAL DEFENSE SCIENCE BASE/MECHANISMS OF ACTION OF CHEMICAL AGENTS AND ANTIDOTES

Work Unit: 232 Immunochemistry of Nerve Agents

Investigators:

Prinicpal: Jerald C. Sadoff, M.D, COL, MC

Associates: Carter L. Diggs, M.D., COL, MC
Bennett M. Kaufman, Ph.D.
Robert Seid, Ph.D.

Problem

Development of immunoprophytoxins for nerve agents using hybridoma technology.

Progress

1. Fusions were performed using splenocytes from mice immunized Soman-Carrier conjugates. These conjugates were prepared at USAMRICD using chloro-GD and various carriers with linkage through the phosphorate moiety. Twenty two hundred hybridomas have been screened by ELISA for reactivity against Soman conjugated to heterologous carriers. Forty six stable hybridomas with reactivity in ELISA have been obtained and all have been cloned. Ascites fluid have been produced on ten of these. All of the hybridomas will be tested for the ability to bind SOMAN in a competitive ELISA assay at USAMRICD within the next two months.

2. The compound methyl, 3-amino, 1,2,2-trimethlypropyl methylphosphohate, a Soman analog with a free phosphorate group was designed at WRAIR and obtained by custom synthesis. This compound has been successfully coupled through the pinacohyl moiety to succinylated bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH). This conjugate has an inert spacer arm. This conjugate with free phosphonate groups in the hapten has a higher probability of eliciting an antibody directed against the active site of Soman than the currently available conjugates coupled to carriers through the phosphohate moiety. Mice have been immunized with these conjugates.

3. In-vitro stimulation systems for production of mouse and human monoclonals have been investigated. The mouse system requires the addition of supernatant from a mixed lymphocyte reaction utility mouse thymocytes. The human

system requires autologous serum, removal of suppressor T cells and supernatant from a mixed lymphocyte reaction between donor lymphocytes and irradiated heterologous lymphocytes. Cometidine coated plates which remove histamine receptor positive suppressor T-cells have been prepared and utilized on this system. In-vitro stimulation in a human system has been performed followed by fusion with the HFB-1 myeloma cell line. The hybridomas are currently being screened for protection of specific antibody. In-vitro stimulation currently appears to be the only feasible approach to production of human hybridomas against nerve agents.

Future Plans

1. Different conjugates are being produced by outside contractors. This conjugate will be used with differing immunization schedules to produce high affinity monoclonals

2. It is scheduled that mice will be given LD_{50} doses of Soman at USAMRICD. The surviving animals will then have their spleen removed. In-vitro stimulation with conjugates and with Soman directly followd by fusion will be performed. Direct fusion on surviving mice will be performed. The sera from surviving animals will be tested for activity against Soman to determine if Soman itself without conjugation is immunogenic.

3. The clones that we have obtained thus far will be tested for specificity of binding to Soman by inhibition with analogues. The affinities will be estimated and the highest affinity clones will be tested in mouse protection assays in collaboration with USAMRICD.

4. Human in-vitro stimulation will continue with Soman conjugates. Human clones will be tested in the same way as the mouse clones.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION: DA 300029	3. DATE OF SUMMARY: 83 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636

2. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS11	EB	233 WWGV
b. CONTRIBUTING	61102A	3M161102BS10		
c. (CONTRIBUTING)	[illegible]	[illegible]		

11. TITLE (Include with Security Classification Code):
(U) Nerve agent antidote screening with invertebrate bioassay systems

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 07	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING 83	1.0	75
FISCAL YEAR — CURRENT 84	2.0	102

19. RESPONSIBLE DOD ORGANIZATION
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NAME:

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Nerve agents; (U) Antidotes; (U) Musca domestica; (U) Atropine; (U) Pyradlioxamine chloride; (U) Sarin; (U) Soman; (U) Tabun; (U) VS

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Develop and evaluate arthropod bioassay systems for screening of potential chemical nerve agent antidotes. Identify the most promising systems, develop testing methodology and conduct comparative evaluations with existing mammalian systems. The development of effective protective measures against chemical warfare agents is a high priority project for the Army and the search for antidotes to the known chemical warfare nerve agents is an important part of that effort. Clearly, realization of the objectives in this work unit may result in development of several rapid, inexpensive arthropod bioassay screening systems for antidotes to CW nerve agents.

24. (U) Conduct a literature review to identify and prioritize the most promising insect systems. Establish new laboratory and insect rearing facilities to support this work. Identify laboratories equipped for CW nerve agent research and obtain permission to conduct research in their facilities. Establish selected insect colonies, develop to conduct research in their facilities. Establish selected insect colonies, develop and refine application procedures, testing methodology and statistical analysis criteriz. Conduct tests and evaluate results so that the most useful and effective screening systems can be brought on-line.

25. (U) 82 10 - 83 09 Renovation of laboratory and office space was completed. Colonies of Musca domestica were established and routine rearing procedures developed. A procedure for injecting M. domestica with test agents was developed and refined. Baseline dose-response data for DFP and atropine was developed with 5-day post pupal eclosion adult females. Hiring attempts to fully staff the project are being continued. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M161102BS11 MEDICAL CHEMICAL DEFENSE SCIENCE BASE/MECHANISMS OF ACTION OF CHEMICAL AGENTS AND ANTIDOTES

Work Unit 233 Nerve Agent Antidote Screening with Invertebrate Bioassay Systems

Investigators:
Principal: Donald R. Roberts, LTC, MSC
Associate: Robert A. Wirtz, CPT, MSC

Problems and Objectives

Development of defensive measures against chemical warfare nerve agents, classified as acetylcholinesterase (AChE) inhibitors (1,2) is a high priority Department of Defense medical research and development effort. AChE is the enzyme which breaks down acetylcholine, the primary neurotransmitter in the mammalian cholinergic nervous system. The blockage of AChE by nerve agents results in the continued presence of excess ACh. This causes repeated firing of target receptors on neurons, muscles and glands which leads to the disruption of neural communication and eventually death (3). Chemical warfare nerve agents with this suspected mode of action include sarin, soman, tabun and VX (1,2). These compounds are nonreversible inhibitors which rapidly and permanently bind to AChE. They also undergo unimolecular dealkylation, a process referred to as"ageing" (4,5). This ageing results in a nonfunctional enzyme-nerve agent complex which cannot be activated using traditional antidote therapy. The suspected mode of action of nerve agents is similar to that of the organophosphate insecticides, which are also nonreversible AChE inhibitors (3).

Therapeutic agents that are currently available are not completely satisfactory in saving life and in reducing physical and mental decrements. The search for new and more effective therapeutic and pretreatment drugs continues. Because of limitations in conducting efficacy studies in humans when one is dealing with organophosphorus nerve agents, testing is almost entirely carried out with in vitro studies or animal models.

As new antidotes or pretreatment drugs are proposed, it will be highly desirable to have a rapid inexpensive bioassay screening system available for drug development. Bioassay systems using intact insects have several distinct advantages over existing models. Most insect systems are rapid and relatively inexpensive with only small amounts of test material and nerve agent required. This can be especially important when candidate

antidotes are custom synthesized and available only in small quantities. The use of small amounts of nerve agent, determined on a ug/kg live body weight, is an added safety factor. Most assays can be brought on line quickly, as no complicated equipment or training are usually required. Costly animal and handling facilities and personnel can be eliminated as less expensive mass rearing procedures have been developed for the arthropods currently under consideration for use. The ability to use large numbers of test insects and/or large sample sizes makes these tests particularly applicable to statistical analysis.

The question as to the applicability of tests conducted on insects to mammalian systems is a valid one due to differences in major organ systems and detoxification, activation and transport mechanisims. However, similar modes of action of nerve agents are suspected in both insects and vertebrates and the presence of similar receptors, enzymes and metabolic systems supports this premise. Once test data are available results can be compared to those of existing noninsect bioassay models and in vitro data to determine the feasibility of using insect systems for antidote screening.

Progress

Considerable progress has been made during the last 12 months of this new-start project. Rooms 2073 and 2075, Building 101, Forest Glen Section, WRAIR, have been renovated to provide office and laboratory space for this project. Colonies of organophosphate susceptible and multi-resistant Musca domestica (house flies) were received from the USDA, ARS Laboratory, Gainsville, FL and routine rearing procedures have been established. A procedure for injecting M. domestica with test agents was developed and refined. Pilot studies using the susceptible fly strain indicate that the LD50 for diisopropyl fluorophosphate (DFP), a nerve agent simulant, is 0.4 to 0.8 mg/kg (7.8 to 15.6 ng/fly) in 5-day post pupal eclosion adult females. The LD50 for atropine sulfate is approximately 70 ug/fly, with surviving insects not resuming normal behavior until one hour or more after injection.

Progress to date, e.g. obtaining and renovating office and laboratory space, purchasing necessary supplies and equipment, establishing rearing procedures for colonies of M. domestica, and conducting preliminary dose-response studies has been accomplished despite consistent failures to hire a professional for this project. The third attempt to hire a professional is currently underway and we are expected to succeed. Once this project is fully staffed, research should proceed at a very rapid pace.

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2. Heilbronn, E. and R. Tolagen. 1965. Toxogonin in sarin, soman and tabun poisoning. Biochem. Pharmacol. 14: 73-77.

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4. Jansz, H.S., D. Brons and M.G.P.J. Warringa. 1959. Chemical nature on the DFP binding site of pseudocholinesterase. Biochem. et Biophys. Acta. 34: 573-575.

5. Berends, F., C.H. Posthumus, J.V.D. Sluys, F.A. Deierkauf. 1959. The chemical basis of the "ageing process" of DFP-inhibited pseudocholinesterase. Biochem. et Biophys. Acta. 34: 576-578.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300294	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORG'N METH'D	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS11	ED	234 WWH9
b. CONTRIBUTING	61102A	3M161102BS10		
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Molecular Biology of Medical Defense Against Chemical Agents

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 002600 Biology 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-house

17. CONTRACT/GRANT

DATES/EFFECTIVE: EXPIRATION:

NUMBER:

TYPE: AMOUNT:

KIND OF AWARD: CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 83	3.0	424
CURRENT FISCAL YEAR 84	3.0	583

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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GENERAL USE

FINA

20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Doctor, B P POC: DA

NAME: Olenick, J G

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Organophosphates; (U) Antidotes; (U) Mode of Action; (U) Receptors; (U) Enzymes

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The objective of this work unit is to study the medical/chemical defense of military personnel against severely intoxicating chemical agents with a view to defining the molecular basis of inhibited cellular processes.

24. (U) Tissue culture and/or live animal methodology will be developed to study the mode of action of chemical agent poisoning. Pharmacokinetics and metabolic profiles of protective or therapeutic drugs also will be studied. Selective enzymatic assays for chemical agents, antidotes and cellular targets will be developed. The relationship and ontogeny of certain enzymes, receptors and neurotransmitters will be studied. Recombinant DNA and gene cloning procedures will be explored to further define ontogenetic relationships and topology of reactive sites.

25. (U) 82 10 - 83 09: Tissue culture method employing a variety of cell systems has been developed to study the mode of action of organophosphates and their antidotes. Aprophen has been found to be able to be hydrolyzed to diphenylpropionic acid and diethylaminoethanol. Diethylaminoethanol, in turn, can undergo acetylation in the presence of cholineacetyltransferase and acetyl-CoA to form a novel false neurotransmitter, diethylamino ethylacetate. The potential ability of this new agent to act pharmacologically at the muscarinic and nicotinic receptors is being explored. With regard to aprophen as an antidote, it was found that it can inhibit the binding of [^{3}H]QNB (quinuclidinyl benzylate) to the muscarinic receptors with an I_{50} of 5 μM, for the N4TG1 neuroblastoma cells and 0.8 μM for the NG108-15 neuroblastoma x glioma hybrid cells. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

Available for restrictive upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAY 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3M161102BS11 MEDICAL CHEMICAL DEFENSE SCIENCE BASE/MECHANISMS OF ACTION OF CHEMICAL AGENTS AND ANTIDOTES

WORK UNIT: 234 Molecular Biology of Medical Defense Against Chemical Agents

INVESTIGATORS:

Principal: Peter K. Chiang, Ph.D.
Associate: B. P. Doctor, Ph.D.; Leo Kazyak
Assistant: Richard K. Gordon, Ph.D.; SP5 George Miura, Ph.D.; F.-Y. Chang, Ph. D.; SFC Evelyn Moore; SP4 Felipe N. Padilla

The objective of this work unit is to study the medical/chemical defense of military personnel against severely toxic chemical agents with a view to defining the molecular basis of inhibited cellular processes. Tissue models are developed for investigating the modes of action of organophosphates and their antidotes on the enzymes and receptors of attendant cholinergic systems. Other physiological and neurochemical parameters that are affected will also be assessed. The following investigations were conducted:

1. Antimuscarinic activity of aprophen.

2. Biotransformation of aprophen.

3. In situ localization of acetylcholinesterase.

4. Role of methylation in cellular processes.

1. Antimuscarinic Activity of Aprophen:

The antimuscarinic activity of aprophen was assayed by the inhibition of binding of the muscarinic antagonist [^{3}H]QNB to the cells. Aprophen blocked the binding of [^{3}H]QNB to the N4TG1 neuroblastoma cells and the NG108-15 neuroblastoma x glioma cells in a dose dependent manner. The I_{50} (minimal concentration that inhibited 50% of the binding) was 5 μM for the N4TG1 cells, and 0.8 μM for the NG108-15 cells. There were about 1.7 x 10^5 muscarinic sites/cell (290 fmol/10^6 cells) in the N4TG1 cells, with a K_D of 13 nM. In the NG108-15 cells, there were 2.0 x 10^5 muscarinic sites/cell (340 fmol/10^6 cells), with a K_D of 10 nM. The K_D values obtained for the two cell lines (>10 nM) were in close agreement with 8 nM for human neutrophils, 1 nM for rat cortex homogenates, and 0.2-0.5 nM for solubilized atrial membranes. If the binding of [^{3}H]QNB were calculated on a mg protein basis, both cell lines would bind approximately 300 fmol/mg, a value comparable to 366-558 fmol of [^{3}H]QNB bound per mg of synaptosomal membranes, and 450 fmol/mg for solubilized atrial membranes. An estimation of 5 x 10^4 muscarinic sites per neutrophil cell has been reported.

In contrast to its inhibitory effect on the muscarinic receptors, aprophen has no discernable effect on the nicotinic receptors of NG108-15 cells when assayed with [^{3}H]tubocurarine. There are about 1 x 10^7

nicotinic sites/cell (16 fmol/10^6cells) in the NG108-15 cells, with a K_D of 5.5 nM. The K_D value for [^{3}H]tubocurarine compared favorably with a K_D of 12 nM for the binding of [^{3}H]acetylcholine to nicotinic receptors of rat brain. Moreover, the amount of [^{3}H]tubocurarine bound to the NG108-15 cells (16 fmol/mg) was close to the finding of 4.6 fmol of [^{3}H]acetylcholine bound to the nicotinic receptors of rat brain. Virtually no nicotinic receptors could be detected in the M4TG1 cells.

2. Biotransformation of aprophen.

Aprophen rapidly disappears upon incubation with primary hepatocytes. This can be explained by the observation that aprophen is readily hydrolyzed to diphenylpropionic acid and diethylaminoethanol by a variety of esterases. Diethylaminoethanol has been found to be able to undergo acetylation in the presence of acetyl-CoA and choline acetyltransferase to form a novel false neurotransmitter, diethylamino ethylacetate. The potential ability of this new agent to act pharmacologically at the muscarinic and nicotinic receptors is being explored.

3. In situ localization of acetylcholinesterase (AChE).

In order to localize organophosphate-poisoned AChE, this program has been initiated. Distinct molecular forms of AChE have been isolated from Torpedo electric organs and characterized biochemically. These include an asymmetric "tailed" species (17S + 13S) and a dimeric hydrophobic species (5.6S). Monoclonal antibodies (MCAb) were raised against Torpedo AChE and have been characterized for antigen specificity. Most antibodies show a high degree of cross reactivity between the different molecular forms of AChE, but two demonstrate selectivity for only one form. MCAb 4 F3 is specific for the asymmetiric species (and is unreactive towards this form of the enzyme following collagenase or trypsin removal of the tail section). MCAb 4E7 exibits 100-fold selectivity for the catalytic subunit of the 5.6S hydrophobic dimeric form of AChE. Using these MCAbs as probes, we were able to examine the distribution of the different forms of AChE in Torpedo electric organ by immunofluorescence and immunoelectron microscopoy. MCAb 4E7 shows a diffuse distribution, staining within the synapse giving the appearance of labeling both the innervated and non-innervated surfaces with approximately equal density. These patterns were confirmed using immunoelectron micorscopy. In contrast, polyclonal antibodies raised against the asymmetric form bind both asymmetric and hydrophobic forms with equal avidity in vitro. Although these polyclonal antibodies do not appear to distinguish between molecular forms in vitro, the antigenic sites they localized in situ are less uniformly distributed than those localized by the monoclonal antibody directed against the hydrophobic dimer only. Localization by both light microscopy and electron microscopy reveal the same pattern of staining.

4. Role of methylation in cellular processes.

a) Aldosterone stimulates methylation reactions and membrane events in cultured toad bladder epithelial cells: The effect of aldosterone (Aldo) on phospholipid (PL) biosynthesis in cultured toad bladder

epithelial cells was studied in cells incubated with [1,2-^{14}C]choline and [methyl-^{3}H]methionine during a 5 hour period. Aldo (10^{-7} M) did not alter the uptake of either precursor, but significantly stimulated the incorporation of both labels into phosphatidylcholine (PC), the only PL labeled. ^{3}H-labeling of PC increases 29% and ^{14}C incorporation into PC increased 34% in cells exposed to Aldo. A similar 30% increase in protein carboxylmethylation occurred in cells treated with Aldo. 3-Deazaadenosine (DZA), a methylation inhibitor, abolished the Aldo-stimulated increase in PC labeling from [^{3}H]methionine. PC labeling from [^{14}C]choline was not affected by DZA. Both basal and Aldo-stimulated protein carboxymethylation were inhibited by DZA. DZA (300 M) caused a mild decrease in basal short circuit current (I_{sc}), but completely inhibited the I_{sc} response to 10^{-7} M Aldo. Inhibition was complete when DZA was added up to 2 hours following exposure to Aldo, and was reversible. Cells previously exposed to Aldo showed a significant increase in I_{sc} within 2 hours following removal of DZA. Therefore, Aldo stimulates PL methylation, protein carboxymethylation and turnover of PC from choline. Inhibition of methylation reactions coincides with the inhibition of I_{sc} response to Aldo. The exact methylation reactions associated with the transport response remain to be elucidated.

b) S-Adenosylhomocysteine hydrolase (AdoHcyase) from hamster liver purification and kinetic properties: DZA is both an inhibitor of and a substrate for AdoHcyase. Its administration to rats results in the accumulation of both AdoHcy and 3-deaza-AdoHcy in the liver, and other tissues. In hamsters, however, the administration of DZA results only in the accumulation of 3-deaza-AdoHcy. In order to investigate the possible reasons for this difference, AdoHcyase has been purified from hamster liver to homogeneity, and some of its kinetic and physical parameters were determined. The molecular weight of the native enzyme is 200,000 with a subunit molecular weight of 48,000. The K_m's for adenosine and DZA are about 1.0 M, and the V_{max}'s are also similar. The K_m for AdoHcy is 1.0 M, or more than ten times smaller than the K_m of the rat liver enzyme. This difference in K_m value may explain the differences in the response of rat and hamster liver to the administration of DZA. AdoHcyase from hamster liver exhibits an interesting kinetic property in that its activity can be affected bimodally by either adenosine or adenosine analogs. At very low concentrations of these analogs, the activity of AdoHcyase can be stimulated by 10-30%, and at higher concentrations these sare analogs become competitive inhibitors.

PUBLICATIONS

(1) Garcia-Castro, I., Mato, J. M., Vasanthakumar, G., Weisman, W. P., Schiffmann, E., and Chiang, P. K. (1983) Paradoxical effects of adenosine on neutrophil chemotaxis. J. Biol. Chem. 258, 4345-4349.

(2) Gordon, R. K., Padilla, F. N., Moore, E., Doctor, B. P., and Chiang, P. K. (1983) Antimuscarinic activity of aprophen. Biochem. Pharmacol., 32, 2979-2981.

(3) Gordon, R. K., Brown, N. D., and Chiang, P. K. (1983) Inhibition of

adenosylmethionine decarboxylase and perturbation of polyamine metabolism by 3-deaza-(±)aristeromycin. Biochem. Biophys. Res. Commun. 114, 505-510.

(4) Guranowski, A. B., Chiang, P. K., and Cantoni, G. L. (1983) 5'-Methylthioadenosine nucleosidase (Lupinus luteus seeds). Methods Enzymol. 94, 365-369.

(5) Kim, I.-K., Zhang, C.-Y., Chiang, P. K., and Cantoni, G. L. (1983) S-Adenosylhomocysteine hydrolase from hamster liver: purification and modulation of its active-site by adenosine analogs. Archiv. Biochem. Biophys. 226, 65-72.

(6) Lucas, D. L., Chiang, P. K., and Wright, D. G. (1983) Induction of human promyelocytic leukemia cells by 3-deazaaristeromycin, an inhibitor of methylation reactions. Fed. Proc. 42, 4351.

(7) Chiang, P. K., Gordon, R. K., Brown, N. D., De Clercq, E., and Montgomery, J. A. (1983) 3-Deazaaristeromycin: a novel antiviral agent that interferes with polamine biosynthesis. Abstr. Med. 34, Am. Chem. Soc. 185 National Meeting.

(8) Wiesmann, W. P., Chiang, P. K., and Johnson, J. P. (1983) Aldersterone stimulates phospholipid methylation in cultured toad urinary bladder epithelial cells. Clin. Res. 31, 445A.

(9) Gordon, R. K., Doctor, B. P., and Chiang, P. K. (1983) Antimuscarinic activity of aprophen. Fed. Proc. 42, 5011.

(10) Chiang, P. K., Wiesmann, W. P., and Johnson, J. P. (1983) Aldosterone stimulates methylation reaction and membrane events in cultured toad urinary bladder epithelial cells. Fed. Proc. 42, 717.

(11) García-Castro, I., Mato, J. M., Vasanthakumar, G., Wiesmann, W. P., Schiffmann, E., and Chiang, P. K. (1983) Paradoxical effects of adenosine on neutrophil chemotaxis. Fed. Proc. 42, 2201.

PRESENTATIONS

(1) Chiang, P. K. New York Cell Biology Club, New York (November 16, 1982)

(2) Chiang, P. K. American Chemical Society, Seattle, Washington (March 23, 1983)

(3) Chiang, P. K. University of British Columbia, Vancouver, Canada (March 24, 1983)

(4) Chiang, P. K. Roswell Park Memorial Institute, Buffalo, N. Y. (May 16, 1983)

(5) Chiang, P. K. NATO International Advanced Study Institute on Targets for The Design of Antiviral Agents, Les Arcs, France (June 19, 1983)

(6) Chiang, P. K. Universite Paris VII, INSERM, Paris, France (June 5, 1983)

(7) Chiang, P. K. G. D. Searle, London, England (July 10, 1983)

PROJECT 3M463750D808

DRUG AND VACCINE DEVELOPMENT

82 10 01	D. Change	U	U		NL	YES / NO	A

WORK UNIT NUMBER: 001 WWMC

(U) Phase II Antimalarial Drug Trials

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

012600 Clinical Pharmacology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	4.0	179
FISCAL YEAR CURRENT 84	3.0	278

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Walter Reed Army Institute of Research
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RESPONSIBLE INDIVIDUAL
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TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

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Div of Experimental Therapeutics
ADDRESS:* Washington, DC 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
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TELEPHONE: (301) 427-5393
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: PAMPLIN, C
NAME: COSGRIFF, T

21. GENERAL USE

Foreign intelligence considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Clinical Pharmacology; (U) Phase II Efficacy; (U) Antimalarial Drugs; (U) Human Volunteer

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security Classification Code.)

23. (U) The technical objective of this work unit is to evaluate the efficacy of new antimalarial drugs in non-immune human volunteers experimentally infected with malaria. Studies are performed in support of the Army Antimalarial Drug Development Program, and are an essential part of each official Investigational New Drug (IND) submission.

24. (U) Normal male volunteers are recruited from the civilian and military (MRVS) population of the greater metropolitan Washington, D.C., area by public advertisement. Each individual receives a thorough medical evaluation and must give his valid, informed consent before being permitted to participate in the study. As a study subject, the volunteer is admitted to an in-patient research facility at Ft. Detrick, inoculated with malaria and treated with the drug or drugs specified in the protocol for each study. Each volunteer is then observed for a sufficient period of time to ensure that he is cured of malaria and is free from any adverse effect from his participation in the study.

25. (U) Studies were continued to determine the lowest effective oral dosage regimen of WR 180,409·H_3PO_4 that produces 100% cure in volunteers with experimentally induced P. falciparum malaria (Smith isolate). Oral doses ranging from 1500 mg to 500 mg were studied. The lowest dose found to be 100% effective was 500 mg followed in 12 hrs by 250 mg. Studies to determine the lowest dose of WR 194,965·H_3PO_4 that produces 100% cure in similarly infected subjects have begun. Three subjects have been treated with a total oral dose of 2250 mg. The follow-up is in progress. One patient continues to receive WR 638 for cystinosis in a study performed in conjunction with the NIH. For technical reports see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

Project: 3M463750D808 DRUG AND VACCINE DEVELOPMENT

Work Unit: 001 Phase II Antimalarial Drug Trials

Investigators:

Principal: Melvin H. Heiffer, Ph.D.

Associate: LTC C. Pamlin, LTC T. Cosgriff, COL C. Canfield, LTC B. Schuster, Dr. L. Fleckenstein, MAJ M. Shmuklarsky, LTC J. Berman, SP5 P. Barr

1. Description.

Phase II clinical studies involve evaluating the efficacy of candidate antimalarial drugs in a limited number of patients subjected to a controlled clinical infection with malaria. These studies are an essential bridge between tolerance studies in healthy, noninfected volunteers and a wide scale study of the curative potential of the new drug in malaria patients. A major aspect of Phase II studies is determination of a curative dose. Pharmacokinetic evaluations of the candidate drugs in man are also performed as they are an essential prerequisite of dosage selection.

2. Progress.

Human efficacy studies for treatment of blood-induced P. falciparum malaria (Smith isolate) treated with WR 180,409·H_3PO_4 were completed at USAMRIID. An additional 15 subjects for a total of 22 total subjects were enrolled in the Phase II study. A total oral dose ranging from 750 mg to 1500 mg given as divided doses 12 hours apart was effective in all subjects. A single subject who received 500 mg (6.4 mg/kg given as a single oral dose) was not cured. Five-hundred mg followed by 250 mg orally 12 hours later was the lowest total dose that was curative in all subjects. On a weight basis the lowest effective dose was 10.2 ± 1.3 mg/kg.

A clinical protocol for the evaluation of the efficacy of WR 194,965·H_3PO_4 against blood-induced infections with the Smith isolate of P. falciparum malaria was begun. The purpose of this protocol is to determine the lowest total dose which is 100% effective in treating this multi-drug resistant isolte of falciparum malaria. To date a total of 3 study subjects have been enrolled in the protocol and treated with 2250 mg of WR 194,965 orally in divided doses of 750 mg at 0, 12 and 24 hours. Follow-up of these study subjects is not complete at this time. Total doses ranging from 2250 mg to 500 mg will be studied in this protocol.

WR 638 continues to be studied in conjunction with the Institute of Child Health and Development at the National Institutes of Health as a treatment for cystinosis. Currently one study subject receives WR 638 on a daily basis at a dose of 130 mg/kg/day. Two study subjects were dropped from this protocol during the course of the year because of non-compliance with the dosing regimens.

3. Future Work.

Efficacy studies of WR 194,965·H_3PO_4 will continue. The patient currently being treated with WR 638 will be continued on the drug during the next year while a suitable sponsor for continued studies is located.

4. Publications.

Cosgriff, T.M., Boudreau, E.F., Pamplin, C.L., Doberstyn, E.B., Desjardins, R.E., and Canfield,C.J.: Evaluation of the antimalarial activity of the phenanthrenemethanol halofantr'ne (WR 171,669). Am. J. Trop. Med. Hyg. 31(6):1075-1079, 1982.

Cosgriff, T.M., Boudreau, E.F., Pamplin, C.L., Berman, J.D., Shmuklarsky, M.J. and Canfield, C.J.: Evaluation of the antimalarial activity of the 4-pyridinemethanol WR 180,409. In preparation.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2527	83 10 01	DD-DR&E(AR)636

TE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORG'N INSTR'N	8A. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	63750A		AC	002 WW05
CONTRIBUTING				
[illegible]	CARDS			

TITLE (Precede with Security Classification Code)

) Evaluation of New Antiparasitic Drugs and Vaccines in the Tropics

SCIENTIFIC AND TECHNOLOGICAL AREAS

0100 Microbiology 002600 Biology

START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
10	CONT	DA			C. In-House

CONTRACT/GRANT

YES/EFFECTIVE:	EXPIRATION:	18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
NUMBER		PRECEDING FISCAL YEAR 83	9.0	854
TYPE:	c. AMOUNT:	CURRENT FISCAL YEAR 84	7.0	1,148
PRIOR AWARD:	f. CUM. AMT.			

19. SPONSORING DOD ORGANIZATION

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NAME: ANDRE,R.G., CHILDS,G.E.,

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FEDERAL USE

FIC

KEYWORDS (Precede EACH with Security Classification Code)

(U) Malaria; (U) Halofantrine; (U) Mefloquine;
) Quinine; (U) Human Volunteer; (U) Doxycycline; (U) Leptospirosis

TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

. (U) The objective of this task is to establish the efficacy of new drugs for both ophylaxis and treatment of tropical infectious diseases of military importance. Particular emphasis is placed on malaria, a disease of worldwide endemicity and resistance to nventional drugs, which continues to cause high attack rates (up to 50%) in unprocted troops. The effect of conventional and experimental antimalarials in treatment, ophylaxis and transmission of drug resistant falciparum malaria will be determined.

. (U) Army investigational antimalarial drugs are compared with standard drugs and new mbinations of standard drugs in the treatment and prophylaxis of drug resistant falciarum malaria in hospitalized human volunteers. Advanced development and field testing new techniques supporting this task will be accomplished. Candidate antimalarial ugs will be evaluated using simian malaria, as a model for human malaria.

. (U) 82 10-83 09 Two prophylaxis studies were initiated comparing mefloquine, chloroine-fansidar, and doxycycline in a trial in the Royal Thai Army, and the second compare floquine, chloroquine, and chloroquine-fansidar in Thai gem miners. Therapeutic and ophylactic trials were initiated in animal models looking at doxycycline in leptospiros. The malaria drug screening program continued using monkeys obtained from the States e chemotherapeutic trials of halofantrine and quinine-tetracycline in RTN Marines and \ troops respectively were continued. The in vitro drug sensitivity testing as completit of the drug trials was also continued. The study of immunosuppressive effects of timalarial drugs on lymphocytes continues. Biochemical targets of drug development oking at ADA and the effect of 2'deoxycoformycin in a primate model was also continued e technical report see Walter Reed Army Institute of Research Annual Progress Report, : 82 - Sep 83.

(Also to contractors upon originator's approval)

FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project Number: 3M463750D808 DRUG AND VACCINE DEVELOPMENT
Title: Evaluation of New Antiparasitic Drugs and Vaccines in the Tropics

Work Unit Number: 002

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1. Evaluation of Plasmodium [illegible] Sporozoite Induced Infection in Captive Born Macaca fascicularis

Problem: India has ceased exportation of rhesus monkeys which are used in the Plasmodium cynomolgi primalarial compound testing model. A systematic evaluation of captive born Macaca fasicularis has not been completed to determine if this species could be used to supplement scarce rhesus monkeys.

PROGRESS: Twenty-five AFRIMS produced cynomolgus have been inoculated with P. cynomolgi sporozoites. The intact cynomolgus does not develop an infection comparable to the rhesus. The splenectomized captive-born cynomolgus monkey appears to be capable of supplementing rhesus in antimalarial compound testing. Relapse occurs (as in rhesus monkeys) after clearance of blood forms with chloroquine given alone or along with a noncurative dose of primaquine or test compound.

FUTURE OBJECTIVES:

1. Gather data on a larger number of splenectomized cynomolgus for significant comparison to the rhesus.

2. Use this model for evaluating potentially toxic compounds.

2. Evaluation of Experimental Antimalarial Drugs for Radical Curative Activity in the Rhesus Monkeys

PROBLEM: The ability of the malarial organism to become resistant to standard therapeutic agents is well known. Primaquine is the primary radical curitive agent at present and its use is accompanied by several adverse effects. This has prompted a search for equally or more effective agents which are less toxic than primaquine.

OBJECTIVES: To test candidate antimalarial compounds for radical curative activity in the sporozoite induced Plasmodium cynomolgi - Rhesus model.

PROGRESS: The mosquito-monkey P. cynomolgi cycle has been reestablished and 45 rhesus and splenectomized cynomolgus monkeys have been used in the antimalarial drug development

program during FY 83. A primaquine baseline study has been completed. Thirteen new compounds have been received for testing. Radical curative screening has been completed on 7 compounds and 25 compounds are currently in progress. Thirty young U.S. origin female rhesus and 17 adult malaria virgin rhesus females from USAMRIID were shipped to AFRIMS for compound screening in FY 83. In FY 84, AFRIMS produced young rhesus will be used for radical curative testing. Potentially toxic compounds are being screened in splenectomized cynomolgus also produced in the AFRIMS breeding colony.

FUTURE OBJECTIVES: To continue screening compounds and attempt to decrease present backlog of untested compounds.

3. Leptospirosis in the Hamster: Chemoprophylaxis in the Acute Infection

PROBLEM: Leptospirosis is a worldwide zoonotic illness that is common in tropical areas and recently has been a cause of outbreaks of acute "flu-like" illness in troops in jungle training exercises. Presently the nonhuman primate is being studied in our laboratory as a model for acute leptospirosis and chemophrophylaxis. A hamster model has been previously described for acute leptospirosis. A clinical, often fatal illness results in hamsters infected with some strains of leptospira. This animal model will be a useful addition to the primate model for testing potential prophylactic drug treatment for leptospirosis. Also the hamster is an ideal laboratory animal for isolating strains of leptospira from samples contaminated with other bacterial or fungal organisms. Proposed studies on the epidemiology of leptospirosis will necessitate the use of hamsters and familiarity with this model for isolation of leptospira from infected water sources.

OBJECTIVES:

1. To characterize the acute Leptospira bataviae infection in the hamster and determine the virulence and infectivity of the organism.

2. To determine the benefit of doxycycline prophylactic treatment in leptospira infected hamsters.

3. To obtain serovar specific antisera for developing an

ELISA method to detect the acute infection.

PROGRESS: Leptospira bataviae isolates from patients in Thailand have been shown to be virulent and produce a chronic renal infection in hamsters with intraperitoneal doses containing as few as 1-10 organisms. A single oral dose of doxycycline prevents a fatal infection but most hamsters develop a chronic leptospira renal infection. Daily treatment for 4 days or more, started at the time of infection or as late as 4 days after infection prevents death as well as chronic renal infection. Similarly, doxycycline treatment of hamsters with a chronic renal infection is effective in eliminating leptospira from the kidney.

FUTURE OBJECTIVES:

1. Sera from hamsters immunized with different strains will be tested using an ELIZA method for the diagnosis of leptospirosis. An antigen of broad cross reactivity for a many serovars will be sought to use in this test.

2. Hamsters will be used for testing water samples for leptospira infection. Hamsters of 40 grams in groups of at least 4 will be injected intraperitoneally to test a given sample.

4. Leptospirosis in the Non-human Primate Model: Chemoprophylaxis and Early Diagnosis of Infection

PROBLEMS: Leptospirosis is a common zoonotic disease found throughout the world. The clinical features in man range from an influenza-like illness to a more severe disease form manifested by continued fever with meningitic symptoms and signs. In some cases infection can lead to renal and hepatic failure, jaundice, and even death. Leptospirosis is frequently found in the tropical areas of the world and recent attention has focused on several outbreaks in soldiers training in jungle areas. Symptomatic treatment and antibiotic therapy are used in the acute illness. However, once symptoms are evident the beneficial effect of antibiotics is questionable. The relatively long recovery period, even with treatment, suggests that prevention is the practical approach in solving the problem of leptospirosis. It is difficult to prevent direct contact with leptospira contaminated water in a tropical environment, especially during military maneuvers. Immunization against specific serovars of leptospira can protect animals but immunization

of man is not practical unless the serovar endemic to the area is identified or a vaccine with broad antiserovar activity is developed. Last year we reported that the experimental infection of monkeys with a local human isolate of the bataviae serovar produced a bacteremia of one to six days, infection of the CSF, and a bacteruria for up to four weeks. An antibody response was detected by microagglutination by one week and peak titers were reached by 3-4 weeks.

OBJECTIVES:

1. To characterize clinical leptospirosis in the non-human primate model.

2. To determine the efficacy of antibiotic treatment as a prophylaxis for the acute infection.

3. To determine if an ELISA method for detecting leptospira antibody or antigenemia is a useful means for obtaining rapid early diagnosis of leptospirosis.

PROGRESS: The inhibition of leptospira by different antibiotics was tested *in vitro*. Doxycycline was more effective than minicycline or tetracycline against three isolates of *L. bataviae*. Dicloxacillin had no effect at tested concentrations up to 4 ug/ml. In a pilot experiment, the serum from 2 monkeys given doxycycline inhibited growth of leptospira *in vitro* when samples were taken as long as 72 hours after a single oral dose (5 mg/kg).

When eight monkeys were treated daily for 10 days with a single dose of doxycycline based on a 2-3 mg/kg equivalent dose for man, there was no bacteremia or the number of days of bacteremia was reduced. In addition, infection of the CSF and chronic renal infection occurred in placebo treated controls but was not seen in infected monkeys treated with doxycycline.

In two other groups of monkeys a single dose of doxycycline was given two hours prior to infection. In 6/8 doxycycline treated monkeys the number of days of bacteremia were less than in controls. In two monkeys the period of bacteremia was similar to infected placebo treated monkeys and one of these developed a chronic renal infection. In the eight placebo treated monkeys, six developed chronic renal infection and leptospira were cultured from the CSF of three. Leptospira were never isolated from the CSF of any

doxycycline treated monkey. An ELISA method for detecting leptospira IgM was developed with Virology. To date the ELISA test has been used for monkeys and can detect IgM antibody to L. baraviae; however in its present form this method is not as sensitive as the microagglutination method.

FUTURE OBJECTIVES:

1. Further develop and refine the ELISA to detect low antibody titers to leptospira.

2. Test sera from monkeys immunized with different serovar antigens by the ELISA method and find an antigen or combination of antigens that will detect serovar infections that are present in Thailand.

5. Effect of Antimalarial Drugs on Human Lymphocyte Response to Mitogenic Lectins

PROBLEM: Since immunosuppression is a characteristic of malaria infection the posibility that an antimalarial agent may itself compromise immune responsiveness becones an important clinical consideration. A drug induced decrease in host immune capacity during malaria infection could result in a prolonged parasite clearance time and subsequent delayed recovery from the disease. Similarily, the compromise to the patient may result in increased susceptibility to intercurrent illness. There is also the concern for malaria endemic populations where suboptimal chemoprophylaxis may combine with the disease itself so as to compromise vaccine employment - especially a prospective malaria vaccine.

PROGRESS: Mitogenic lectin induced lymphocyte blast transformation provides an established assay for evaluation of cellular immune responsiveness. We have standardized an in vitro mitogenic lectin assay to assess whether selected antimalarial drugs suppress cellular immune responsiveness in human lymphocytes. Preliminary studies show that the new antimalarial drugs, mefloquine and halofantrine, suppress normal lymphocyte response to mitogenic lectins (phytohemagglutinin, Concanavalin A and pokeweed mitogen). These drugs also suppress responsiveness of mononuclear cells isolated from malaria patients.

FUTURE OBJECTIVES: These studies represent in vitro conditions and may not accurately reflect the in vivo situation-especially when metabolites of a specific drug

occur. A combination in vitro/in vivo, test system has been proposed as a more predictive measure for assessing the effect of antimalarial drugs on immune responsiveness.

6. Adenosine Deaminase in Malaria Infection: Effect of 2'-Deoxycoformycin in vivo

PROBLEM: Purine nucleotides are required by the rapidly proliferating malaria parasite for both energy metabolism and nucleic acid synthesis. The malaria parasite cannot synthesize purines de novo and depends for its intraerythrocytic (IE) growth and development on salvage of purine bases from the host RBC and extracellular environment. We have shown with Plasmodium falciparum, in vitro, that hypoxanthine is utilized as a purine base precursor for parasite synthesis of adenosine and guanosine nucleotides and that specific inhibition of these synthetic pathways leads to parasite destruction. Whether hypoxanthine is the malaria parasites preferred substrate in vivo is not known. An increase in adenosine deaminase (ADA) activity, however, is an obvious means for production of IE hypoxanthine. Increased availability of hypoxanthine would be a natural consequence of adenosine metabolism in the mature erythrocyte since this cell lacks the enzyme xanthine oxidase. Conversely, inhibition of ADA activity could act to deprive the rapidly growing IE malaria parasite of a readily accessible hypoxanthine pool for purine nucleotide synthesis.

PROGRESS: Adenosine deaminase from both human P. falciparum and monkey P. knowlesi has been characterized by an immunoassay. Host RBC ADA enzyme was precipitated with rabbit anti-human RBC ADA antibody bound to S. aureus. The non-immunoreactive parasite enzyme was recovered in the supernatant. The parasite enzyme has been characterized for its kinetic properties and response to inhibitors. Deoxycoformycin inhibited both parasite and host ADA whereas the competitive ADA inhibitor erythro-9- (2-hydroxyl-3-nonyl) -adenine (EHNA) was ineffective for the parasite enzyme. Deoxycoformycin administed to P. knowlesi infected rhesus monkeys produced a dramatic reduction in parasitemia implicating catabolism of adenosine as an important source of parasite hypoxanthine. Infection was not, however, eliminated by deoxycoformycin in vivo suggesting stage specificity of the agent or an alternative source of hypoxanthine once infection has progressed.

FUTURE OBJECTIVES: The project should be continued. The

central role of hypoxanthine in malaria parasite purine metabolism provides a unique biochemical focus for antimalarial drug design. A series of new purine inhibitors have been developed and will be tested against P. falciparum in vitro. Further studies will be done on the question of stage-specificity for deoxycoformycin and the role of ADA in parasite development and proliferation.

7. In vitroAntimalarial Drug Sensitivity Testing

PROBLEM: In Thailand, Plasmodium falciparum is now resistant to conventional antimalarial drugs. This resistance varies from almost complete in the case of chloroquine and other 4-aminoquinolines and pyrimethamine/sulfadoxine to moderate but increasing for quinine. Quinine at high therapeutic dose continued to be effective when combined with tetracycline. Two new antimalarial drugs, mefloquine and halofantrine, have been introduced into Thailand and are now in various stages of evaluation and development. In vitro antimalarial drug sensitivity testing provides an objective means of quantifying dose-response characteristics for individual drugs and thus the identification of resistance patterns in Thailand.

PROGRESS: A radioisotope microdilution technique has been adapted to test antimalarial activity in vitro under field conditions. The technique was standardized in the central Bangkok laboratory. The technique is based on incorporation of (3 H) hypoxanthine by parasitized RBC in microculture. Inhibition of uptake of (3 H) hypoxanthine by the parasites serves as an indicator of antimalarial activity. This technique has proven more sensitive and precise than traditional microscopic methods. It also permits large scale testing with fewer personnel. Data computation and records storage has been automated.

Antimalarial drugs are being tested in support of Phase III clinical evaluation studies and other field medical protocols. The drugs are: mefloquine, halofantrine, WR 180409, quinine, chloroquine, pyrimethamine and sulfadoxine. The data from in vitro testing has helped accomplish the following: (1) provide in vitro/in vivo correlation of antimalarial drug response; (2) establish base-line quantitative data (ID 50); (3) permit identification and collection of drug resistant malaria strains; (4) allow comparative testing of malaria strains from treatment

failures when they occur; and, (5) support comparative epidemiological studies. Methods have been established for use of the radioisotope procedure in the field. Morphological field tests have been developed for testing of antifols. A cryobank has been established for preservation of reference strains. Additionally, work has begun on cloning of mefloquine resistant strains of P. falciparum.

FUTURE OBJECTIVES: Antimalarial drug resistance is an on-going problem. It is essential that antimalarial drug sensitvity testing be done on a continuing basis to support clinical trails and provide base-line epidemiological data on drug resistance in Thailand.

P. The Treatment of Plasmodium falciparum Malaria with Halofantrine, a Phenanthrenemethanol

PROBLEM: The rapid development of drug resistance in P. falciparum malaria necessitates the continuing process of new drug development for potent blood schizonticides which could replace mefloquine as an antimalarial treatment drug. Halofantrine (WR 171,669) is in Phase II testing.

In clinical Phase II studies performed in 27 nonimmune subjects infected with Vietnam Smith strain P. falciparum, all 14 subjects who received 1000-1500 mg as split doses over a single day were cured. Only 3 RI recrudescences occurred in single doses of 1000 mg or 1500 mg. In addition the remaining 10 patients who received higher doses of the drug over a longer time period were all cured (1).

The important task was to determine field efficacy of this new antimalarial drug. Therefore 1500 mg split dose over one day was compared against mefloquine as single dose in a treatment trial of multi-drug resistant P. falciparum malaria on the Thai-Kampuchean border.

PROGRESS: From October 15, 1982 - October 15, 1983 at Ft. Taksin, Chantaburi in a population of Royal Thai Marines and volunteer soldiers naturally infected with P. falciparum malaria a double blind randomized treatment trial was conducted. These soldiers had parasite counts between 1000-100,000/mm3, and had no GI, renal or cerebral complications. They were dosed then observed as hospitalized patients in a non-malarious area for 21 days post treatment and were examined again at day 28 after returning to their units.

Fifty-nine patients were dosed with Halofatrine at 500 mg Q 6 hr x 3. 53/59 patients were cured (90%). There were 6 RI recrudescences. (1 day 12, 3 day 21, 1 day 26, and 1 day 28).

Thirty-eight patients were treated with mefloquine single dose. Twenty patients received a 1500 mg dose and 18 patients received a 1000 mg dose. 20/20 patients were cured at the 1500 mg dose (100%), and 17/18 patients were cured at the 1000 mg dose (94% cure rate). There was 1 RI recrudescence at day 28. The comparative parasite clearance times, (PCT), fever clearance times (FCT), initial parasite counts (IPC) and side effects are out lined below.

	Geometric Mean IPC/(per mm3)	Mean PCT	Mean FCT
Halo 500 mg q 6 hr x 3 n=59	14,893	75 hr	54.6 hr
Mefloquine 1500 mg n=20	12,745	64 hr	34.3 hr
Mefloquine 1000 mg n=18	18,375	72 hr	53 hr

	Vomiting	Nausea	Diarrhea	Abdominal Pain	None
Halo 500 mg every 6 hr x 3 n=59	20%	29%	22%	8.4%	42%
Mefloquine 1500 mg n=20	20%	20%	45%	5%	40%
Mefloquine 1000 mg n=18	28%	11%	39%	0	67%

FUTURE OBJECTIVES: In order to elucidate the degree of cross-resistance between mefloquine and halofantrine, a treatment trial should be performed with halofantrine patients receiving mefloquine after recrudescence and mefloquine failures receiving halofantrine treatment. Blood levels of halofantrinre should be done on all future halofantrine patients at day 1, 3 and 5.

A pharmacokinetic study of halofantrine in patients infected with P. falciparum would develop guidelines to judge the adequacy of blood level in malaria patients treated with halofantrine.

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9. The Comparison of Mefloquine and Doxycycline as Clinical Prophylactic Agents for Falciparum and Vivax Malaria in Royal Thai Army Troops Assigned to the Thai-Kampuchean Border

PROBLEM: Antimalarial prophylaxis in South East Asia has been of great concern with the emergence of chloroquine resistance in the 60's and 70's and Fansidar resistance in the 80's. The last meaningful prophylactic study was perfomed by AFRIMS in Prachinburi Province in 1976 comparing Fansidar and Mefloquine (1). It revealed a greater than 95% protection rate for both Fansidar and Mefloquine given either weekly or every two weeks using a double dose against P. falciparum . Fansidar had a 91%-93% protection rate against P. vivax . Current CDC and WHO recommendation for Thailand are Chloroquine plus Fansidar administered on a weekly basis.

We examined the prophylactic efficacy of three antimalarial drugs in a highly endemic area.

PROGRESS: The duration of this study was 14 weeks from 5 July 1983 - 14 October 1983. Three hundred and twenty two patients were enrolled from four sites along the Kampuchean border (Khao Tangoc, Khao Sarapee, Khao Din, and Khun Phol). They were randomly assigned to one of three prophylactic groups- mefloquine 250 mg weekly, chloroquine 300 mg base plus fansidar (500 mg sulfadoxine with 25 mg pyrimethamine) or doxycycline 200 mg 2 x/week.

Forty-three patients have been positive to date out of 322 patients. None were in the mefloquine group. Attack rates for each drug group will be calculated and the significant difference between groups will be noted.

FUTURE OBJECTIVES:

1. Mefloquine prophylaxis on a weekly basis is 100% protective even in this area of multi-drug resistant malaria.

2. Doxycycline should be evaluated as a 100 mg/day prophylactic dose.

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10. The Pharmacokinetics of Intravenous Quinine in Patients with Naturally Acquired Falciparum Malaria

PROBLEM: Quinine continues to hold the distinction of being the only parenteral treatment drug for chloroquine resistant P. falciparum . Intravenous administration of quinine is essential for treating critically ill patients. The success of quinine treatment depends on achieving adequate blood levels of the drug. There are known to be large interindividual variations in plasma quinine levels and we hope to identify the major factors accounting for such variability in quinine disposition in patients undergoing intravenous therapy.

PROGRESS: One IV infusion of quinine HCL 650 mg , in 500 ml of normal saline was administered over two hours and serial blood and urine samples were collected over a 26 hour period. The final five of nineteen patients completed this study between Oct 1982-Oct 1983. There was one RII resistant patient to the followup therapy of quinine 650 mg tid x 3 days with tetracycline 500 mg-250mg-250 mg x 7 days.

The study was concluded and awaits High Performance Liquid Chromatography analysis of the serum and urine quinine levels and the synthesis of a pharmacokinetic curve.

The arithmetric mean initial parasite count of the group was 31,284 per mm3. The mean parasite clearance time was 97.2 hours and the mean fever clearance time was 87 hours, following a treatment program of quinine 650 mg tid x 3 days

and tetracycline 500 mg-250 mg-250 mg tid x 7 days.

FUTURE OBJECTIVES: Pending data analysis.

11. The Comparison of Chloroquine, Chloroquine-Fansidar and Mefloquine as Clinical Prophylactic Agents for P. falciparum and P. vivax Malaria in Thai Gem Miners Along the Thai-Kampuchean Border

PROBLEM: The goal of this study was to assess if chloroquine plus fansidar has a prophylactic efficacy superior to chloroquine alone, and to compare both to the best available antimalarial, mefloquine.

PROGRESS: This study began on July 21, 1982 in Borai, Thailand. As of 15 October 1983, 199 subjects had been enrolled in this prophylactic trial. Patients have been randomly assigned to one of three drug groups (1) chloroquine (300 mg base/weekly) plus fansidar (1000 mg sulfadoxine and 50 mg of pyrimethamine) every 2 weeks, (2) chloroquine 300 mg base weekly or (3) mefloquine 500 mg every two weeks. Medications are administered in a double-blinded fashion. Forty-five patients were positive to date: 5/45 on week 1, 24/45 on week 2, 3/45 on week 3, 5/45 on week 4, 1/45 on week 5, 2/45 on week 6, 2/45 on week 8, 3/45 on week 10, all with P. falciparum . Fifty-nine patients were discharged from the study due to poor compliance with followup visits. Seventy-seven patients continue in the study to date.

FUTURE OBJECTIVES: Our goal is to follow 450 patients to either positivity or to a complete 14 week follow up.

12. Comparative Bioavailability and Renal Clearance of the Combination of Quinine and Tetracycline Given Simultaneously or Sequentially

PROBLEM: To evaluate the bioavailability of tetracycline when administered sequentially or simultaneously with quinine and to assess the effect quinine has on the renal clearance of tetracycline. Also to elucidate the effect simultaneous tetracycline has on serum levels of quinine.

PROGRESS: A total of 25 patients comprised this study population at Phrabuddhabhat Hospital in central Thailand. Eleven of twenty-five patients were treated between October 1982 and October 1983. The clinical portion of the study is

now complete and awaits biochemical and pharmacokinetic analysis at WRAIR.

The mean age of both sequential (Group I) and simultaneous (Group II) Q-T was 28 years old. The mean weight for group I was 53 kg and for Group II was 57 kg.

Side effects were not significantly different in each group.

	Mean Parasite Clearance Time	Fever Clearance Time	Mean Initial Parasite Count
Quinine 650 mg x 3 days followed by Tetracycline 1 gm per day x 7 days (Group I) n = 12	92 hr	66 hr	21,780
Quinine 650 mg x 3 days together with Tetracycline 1 mg per day x 7 days (Group II) n = 13	88 hr	59 hr	18,864

FUTURE OBJECTIVES: Pending pharmacokinetic analysis. Tinnitus was universal, perhaps indicative of adequate blood levels, while blurred vision as a sign of drug toxicity was seen in three patients (two Group I and one Group II).

13. The Treatment of P. falciparum Malaria with a Combination of Quinine and Tetracycline

PROBLEM: To determine the efficacy of the combination of quinine and tetracycline in various treatment regimens and to compare them to mefloquine for efficacy and severity of side effects. All patients were randomized and treated with one of four drug regimens: mefloquine 1500 mg single dose, quinine 650 mg every 8 hr x 6 days and tetracycline 500 mg every 8 hr x 6 days (Q6 T6), quinine 650 mg every 8 hr x 3 days and tetracycline 500 mg every 8 hr x 7 days (TQ3 T7) or quinine 650 mg loading dose followed by quinine 325 mg every6 hr x 3 days plus tetracycline 250 mg every 6 hr x 7 days (Q3 T7). The patients were examined daily for symptoms or physical findings and hospitalized for 21 days of

a 28 day follow up period in a non-malarious area.

PROGRESS:

	Arithmetic Mean Initial Parasite Count	Mean Parasite Clearance Time	Mean Fever Clearance Time	Cure Rate	Recrudescences
Mefloquine n = 26 19/26 between Oct 82-Oct83	23,033 per mm3	65.2 hr	40.4 hr	92% (24/26)	1 RI day 21 1 RII not cleared in 14 days
Q6 T6 n = 42 18/42 between Oct 82-Oct 83	23,403 per mm3	86.5 hr	58.5 hr	100% (42/42)	4 P. vivax relapses 1 day 21 2 day 29 1 day 28
Q3 T7 High Dose n = 20 18/20 between Oct 82-Oct 83	29,933 per mm3	92.5 hr	64 hr	90% (17/19)	2 RI day 28 1 lost to followup day 21
Q3 T7 Low Dose n = 15 15/15 between Oct 82-Oct 83	21,253 per mm3	106 hr	57 hr	93% (14/15)	1 RI day 28 2 P. vivax 1 day 27 1 day 28

FUTURE OBJECTIVES: The study will be continued until 40 patients are in each group. To date mefloquine has comparable efficacy with Q3 T7 (high or low dose).

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Title: Evaluation of New Antiparasitic Drugs and Vaccines in the Tropics
Work Unit Number: 002

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6448	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M163750D808	AB	003 WWGG
b. CONTRIBUTING				
c. XXXXXXXX	ICARDS			

11. TITLE (Precede with Security Classification Code)
(U) Advanced Vaccine Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
58 05	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PREVIOUS 83	3.0	735
FISCAL YEAR CURRENT 84	3.0	728

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: ALTIERI, P L
NAME: POWELL, C POC: DA

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede each with Security Classification Code) (U) biological products; (U) typhoid-Shigella hybrid vaccine; (U) E. coli vaccines; (U) Leishmania diagnostic skin test antigen; (U) Meningococcal B protein-polysaccharide vaccine; (U) Bioassay; (U) Freeze-drying

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Precede with Security Classification Code)

23. (U) This work unit is concerned with development of manufacturing methods and production of new vaccines for military use and with modification of existing biologicals to increase effectiveness, reduce reactivity, to afford greater stability and to minimize logistic requirements.

24. (U) Increased effectiveness and reduced reactivity are pursued by applying new physical and chemical methods to processing. Improvement in stability and reduction of logistic requirements are achieved by application of modern freeze-drying and packaging techniques.

25. (U) 82 10 - 83 09 Investigations on the development of new and improved biologics for military use have continued. Eleven meningococcal protein-polysaccharide vaccines have been prepared, tested, and made available for human tests and studies on improved methods of production initiated. An oral Escherichia coli pilus vaccine has been made available for human studies and work initiated on producing two additional lots comparing the effect of methods of inactivation on yield and immunogenicity. A second lot of a Salmonella typhosa-Shigella sonnei, live oral, freeze-dried vaccine using a new hybrid strain is currently in the production stage. Production of certified seed lots of a strain of Leishmania has been completed. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

* Applicable to contractor (non-government) summary

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M4637500808 DRUG AND VACCINE DEVELOPMENT

WORK UNIT 003 ADVANCED VACCINE DEVELOPMENT

Investigators:

Principal: Sanford L. Berman, Ph.D.

Associates: Patricia L. Altieri
Calvin Powell

Problems and Objectives

Investigations on the development of new and improved biological products for human use have continued. Eleven different meningococcal vaccines were prepared, tested and made available for field studies. Work was also initiated on improving the production methods for these products. An oral Escherichia coli pilus vaccine was produced and made available for field studies and work was initiated on the production of two new lots of vaccine comparing methods of inactivation and the effect on immunogenicity. A second lot of a Salmonella typhosa-Shigella sonnei hybrid oral, live, attenuated vaccine is currently being produced using a hybrid seed strain more stable in genetic character than the first lot made available for field tests. Certified, tested and stable Leishmania seed preparations were made available for studies on the production techniques for a diagnostic skin test antigen.

Progress

Meningococcal B strain proteins were isolated from two different B strain cultures and combined in various formulations with A, C, Y, B, and W-135 purified polysaccharides. Eleven meningococcal protein-polysaccharide vaccines were prepared, tested, and made available for field studies. Studies were also initiated on comparing the effect of formalin inactivation and extraction with empigin BB detergent on resultant B strain protein products. Preliminary results indicate a significant increase in yield compared to previous methods and these materials are currently being analysed as to chemical make-up, specificity, size, and pyrogenicity. Methods were developed for obtaining pure culture agar grown harvests of E. coli for the subsequent preparation of purified pilus oral vaccines and a lot was prepared, tested, and made available for field studies. Studies were also initiated using formalin for inactivation of the E. coli instead of irradiation and both products compared in rabbit immunogenicity tests appeared equal. Formalin inactivation would make a safer production method, inactivating the E. coli before purification procedures were employed

rather than at the end of the procedure as required with the use of irradiation inactivation. Currently, a formalin-inactivated and an irradiated lot of pilus vaccine are being prepared for human trials. Using a genetically more stable strain of a S. typhosa-S. sonnei hybrid, production of a second lot of a Typhus-Shigella hybrid vaccine was initiated. This material will be tested and made available for field studies. Seed cultures of the Leishmania parasite were prepared, tested, and found suitable for production of a Leishmania skin test antigen. The frozen seed materials were made available to the Department of Parasitology, Division of Experimental Therapeutics, for studies on techniques for producing the skin test antigen.

Recommendations

The direction of future work with the meningococcal vaccines will depend on the results of the field trials with the current vaccines available. If any show promise, production of a single large lot will be required for future field trials. Efforts to improve production techniques for the meningococcal vaccines will continue. Production of the 2 lots of purified E. coli pilus vaccines will continue and after safety testing, these will be made available for tests in humans. It is also expected that a whole E. coli organism vaccine will be produced for field tests. The production and testing of the Typhoid-Shigella hybrid vaccine will be completed and preparation of other hybrid vaccines will be attempted. The Department of Biologics Research will continue to provide production and freeze-drying support to other investigators as required.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OG 7009	2. DATE OF SUMMARY: 83 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUM'RY: 82 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DRG'N INSTR'N: NL	9a. SPECIFIC DATA - CONTRACTOR ACCESS: ☒ YES ☐ NO	9b. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D808	AB	004 WWGP
b. CONTRIBUTING				
c. XXXXXXXX	Cards			

11. TITLE (Procede with Security Classification Code):
(U) Gonococcal Vaccine Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
002600 Biology 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 06	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PRECEDING FISCAL YEAR 83	4.0	500
CURRENT FISCAL YEAR 84	5.0	306

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NAME: Piziak, M POC: DA
NAME:

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Neisseria; (U) Gonorrhea; (U) Gonococcal Vaccine; (U) Antigen; (U) Immunity

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide individual paragraphs identified by number. Precede text with Security Classification Code.)

23. (U) To develop a gonococcal vaccine. Gonorrhea has reached epidemic proportions in field troops in some areas (20 cases/1000/day) and gonococcal strains have developed resistance to penicillin as well as second line drugs.

24. (U) The general approach is to study and determine the immunologic response to naturally occurring gonococcal infections, determine the gonococcal antigen(s) responsible for that immunologic response, correlate these studies with natural disease, and then develop that antigen(s) as a vaccine candidate. Gonococcal pili which function to attach the gonococcus to epithelial mucosal cells, have been isolated and purified. Antibodies directed against gonococcal pili block the attachment of gonococci to epithelial cells. A prototype gonococcal pilus vaccine has been tested in humans and has been found to be safe and immunogenic. Field studies to determine vaccine efficacy, antibody level correlates and antigenic variation are planned. It is anticipated that a multi-antigen vaccine will eventually be developed.

25. (U) 82 10-83 09 A field trial to test the efficacy of P32 vaccine was conducted in US troops in ROK January-March 1983. 3252 volunteers entered the double blind, placebo-controlled trial (50 percent vaccine, 50 percent placebo). 93 percent completed the 8 week study. Vaccinees received 100 micrograms dose 2 weeks apart. Serum and secretion were collected at 2 week intervals. Treatment facilities were manned to evaluate venereal disease cases. The vaccine was well tolerated. Operationally, the study was a success. There were approximately 200 cases of GC defined. Preliminary results indicated the vaccine offered little, if any, protection during the trial period (For technical report see Walter Reed Army Institute of Research Progress Report, 1 Oct 82 - 30 Sep 83).

*Available to contractors upon originator's approval

Project 3M463750D808 DRUG AND VACCINE DEVELOPMENT

Work Unit 004: Gonococcal Vaccine Development

Investigators:

Principals: COL Edmund C. Tramont, MC
Samuel B. Formal, Ph.D.

Associates: COL Jerald C. Sadoff, MC
MAJ John Boslego, MC
MAJ Raymond Chung, MC
CPT Myron Piziak, MSC
CPT Daniel McChesney, MSC

Problem

The incidence of gonorrhea in military populations is highest in young adults between the ages 18 and 30 years, the bulk of the military population. In some parts of the world where U.S. troops are stationed, attack rates of gonorrhea are 500 per 1000 per year and it is estimated that up to 80% of enlisted troops will contract gonorrhea at least once during their tour of duty. The highest prevalence of penicillin resistant gonococcal strains occur in those parts of the world where military troops are stationed. For example, greater than 75% of the gonococcal strains now being isolated in Subic Bay, Philippines are resistant to penicillin. Resistance to spectinomycin has also recently been reported. The objectives of the WRAIR program are to determine the antigens of the gonococcus that elicit an immune response, to study the mechanisms of immunity to the gonococcus and to develop vaccines to protect against clinical disease.

Progress

A field trial protocol to test the efficacy of P32 gonococcal pilus vaccine was developed and approved by standard scientific and human use committees. Because of the nature of venereal disease and the potential for adverse publicity, review of the protocol by the Sec Army as well as the Dept of Defense was needed. Approval was finally obtained from the Deputy Sec Defense for a worldwide, tri-service testing program. US military personnel stationed in the Republic of Korea were selected as the initial field trial candidates. The field trial occurred between Jan-Mar 1983. A total of 3252 volunteers entered the double-blind, placebo controlled study (50% vaccine, 50% placebo). 93% completed the 8 week trial period. The vaccinees received 100 ug of vaccine I.D. on

day 1 and a booster on day 14. Serum and local secretions were collected at 0,2,4,6 and 8 wks. Medical treatment facilities were manned by physician assistants endogenous to the study team during the trial to evaluate all potential venereal disease cases. The field trial was an operational success. An adequate number of volunteers, good follow-up, good laboratory support and adequate disease surveillance were all accomplished. Cooperation between medical and line units was optimal. The vaccine was well tolerated. No major systemic reactions occurred. There was no adverse publicity. The ROK government was supportive of the study. Approximately 200 cases of gonorrhea occurred in study patients and these were evenly divided between the vaccine group and the placebo group suggesting little vaccine efficacy. Laboratory evaluation of strains, serum, secretion is underway.

Future Studies

1) Assay serum and secretions for antibody levels. Look for protection with high antibody level group.

2) Do antibiotic sensitivity tests on all isolates.

3) Analyze and compare the antigenic determinants of the pili from the infecting strains with the vaccine pilus.

4) Devise a 2nd generation vaccine composed of mixed pilus types or of a more immunogenic common determinants.

5) Investigate local immunization techniques.

6) Second generation vaccine field trial FY 85.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6766	83 10 01	DD-DR&E(AR)436

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORGN MATH'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M163750D808	AB	005 WWGQ
b. CONTRIBUTING	62770A	3M162770A871		
c. [illegible]	CARDS			

11. TITLE (Provide with Security Classification Code)

(U) Role of Polysaccharide Antigens in Immunity

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMING ORGANIZATION
80 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	2.0	486
	CURRENT 84	2.0	470

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Boslego, J
NAME: Brandt, B

POC:DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Vaccines; (U) Human Volunteers; (U) Meningococci; (U) Pseudomonas

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Infectious diseases continue to be a threat to military operations. Effective vaccines are a means to control infections, and several have reached the stage of development which requires preliminary testing in human beings for safety and antigenicity. Current emphasis is on the testing of meningococcal vaccines. Preliminary safety and antigenicity studies in human beings of experimental vaccines are necessary before efficacy studies of experimental vaccines can be undertaken.

24. (U) Experimental vaccines, consisting of purified products extracted from bacteria, are prepared in pilot lots by the Department of Biologic Products, WRAIR. These are tested for safety and antigenicity in the laboratory. Following review by the SGO and the Bureau of Biologics, FDA and with the consent and cooperation of Field Commanders, these vaccines are tested in soldier volunteers for safety and antigenicity.

25. (U) 82 10-83 09 Clinical testing of the meningococcal tetravalent A,C,Y,W135 vaccine was completed. Results indicated that the vaccine is safe and antigenic and that the dose could be reduced from 200 to 60 micrograms without reduction in antigenicity. This vaccine has now been licensed and its routine use in military recruits was initiated in FY83. Group B meningococcal disease remains a problem. Five new lots of meningococcal group B vaccine including a combined group B-tetravalent vaccine were prepared for human use and characterized with respect to composition, purity, safety and antigenicity. These vaccines consist of serotype protein-capsular polysaccharide complexes and were prepared using improved protein purification methods which results in lower levels of contaminating endotoxin. These vaccines are ready for clinical testing. (For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83).

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M463750D808 DRUG & VACCINE DEVELOPMENT

Work Unit 005 : Role of Polysaccharide Antigens in Immunity

Investigators: Wendell D. Zollinger, Ph.D.
Samuel B. Formal, Ph.D.
Brenda Brandt, MS

Problem

Infectious diseases continue to be a threat to military operations. Effective vaccines are a means to control infections, and several have reached the stage of development which requires preliminary testing in human beings for safety and antigenicity. Current emphasis is on the testing of meningococcal group B and tetravalent A,C, Y, W-135 vaccines. Preliminary safety and antigenicity studies in human beings of experimental vaccines are necessary before efficacy studies of experimental vaccines can be undertaken. Experimental vaccines, consisting of purified products extracted from bacteria, are prepared in pilot lots by the Dept. of Biologic Research, WRAIR. These are tested for safety and antigenicity in the laboratory. Following review by the SGO and the Bureau of Biologics, FDA and with the consent and cooperation of Field Commanders, these experimental products are tested in soldier volunteers for safety and antigenicity.

Progress

The relationship of dose to the reactogenicity and immunogenicity of meningococcal tetravalent A,C,Y, W135 vaccine was studied in Army recruits at Ft. Dix, NJ. The commercial vaccine which has recently been licensed and is now being given to all new military recruits was used in this study. A total of 121 volunteers received the standard 200 ug dose and 319 received a reduced dose of 60 ug of the vaccine. An additional 118 nonvolunteers who received the standard 200 ug dose were monitored for side effects. The vaccines were well tolerated; no major systemic reactions occurred. The larger dose produced a slightly larger area of erythema and slightly more soreness at the vaccination site than the smaller dose, but other parameters such as frequency of sore arms, induration and systemic complaints were the same for both doses. The results suggest little clinical difference between the vaccine doses in terms of side effects. Those volunteers who received the 200 ug dose appeared to

respond with higher binding antibody to A and C polysaccharides, but both groups responded equally well with respect to bactericidal antibody.

A candidate meningococcal group B vaccine consisting of capsular polysaccharide noncovalently complexed to the serotype proteins of the outer membrane has shown promise of being an effective vaccine in previous safety and immunogenicity studies in human beings. Available evidence indicates, however, that this vaccine may provide only type specific protection. This requires that a group B vaccine of this type contains serotype proteins that are representative of the strains causing disease in the target population. A survey of serotypes of group B case strains (90) and carrier strains (350) recently isolated from U.S. Army personnel and from Norway and South Africa was conducted by a newly developed method using monoclonal antibodies as serotyping reagents. The results indicated that serotype 2b and 15 are presently the most common. Since our previous group B vaccines were prepared from a serotype 2a strain, new vaccines (9 lots with different compositions and characteristics) were prepared for human use by complexing serotype 2b and 15 proteins with either group B polysaccharide or the licensed tetravalent A,C,Y,W135 vaccine. The latter combination will be tested as a pentavalent A,B,C,Y,W135 vaccine. These new vaccines were prepared using newly developed procedures which reduced substantially the level of contaminating lipopolysaccharide. They were all characterized with respect to composition and physical properties and tested for safety and immunogenicity in animals. They are now ready for safety and immunogenicity testing in human beings.

Future Plans

Development of the tetravalent A,C,Y, W135 vaccine is essentially complete except for proof of efficacy of the Y and W135 components. Since the disease rate for Y and W135 is too low for a controlled efficacy trial to be practical and there is every indication the vaccine will be effective against all four serogroups evidence of efficacy will be obtained by monitoring the incidence of vaccine failures among recruits who are receiving the vaccine on a routine basis. The pentavlent vaccine containing four capsular polysaccharides (A,C,Y,W135) complexed to serotype 2b and 15 outer membrane proteins will be evaluated for safety and immunogenicity in volunteers. Several additional lots of this type will be manufactured with modifications as necessary to achieve

optimal immune response and minimal reactogenicity. A large lot of vaccine will then be produced by a drug company on contract. This lot will be used in an attempt to obtain efficacy data for group B disease. The serotype of meningococcal strains causing group B disease will be monitored on a continuing basis to determine if additional or different proteins should be included in the vaccine formulation.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA 302684	2. DATE OF SUMMARY: 83 10 01		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 83 06 15	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISP INSTR: NL	8a. SPECIFIC DATA CONTRACTOR ACCESS: ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT
10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	63750A	3M463750D808		AI	007	WWK3	
b. CONTRIBUTING							
c. CONTRIBUTING	DARPC						

11. TITLE (Precede with Security Classification Code)

(U) Evaluation of prophylactic drugs and vaccines against diseases of military importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 102600 Clinical Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
83 06	CONT	DA			C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	0.8	25
	CURRENT 84	0.8	25

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NAME: Kelley, P W

POC: DA

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Clinical Pharmacology; (U) Human Volunteer; (U) Biological Products; (U) Epidemiology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To assess the suitability of certain military population groups such as Special Forces, Rangers, or Jungle Operations Training Center trainees as study groups for vaccine and drug prophylaxis trials. To conduct field trials of prophylactic agents. To evaluate compliance of military populations with prophylactic regimens.
24. (U) Contemporary epidemiologic methods are applied to accomplish the above objectives. Multi-disciplinary collaborative approaches are emphasized in the conduct of placebo-controlled double-blind studies of safety, immunogenicity, and efficacy of vaccines and prophylactic drugs. Studies are normally done using soldier-volunteers.
25. (U) 8210 This fall a new policy was established by the Army Surgeon General for weekly administration 8309 to trainees at the Jungle Operations Training Center, Ft. Sherman, Panama of doxycycline to prevent leptospirosis. We have undertaken a prospective study to assess aspects of compliance and the incidence of side effects in the operational setting. Pre-deployment sera has been obtained from two U.S. Marine battalions. The post-deployment evaluations to gather the required data and to confirm the efficacy of doxycycline prophylaxis are pending.

In collaboration with the Department of Bacterial Diseases, basic trainees at Ft. Benning were, through analysis of acquired data bases, determined to be a suitable population for field testing of a pentavalent meningococcal vaccine incorporating group B antigen. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A AND 1498-1 MAR 88 (FOR ARMY USE) ARE OBSOLETE.

Project 3M463750D808 Development of Drugs and Vaccines Against Militarily Important Diseases

Work Unit 007 Field Evaluation of Prophylactic Drugs and Vaccines Against Diseases of Military Importance

Investigators.
Principal:
COL Richard N. Miller, MC
Associate: LTC Ernest T. Takafuji, MC
CPT Patrick W. Kelley, MC

Objective: To assess the suitability of certain military population groups such as Special Forces, Rangers, or Jungle Operations Training Center trainees as study groups for vaccine and drug prophylaxis trials. To conduct field trials of prophylactic agents. To evaluate compliance of military populations with prophylactic regimens.

Progress:

1. Doxycycline Prophylaxis of Leptospirosis: A new policy has been established by the Army Surgeon General for weekly administration of doxycycline prophylaxis against leptospirosis during the fall high risk season to trainees at the Jungle Operations Training Center, Ft. Sherman, Panama. We have undertaken a prospective study to assess aspects of compliance and the incidence of side effects in the operational setting. Pre-deployment sera has been obtained from two U.S. Marine battalions. The post-deployment evaluations are pending.

2. Meningococcal Meningitis Group B Vaccine: In collaboration with the Department of Bacterial Diseases, a suitable population was identified for field testing of a pentavalent meningococcal meningitis vaccine incorporating group B antigen.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

	DA305150	83 10 01					
	A. NEW	U	U		NL		
	63750A	3M463750D808		AJ	008	WRSE	
	CAPES						

(U) Hepatitis A vaccine development

010100 Microbiology 002600 Biology 003500 Clinical Medicine

83 10	CONT	DA		C. In-house	
		FISCAL YEAR 83	0.0	00	
		FISCAL YEAR 84	3.0	57	

Walter Reed Army Institute of Research
Washington, DC 20307

TOP, F H JR
202-576-3551

FINA

Walter Reed Army Institute of Research
Div of Comm Dis & Immunology
Washington, DC 20307

Eckels, K H
301-427-5208

Dubois, D R

POC: DA

(U) hepatitis virus; (U) hepatitis vaccine; (U) inactivated vaccine

23. (U) Hepatitis A virus has caused epidemic hepatitis in American soldiers in every war and is the most prevalent form of viral hepatitis that will disrupt normal functioning of a whole military unit. Presently, the only immunoprophylaxis available is temporary passive immunization with immune serum globulin. This work unit is concerned with the development of a formalin-inactivated vaccine for hepatitis A virus which will confer protective, active immunity to infection. The first lot of vaccine will be produced on a pilot scale so that it may be used for clinical safety and efficacy trials in human subjects.

24. (U) A strain of human hepatitis A virus designated HM-175 previously passaged in BSC-1 or MRC-5 cells will be passaged one more time in these cells so that two master seeds can be prepared. The master seed with the highest infectivity titer and which has passed all safety tests will be used to make a production seed and a lot of vaccine (approximately 15 liters). The viral harvest for the vaccine will consist of a series of supernatant fluid collections with each being inactivated with 1:4000 formalin over a period of 96 hrs. The first lot of vaccine will undergo safety testing including primate inoculation prior to human trials.

25. (U) New.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 83 (FOR ARMY USE) ARE OBSOLETE.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 303 137	2. DATE OF SUMMARY* 83 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY: A New	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8a. ORG'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M463750D808	AB	009 WWGK
b. CONTRIBUTING				
c. CONTRIBUTING	CARDS			

11. TITLE (Include with Security Classification Code)*
(U) Shigella Vaccines

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:*
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	1.0	228
FISCAL YEAR CURRENT 84	1.0	241

19. RESPONSIBLE DOD ORGANIZATION
NAME:* Walter Reed Army Institute of Research
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TELEPHONE: (202) 576-3344
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign intelligence considered

POC: DA

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Attenuated Vaccines; (U) Human Volunteers; (U) Shigella; (U) Salmonella

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Provide text of each with Security Classification Code.)

23. (U) Infectious diarrheal diseases continue to be a threat to military operations. Effective vaccines are a means to control some of these infections. Experimental products require safety testing in volunteers and field tests for efficacy. Current emphasis is on a preparation designed to protect against both typhoid fever and dysentery caused by Shigella sonnei.

24. (U) Experimental oral vaccines consisting of living attenuated bacteria are prepared in pilot lots by the Department of Biologics Research Department, WRAIR. These are tested for safety and antigenicity in the laboratory. Following review by the Surgeon General's Office and the Bureau of Biologics, FDA, these products are tested in volunteers for safety and antigenicity. Upon successful completion of these studies commerically prepared lots are tested for efficacy in suitable populations.

25. (U) 82 10 - 83 09 Safety tests of the Salmonella typhi Shigella sonnei vaccine strain have been complete. An initial efficacy trial involving 18 volunteers gave evidence of protection. An E. coli K-12 strain carrying S. flexneri chromosomal and plasmid genes and expressing tne antigens of S. flexneri 2a protected monkeys against challenge with virulent S. flexneri 2a. (For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83).

*Available to contractors upon originator's approval

Project 3M463750D808 DRUG AND VACCINE DEVELOPMENT.

Work Unit 009 : Shigella Vaccines

Investigators:

Principal: Samuel B. Formal, Ph.D.
Associates: Edmund C. Tramont, M.D.

Problem

Shigellosis continues to be a military problem. Parenterally administered vaccines are not effective. Evidence has accumulated which indicate that oral immunization can be effective. The purpose of this work unit is to develop living attenuated oral shigella vaccines.

Progress

A total of 19 volunteers have ingested the *S. typhi-S. sonnei* vaccine strain in doses ranging from 1×10^{10} cells. One person fed the highest dose experienced transient mild abdominal symptoms. An initial efficacy trial, carried out at the University of Maryland, involving 18 volunteers has been carried out. Following challenge with virulent *S. sonnei* 6 of 8 (75%) control individuals became ill while 2 of 10 (20%) vaccinees developed signs of illness.

A second candidate vaccine has been prepared using *E. coli* strain K-12 which has inherited chromosomal and plasmid genes from *S. flexneri*. A strain expressing the antigens of *S. flexneri* 2a significantly protected monkeys against challenge.

Bibliography

None

PROJECT 3M162770A870

RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

RESEARCH AND TECHNOLO WORK UNIT SUMMARY | DA0G1295 | 83 10 01 | DD-DR&E(AR)636

2. DATE PREV SUMRY	3. KIND OF SUMRY	4. SUMMARY SCTY	5. WORK SECURITY	6. REGRADING	7a. GRADE/GR ENTERED	7b. SPECIFIC DATA CONTRACTOR ACCESS	8. LEVEL OF SUM
821001	D. Change	U	U		CY	[X] YES [ ] NO	A. WORK UNIT

9a. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	00	041 WWG6
b. CONTRIBUTING	61101A	3A161101A91C	00	121
c. CONTRIBUTING	STOG 82/83-6.2.3			

9. TITLE: (U) Identification of Trypanosoma rhodesiense Protective Antigens

10. SCIENTIFIC AND TECHNOLOGICAL AREAS: 0613 Microbiology 0603 Biology

11. START DATE	12. ESTIMATED COMPLETION DATE	13. FUNDING AGENCY	14. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

15. CONTRACT/GRANT
a. DATE/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

16. RESOURCES ESTIMATE

		a. PROFESSIONAL MAN YRS	b. FUNDS ($ thousands)
FISCAL YEAR	PRECEDING 83	1.0	250
	CURRENT 84	2.0	335

17. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: (202) 576-3544
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Esser, K
NAME: POC: DA

19. GENERAL USE
H
Foreign Intelligence Considered

20. KEYWORDS: (U) Lab Animals (U) Mice (U) RAM I
(U) Vaccine; (U) Trypanosomiasis; (U) Monoclonal Antibody; (U) Antigen

21. TECHNICAL OBJECTIVE, 22. APPROACH, 23. PROGRESS (Provide brief individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) African sleeping sickness, a potential threat to military operations in Africa, has reached epidemic proportions in some areas. Currently no prophylaxix is available and the chemotherapeutic agents are toxic. The objective of the current work is to identify protective antigens of Trypanosoma rhodesiense. This work will be the basis for vaccine development.

24. (U) These studies employ an animal model to investigate immunity to both the infective insect form and blood form of the parasite. To identify the antigen types involved in immunity, monoclonal antibodies are prepared as markers for specific antigens. These reagents are used for the antigen type analysis of parasites from the field and will also provide the means to isolate specific antigens.

25. (U) 82 10-83 09 Monoclonal antibodies were generated which identified and neutralized 13 distinct metacyclic (insect) forms of T. rhodesiense. Genes coding for 2 of these antigens have been cloned and sequenced. Mice have been immunized with peptides corresponding to conserved regions of amino acid sequences coded for by these cloned genes. Resistance to trypanosome infection in these mice is now being assessed. Monoclonal antibodies have been used to identify previously unidentified surface antigens of trypanosomes which might be the basis for a vaccine. This research will be continued as part of the Institute's integrated research program. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 83 AND 1498-1 1 MAR 86 FOR ARMY (L) ARE OBSOLETE.

PROJECT 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

Work Unit 041 Identification of *Trypanosoma rhodesiense* Protective Antigens

Investigators:

Principals: Dr. Klaus M. Esser
LTC Wayne T. Hockmeyer, MSC

Associates: Dr. Bruce T. Hall
CPT Donald E. Burgess, MSC
Mr. Maurice J. Schoenbechler
Mr. William L. Bowie
SP5 Margaret Meadows

Problem and Objective:

African trypanosomiasis poses significant health hazards to troops operating in endemic areas. This is a progressive, generally fatal disease transmitted by the bite of infected tsetse flies. The fly vector and the causative protozoan parasite are prevalent throughout 30% of Africa. The current level of reported human disease is not high due primarily to restricted land use patterns and vector control measures in highly populated areas. However, the potential of this disease is evident from previous epidemics in which 20 - 30% of the population in some areas died. Although data on the risk of infection for military troops deployed in endemic areas is not available, a high incidence of infection would be expected. Currently no prophylaxis is available and therapeutic drugs are toxic. Vaccination against African trypanosomiasis is theoretically possible. Protection against infection with a single trypanosome antigen type can easily be achieved by immunization with attenuated parasites or purified antigens. However, multiple antigen types of the parasite are present in the fly vector and a large number arise by antigenic variation in the host. The objective of this work unit is the identification and isolation of the antigens which can elicit a protective immune response against the infective insect form of the parasite.

Progress:

Monoclonal antibodies (MAbs) which identify and neutralize infectivity of fourteen metacyclic (infective insect form) antigen types have been generated. In sum, these MAbs provide partial immunity against metacyclic infection in experimental systems and therefore are considered to be specific for antigens important in vaccine development. These MAbs have been used in efforts to clone the genes which code for metacyclic antigens in collaboration with Dr. John Donelson at the University of Iowa. Currently, six cloned genes have been identified which are likely candidates for metacyclic surface antigen coding sequences. These genes have been inserted into an E. coli system which allows expression of the protein for which each gene codes. These trypanosome proteins produced in E. coli have recently been used to immunize mice. If immunization studies confirm the identity of these gene products, then these cloned genes will provide a means to synthesize antigens for vaccine development.

Studies have been done to determine if metacyclic trypanosome in a given endemic area are antigenically stable over time. Trypanosomes isolated from naturally infected humans in the Lambwe Valley, Kenya over the period 1974 - 1981 were used to infect laboratory reared tsetse flies. MAb analysis of metacyclics from these flies has demonstrated expression of the same metacyclic antigen types over this eight year period. The lack of any observed "antigenic drift" with time suggests that polyvalent vaccine development may be possible.

An alternative to polyvalent vaccination directed against different metacyclic antigen types, is development of a vaccine targeted to antigenic determinants shared among different trypanosome antigen types. Two approaches to this possiblility have been pursued. First, seven synthetic peptides corresponding to regions of highly conserved amino acid sequences of the major trypanosome variable surface proteins have been produced and used to immunize mice. Anti-trypanosome activity of antibody produced in these mice is now being assessed. Alternatively, identification of surface protein molecules which are shared among different trypanosome antigen types is being attempted. MAbs have been produced which appear to bind to the surface of live trypanosomes of several different VATs. If these MAbs can protect against trypanosome infection or if immunization with the target antigen is protective, the vaccine development will proceed along these lines.

Recommendations:

In view of the finding that metacyclic heterogeneity appears to be restricted and that experimental immunization is possible, further work is indicated for the identification of antigens involved in eliciting a broad-spectrum immunity. Also, continued analysis of metacyclics from a range of different trypanosome isolates is necessary to determine the degree of metacyclic hetergeneity in a particular endemic area. Direct analysis of metacyclics present in tsetse flies in endemic areas will allow confirmation of key laboratory findings. Monoclonal antibodies will continue to be the major tool for these studies. Refinement of serodiagnostic techniques is also needed to provide a clinically useful level of sensitivity and specificity. Synthetic peptide immunization studies should be continued on an expanded scale. This work will be relevant for trypanosomiasis vaccine development and also for establishing critical groundwork for production of synthetic vaccines is general.

Presentations:

1. A comparison of the epitope specificity and biological activities of protective and non-protective monoclonal antibodies specific for the WRATat 1 clone of Trypanosome rhodesiense. T. Hall and K. Esser. American Society of Tropical Medicine and Hygiene, 31st Annual Meeting, November 1982.

2. Comparison of the reactivities of variable antigen type (VAT) specific rabbit antisera and monoclonal antibodies (MAbs) with Trypanosoma brucei rhodesiense. D. Burgess, B. Welde, G. Campbell and K. Esser. American Society of Tropical Medicine and Hygiene, 31st Annual Meeting, November 1982.

3. African trypanosomiasis: protective immunity and target antigens. K. Esser. American Society for Microbiology: Impact of Monoclonal Antibodies on the Diagnosis and Prevention of Parasitic Disease, March, 1983.

4. Systematic identification of Trypanosoma brucei rhodesiense metacyclic variable antigen types (M-VATs) important for protective immunity against tsetse fly challenge. M. Schoenbechler, J. Gingrich and K. Esser. Federation of American Societies of Experimental Biology, April, 1980.

5. Topological mapping of protective and non-protective epitopes on the variant-specific surface glycoprotein of the WRATat 1 clone of Trypanosoma rhodesiense. T. Hall and K. Esser. Federation of American Societies for Experimental Biology, April, 1983.

6. Variable antigen type (VAT) composition of Trypanosoma brucei rhodesiense: discrepancy between results using VAT-specific monoclonal antibodies and rabbit antisera. D. Burgess. Gordon Research Conferences: Immunological and Molecular Aspects of Parasitism, August, 1983.

7. Restricted metacyclic heterogeneity in Trypanosoma brucei rhodesiense. K. Esser. Gordon Research Conference: Immunological and Molecular Aspects of Parasitism, August, 1983.

8. Epitope mapping of the variant-specific surface glycoprotein of the WRATat 1 clone of Trypanosoma rhodesiense. T. Hall. Gordon Research Conferences: Immunological and Molecular Aspects of Parasitism, August, 1983.

Publications:

1. Trypanosoma rhodesiense blood forms express all antigen specificities relevant to protection against metacyclic (insect form) challenge. K. Esser, M. Schoenbechler and J. Gingrich. J. Immunol. 129:1715-1718. (1982).

2. African sleeping sickness: new evidence that mature tsetse flies (Glossina morsitans) can become potent vectors. Trans. R. Soc. Trop. Med. Hyg. 76:479-481. (1982).

3. Topological mapping of protective and non-protective epitopes on the variant-specific surface glycoprotein of the WRATat 1 clone of Trypanosoma brucei rhodesiense. T. Hall and K. Esser (Submitted for Publication).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA OB 6489	83 10 01	DD DR&E(AR)613
2. DATE PREV SUM'RY: 82 10 01	3. KIND OF SUMMARY: D. Change	4. SUMMARY SCTY: U	5. WORK SECURITY: U	7. REGRADING:	8. DISP'N INSTR'N: NL	9a. SPECIFIC DATA - CONTRACTOR ACCESS: ☒ YES ☐ NO	9b. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AI	072 WWK2
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG	3M162	072	

11. TITLE (Procede with Security Classification Code):

(U) Assessment of Infectious Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

003500 Clinical Medicine 005900 Environmental Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
7- 97	CONT	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PRECEDING FISCAL YEAR 83	3.0	204
CURRENT FISCAL YEAR 84	3.0	155

19. RESPONSIBLE DOD ORGANIZATION

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20. GENERAL USE

FINA

20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Takafuji, E T

NAME: Lednar, W M

POC: DA

21. KEYWORDS (Provide EACH with Security Classification Code):

(U) Epidemiology; (U) Infectious Disease; (U) Risk Assessment; (U) Data Bases

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To identify, define, and study known and potential causes of disability in military populations using relevant, existing epidemiologic techniques and developing appropriate new methodology. To apply this information to the assessment, prevention, and control of diseases in military populations.

24. (U) Contemporary epidemiologic methods are applied to causes of disability in military populations. Multidisciplinary collaborative approaches are utilized and new methods developed as required.

25. (U) 8210-8309. Analyses of the following studies are in progress: assessment of the incidence of tropical diseases in soldiers training at the Jungle Operations Training Center, Ft. Sherman, Panama; prophylaxis of leptospirosis with doxycycline; etiologies for eosinophilia in individuals exposed to the Panamanian jungle; epidemiologic and clinical characteristics of two military hepatitis A outbreaks; hepatitis B transmission among inmates at the US Disciplinary Barracks; hepatitis B risk within military occupations; prevalence of penicillinase-producing Neisseria gonorrhoeae in US Army populations; disease surveillance of Special Forces and special operations personnel deployed overseas; a tuberculosis outbreak at a military hospital laboratory; disease surveillance during the Brightstar '83 military exercise; the routes of shigella transmission during a deployment associated outbreak; the epidemiology of Neisseria meningitidis group B in basic trainees at Ft. Benning. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

* Available to contractors upon requestor's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND MAY BE USED UNTIL EXHAUSTED

Project 3M162770A8'0 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

Work Unit 072 Assessment of Infectious Diseases of Military Importance

Investigators.
Principal:
COL Richard N. Miller, MC
Associate: LTC Ernest T. Takafuji, MC
LTC James W. Kirkpatrick, MC
MAJ Mary K. McKenna, ANC
MAJ Wayne M. Lednar, MC
CPT Jose L. Sanchez, MC
CPT Patrick W. Kelley, MC
MAJ Adeline G. Washington, ANC
Dr. Lytt I. Gardner

Objective: To assess the actual or potential impact of selected diseases of military importance. Military importance is determined by examining existing or historical morbidity and mortality data or analysis of potential threats. The studies are primarily epidemiologic in nature and represent cooperative efforts with other divisions of WRAIR and USAMRDC.

Progress:

1. Disease Surveillance Following Jungle Training: Medical surveillance was conducted throughout the year on over 4000 soldiers and marines training at the Jungle Operations Training Center (JOTC) at Fort Sherman, Panama. This effort involving serological screening and questionnaire evaluations identified continuing problems with leptospirosis, leishmaniasis, Venezuelan Equine Encephalomyelitis, and intestinal parasites. This surveillance effort has resulted in a better understanding of infectious diseases associated with jungle exposure.

2. Doxycycline Prophylaxis Study: In response to the leptospirosis problem first identified among jungle trainees in the fall of 1981, the Division of Preventive Medicine conducted a double-blind,

randomized, placebo-controlled field trial to determine if doxycycline was an effective chemoprophylactic agent against this infection. Doxycycline (200mg) or placebo was administered orally on a weekly basis and at the completion of training to 940 volunteers from two battalions deployed to Panama for approximately three weeks of jungle training. Twenty cases of leptospirosis occurred in the placebo group (attack rate of 4.2%) compared to only one case in the doxycycline group (attack rate of 0.2%. $p<0.001$), yielding an efficacy of 95 percent. This study demonstrated for the first time the value of doxycycline as a prophylactic agent against leptospirosis. These findings were presented to the Armed Forces Epidemiology Board, after which the Surgeon General promulgated a policy of weekly doxycycline prophylaxis for JOTC trainees during the high risk fall season. This policy was instituted in September 1983.

3. Evaluation of Eosinophilia Associated with Jungle Training: Eosinophilia and gastrointestinal complaints of abdominal pain and diarrhea have been recognized in soldiers training in Panama. Helminthic infections have been documented in some of these soldiers, but the source(s) of infection has been unclear. In September 1983, a team from the Division of Preventive Medicine was deployed to the JOTC to determine the extent of the problem and to identify possible environmental sources of infection. Laboratory records and personal medical records for JOTC cadre and their dependents were reviewed. Military and civilian personnel stationed at Ft. Sherman were screened for evidence of intestinal parasitic infections and eosinophilia, and Panamanian families living in heavily used training areas were screened. Animals were collected from different areas on the Ft. Sherman Military Reservation and evaluated.

The screening of laboratory and medical records identified some cases of eosinophilia. Five of 14 cadre instructors with frequent jungle exposure had eosinophilia greater than 10%; stool evaluations are pending. Over 90% of the Panamanians living in the training areas were found to be heavily infected with hookworm, Strongyloides stercoralis, and/or Trichuris trichiura. At least four cases of cutaneous leishmaniasis were also identified among these Panamanians. Specimens obtained from animals are still being analyzed.

4. Hepatitis: The analysis of data from three hepatitis outbreaks that occurred during FY 82 continued. Serological analyses of blood from the April-May 1982 United States Disciplinary Barracks outbreak due to hepatitis A virus (HAV) also identified 23 inmates acutely infected with hepatitis B virus (HBV). Eighteen percent of these acutely infected inmates had been imprisoned more than two years. These findings, together with a serological pattern suggesting resolving infection and a 2.9% prevalence of surface antigenemia among arriving inmates, are consistent with intraprison transmission of HBV.

A one-year follow-up of arriving inmates for HBV seroconversion is underway to document the risk of intraprison transmission and the need for immunoprophylaxis with the hepatitis B vaccine. Analyses of this outbreak and the July 1982 Grafenwoehr Germany hepatitis A outbreak showed that HAV infection in adults may be more symptomatic than previously believed. Follow-up evaluation 8 weeks after ISG revealed that post-exposure prophylaxis rapidly terminated subsequent spread of HAV.

In the fall of 1982 the Epidemiology Consultant Service (EPICON) investigated an outbreak of hepatitis A in the 205th Infantry Brigade of the U.S. Army Reserve Command following the unit's training at Ft. McCoy, WI. Attack rates of 4.2% and 3.0% were documented in the two most heavily affected battalions. Foodborne transmission was suspected but no specific food item could be incriminated. Unsatisfactory field sanitation practices and problems in personal hygiene may have also contributed to the epidemic.

An analysis of the association between military occupation and hospitalization in 1980 in a U.S. Army hospital for hepatitis B was undertaken. Excess hospitalization rates were found for enlisted hospital employees regularly exposed to blood. The differences between the rates for high risk occupations, combat arms, and combat support personnel were small and statistically non-significant. Medical personnel in Korea exposed to blood had HBV hospitalization rates more than twice that observed among similarily exposed medical personnel in CONUS ($p<0.07$). Medical personnel assigned to Korea had rates which were 50 percent higher than rates among medical personnel in Europe. The explanation for the excess in HBV risk seen among medical personnel in Korea is currently under study.

5. Cardiovascular Disease Studies: The Division of Preventive Medicine has been assisting the Munson Army Community Hospital, Ft. Leavenworth, KS, and the U.S. Army Surgeon General's Task Force on Fitness in the design and analysis of studies directed at assessing the cardiovascular disease risk among students at the Command and General Staff College (CGSC). Secondary screening for occult coronary disease and cardiac dysrythmias emergent upon graded exercise testing (treadmills) was performed in 1982 and 1983. During 1982, follow-up of asymptomatic soldiers under age 40 with abnormal stress ECG's identified no individuals with occult coronary disease. A similar evaluation will be performed on CGSC students with high and low cardiovascular risk profiles to assess the effectiveness of a health risk appraisal to predict abnormalities in graded exercise tests. An evaluation of the cardiovascular risk factor intervention program for CGSC students with hypertension, elevated serum cholesterol, and/or cigarette smoking was begun.

6: Evaluation of Military Weight Tables: Revised weight for height screening tables were published in AR 600-9, effective 15 April 1983. The validity of these tables to identify obese soldiers evaluated by extensive anthropometric assessment (sum of four skinfolds - triceps, biceps, subscapular, and suprailiac) was evaluated. Results of selected "administrative and anthropometric assessments" requested by commanders for soldiers within the screening tables' limits were also assessed. Forty thousand recruits undergoing Basic Combat Training (BCT) at Army installations during January-June 1983 were assessed for height and weight compliance with the screening tables and performance (pass/fail) in BCT. No consistent performance pattern emerged for meeting/failing AR 600-9 obesity standards.

7. Sexually Transmitted Diseases in the Army: The Division of Preventive Medicine continued to monitor the incidence of gonorrhea in the Army and specifically the incidence of penicillinase-producing Neissseria gonorrhea (PPNG). The prevalence of PPNG in Korea was followed and was noted to decrease following institution of spectinomycin as the first drug-of-choice in the treatment of uncomplicated gonorrhea. A study is in progress at Ft. Bragg to define the epidemiologic characteristics associated with STD infection and risk factors.

8. Disease Surveillance of Special Forces Units and Special Operations: Support in the monitoring of febrile illnesses of Special Forces personnel and other military personnel deployed to overseas areas continued to be provided by the Division of Preventive Medicine. Support was provided to the U.S. Central Command, Multinational Peace Keeping Force and Observers (MFO), and military operations in Central America. No unusual etiologies of disease were identified in FY 83.

9. Tuberculosis: During January and February 1983, an EPICON team investigated a suspected outbreak of tuberculosis in the microbiology section of the Department of Pathology at the Walter Reed Army Medical Center. In the fall of 1982, 5 of 26 employees from that section were identified as new skin test converters including an employee who subsequently was found to have active tuberculosis. The investigation suggested possible acquisition of infection within the laboratory setting and a possible association for some cases with work in the mycology/tuberculosis section. No ongoing tuberculosis transmission was identified at the time of the investigation and no subsequent cases have occurred.

10. Brightstar '83 Disease Surveillance: During August and September 1983, a major military exercise involving approximately 5500 U.S. troops was conducted in Egypt, Sudan, Somalia, and Oman. The Problem Definition and Assessment Team (PDA) was deployed for the first time with Third U.S. Army to Cairo-West to provide on-site assistance in the identification of potential disease threats in the area. Disease surveillance during the exercise was conducted with the assistance of a portable microcomputer monitoring hospitalizations and outpatient medical visits. Sick call log sheets listing diagnoses and diagnostic categories were utilized by treatment facilities. Through the collection and analysis of sick call/hospitalization data, it was possible to calculate daily attack rates for disease categories and monitor morbidity during the exercise. This effort is part of a continuing interest of the Division of Preventive Medicine to monitor non-battle illnesses in the field.

11. Shigellosis Outbreak During Brightstar '83: During Brightstar '83, the 82nd Airborne Division experienced an outbreak of shigellosis involving approximately 95 individuals (attack rate of 20%).

Twenty-five soldiers were hospitalized. The responsible organism was Shigella dysenteriae, subtype 4. The epidemiologic investigation was conducted by the PDA team, and a follow-up evaluation was made by the Division of Preventive Medicine after the unit returned to Ft. Bragg. Numerous deficiencies in the handling of potable water supplies were documented, and a waterborne mode of transmission was suggested. Analysis of data from this outbreak is in progress.

12. Meningococcal Disease: In late July 1983, two cases of meningococcal meningitis occurred among basic trainees at Ft. Benning. A review of cases and meningococcal specimens submitted to WRAIR revealed that Fort Benning accounted for an unusually large number of isolates of Neisseria meningitidis over the past two years and that every isolate from Benning in the last 18 months was of an identical antigenic strain: group B, subtype 2b. In contrast to Ft. Benning, records for the 7 other basic training centers showed significantly lower rates of meningitis and a heterogeneous mix of serogroups. All of the Ft. Benning cases in the last 18 months were members of one training brigade on post. The outbreak is currently under investigation.

Formal Presentations:

1. "Prospective Epidemiologic Studies" Uniformed Services University of the Health Sciences, Bethesda, MD, 28 October 1983, Lytt I. Gardner, Ph.D.

2. "A Review of the Epidemiology of Leptospirosis Associated with Jungle Training in Panama", Society of Medical Consultants to the Armed Forces, Uniformed Services University of the Health Sciences, Bethesda, MD, 13 November 1982, Wayne M. Lednar, MC.

3. "Epidemiology and Clinical Characteristics of Leptospirosis Among Soldiers Training in Panama" Grand Rounds in Preventive Medicine, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD. March 1983, MAJ Wayne M. Lednar, MC.

4. "Outbreaks of Leptospirosis in 1983" Leptospirosis Workshop II, Walter Reed Army Institute of Research, Washington, D.C., 2 March 1983, MAJ Wayne M. Lednar, MC, LTC N. Joe Thompson, MC, MAJ Miriam L. Fields, MC.

5. "1982 Doxycycline Prophylaxis Study" Leptospirosis Workshop II, Walter Reed Army Institute of Research, Washington, D.C. 2 March 1983, LTC Ernest T. Takafuji, MC, LTC James W. Kirkpatrick, MC, COL Richard N. Miller, MC, MAJ Jerome J. Karwacki, MC, CPT Patrick W. Kelley, MC, Michael R. Gray, M.S., MAJ K. Mills McNeill, MC, MAJ Harold L. Timboe, MC, CPT Robert Kane, MC, CPT Jose L. Sanchez, MC.

6. "1982 Doxycycline Prophylaxis Study" Armed Forces Epidemiology Board, Walter Reed Army Institute of Research, Washington, D.C.. 11 March 1983, LTC Ernest T. Takafuji, MC, et al.

7. "Infectious Disease Problems in Panama" Tropical Medicine Dinner Club, The Johns Hopkins University, Baltimore, MD, 23 March 1983, LTC Ernest T. Takafuji, MC.

8. "The Epidemiology Consultant Service" Preventive Medicine Managment Course, Academy of Health Sciences, Ft. Sam Houston, TX, 30 March 1983, MAJ Wayne M. Lednar, MC.

9. "Medical Problems Associated with the Jungle Operations Training Center" FORSCOM Surgeon's Conference, Atlanta, GA, 19 April 1983, LTC Ernest T. Takafuji, MC.

10. "When Is It Too Late to Investigate an Outbreak?" Society for Epidemiologic Research, Winnipeg, Manitoba, June 1983, MAJ Wayne M. Lednar, MC, MAJ Robert R. Redfield, MC, CPT Patrick W. Kelley, MC, MAJ Steven Hayne, MC, COL Richard N. Miller, MC.

11. "Hepatitis B Hospitalizations in High Risk Occupations" Society for Epidemiologic Research, Winnepeg, Manitoba, 15 June 1983, Lytt I. Gardner, Ph.D., MAJ Wayne M. Lednar, MC, MAJ Robert R. Redfield, MC, COL Richard N. Miller, MC.

12. "Leptospirosis" Uniformed Services University of the Health Sciences, 23 June 1983, LTC Ernest T. Takafuji, MC.

13. "Infectious Disease Threats Associated with the Jungle Operations Training Center" Tropical Medicine Course, Walter Reed Army Institute of Research, 4 August 1983, LTC Ernest T. Takafuji, MC.

14. "A Cardiovascular Risk Factor Reduction Protocol" Munson Army Community Hospital, Ft. Leavenworth, KS, 29 August 1983, Lytt I. Gardner, Ph.D.

15. "Military Medical Surveillance" Armed Forces Epidemiology Board, Walter Reed Army Institute of Research, Washington, D.C., 9 September 1983, CPT Patrick W. Kelley, MC.

Bibliography:

1. Takafuji, E.T., Kirkpatrick, J.W., Miller, R.N., Karwacki, J.J., Kelley, P.W., Gray, M.R., McNeill, K.M., Timboe, H.L., Kane, R.E., Sanchez, J.L. " An Efficacy Trial of Doxycycline Chemoprophylaxis Against Leptospirosis." The New England Journal of Medicine (in press).

2. Gardner, L., Lednar, W., Redfield, R., Miller, R. "Hepatitis B Hospitalizations in High Risk Occupations." American Journal of Epidemiology, 118 (3):410, September 1983, (abstract).

3. Lednar, W., Redfield, R., Kelley, P., Hayne, S., Miller, R. "When Is It Too Late to Investigate an Outbreak?" American Journal of Epidemiology, 118 (3): 427, September 1983, (abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA OG 6760	83 10 01		DD-DRAE(AR)635
3. DATE PREV SUMRY: 82 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8A. DISPH INSTR'N: NL	8B. SPECIFIC DATA: CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT
10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
A. PRIMARY	62770A	3M162770A870		AN	073	WW02	
B. CONTRIBUTING							
C. CONTRIBUTING							

11. TITLE (Precede with Security Classification Code):

(U) Threat Assessment of Diseases of Military Importance in the Tropics

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

910100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

A. DATES/EFFECTIVE: EXPIRATION:

B. NUMBER:

C. TYPE: D. AMOUNT:

E. KIND OF AWARD: F. CUM. AMT.

18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	B. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	9.0	632
	CURRENT 84	8.0	1,259

19. RESPONSIBLE DOD ORGANIZATION

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PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: ECHEVERRIA, P E BURKE, D S POC: DA

21. GENERAL USE

FINA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Malaria; (U) Diarrhea; (U) Chancroid; (U) Vectors; (U) Dengue

23. TECHNICAL OBJECTIVE; 24. APPROACH; 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code)

23. (U) The technical objective is to assess the risk of various tropical diseases to military troops and operations, and to determine the potential mortality and morbidity of military personnel undertaking operations in the tropics.

24. (U) This requires defining the ecology, epidemiology, and etiology of various tropical diseases through the development of new or improved technologies related to field studies, in vitro cultivation, microbiological assays, vector colonization, serological procedures, and other necessary approaches.

25. (U) 82 10-83 09 A serosurvey of rodents and humans was done looking for evidence of infection with hantaan virus and this will be expanded to look for risk factors and disease in humans. Japanese encephalitis studies were done in Kampangphet to isolate the virus and acquire strains from humans, swine, and mosquitoes. Sentinal pigs were placed in the communities to determine the rate of seroconversions as well as to obtain isolated from various parts of the country. Studies on malaria susceptibility of colony reared members of the various sibling species were continued as was the detrimention of the detrimental effects of malaria infection on these possible vectors. Techniques to differentiate the sibling species by electrophoretic, cytogenetic, cross mating and morphological characteristics continued to be explored and developed. A new monoclonal RIA test to detect and differentiate the species of sporozoites in wild caught mosquitoes is being field tested and will be used in a long term study of the correlation of the sporo[illegible] rate in a rural village where transmission is occurring. Mosquito survey and taxonomic work continues with new collections from southern Thailand and Malaysia. The long term study of dengue fever as a cause of PUO at Children's Hospital continues. The role of rotavirus as a cause of adult diarrhea was completed. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82-30 Sep

* Available to contractors upon presentation of request

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE

Work Unit: 073: Threat Assessment of Diseases of Military Importance in the Tropics

Investigators: COL Michael W. Benenson, MC; LTC Donald S. Burke, MC; LTC Charles H. Hoke, MC; LTC George S. Ward, VC; LTC Peter E. Echeverria, MC; MAJ Michael R. Elwell, VC; MAJ Richard G. Andre, MSC; MAJ David N. Taylor, MC; CPT Ronald M. Rosenberg, MSC; Ananda Nisalak, M.D.; Rapin Snitbhan, M.D.; Chiraphun Duangmani, M.D.; Markpol Tingpalapong, DVM

1. Dengue as an Etiology of Undifferentiated Fevers at Bangkok Children's Hospital, 1983

PROBLEM: To determine the relative importance of dengue virus as the etiology of undifferentiated fevers in children in Bangkok.

METHOD: The outpatient clinic of the Children's Hospital, Bangkok is visited each day. Blood specimens and clinical information are collected from a sample of children with fevers. From 10 to 14 days later, follow-up specimens are collected. Acute specimens are cultured for dengue virus. HAI serology for dengue is done on each serum.

PROGRESS: This study has been in progress since 1979, excluding 1982 (Table 1). During that time, a total of 523 cases were evaluated. 25% of the fully evaluated cases had evidence of flavivirus as the etiology of fever.

Table 1. Dengue isolations and serology from cases of undifferentiated fever at Bangkok Children's Hospital, 1979-1983.

YEAR	CASES EVALUATED	SEROTYPE 1	2	3	4	SEROCONVERSIONS PRIMARY	SECONDARY	FLAVI	NOT DENGUE	% ISOLATES
1979	166	1	8			1	10	155		9%
1980	256	36	25	4	1	42	52	169		36%
1981	36	4				5	4	26		26%
1982	Not done									
1983	59	Not complete				4	11	44		26%
Total:	523	43	33	4	5	52	77			25%

Overall, dengue 1 has been the predominant isolate, in contrast to the predominance of dengue 2 in DHF patients. Most of the isolates occurred in 1980, a year in which a record number of DHF cases and isolates occurred. In that year, 36% of children cultured yielded a dengue isolate, attesting to the fact that much of the increased load of febrile children was due to the dengue epidemic.

CONCLUSION:

1. 25% of undifferentiated fevers in children are

attributable to flavivirus infections, presumably dengue.

2. Dengue 1 and 2 are the most frequent isolates, accounting for 94% of isolates.

3. In contrast, dengue 2 is a relatively more common isolate from DHF cases seen at the same hospital.

FUTURE OBJECTIVES: This study represents a long term surveillance of dengue isolates in patients with a clinical syndrome likely to be seen in US troops affected by epidemic dengue. Moreover it allows longitudinal determination of serotypes of dengue virus causing dengue fever. Comparison of these data with those from the DHF study will allow determination of the relative importance of dengue serotypes in the two syndromes. The longitudinal study should be continued and expanded to include more epidemiological evaluation.

2. An Epidemic of Dengue Fever Due to Dengue 1 in a Rural Thai Village

PROBLEM: To determine the etiology and characteristics of an outbreak of an illness characterized by fever and rash in rural northern Thailand.

BACKGROUND: On 20 July, 1983 the AFRIMS Department of Virology was requested to assist in the laboratory aspects of an investigation of an outbreak of an epidemic of fever and rash in Phayao, a province in the extreme north of Thailand.

PROGRESS: In the village where the outbreak occurred, 25% of the total of 523 persons reported an illness that met the case definition of fever or history of fever plus headache, myalgia or rash. The epidemic curve revealed a sharp increase in cases beginning in the middle of June and peaking in early July. The epidemic was characterized by fever (100%), myalgia (85%), headache (81%) and rash (80%). Hemorrhagic manifestations were seen in very few cases. Age specific attack rates revealed that from 20 to 30 % of individuals in each age group over 1, including 20% of those over 60 years of age were involved.
Evidence that the outbreak was due to dengue included the following (in order of their completion):

1. Significant difference in the HAI dengue 2 GMT's of cases and controls in the bleeding from the initial days of

investigation (Controls = 10 (sd=4.4,n=8), Cases = 60 (sd=9.1,n=30), $p < .02$ by Student's t test).

2. Significantly more cases than well controls experienced HAI rises. (21 of 30 cases vs. 2 of 8 controls had HAI rises, p=.03 by Fisher's exact test.)

3. Significant rise in HAI titer in cases between acute and convalescent sera (acute GMT = 30 (sd = 8), convalescent GMT = 251 (sd = 10), $p < .001$).

4. Isolation of dengue 1 from acute sera of seven human cases (Identified by ELISA).

In addition to confirming the etiology of the epidemic, several other aspects of the diagnosis of dengue epidemics were investigated:

1. Comparison of HAI and ELISA definition of primary and secondary cases. In 22 infected patients, paired sera were available. By HAI, primary infections were those with low or absent antibody in S1, 4 fold rise in S2, but no titer > 160. Secondary infections were those with high titered antibody against several antigens in S2. By ELISA, primary infections were those with MAC index > GAC index, while in secondary infections, GAC > MAC. In 21 out of 22 sera, there was concordance between these two classifications, suggesting that either method may be used.

CONCLUSIONS:

1. An outbreak of dengue fever due to dengue 1 occurred in a sharp epidemic in all age groups of a rural population, suggesting that more than 60 years had passed since this serotype was last introduced into the population.

2. Presumably, any cohort of individuals previously unexposed to the circulating serotype would be susceptible to such an epidemic.

3. Outbreaks of dengue, like epidemics of influenza, may be diagnosed during a single visit by bleeding a number of cases and non-cases and comparing HAI GMT's using a t-test. Confirmation of etiology may await more detailed serology and virus isolation.

FUTURE OBJECTIVES:

1. More information on the transmission of dengue in rural Thailand and the relationship between the strains causing dengue fever and dengue hemorrhagic fever is needed to understand the complex ecological interrelationships.

2. Because of the possibility that this sort of outbreak could have serious adverse effects on military operations in any area of dengue transmission, field studies such as these should be pursued.

3. Flavivirus transmission in rural Thailand.

PROBLEM: To determine the risk fo flavivirus infection and the environmental risk factors influencing transmission of flaviviruses in rural Thailand.

PROGRESS: Four villages in a province of Thailand in which Japanese encephalitis was known to be prevalent were selected. In May, 1983, elementary school children in these villages were enrolled and bled. Sera were screened for JE antibody at dilutions of 10 and 20. Seronegative children (felt to be at most risk for infection), plus a random sample of seropositive children, were selected for detailed epidemiological evaluation. During June and July, the study children were visited, their mothers questioned about the presence of various risk factors, and their homes and environs examined. In addition, human mosquito biting collections were perfomed during the period of likely transmission. In September, the children were bled again. HAI antibody levels against four dengue serotypes and JE were determined on paired sera to determine who had been infected by flavivirus. Plaque reduction neutralization tests on sera and virus isolation from selected mosquito pools is planned.

PRELIMINARY RESULTS: A total of 1,604 children with a mean age of 9 were bled. The screening HAI revealed that 329 children had anti-JE titers of $\leq$ 10. These children, plus an additional 84 seropositive controls were selected for prospective evaluation of risk factors. Homes of all but 20 of the children were located. Of the first 101 children in which complete pairs of sera were obtained, 22% had a four-fold rise against at least one of the flaviviruses tested. Two had antibody rises against only JE, and one against dengue 4 only, suggesting that these two agents were circulating. No cases of encephalitis occurred in the study population, although many cases occurred elsewhere in the province during the study period. A relationship between

preexisting antibody was suggested, with 33% of individuals with preseason titers $\leq$ 40 becoming infected during the rainy season (Table 1).

Table 1. Realtionship of pre-season anti-JE antibody level to liklihood of flavivirus infection during the rainy season.

			INFECTED*	
			YES	NO
Anti JE titer in May, 1983	$\leq$	40	15	30
	>	40	7	49

Chi square=6.35, df=1, p=.012.

* Four fold rise in anti-JE HAI antibody.

The relationship of these seroconversions to the presence of epidemiological factors and the the presence of infectious vector mosquitoes remains to be determined.

CONCLUSIONS:

1. The preliminary results suggest that 24% of children studied became infected by a flavivirus in a three month period.

2. Children with lower HAI titers were more likely to become infected during the rainy season.

FUTURE OBJECTIVE: This study may reveal important information about the transmission of flaviviruses in a rural setting. The study should be completed and followup studies planned.

4. Outbreaks of encephalitis in Thailand, 1982-1983

PROBLEM: To determine the proportion of cases of acute encephalitis which is due to Japanese encephalitis virus using the JE MAC ELISA test.

BACKGROUND: Encephalitis, probably due chiefly to Japanese encephalitis virus, continues to occur in Thailand some 17 years after its demonstration in humans. Diagnosis is not possible in most of the regional hospitals, and when it is attempted, the HAI method is used. This method is unreliable

because 1. Paired sera are required, thus negating the possibility of a diagnosis in rapidly faral cases, and '. The widespread presence of dengue causes confusing cro.; reactions in secondary infections that make differentiation between dengue and JE impossible in any second flavivirus infection. In recent years, this laboratory has developed a MAC ELISA for diagnosis of JE (Burke, DS., et al. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J Clin Microbiol, pp 1034-1042, (1982).) which is positive in 80% of patients at the time of admission, and in 100% within three days. Moreover, since the test measures CSF antibodies, the presence of large amounts of flavivirus antibody in the serum do not interfere. Thus the test has the potential of providing a specific diagnosis in many cases where HAI serology is inadequate. Its usefulness in the field suggests that it is a test which will be very helpful in clarifying the etiology of the ongoing problem of encephalitis in Thailand.

To further evaluate the usefulness of this test, we have sought outbreaks of encephalitis and investigated them ourselves, or encouraged their investigation by Ministry of Public Health epidemiology trainees.

PROGRESS: Outbreaks of encephalitis occurring during the rainy season were sought prospectively based on the reporting of encephalitis cases in the national surveillance system. Four such outbreaks were investigated: one each in the provinces of Kampangphet, Uttaradit, Chaingmai, and Yasorhon.

RESULTS:

1. Kampangphet : For the third consecutive year, (1981, 82, and 83) we conducted epidemiological and clinical studies of Japanese encephalitis in Kampangphet Province, Thailand. During the three years of the study, 40, 41, and 66 cases occurred, respectively, marking 1983 as a year of particularly intense JE activity in that province. The 1983 epidemic began abruptly in mid June and rose to a sharp peak during the week of 13-19 July (Table 1). In that week alone, 14 encephalitis cases, of which 11 were confirmed as due to JEV, were admitted to the hospital requiring the dedication of a special hospital ward exclusively for the care of encephalitis patients. By the third week in August, admissions due to encephalitis were decreasing.

Table 1. Weekly number of cases of encephalitis in Kampangphet province, 1983

Week	Cases	JE*
6/15-6/21	3	2
6/22-6/28	8	5
6/29-7/ 5	7	6
7/ 6-7/12	7	4
7/13-7/19	15	11
7/20-7/26	10	10
7/27-8/ 2	4	4
8/ 3-8/ 9	7	4
8/10-8/16	5	3
TOTAL	66	49

* confirmed by anti JE IgM in CSF

Of the 66 cases in 1983, Japanese encephalitis was established as the etiology of 49 by detection of IgM anti-JE antibody in the admission cerebrospinal fluid with a JE MAC ELISA test. Twelve confirmed cases were fatal, for a case fatality ratio of 24%. Post-mortem brain biopsies or autopsies were performed in 10 of the 18 fatal encephalitis cases, and JE virus was isolated from brain tissue in at least 8 of these cases. Cultures of CSF, performed by inoculating AP-61 (Aedes pseudoscutellaris) cells at the bedside, yielded JE virus in 5 out of 47 attempts from confirmed cases.(Table 2)

Table 2. Isolations of JEV in Kampangphet, 1983

Source	Number Attempted	Number Positive
Brain	10	8
CSF	47	5
Pig	4	1

There are approximately 560,000 people in Kampangphet Province, thus the attack rate for JE was 7 cases per 100,000 population. Age specific attack rates were highest in 6-10 (22/100,000) and 1-5 year olds (21/100,000). Within the province, the geographic distribution of the 1983 cases extended approximately 50km further south than in 1981 or 1982, suggesting some southward movement of the disease.

We conclude that:

1. Japanese encephalitis epidemics continue to recur on an annual basis in Thailand.

2. 82% of cases of encephalitis were confirmed as due to JEV.

3. Age specific attack rates are such that any immunization programs for JE must be targeted at pre-school age, as well as school age children.

4. The diagnosis of JEV encephalitis can be confirmed by post-mortem needle brain biopsy in a high percentage of fatal cases.

5. The technique of bedside inoculation of CSF into AP-61 cells yields a virus isolate in 10% of cases.

2. Uttaradit: Uttaradit is a province in the northern part of Thailand which has generally experienced some of the highest rates of encephalitis in Thailand. Encephalitis cases were diagnosed by the pediatrician at the provincial hospital. Serum and CSF specimens were collected and sent to AFRIMS for testing using the JE MAC ELISA test.

The monthly number of cases demonstrated the typical sharp July peak seen in virtually every outbreak which has been studied in recent years. (Table 3)

Table 3. Monthly number of encephalitis cases seen in Uttaradit in 1983

Month	No. cases	No. fatal
Jan	2	0
Feb	3	0
Mar	1	0
Apr	4	1
May	1	0
Jun	16	2
Jul	59	10
Aug	17	0
Sep	3	0
TOTAL:	106	13

Clinical specimens were collected on 31 cases and sent to AFRIMS. Of 29 with paired sera, 25 had a positive test for anti JE IgM. Of the other 5, only two had a firm diagnosis of encephalitis, one was felt to be a reaction to

typhoid vaccine, one was accompanied by a rash and felt to be due to another agent, and one was a brain tumor. Thus of 27 encephalitis patients, 25 (93%) had evidence of acute JE infection as the etiology.

3. Chiangmai: On July 20, 1983, Drs. Supramit and Surachai of the Thai Ministry of Public Health came to AFRIMS to discuss their plans for an investigation of an outbreak of encephalitis in Chaingmai, a province in northern Thailand long known to contain many cases of encephalitis each year. Discussion of the optimum specimens for diagnosis ensued. In addition to the requirement for CSF for the MAC ELISA test, the recent success in isolation of JE virus from the CSF of acute cases suggested that field innoculation of AP-61 cells should be attempted.

CSF specimens were received on 17 individuals and from 33 well family members of cases. JE MAC ELISAS were strongly positive in 14 of the 17 cases (82%). Of the 33 well contacts of cases, 5 (15%) had evidence of JE IgM in their serum, suggesting that they had been recently exposed and infected, but had not developed encephalitis. Of the 17 cell cultures received back at the lab, only 4 were not heavily contaminated. These 4 yeilded no evidence of JE virus.

CONCLUSIONS:

1. JE virus accounted for 82% of cases of encephalitis tested in this outbreak in Chaingmai.

2. 15% of asymptomatic controls had evidence of recent flavivirus infection, suggesting that transmission to humans is quite intense.

3. Field innoculation of cell cultures for isollation of JE from CSF was unsuccessful in this case, but the technique should be improved and retried under more felicitious circumstances.

4. Yasothon: In a recent review of encephalitis in Thailand, the province of Yasothon had the highest mean rates of any province of reported encephalitis during the last decade. (Table 4)

Table 4. Provinces in which the encephalitis rates were among the 10
highest for six or more of the 12 years of surveillance between 1970 and 1981.

Province	Region	No. yrs in top 10	Mean Annual rate
Yasothon	NE	11	12.8
Kamphangphet	N	11	12.4
Chiangmai	N	11	9.4
Chiangrai	N	10	12.1
Nan	N	9	12.1
Lampang	N	7	6.8
Lamphun	N	7	6.8
Uttaradit	N	6	10.9

Because Yasothon was far from any other province which had high encephalitis rates, its own high rate was a prominant feature of maps showing encephalitis rates by province. Moreover, pressure had been growing from local groups for the government to institute control programs. Therefore a request was made of local surveillance officials that they provide more detailed information and specimens from cases of encephalitis.

RESULTS: During the surveillance period, 4 cases of possible encephalitis occurred. These specimens have not been sent to AFRIMS for analysis as of this time. However, even if they are all positive, the rate will be less than 10% of most previously reported rates.

CONCLUSION: One result of improved diagnositic tools is improved surveillance. In this case, the availability of an improved diagnostic test occurred simultaneously with the marked reduction in reported encephalitis cases from the province.

SUMMARY: These 4 investigations (Table 5) reveal that JE continues as a serious health problem in northern provinces, but not in Yasothon. About 80% fo cases of encephalitis occurring in the rainy season are due to JEV

Table 5. Summary of epidemiological investigations of JE, 1983

Province	# cases	# tested	# with JEV infection	%
Kamphangphet	66	66	49	74
Uttaradit	76	27	25	93

Chiangmai		17	14	82
Yasothon	4	—		

FUTUTE OBJECTIVES: Control measures for this disease are urgently needed in northern Thailand and should be evaluated.

5. Seroconversion of Pigs in Northeast Thailand to JEV

OBJECTIVE: To determine in swine the monthly seroconversion to JEV in Northeast Thailand.

PROBLEM: Pigs are known to be an amplifying host for JEV. Studies on pig seroconversion rates in Chiangmai, Japan, and Sarawak have shown high rates of seroconversion that are not constant throughout the year. Infection in pigs are a means for monitering the JE season. Increased seroconversion in pigs is seen when JE cases occur in man. Chiangmai in the north is an area with a high human attack rate. However in Southern Thailand there are many pigs with JE antibody and few reported cases of JE in man. Multiple factors may account for this including climate and vector differences and differences in strains of virus. This study at Sri Kiu pig farm near Korat will allow calculation of seroconversion rate in a province with an intermediate attack rate. This information will help in determining the importance of swine in JE transmission and possible differences in various locations in Thailand. Attempts will be made to isolate virus from these pigs so it can be compared with recent isolates from an area where clinical JE infection in man is common.

PROGRESS: The testing of pigs for HAI antibody to JEV began on 15 June 1983. Twenty pigs, three months of age were bled and 12 had positive HAI titers. To date, 4 months into the study, the average monthly seroconversion has been 23.3% (range 17-28%).

6. Hepatitis A Outbreaks in Thailand, 1982-1983

PROBLEM: In the past two years, two epidemics of Hepatitis were investigated. Both epidemics were investigated because initial data suggested that young adults were most heavily affected, a characteristic that was thought to suggest non-A non-B hepatitis. Unexpectedly, both outbreaks were due primarily to Hepatitis A virus, suggesting that susceptible

cohorts of young adults are developing as sanitation improves in Thailand. The first outbreak may have been due to both A and NANB viruses.

1. ACUTE HEPATITIS IN NAKORN SAWAN: In November, 1982, routine surveillance of hepatitis indicated a marked increase in reported cases in the province of Nakorn Sawan, about 200 kilometers north of Bangkok. An investigation by the Ministry of Public Health epidemiology trainees and AFRIMS was initiated. Surveillance of acute hepatitis cases was instituted at the provincial hospital, and convalescing cases which had been previously reported in the national surveillance were sought. Controls for cases seen at the hospital were chosen from patients seen with traumatic illnesses.

Seventy-nine convalescent cases and 21 acute cases were found in the surveillance program. The epidemic lasted from May until November. A peak in hepatitis A cases occurred in August, while a peak in apparent non-A non-B cases occurred in October. Cases were concentrated in a neighborhood where the water supply to several blocks of shop houses was compromised by poor quality, submerged, leaking pipes and considerable poorly-drained, standing water. It was hypothesized that the disease had been transmitted through the drinking water, and recommendations for improving the quality of the water supply were made.

Laboratory studies revealed that 63% of convalescent cases, but only 20 % of controls, were positive for IgM anti HAV by radioimmunoassay ($p<.01$). Only 1% of cases and no controls had evidence of hepatitis B infection. Among the patients found in the prospective surveillance, 29% of cases and 22% of controls had evidence of hepatitis A. The incidence of hepatitis A was highest in 10 to 19 year olds. 72% of acute hepatitis cases in this age range had evidence of hepatitis A infection. In older patients, about 40% of those with hepatitis had evidence of neither A nor B, suggesting that a form of non A non B hepatitis may have occurred in conjunction with the hepatitis A epidemic. When possible, stool specimens had been collected from early cases with the intention of seeking an infectious agent if the case appeared to be due to a non-A non-B hepatitis. In one case of NANB hepatitis, such a specimen was collected. Immune electron microscopy revealed the presence of a small number of 27 nm particles consistant with hepatitis A virus or non-A non-B hepatitis.

2. HEPATITIS A IN CHIANGRAI, THAILAND. In August of 1983, investigators from the Thai Ministry of Public Health epidemiology program, requested the assistance of the AFRIMS department of virology in investigating an epidemic of hepatitis in Chiangrai, a province in the far northern part of Thailand.

The largest number of cases occurred in August, when 61 cases occurred. Again, most cases were in young adults between 15 and 24 years of age. Blood specimens from 24 acute cases were obtained. Of these, 20 contained IgM anti HAV, suggesting that hepatitis A virus was the etiologic agent. Epidemiological studies are in progress.

CONCLUSIONS:

1. These two outbreaks suggest that hepatitis A can no longer be considered exclusively a disease of children in Thailand. Presumably, as sanitation improves, the numbers of children exposed to hepatitis A virus in young childhood is lessening. As a result, cohorts of adults are developing.

2. The first outbreak suggests that NANB hepatitis may occur in conjunction with hepatitis A in young adult populations in Thailand.

FUTURE OBJECTIVES:

1. Further studies to isolate the agent(s) and define the epidemiology of NANB hepatitis in Thailand are needed. Either investigations of acute outbreaks or prospective surveillance among high risk groups, such as those along the Cambodian border, would be appropriate.

2. With respect to efficacy trials of hepatitis A vaccine in Thailand, attack rates in younger children may be lower than previously thought, while those in older people may be higher. Vaccine protocols should take these findings into account.

7. Rotavirus as a Cause of Severe Gastroenteritis in Adults

OBJECTIVE: To determine the role of rotavirus as a cause of gastroenteritis in adults in Thailand.

PROGRESS: Rotavirus was identified as the only etiologic agent in five percent (28/526) of adults with diarrhea who were admitted to Ramrasnaradora Hospital in Nonthaburi, Thailand during a one year period. Infection was determined by detection of rotavirus in diarrheal stools by ELISA accompanied by a greater than fourfold rise in serum CF and RIA antibody titers to rotavirus. Adults with clinical rotavirus infections were as severely ill as patients with most bacterial enteric infections; only patients with cholera passed more watery stools and were more dehydrated than those with rotavirus infections. Only two of the 28 adults with rotavirus infections had known recent contact with young children with diarrhea. Rotavirus infections in these adults occurred most frequently in the cooler, drier months in Thailand than during the rest of the year. In some settings, rotavirus should be considered in the differential diagnosis of severe diarrhea in adults as well as in young children.

FUTURE OBJECTIVES: To collect rotavirus from infants and adults and compare the serology and immunological response for the different serotypes of rotavirus.

5. Prevalence of Heat-stable II Enterotoxigenic Escherichia coli in Pigs, Water, and People at Farms in Thailand as Determined by DNA Hybridization

OBJECTIVE: To define the prevalence of heat-stable II enterotoxigenic Escherichia coli in pigs, water, and people at farms in Thailand as determined by DNA hybridization.

PROGRESS: The DNA hybridization assay employing a 460 base pair fragment of DNA encoding for ST-II enterotoxin was used to determine the prevalence of heat-stable II enterotoxigenic Escherichia coli (ETEC) in pigs, people, and water at 57 farms in Sri Racha, Thailand. ST-II ETEC infections were found in 24% (13/54) of 2145 suckling pigs, none of 560 weanling, or 55 adult pigs examined. Evidence of ST-II ETEC was found in pigs at 13 of 32 (41%) farms with suckling pigs with diarrhea and at three of 25 (12%) farms with pigs of the same age without diarrhea ($p<0.025$). ST-II ETEC was detected in water collected from three of 57 clay jars used to store bathing water at three pig farms, in one jar outside of the barn, and from one asymptomatic farmer at a pig farm. Evidence of this organism was not found in 245 other individuals living at the pig farms, or in 229 inhabitants and 114 water specimens at tapioca farms nearby. ST-II ETEC

was associated with young pigs with diarrhea in Thailand, but was infrequent in man.

FUTURE OBJECTIVE: Study completed.

9. Mosquito Survey and Taxonomic Studies

PROBLEM: To elucidate the mosquito fauna of Thailand and Southeast Asia. Primary emphasis is put on the determination of diagnostic characters that separate the vector species and groups containing vector species that transmit parasites detrimental to humans.

PROGRESS: During the past year, morphological studies were continued on the sibling species of the balabacensis and the maculatus complexes. These studies are being done in collaboration with the WRAIR Biosystmatics Unit at the Smithsonian Institution. Manuscripts are in preparation to describe new species in the balabacensis complex, with the members currently being refered to as Anopheles dirus A, B, C, or D. Discriminating morphological characters have been found that will differentiate some of the new species in the maculatus complex. A key has been prepared and is included in a manuscript still under preparation (1). Work on the Aedes (Finlaya) manuscript continues with over 22 plates having been completed. Progeny rearings of vector species from many locations in Thailand and several locations in We Malaysia have been completed and correlated with genetic identifications.

FUTURE OBJECTIVES: Taxonomic studies on members of the balabacensis and maculatus complexes will continue. Morphological characters to discriminate field-collected An. dirus A, B, C, and D will be tested with populations in Thailand and Malaysia. The key to separate the maculatus complex also will be tested in the field with large population samples. The final ten plates for the Aedes (Finlaya) manuscript will be prepared.

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10. Mosquito Cytogenetic, Electrophoretic and Cross Mating Studies

PROBLEM: To define and delimit the taxa in the vector Anopheles species complexes by cytogenetic, electrophoretic and cross mating techniques for the following reasons: a. to check against current morphological species concepts; b. as an accurate determination of chromosomal polymorphisms and genetic variations in natural populations of malaria vector species and/or suspected vector species; and c. to correlate genetic variations in natural populations of the vector species with habitat differences, innate susceptibility to the human malarias, and behavioral patterns that may facilitate more effective control measures in the future.

PROGRESS: Analyses of metaphase chromosomes of the four taxa of the Anopheles balabacensis complex (An. dirus A, B, and C, An. rakasagoensis,) using the Hoechst 33258 fluorescent staining technique have revealed remarkable differences in fluorescent banding patterns of the sex chromosomes, particulary the Y chromosome of these taxa. These differences are mainly the amount and distribution of constitutive heterochromatin. Two largely independent studies of chromosomes from natural populations of Anopheles maculatus provide evidence for several genetic species within the taxon. Polytene chromosome variation shows four different rearrangements of arm 2 and three rearrangements of the X chromosome. There is unequivocal evidence for three species within this complex. The progeny of five isofemale lines of Anopheles indefinitus exhibited a supernumerary (B) chromosome. Some preparations of the B-chromosome manifested 2 sister chromatids indicating some normal duplication and segregation. Electromorphic variation for some esterases and 6-phosphogluconate dehydrogenase enzymes in An. maculatus is controlled by four loci which are unlinked to sex. Esterase loci are linked to each other; Est 1-36%-Est4-16.5%-Est 3; but unlinked to Pgd 2. Linkage data were obtained by selfing F-1 from selected parents and analysing genotypes in the F-2. Cross mating experiments have provided additional evidence for the species status of members in the balabacensis and maculatus complexes.

FUTURE OBJECTIVES: Large samples from natural populations of members of the balabacensis and maculatus complexes will be collected and analyzed by cytogenetic and electrophoretic techniques. Correlations with morphology will be attempted. Recombinant DNA techniques will be tried to differentiate members of the balabacensis complex.

11. Comparative Susceptibility of Known and Suspected Species/Strains of Anopheles to Plasmodium Parasites

PROBLEMS: The objectives of this investigation are as follows: a. to determine and compare the susceptibility of primary and potential secondary vectors of malaria to Plasmodium parasites; b. to delineate the development of malaria parasites in Anopheles species with varying degrees of susceptibility; and c. to observe the feeding behavior of colonized vectors of human malaria under laboratory conditions.

PROGRESS: Studies on the susceptibility of various anophelines to human malaria continued this year at the Malaria Control Center, Tha Muang, Kanchanaburi Province. Anopheles dirus A was compared to other colonized Thai Anopheles species/forms in paired-feeding experiments. Thirty-two patients infected with falciparum malaria gave rise to infection in dirus A. Over three-quarters of the mosquitoes dissected had developed oocysts, but only forty percent had positive salivary glands. Half of the other mosquitoes (dirus B and C, maculatus A and B) became infected and of those, forty-one percent had sporozoites. Results from forty-five paired feeds on Plasmodium vivax patients showed that the mosquitoes were more susceptible to this parasite. Overall, dirus A was more susceptible to human malaria parasites than the other species/forms tested.

FUTURE OBJECTIVES: It is planned to continue this study next year in order to test the susceptibility of the new species in the balabacensis complex and the maculatus complex. Known genetic lines will be used to determine the importance of these sibling species in the natural history of malaria in Thailand.

12. Detrimental Effects of Plasmodium Infections on the Survival Rate of Anopheles dirus

PROBLEM: The objectives of this study are as follows: a. to determine if the longevity of mosquitoes infected with Plasmodium is different to a significant degree from that of uninfected mosquitoes; b. to determine if the longevity among mosquitoes with heavy or light infection rates is

significantly different; and c. to determine if the longevity of mosquitoes infected with different species of Plasmodium is different significantly among groups.

PROGRESS: An investigation of the effects of human malaria parasites on the longevity of Anopheles dirus continued this year. Over one hundred and fifty lots of mosquitoes have been fed on Plasmodium vivax patients. One hundred and ten lots of mosquitoes were allowed to feed on P. falciparum patients. About 40% of the feeds were positive. Control lots of mosquitoes were fed on uninfected volunteers on the day of the patient feed. Survival of control mosquitoes and lightly infected mosquitoes was excellent, with some mosquitoes living more than seventy days. Heavily infected mosquitoes usually died within one month. A manuscript on the correlation of survival rates of An. dirus with different infection densites of P. cynomolgi was revised and is in press (1).

FUTURE OBJECTIVES: Data from these experiments will be entered onto the computer for statistical analysis. Feeds will continue to be made during the next year to increase the number of infected comparisons.

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13. Identification of Field-Collected Sporozoites

PROBLEM: Four different tests are available to potentially identify Plasmodium sporozoites in mosquito salivary glands: a. circum-sporozoite precipitin test; b. immunofluorescent antibody test; c. radioimmuno assay test; and d. enzyme-linked immunosorbent assay test. The objectives of this study are to provide Plasmodium falciparum and P. vivax sporozoites to WRAIR for the development and improvement of these tests, to evaluate these four tests in the laboratory with mosquitoes infected with Thai strains of Plasmodium, and to adapt the tests for use in the field to identify natural infections in vector anophelines.

PROGRESS: Anopheles dirus females were allowed to feed on

patients infected with Plasmodium falciparum and P. vivax parasites. After 14 days the mosquito salivary glands were removed and the sporozoites harvested, or mosquitoes were frozen, or mosquitoes were killed and dried. Plasmodium vivax sporozoites were inoculated into BALB/C mice. Each mouse received an initial inoculum and four boosters over a period of three months. The mice were sent then to WRAIR for the development of monoclonal antibodies to be used in an ELISA test. Mosquitoes infected with P. falciparum sporozoites were sent to WRAIR for evaluation of a new ELISA test and were sent also to NIH for evaluation of the RIA test (1). Mosquitoes infected with P. vivax also were sent for evaluation of the tests. Harvested sporozoites were spotted onto IFA slides for monoclonal antibody analysis and rabbit antiserum specificity evaluation. One An. dirus female, captured during a human biting collection, was found to have sporozoite-infected salivary glands. These sporozoites were tested using the IFA and gave a positive reaction when exposed to a P. falciparum monoclonal antibody. Manuscripts describing the ELISA test for both species of Plasmodium are under preparation at WRAIR (2).

FUTURE OBJECTIVES: During the next year, evaluation of the different tests will continue. Emphasis will be placed on field-testing the ELISA technique and its applicability to use at a longitudinal malaria study site. Tests will be run to quantify the specificity and sensitivity of the ELISA for both falciparum and vivax malaria sporozoites against local Thai strains of parasites and vectors. The ELISA test will be used to determine the density of anophelines infected with human malaria sporozoites in a village situation.

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14. Correlation of Sporozoite to Gametocyte Ratios in a Village Situation

PROBLEM: The objectives of this study are as follows: a. determine the monthly gametocyte prevalance for Plasmodium vivax and P. falciparum ; b. determine the species of sporozoites found in man-biting Anopheles in the same community using the IFA or ELISA tests; and c. compare species ratios for gametocytes with species ratios for sporozoites.

PROGRESS: Much of the year was spent in a search for a suitable study site which had hyperendemic malaria transmission. Villages in Chumporn, Ranong, Pak Chong, Tak, Petchabun, Trad, and Chantaburi were examined. In May, a village in Chantaburi was found that possessed all the attributes needed to support this investigation. The study site selected was Village 7, Baan Phluang, Amphoe Makaam, Chantaburi Province. Starting in June, monthly blood smears were taken from a population of 200 people. The monthly prevalence of malaria ranged from fifteen to forty-five percent depending on the transmission season, which in this village appears to be different from the predicted season. Mosquito collections showed the three major vectors, Anopheles dirus, maculatus, and minimus, to be present in the village. Several mosquito species have been found to carry oocysts, but only An. dirus has been found with infected salivary glands. The sporozoites were identified as P. falciparum by the IFA test.

FUTURE OBJECTIVES: This study will continue for at least a year. The ELISA test for sporozoite identification will be evaluated in this village. Larval breeding areas will be determined, mapped, and correlated with habitat characteristics.

15. Ectoparasite and Rickettsia tsutsugamushi Studies in Thailand

PROBLEM: The objectives are to establish and describe ectoparasites that are or are potential vectors of human parasites or pathogens of human disease in Thailand, and to delineate the distribution of natural populations of larval mites infected with Rickettsia tsutsugamushi in Thailand.

PROGRESS: A checklist of the ticks occuring in Thailand has been revised and published (1). The Genus Miyatrombicula was redefined and a new species was described. This paper is currently in press (2). Illustrations of new species of the

Genus Leptotrombidium have been prepared and a manuscript is in preparation. Collections of potentially infected chiggers were conducted at Khao Yai, Sakarat, and Pak Thong Chi. The chiggers were sent to USAMRU in Malaysia for possible colonization and determination of infectivity status.

FUTURE OBJECTIVES: Because several new species of Leptotrombidium have been discovered and were found to carry R. tsutsugamushi, a collaborative study with the USAMRU Lab in Kuala Lumpur is being planned. This study would determine the role these new species play in the transmission of scrub typhus. Chromosome studies of L. deliensis also are proposed to determine if this species occurs in Thailand or if it is a sibling species and possibly not important in disease transmission.

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1o. Serosurvey and Virus Isolation from Rodents to Determine the Hantaan Virus Presence in Thailand

PROBLEM: Recent studies have found Hantaan virus to be the causative agent of Korean hemorrhagic fever (KHF), a disease syndrome of significance in Korea and Manchuria and of potential military significance in the USSR, the Balkans, parts of Western Europe and Scandinavia. Evidence has recently been obtained in Seoul, Korea that urban Rattus also are chronically infected with Hantaan virus. Cases of KHF in man have now been linked to infected wild rats in urban Seoul and Osaka, Japan. In addition, antibodies to Hantaan virus have been found in Rattus captured near the docks in Japan, Korea and the United States. Chronic infection, rats and international shipping thus provide a likely chain which may have disseminated this virus worldwide. Thus the potential for this agent to cause human disease may be far greater and more widespread than is presently appreciated. Last year we reported that rodents trapped at Klong Toey port in Bangkok

had antibody to Hantaan virus. In this preliminary study, approximately 20% of the bandicoots (B. indicus) tested had antibody titers to Hantaan. Natural infection with Hantaan virus has not been previously reported in this rodent species.

OBJECTIVES:

1. To identify areas in Thailand where rodents have antibody for Hantaan virus.

2. To test human sera from areas with rodent infection to determine if there is serological evidence of human infection.

3. To isolate Hantaan virus from tissues of rodents in endemic areas.

PROGRESS: Rodent trapping has been completed at Klong Toey (Bangkok Port, Sriracha, and Bangpakong ports. Of 235 rats (R. rattus and R. norwegicus) trapped in these locations, 10(4.3%) has positive antibody titers $\geq$ 1:32. However, 8/45 (17.8%) bandicoots, (Bandicota indicus) that were trapped in Bangkok had titers $\geq$ 1:32. A trapping of bandicoots near Kanchanaburi resulted in 6/21(28.6%) with titers $\geq$ 1:32. In addition, 30 residents living in the immediate area of the trapping were tested and 10/30(33.3%) had titers $\geq$ 1:32.

FUTURE OBJECTIVES:

1. Tissue samples (lung, spleen, kidney, urine) from bandicoots will be tested for virus antigens by FA and virus isolation attempt will be made.

2. A group of 35 serum samples from residents at the Klong Toey trapping site who frequently trap and eat bandicoots will be tested for Hantaan antibody.

3. A study of the residents in Kanchanaburi is planned to determine if there is evidence of a HFRF like illness in those people with serologic evidence of Hantaan or Hantaan-like virus infection.

Project Number: 3M162770A870
Title: Threat Assessment of Diseases of Military Importance in the Tropics
Work Unit Number: 073

Publications:

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY		DA 05 674	83 10 01	DD-DR&E(AR)1498
1. DATE PREV SUMMARY: 82 10 01	2. KIND OF SUMMARY: D. Change	3. SECURITY: U	4. WORK SECURITY: U	5. PERFORMING:	6. INSTITUTION: CX	6a. SPECIFIC DATA - CONTRACTOR ACCESS: ☒ YES ☐ NO	7. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AD	042 WWGW
b. SECONDARY	62770A	3M162770A871	AH	
c. (Not Applicable)	(N/A)			

11. TITLE: (U) Biosystematics of Arthropods of Military Medical Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 0603 Biology; 0605 Environmental Biology

13. START DATE	14. EST. DATE COMPLETE: CONT	15. FUNDING AGENCY: DA	16. PERFORMANCE METHOD: C. In-House
80 10			

17. CONTRACT/GRANT

a. DATES/EFFECTIVE:	EXPIRATION:
b. NUMBER:	
c. TYPE:	d. AMOUNT:
e. KIND OF AWARD:	f. CUM. AMT.:

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PREVIOUS FISCAL YEAR 83	3.0	295
CURRENT FISCAL YEAR 84	4.0	472

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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TELEPHONE: 202-357-1856
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
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NAME: Zavortink, T J

21. GENERAL USE
H
Foreign Intelligence Considered

POC: D.

22. KEYWORDS: (U) Biosystematics; (U) Disease Vectors; (U) Arthropods; (U) Mosquitoes; (U) Epidemiology; (U) Malaria; (U) Arboviruses

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief statement of work security classification code):

23. (U) Conduct biosystematic research of important arthropod groups in support of epidemiological studies and disease control strategies of importance to the military. Disease vector groups currently under investigation are (1) Anopheles of Orient and Middle East (malaria, (2) Culex (Culex) of Middle East (arboviruses and filariasis), (3) Aedes (Stegomyia) Afrotropical Region (arboviruses) and (4) Trichoprosopon of the Neotropics (arboviruses). Build a computer data base from over 1,000,000 mosquito specimens and collection records in the Smithsonian Institution.

24. (U) Comparative morphological study of medically important mosquito groups in regions of military interest, with biological, cytogenetic, electrophoretic and cross-mating studies of vector populations, and correlation of all data to provide (1) descriptions and illustrations of species, (2) development of effective identification keys, and (3) information about medical importance of the species. File biological data for museum mosquito specimens in SELGEM computer program to facilitate biosystematic research and to understand vector behavioral patterns.

25. (U) 82 10 - 83 00 The Dirus Complex in the Southeast Asian Leucosphyrus Group now known to consist of 7 confirmed species. Anopheles maculatus found to consist of 4-5 cytogenetic species in Southeast Asia. A new species of Culex was found in Israel and a new species of Anopheles was collected in Egypt. Nine new species now recognized in Afrotropical Aedes (Stegomyia), and 8 new species are recognized in the Neotropical Trichoprosopon. Incorporated 4543 collection forms and the data for 116,698 specimens into the computer data base. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sept 83.

* Entries in capitals upon initial approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. (DD FORMS 1498, NOV 83)

Project 3M162770A870 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 042 Biosystematics of Arthropods of Military Medical Importance

Investigators:
Principal: Bruce A. Harrison, LTC, MSC
Assistant: Ralph E. Harbach, CPT, MSC; E.L. Peyton; Y.-M. Huang, Ph.D.; Thomas J. Zavortink, Ph.D.
Associate: SP4 Richard Soltero, Thomas V. Gaffigan, Charlotte Burnett, James E. Pecor, Dolores Chalfont

Problem

Epidemiological studies and disease control strategies involving arthropod-borne pathogens are dependent upon biosystematic research support to provide accurate identifications of arthropod vectors and reservoirs. The objectives of biosystematic research of medically important arthropod groups are (1) to resolve systematic problems by identifying all of the species in these groups, (2) to describe and illustrate all the species in these groups, (3) to develop effective keys for identifying all of the species under study in their 4 primary life stages, (4) to analyze biological and ecological data useful in understanding the epidemiology of diseases and in the control of vector species, (5) to provide data concerning the medical importance of each species under study, and (6) to train personnel in field and laboratory techniques and in systematic research methods. All current research efforts are focused on mosquito vectors groups known to be significantly involved in the transmission of human pathogens: (1) malaria vector-groups of the Oriental Region [Leucosphyrus and Maculatus Groups of Anopheles (Cellia)], and of northern Africa and southwest Asia (genus Anopheles), (2) arbovirus and filaria vectors of northern Africa and southwest Asia [Culex (Culex)], and of the Ethiopian Region [Aedes (Stegomyia)], and (3) arbovirus vectors of the Neotropical Region (genus Trichoprosopon). An effort accompanying these studies, is the development of a computer based master file of detailed systematic and ecologic data for the one million plus mosquito specimens and their collection records in the National Museum of Natural History. This effort is directed at providing easily accessible and coordinated ecological, distributional and taxonomic data to military, public health and other scientific and environmental personnel or organizations for use in epidemiological or vector control schemes.

Progress

Considerable progress was made during the year on a revision

of the Leucosphyrus Group of Anopheles (Cellia). This study, a collaborative effort involving researchers at the AFRIMS Laboratory and at Mahidol University in Bangkok, Thailand, has intensified over the last year and has shown that the species originally called balabacensis balabacensis in mainland countries and Taiwan in the Orient actually represents at least 7 sibling species in the Dirus Complex (dirus A, B, C, D, E, Con Son Form and takasagoensis). The separate species status of C, D and E was only suspected until this year, when cross-mating and cytogenetic studies in Bangkok revealed hybrid sterility in crosses and distinctive heterochromatin and polytene banding patterns on the chromosomes. Of the 7 sibling members in this complex, dirus A, B, C and D are all found in Thailand where it is not uncommon to find 2-3 species together. The epidemiological implications of these findings for malaria transmission may be far reaching and other studies are in progress to determine the impact of sibling species differences on human malaria transmission in Thailand. The Dirus Complex, when combined with the Balabacensis Complex, i.e., balabacensis, baisasi and introlatus, forms an assemblage of 10 valid species from what was considered one species with 2 subspecies and one form (balabacensis and subspecies baisasi and introlatus, and the Frasers Hill form) only 6 years ago. This example of sibling speciation is already larger than the very popular Gambiae Complex in Africa, and is approaching the size of the famous Maculipennis Complex in the Palearctic Region. Sibling species in Anopheles have been detected at an ever increasing rate over the last 10 years by the use of rapid biochemical and cytogenetic techniques. However, the discovery of the Dirus Complex is unique in that all of the species in the complex were initially separated and recognized by modern morphological studies at the Walter Reed Biosystematics Unit (WRBU), and then were confirmed as distinct species by biochemical, cytogenetic or crossing studies at a later time. Anopheles dirus C, a species currently known only from Thailand, probably has the best morphological characters for species separation. This species is easily recognized in the adult, pupal and larval stages, and a manuscript naming and describing this species is nearing completion. A manuscript naming and describing dirus D is also nearly finished. During this period the larval and pupal stages of Anopheles elegans, another member of the Leucosphyrus Group, were described and illustrated and the paper is currently in press. Intraspecific variation was also detected in the amounts and position of heterochromatin bands in the mitotic chromosomes of dirus A in Thailand, and this paper is in press.

Collaborative efforts with AFRIMS and Mahidol personnel in Bangkok, Thailand, have also established that the Southeast Asian taxon, Anopheles maculatus, is actually a sibling complex of 4-5 cytogenetic species. These findings, based on 2 separate studies

utilizing polytene chromosome banding patterns and heterochromatin banding patterns on mitotic chromosomes, have been combined into a single definitive paper, which is currently in press. One collaborating scientist at AFRIMS has also discovered good adult and egg morphological characters to recognize one of the new species in Thailand and these characters are being checked at the WRBU on specimens in the National Museum of Natural History. These findings offer an explanation for the long standing puzzle of why maculatus in Malaysia is such an effective vector of human malaria parasites, but a poor or non-vector in other mainland Asian countries. In fact, one of the sibling species in Thailand is identical to Malaysian maculatus, and this species is most abundant in southern Thailand where Plasmodium parasites have been reported from "maculatus."

The study and examination of Anopheles specimens from northern Africa and southwest Asia continued, but is still hampered by insufficient specimens. A collecting trip to Egypt and Israel (see below) provided 531 urgently needed adults of 8 Anopheles species, most with associated immature skins. Included in these were 65 specimens of a new species from Egypt. Some of these specimens will be used as the type-series in a forthcoming description of the species by collaborating Egyptian researchers at Ain Shams University in Cairo.

Excellent progress was made during the year on the revision of the Culex (Culex) of northern Africa and Southwest Asia. Of the 21 species involved in this study, several thousand adults of 9 species (most with associated immature skins) were collected during the highly successful collection trip to Egypt and Israel. These specimens, particularly the immature stages, were urgently needed so that illustrations, setal counts, descriptions and keys could be prepared. To date, the adult descriptions, pupal chaetotaxy charts and pupal illustrations have been completed for 16 species. Specimens were reared from a collection in Rosetta (Rashid), Egypt, that will be used in designating a Neotype for Culex molestus, a controversial variant of the filarial and arbovirus vector, Culex pipiens. A large number of Culex pipiens and Culex torrentium were reared with associated skins from 2 collections made in Sweden. These specimens will help resolve identification problems with these 2 species in Europe, and provide a type-series for a forthcoming Neotype designation of Culex pipiens. A new species of Culex (Neoculex) was found in specimens preserved at Ben Gurion University, Beer Sheva, Israel, and a manuscript coauthored by an Israeli collaborator is in preparation. During the year an exceptionally good preservation method for adults, i.e., freeze-drying, was described and published. A number of very valuable taxonomic notes were published in a paper about Culex (Culex) types that were

examined in several European museums. Live specimens of Culex perexiguus from Egypt were shipped to a collaborator in South Africa for crossing experiments to determine if this species is conspecific with the arbovirus vector Culex univittatus.

Taxonomic efforts focused on the African Aedes (Stegomyia) arbovirus vectors were greatly enhanced by additional specimens collected in or received from Cameroon, Kenya, and South Africa. Nine new species are currently recognized in the important Africanus, Simpsoni, Poweri and Pseudonigeria Subgroups of Aedes (Stegomyia). These new species plus the recently elevated species, lilii and bromeliae, will force drastic changes in the names of species that have been implicated and published in the past as arbovirus vectors in Africa. Examples of changes are: (1) the species previously called simpsoni in Uganda that bites man and was incriminated as an enzootic Yellow Fever vector in primates, is actually bromeliae, and the non-human biting species called simpsoni in Uganda is probably lilii. Aedes simpsoni is now known to be restricted to the Transvaal region of South Africa and to southern Zimbabwe; and (2) the species previously called africanus in eastern Africa and incriminated as a primary vector of Yellow Fever during large human epidemics in Ethiopia, is actually a new species, while true africanus is restricted to western Africa. This knowledge has become evident only because of several successful collecting trips to various countries in Africa over the last 4 years. Additional trips are planned to other critical areas in Africa. One major benefit of these trips is the close professional working relations that have been established with French researchers in ORSTOM institutes, several South African researchers and with personnel of the Division of Vector-Borne Diseases, Nairobi, Kenya.

Research of the Neotropical mosquito genus, Trichoprosopon, has proceeded at a very good rate. Currently, descriptions have been completed for the larvae, pupae and male genitalia of all 22 species. In addition the descriptions for the adults are complete for over half of these species, including the 8 new species found to date. Most of the required illustrations are in the correction phase, with only a few originals needed. Keys have been completed for larvae, pupae, adults and genitalia of all species. During the year a paper outlining the biology and medical significance of Trichoprosopon digitatum was published. In addition, 2 new species of Wyeomyia were discovered in Venezuela and are being described. Also, a new genus is being described for 2 species previously assigned to Topomyia in Southeast Asia.

All of the specimens of African Aedes (Neomelaniconion) on hand at the National Museum of Natural History were sorted in preparation for a future revisionary study. At least one species

of this group of Aedes was recently incriminated in the trans-ovarial transmission of Rift Valley Fever (RVF) virus in Kenya.

A total of 278 man-days involving 3 professionals and 2 technicians were devoted to 4 major field trips during the year: Egypt-Israel 68 days, Cameroon and Kenya 90 days, Peru 45 days and the last 7 days of the fiscal year in a trip to Senegal. The last trip will be covered in next year's annual report. The other 3 trips were highly productive, collecting over 6,702 urgently needed adults with over 11,913 associated immature skins and larvae from areas of critical importance to ongoing studies. Very little material exists in U.S. or international repositories from these areas, and the ongoing studies would have been cursory at best without these and additional future trips. While in these countries intensive searches were made for collections of specimens in previously unknown repositories, these searches were very successful and hundreds of valuable specimens were located and examined, including previously lost type-series of several species. In addition to specimens, these trips were very important politically for the WRBU. On site training courses were provided for the local personnel ard long term collaborative arrangements were established with professional researchers in Egypt, Israel and Kenya that should allow continued joint productive efforts in these countries in the future.

A total of 25,696 specimens were received by the unit as gifts, transfers, loans, etc., for deposition in the National Museum of Natural History, or for use in ongoing studies and then return to the loaning institution. Outgoing loans, exchanges, gifts, only involved 364 specimens. A total of 202 specimens were identified as a service to the USDA Systematic Entomology Laboratory. Several hundred Anopheles (Nyssorhynchus) specimens from the eastern slopes of the Andes in South America were identified for public health organizations in Colombia and Peru. In addition, several unit personnel visited Mt. Gretna, PA, and made collections of Anopheles to help resolve the identity of a species originally described from this site.

The computer data base was increased by 4,543 collection records and 116,698 specimens during the year. These entries brought the geographic file for Mexico and Central America up to date, and completed nearly half of the available entries for the South American file. Service demands for information about mosquitoes and the production of maps based on data in the master file increased significantly during the year. Over 150 requests for services from DOD and other organizations were filled.

Personnel for the unit were also heavily involved in other

aspects of biosystematic and medical entomology research. (1) One member organized a symposium on "Mosquito Biosystematics" at the annual meeting of the American Mosquito Control Association, Lake Buena Vista, Florida. (2) One member was appointed to a National Research Council peer review panel on vector control. (3) One member chaired a meeting involving USDA, DOD and Smithsonian scientists, on the current status of Biosystematics of Medically Important Arthropods. (4) One member translated several publications of importance to the unit from Chinese to English, while another member translated several Spanish publications to English. (5) The professional staff reviewed 51 manuscripts being submitted for publication in internationally recognized periodicals, and 13 research proposals being submitted to the National Research Council, the World Health Organization (WHO), the U.S. Agency for International Development (USAID) and the U.S. Army Medical Research and Development Command.

Difficulties Encountered

One problem seriously hampered the productivity of the unit, and will seriously impact on next year's productivity. The scientific illustrator's slot was unoccupied for 8 months and is still unoccupied. Efforts to offset this problem included a short contract for illustrations and close coordination/contact with the civilian personnel office in trying to fill the position. Despite these actions, a number of manuscripts were and still are being delayed for lack of illustrations, and all of the major revisions are being hindered because of this problem.

Future Plans

Every effort will be made to eliminate the illustration problem. Research currently in progress will continue and efforts to obtain adequate specimens for these studies are expected to result in at least 3-4 collection trips, probably to Turkey and/or Israel, Ivory Coast and Sierra Leone, Peru and Bolivia (at no cost to WRAIR). Efforts will intensify to develop a capability to identify specimens of the Albimanus Section of Anopheles (Nyssorhynchus) from the eastern slopes of the Andes Mountains in South America. It is anticipated that these last efforts will be assisted by a collaborative research effort with CENETROP in Bolivia. Ongoing collaborative efforts with Egypt and Israel, the WRAIR overseas laboratories and the Navy overseas laboratories will continue. In addition, it is anticipated that taxonomic assistance will be provided to a malaria research effort being started in Papua. Sorting and work will proceed as specimens become available on the Aedes (Neomelaniconion) species found in "Dambos" in Africa.

Formal Presentations

Harrison, B.A. 1983. Impact of sibling species complexes on the study and knowledge of the epidemiology of vector-borne diseases. Seminar presented at Johns Hopkins University, 22 Feb, Baltimore, MD.

Harbach, R.E. (with B.A. Harrison). 1983. Freeze-drying adult mosquitoes for taxonomic study. Presented at Annual Meeting of American Mosquito Control Association, 27 Feb - 3 Mar, Lake Buena Vista, FL.

Harrison, B.A. (with R. Rattanarithikul, K. Mongkolpanya, T.A. Klein and E.L. Peyton). 1983. New mosquito records for Thailand, with notes on other uncommon or important species (Diptera: Culicidae). Presented at Annual Meeting of American Mosquito Control Association, 27 Feb - 3 Mar, Lake Buena Vista, FL.

Peyton, E.L. (with D.R. Roberts, F.P. Pinheiro, R. Vargas and F. Balderama). 1983. Mosquito collections from a remote unstudied area of southeastern Bolivia. Presented at Annual Meeting of American Mosquito Control Association, 27 Feb - 3 Mar, Lake Buena Vista, FL.

Zavortink, T.J. (with D.R. Roberts and A.L. Hoch). 1983. Trichoprosopon digitatum - morphology, biology, and potential medical importance. Presented at Annual Meeting of American Mosquito Control Association, 27 Feb - 3 Mar, Lake Buena Vista, FL.

Harrison, B.A. and R.E. Harbach. 1983. Mosquito morphology, characters for separating Egyptian mosquitoes, preservation of specimens and the Smithsonian system for taxonomic studies. Training session presented at Ain Shams University Research and Training Center on Disease Vectors, 8 May, Cairo, Egypt.

Harrison, B.A. 1983. Impact of sibling species complexes on our concepts of tropical infectious disease epidemiology. Seminar presented at U.S. Navy Medical Research Unit No. 3 (NAMRU 3), 10 May, Cairo, Egypt.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6538	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORDER NUMBER	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM	10. WORK UNIT
82 10 01	D. Change	U	U		CX	YES NO		

11. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AA	043 WWGM
b. [illegible]	62770A	3M162770A871	AA	
c. [illegible]	STOG 82/83-6	2/3		

12. TITLE (Procede with Security Classification Code)

(U) Characteristics of Artenuated Dengue Viruses

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

0613 Microbiology 0603 Biology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY			17. PERFORMANCE METHOD
75 07	CONT	DA			C. In-House

18. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

19. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	2.0	268
FISCAL YEAR CURRENT 84	2.0	255

20. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

H
Foreign Intelligence Considered

22. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: HARRISON, V R
NAME: SUMMERS, P L POC: DA

23. KAM 1 (C) Lab Animals (U) Attenuation; (U) volunteer; (U) Dengue; (U) Vaccina; (U) Immunity; (U) Cell culture (U) Monkeys

24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Provide brief explanation of progress achieved during this work unit and cumulative through last reported fiscal year. Indicate Classification Code.)

22. (U) The objective is development, production, and assay of live-attenuated vaccines against classical strains of dengue viruses. The major types (1,2,3, and 4) of this virus are endemic throughout populated areas of the world, and although mortality rates are low, the incapacitation effected by these viruses and their associated sequelae could have serious impact on military time tables and troop mobility.

24. (U) Selected strains of dengue viruses are subjected to multiple passages and frequent cloning in tissue culture systems, to produce pure progeny characterized by reduced virulence and adequate antigenicity, that will serve as candidate vaccine seed viruses.

25. (U) 82 10 - 83 09 1. Several temperature sensitive clones of dengue-3 virus, passaged and cloned in C6/36 mosquito cells followed by vaccine production in monkey diploid cells, were immunogenic in rhesus monkeys. All the clones produced a diminished viremia compared to the parent virus but only one clone, designated 24/28, was phenotypically stable after monkey passage. A lot of vaccine has been produced for clone 24/28 and further monkey testing will follow. 2. Vaccines cannot be made using C6/36 mosquito cells as the substrate because of allergenic proteins contained in the supernatant fluids of these cultures. Other mosquito cell lines including three lines from Toxorhynchites amboinensis mosquitoes were also found to contain allergenic proteins that were reactive when inoculated into monkeys intradermally. These findings indicated a high degree of cross-reactivity associated with the allergenic components of these cultures. 3. Enhancing antibodies were found in sera from human volunteers with yellow fever vaccination. An in vitro system employing elutriated monocytes as the target cells was sensitive for detecting low levels of these antibodies which enhanced dengue-2 virus infection. Further studies will correlate titer of enhancing antibodies in these individuals with response to dengue-2 vaccine. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

* Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DA FORM 4466 (APR 80) AND 1498-1 MAR 83 FOR ARMY USE ARE OBSOLETE. (ADP1 [illegible])

PROJECT 3M162770A870 PREVENTION OF MILITARY DISEASE HAZARDS

WORK UNIT 043 CHARACTERISTICS OF ATTENUATED DENGUE VIRUSES

Investigators:

Principal: Kenneth H. Eckels, Ph.D.

Associates: Doria R. Dubois
Venton R. Harrison
Peter L. Summers

Problems and Objectives

The project involves the development, production, and assay of live-attenuated vaccines against various strains of dengue viruses. Dengue isolates are selected from suitable sources and subjected to multiple passage and frequent cloning in cell culture systems. Pure clones of virus are screened for various markers of attenuation, including temperature sensitivity, small plaque size, lowered intracerebral virulence in mice and reduced peripheral virulence in monkeys. If the selected clones are also immunogenic in monkeys, they will serve as candidate viruses for the production of experimental seed and vaccine lots.

Progress

Four dengue-3 (DEN-3) temperature sensitive virus clones were isolated in C6/36 cell culture with a final passage in FRhL diploid cells. The four clones, when inoculated in rhesus monkeys, had reduced virulence compared to the parent DEN-3 virus. All of the clones infected monkeys and resulted in antibody formation that protected the monkeys against challenge with the parent virus. Only one of the clones (24/28) was completely stable in monkeys when viremia isolates were studied for loss of temperature sensitivity and plaque size phenotypes. A production seed for clone 24/28 was prepared in C6/36 cells and freeze-dried. The production seed was used to make the first lot of vaccine for candidate clone 24/28 in FRhL cells which will be tested in the next fiscal year.

It was previously demonstrated that a C6/36 sham vaccine using uninfected cell culture fluids caused immediate hypersensitivity reactions in humans and monkeys (AR 1982, Scott et al., 1983). Other mosquito cell lines including three derived from Toxorynchites amboinensis tissues (TA-9, TA-42, and TRA-284) were used to prepare sham vaccines. All of these mosquito cell lines were reactive in monkeys by direct intradermal inoculation. This indicated that

the allergenic protein in these preparations was similar to that found for C6/36 cells and may be highly cross-reactive with cytophilic antibodies that are common in monkeys and humans.

Due to higher infection rates and higher titers of DEN-2 antibodies in yellow fever-immune recipient of the DEN-2/S-1 vaccine, a screen of pre-vaccine sera was initiated to detect the presence of enhancing antibodies for DEN-2 virus. A group of volunteers involved in a vaccine efficacy trial at Fort Bragg were studied and their pre-vaccine sera used in an assay incorporating elutriated human monocytes as the target cells. Preliminary results indicated that the elutriated (fresh) monocytes were superior to a lymphocyte line (U-937) that was used previously to detect enhancing antibody and that low levels of these antibodies were found in the yellow-fever immune subjects. Correlation with pre and post-vaccine antibody titers and clinical symptomatology will be addressed after all data is collected.

Various stabilizers were evaluated for maintenance of the infectivity of DEN-2 and DEN-3 preparations. Previously, human serum albumin (HSA) was the sole stabilizer for the DEN-2/S-1 vaccine and was adequate for maintaining infectivity levels over freezing, drying, and storage of the vaccine up to 6 years. The DEN-3 vaccine clone 24/28 and other DEN-3 seeds suffer a loss of infectivity if HSA alone is used as a stabilizer. Combinations of HSA with various sugars including maltose and lactose indicate that the sugar-HSA combination is far superior than HSA alone. Gelatin as a substitute for HSA has also shown promise when it is used in combination with lactose as a stabilizer.

Seeds for DEN-2 and DEN-4 viruses that are suitable as human "challenge" viruses were tested for safety and freedom from adventitious agents. The testing was performed under contract by the University of Hawaii School of Medicine. All tests indicated that the seeds were safe for use by criteria established for live vaccines.

Recommendations

A DEN-4 vaccine (H-241 PDK35 PD3 FRhL-3) tested in 5 human volunteers has been discontinued. Caribbean DEN-4 isolates as well as viremic blood samples taken from a recipient of the DEN-4 H-241 vaccine will be used as starting seeds for the development of new DEN-4 vaccines. This work is currently under contract to the University of Hawaii School of Medicine with collaboration by this laboratory.

A DEN-3 vaccine candidate (clone 24/28) will be further evaluated in cross-challenge experiments in monkeys using various strains of DEN-3 viruses. Protection and the stimulation of neutralizing antibodies will be evaluated prior to the next phase of testing which will be neurovirulence tests of the vaccine clone.

A DEN-1 vaccine (45AZ5) which has undergone safety testing will be tested on a small scale in human subjects. This laboratory will collaborate on the virological studies of the vaccine during the human testing.

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1. Scott, R. McN., Shelton, A.L., Eckels, K.H., Bancroft, W.H., Summers, R.J., and Russell, P.K. 1983. Human hypersensitivity to a sham vaccine prepared from mosquito cell culture fluids (submitted to the Journal of Immunology).

2. Scott, R. McN., Eckels, K.H., Bancroft, W.H., Summers, P.L., McCown, J.M., Anderson, J.H., and Russell, P.K. 1983. Dengue-2 vaccine: dose response in subjects with and without yellow fever antibody. J. Infect. Dis. (accepted for publication).

3. Summers, P.L., Eckels, K.H., Dalrymple, J.M., Scott, R. McN., and Boyd, V. Ann. 1983. Antibody response to dengue-2 vaccine measured by two different radioimmunoassay methods (submitted to the Journal of Clinical Microbiology).

4. Eckels, K.H., Scott, R. McN., Bancroft, W.H., Brown, J., Dubois, D.R., Summers, P.L., Russell, P.K., and Halstead, S.B. 1983. Human response to immunization with a candidate, live-attenuated dengue type 4 (H-241) virus vaccine (accepted for publication in the American Journal of Tropical Medicine and Hygiene).

5. Bancroft, W.H., Scott, R. McN., Eckels, K.H., and Hope, C.H. 1983. Safety and immunogenicity of a dengue-2 vaccine (submitted to the Journal of Infectious Diseases).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

DA OA 6442 | 83 10 01 | DD-DR&E(AR)636

3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. MATERIEL	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM	A. WORK UNIT
82 10 01	CD Change	U	U	DA	CX	☒ YES ☐ NO		

10. PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
62770A	3M162770A871	AE	044 WRGD

11. TITLE (Procedure, Method, or Technique Classification Code)

Rickettsial Diseases of Military Personnel

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
55 08	Cont	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: | EXPIRATION:

b. NUMBER: NA

c. TYPE: | d. AMOUNT:

e. KIND OF AWARD: | f. CUM. AMT.

18. RESOURCES ESTIMATE	A. PROFESSIONAL MAN YRS	B. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	4.0	550
FISCAL YEAR CURRENT 84	5.0	445

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: Walter Reed Army Institute of Research

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20. PERFORMING ORGANIZATION

a. NAME: Walter Reed Army Institute of Research
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SOCIAL SECURITY/ACCOUNT NUMBER:

d. ASSOCIATE INVESTIGATORS POC: DA

NAME: Jerrells, T R Rice, R M

NAME: MacMillan, J G Jarboe, D L Mentes, W H

21. GENERAL USE

H

Foreign Intelligence Considered

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Rickettsial Infections; (U) Laboratory Diagnosis; (U) Vaccines; (U) Epidemiology (U) Lab Animals (U) Mice (U) RAM I

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code)

23. (U) Develop experimental rickettsial immunogens; define the pathology of rickettsial infections in laboratory animals to include subhuman primates; determine the sequence of events leading to immunity following vaccination or infection. These studies are aimed at development of vaccines that will protect deployed military troops, and development of immunoassays to evaluate the extent of immunity induced by vaccination.

24. (U) Gamma irradiation of rickettsiae to produce attenuated immunogens. Evaluate tissue culture-propagated strains for use as immunogens to provide protection against scrub typhus infection. Analyze correlates of lymphocyte recognition to determine the adequacy of immune response. Correlation of subhuman primate response to that seen in the murine model. Determine the genetic basis of resistance and sensitivity of the mouse model to scrub typhus infection.

25. (U) 82 10 - 83 09 Comparative studies indicated that 4 to 10 times more cell culture derived rickettsiae are needed to give the same level of protection as yolk sac derived organisms under the same conditions. Lipovant was the most promising adjuvant when humoral and lymphoproliferative responses were quantitated against the appropriate controls. The T-lymphocyte nature of gamma interferon production to R. tsutsugamushi antigens was demonstrated when athymic mices failed to produce gamma interferon while euthymic controls did under similar treatment. Initial data indicate that gamma interferon may be indicative of antigen responsive lymphocytes circulating in immunized animals. Immunological evaluation of a small number of cynomologus monkeys following infection with Karp strain R. tsutsugamushi organisms demonstrated our operational capacity to measure humoral reactivity by indirect immunofluorescence and radioimmunoassay. Cellular responses were followed by lymphocyte proliferation to rickettsial antigens and lymphokine (gamma interferon) production. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

DD FORM 1498 (MAR 88) PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A (1NOV 68) AND 1498-1 (MAR 88) (FOR ARMY USE) ARE OBSOLETE

Project 3M162770A870 PREVENTION OF MILITARY DISEASES HAZARDS

Work Unit 044 Rickettsial Diseases of Military Personnel

Investigators: COL Kenneth W. Hedlund, MC; Thomas R. Jerrells, PhD.; MAJ Robert M. Rice, V.C.; CPT James G. MacMillan, V.C.

Associates: SP5 September Bodden; SP4 Miriam R. Pedersen; SP4 Stephan Platt; SP4 Peter Pond

Problems and Objectives

Investigations have been aimed at the production of safe and useful immunogens which can in turn be used to develop an efficacious rickettsial vaccine to protect troops in areas of endemic disease. One early aspect of the work has been to define the pathogenesis of rickettsial disease in laboratory animals and to determine the sequence of events leading to immunity following vaccination or infection. Since 1978, the immunogen which has received the greatest attention has been whole rickettsiae derived either from embryonated yolk sacs or primary chick embryo tissue culture cells inactivated by gamma irradiation. Methodologies for studying immune mechanisms developed in the murine model have been adapted to a primate model. These assays for both humoral and cell mediated immune response have been used successfully in following infections and will be critically important in evaluating the efficacy of any new potential vaccine. The most difficult task before us is to chose from the presently available forms of rickettsial immunogens those that will elicit an optimum protective response. At present, there is an absence of specific knowledge as to the immunodominant components that elicit this protection. Our knowledge of cell circuitry and lymphokine kinetics is rapidly expanding and been highly productive. We are still moving to the position where we can selectively elicit and clinically exploit these phenomena by correctly "chosing and packaging" the components of the scrub vaccine.

Progress

Primary chick embryo cell culture derived Rickettsia tsutsugamushi was partially purified by a series of differential centrifugations, irradiated with 300 Krads gamma-irradiation and stored at -70°C until used. Characterization of individual as well as pooled lots included determination of relative antigenicity, number of intact rickettsiae, and mouse protection from

v able homologus challenge. Data showed that mice could be protected by the gamma-irradiated cell culture derived vaccine. Previously published research from this department indicated that 3 vaccinations of 1.6×10^6 yolk sac derived rickettsiae were needed to protect 100% of mice. Current investigation indicates that 4 to 10 times more of the cell culture derived rickettsiae are needed to give the same protection under the same conditions. Comparable humoral and cell mediated immunity were induced with the tissue culture vaccine when compared to previously reported work with gamma-irradiated yolk sac vaccine. No individual mehtod of characterization was preferred. All three gave pertinent quantitative and qualtative information concerning the individual or pooled lots of produced vaccine. Of the techniques used, the RIA was the most sensitive and characterizes the vaccine according to its antigencity. Specific activity (SA, units of antigenicity/mg of protein) is a measure of purity. A SA of <5000 did not protect 50% of the mice from viable challenge whereas individual or pooled lots >5000 U/mg did protect mice.

A dilution of a pooled lot of cell culture derived vaccine which gave less than 50% protection was adjuvantized with Lipovant (L), Complete Freund's Adjuvant (CFA), 20% yolk sac (YS), Snyder's buffer (B, nonadjuvantized vaccine), or Glucan (G). Humoral antibody response, cell mediated immunity, and protection from homologus challenge were all potentiated when adjuvantized vaccine was given intraperitoneally. G, L, YS, and CFA gave 80-100% protection from homologus challenge when given intrapertitoneally while the vaccine alone gave less than 50% protection. Humoral antibody response was 8 times or greater than that for the vaccine alone when adjuvantized with G, L, or CFA. Lymphocyte proliferation assays were done for B, L, and YS. All were positive indicating that they stimulated the cellular component of the immune system, however values for L were significantly greater than the others. Subcutaneous vaccinations, with gamma irradiated organisms; however, did not drive the immune response or protect mice from homologous challenge.

Subcutaneous inoculation of mice with viable R. tsutsugamushi: results in the development of a long-lived chronic infection. In unpublished studies Groves and Osterman demonstrated that mice infected in this manner harbored viable rickettsiae for the life of the animal. In more recent studies infection by the subcutaneous route results in the development of both humoral and cell-mediated immunity. In many infectious disease models, e.g., Brucella infections of mice, the development of chronic infections is associated with an immunosuppression which may temper or delay the immune response thus allowing some organisms to establish the chronic infection. To study this in

experimental scrub typhus, mice undergoing the development of chronic infection with various strains of R. tsutsugamushi, were evaluated for immunocompetency. At various times after infection lymphocyte responses to T- and B-cell mitogens as well as in a mixed lymphocyte response (MLR) were monitored. It was found that at 10 to 21 days after infection these in vitro responses were significantly lower than in age matched controls. It was also found that infected animals produced depressed responses in vivo after immunization with sheep erythrocytes or TNP-Ficoll as measured by a modification of the Jerne plaque assay. Suppression of proliferative responses in vitro could be reversed by depletion of adherent cells or by the addition of indomethacin to cultures thus raising the possibility of a prostaglandin producing macrophage as the effector cell in in vitro suppression. Examination of the spleens at the height of immunosuppression revealed a marked splenomegaly due mostly to an inflammatory (peroxidase positive) macrophage influx. Interestingly, macrophages isolated from the spleens of infected animals appeared to be activated as measured by tumor cell cytolysis and cytostasis as well as by antimicrobial activity. These data would suggest that macrophages which are activated during the process of clearing the SQ infection may be partially responsible for the observed immunosuppression.

It has been previously established that C3H/HeDub mice immunized s.c. with 1000 MLD_{50} of R. tsutsugamushi strain Gilliam produce immune (gamma) interferon (IFN) after administration of homologous antigen in vivo or in vitro. The further role of γIFN during infection has been investigated and salient features of the research are provided:

1. A role for immune interferon was established during active rechallenge of immune animals. IFN was found to be produced during the response leading to rickettsial clearance of animals challenged both i.v. and ip. with 10,000 MLD_{50} of the Gilliam strain. Bimodal kinetics of the IFN response are thought to reflect the inital proliferation of rickettsiae (early IFN peak) and immune clearance of the challenge (late IFN peak).

2. The development of the ability to produce IFN after a s.c. infection was found to be initiated in local-draining inguinal lymph nodes, which decreased in responsiveness as splenic lymphocytes were demonstrated to be capable of responding. The IFN response which was paralleled by the ability of lymphocytes to respond by proliferation to immunizing antigen (LP) was shown to be a long lived response (up to 60 days post infection). The cell type responsible for

antigen-specific IFN producton in the spleen was a lymphocyte bearing Thy 1.2 and Lyt $1^{+}2^{-}$, 3^{-} antigens. The IFN response of this cell population was dependent on a source of spleen adherent cells, which presumably functioned as antigen presenting cells. The T-lymphocyte nature of IFNγ production to R.tsutsugamushi antigens was further shown when athymic BALB/C (nu/nu) mice failed to produce IFNγ after infection while euthymic BALB/C control animals were found to produce IFN in vitro after similar treatment.

3. Immune IFN has also been studied in the lethal infection of C3H/HeDub mice after i.p. challenge of naive animals with the Gilliam strain of R. tsutsugamushi. Serum levels of IFN were found to be low until the late and final days of infection. This response was found to be similar to the in vitro IFN responses of spleen and mesenteric lymph node cells, however these cells were found to be capable of producing much higher levels of IFN just before death, with or without (spontaneous) antigen administration. Lymphocyte proliferative responses of these animals to antigen developed about the same time as the IFN response but were totally suppressed at the time of death, as were LP responses to nonspecific T-cell mitogens (CON A). These results suggest that IFN is produced in an acute infection of laboratory animals and may act in a negative manner. Further study is planned to investigate the role of IFNγ in acute infection of mice.

4. IFNγ production was used in conjunction with LP responses to monitor cellular immunity in naive, previously infected, and vaccinated cynomologous monkeys. IFNγ was found in lymphocyte culture supernatants 14 days after i.d. infection with the Karp strain which also correleted with detectable plasma level of IFN. IFN responses from PBL cultures declined through day 56 of infection at which time a state of nonresponsiveness was observed. Initial studies using vaccinated animals showed that animals did produce IFNγ in vivo (plasma) after challenge with viable rickettsiae. Preliminary studies are still currently in progress, but initial data indicate that IFN may be indicative of antigen responsive lymphocytes circulating in immunized monkeys.

Previous work has established that genetic resistance to the Gilliam strain of R. tsutsugamushi is associated not with the sheer quantity of the macrophage inflammatory response but with the type of macrophage responding to the infection. To extend these observations the inflammatory exudates of resistant (C3H/Rv) and susceptible (C3H/HeDub) mice were examined for macrophages with I-region-associated antigens (Ia). Although susceptible

C3H/HeDub mice produced a greater exudate the C3H/Rv mice produced an exudate consisting mostly of Ia bearing macrophages. As expected, the exudate cells from C3H/Rv mice were superior to an equal number of cells obtained from C3H/HeDub mice in terms of presentation of antigen to immune lymphocytes. In further studies, immunized mice produced an inflammatory exudate consisting predominantly of Ia bearing macrophages regardless of the genetic background demonstrating that C3H/HeDub mice do not inherently lack the ability to produce an Ia positive exudate and also emphasize the importance of Ia antigen bearing macrophages in immunologically acquired resistance to reinfection. Interestingly, it would appear that in susceptible animals the Ia positive macrophage response is suppressed late in the infection. This is more striking as it has been shown that large amounts of immune interferon is produced. As it is thought that this molecule is primarily responsible for induction of Ia in macrophages these two pieces of data suggest that an active suppressive event is occuring in these animals which is blocking the induction of Ia molecules on the surface of inflammatory cells.

A major effort this fiscal year was the study of primary R. tsutsugamushi infection of primates. In these studies, adult cynomolgus (Maca fascicularis) monkeys were infected with rickettsiae, which were grown in embryonated eggs, and followed for immunological responsiveness and clinical signs of infection. Animals infected with the Karp strain of R. tsutsugamushi by intradermal inoculation reproducibly developed eschars at the site of infection from 5-7 days after infection. Draining lymph nodes also demonstrated lymphadenopathy after infection. The majority of animals infected intradermally developed an elevated body temperature after infection although only a few animals reached 40°C which has traditionally been considered a febrile response in this species. In contrast to these findings, animals which were infected with similar or higher doses of rickettsiae administered subcutaneously did not develop any noticable response at the site of infection or any marked change in temperature.

Immunological evaluation of these animals consisted of serum antibody titers measured by IFA and an RIA, peripheral blood lymphocyte proliferation to rickettsial antigen, and lymphokine (gamma interferon) production in lymphocyte culture supernatants. Serum antibody was first detectable from 7-10 days after infection and using isotype specific reagents in the RIA was found to consist primarily of IgM. Relatively high titers of IgG antibody developed by 30 days after infection. Representative animals from this study will be retained and followed for an extended time to determine the longevity of the antibody produced in response to the initial infection as well as any changes in antigen specificity. Antigen responsiveness of circulating T-lymphocytes as

measured by antigen specific proliferation developed roughly in parallel with the antibody response. As has been noted in other animal models, the early T-lymphocyte response demonstrated a great deal of strain cross-reactivity although the homologous antigen response was generally the greatest. Interac-tion of antigen and immune lymphocytes was also evident by the production of gamma interferon in culture supernatants. Gamma interferon was also demonstrable in sera of infected animals and may be involved in rickettsial clearance as has been shown in the mouse. In a similar study, monkeys which were experimentally infected with R. tsutsugamushi in Malaysia in 1977-1978 were evaluated for circulating antibody and long-lived antigen responsive lymphocytes using the above techniques. None of these animals were found to have antibody even using the RIA. However, the majority of monkeys tested were found to respond to the homologous infecting antigen by lymphocyte proliferation. These responses were only to the homologous antigen and no cross-reactivity was noted. These animals were also challenged and followed as before. As would be predicted, these animals responded similarly to naive monkeys in terms of clinical signs of infection. Immunologically a typical anamnestic antibody response was noted suggesting long-lived B-cell memory was present. Lymphoproliferative responses were developed at essentially the same rate as in naive animals. In these studies, two animals from the initial studies were rechallenged approximately 6 months after the initial infection and showed no signs of infection and an anamnestic response as expected.

Recommendations

The feasibility of a scrub typhus vaccine is based on the development of specific immunity to R. tsutsugamushi during the course of the natural infection. Most vaccine attempts have failed to induce a significant degree of artificial immunity until the uses of volk sac derived gamma irradiated whole organisms was employed in mice by Osterman et al. Our goal is the development of an optimized tissues culture methodology which will allow for predictably consistent yields of rickettsiae to be used in production of vaccine lots. Having biochecmically and biologically characterized the vaccine preparations we will define those conditions necessary to maximize the immune response either to the inactivated whole organism or subunits of the organism. The role of adjuvants will be considered. We have established in mice and monkeys, an operational capacity to assay cell mediated and humoral responses to scrub infections. The next step will be to use these same assays to follow the primate's response to the irradiated vaccine before and after an homologous infectious challenge.

Our studies on the natures of cell mediated and humoral responses and the roles of lymphokines both as mediators of effector mechanisms and as indicators of protective immunity will continue.

Formal Presentations

1. Palmer, B.A.. Hetrick, F.M., and Jerrells, T.R.: Immune Interferon Production by Mice Immune to Rickettsia tsutsugamushi after Rickettsial Challenge or Antigen Administration. 67th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Illinois, April 1983.

2. Palmer, B.A., Hetrick, F.M., and Jerrells, T.R.: Development of Immune Interferon Production and Cell-Mediated Immunity Following Immunization of C3H/He Mice with Rickettsia tsutsugamushi. 5th International Congress of Immunology, Kyoto, Japan, August 1983.

3. Jerrells, T.R.: Suppression of Lymphocyte Function Concomitant with Macrophage Activation as a Result of Chronic Infection with Rickettsia tsutsugamushi. 15th International Leucocyte Culture Conference, Asilomar, California, Dec. 1982.

4. Jerrells, T.R.: Ia^+ Macrophage Response Differences in Strains of C3H Mice Genetically Susceptible or Resistant to Infection with R. tsutsugamushi. 67th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Illinois, April 1983.

5. Jerrells, T.R., Palmer, B.A. and MacMillan, J.G.: Cellular Mechanisms of Innate and Acquired Immunity to Rickettsia tsutsugamushi. RML workshop in Molecular Microbiology, Rockey Mountain Laboratory, Hamilton, Montana, June 1983.

6. Jerrells, T.R.: Mechanism of Innate and Acquired Resistance to Rickettsia tsutsugamushi in a Murine Model: Role of Ia Positive Macrophages. 5th International Congress of Immunology, Kyoto, Japan, August 1983.

Bibliographies

1. Jerrells, Thomas R., Bennie A. Palmer and Joseph V. Osterman 1983. Gamma-irradiated scrub typhus immunogens: Development of Cell-Mediatd Immunity After Vaccination of Inbred Mice. Inf. Immun. 37:1066-1073.

2. Jerrells, Thomas R. and Joseph V. Osterman 1983. Development of Specific and Cross Reactive Lymphocyte Proliferative Responses During Chronic Immunizing Infections with Rickettsia tsutsugamushi. Inf. Immun. 40:147-150.

3. Jerrells, Thomas R. and Christine Eisemann 1983. Role of T-Lymphocytes in Antibody Production to Antigens of Rickettsia tsutsugamushi and Other Rickettsia species. Inf. Immun. 41:666-674.

4. Jerrells, Thomas R. and Joseph V. Osterman, 1983. Parameters of Cellular Immunity in Acute and Chronic Rickettsia tsutsugamushi Infections of Inbred Mice. pp.355-360. In T.K. Eisenstein and P. Actor (eds.), Host Defenses to Intracellula Pathogens. Plenum Publishing Corporation.

5. Jerrells, Thomas R. 1983. Association of the Inflammatory Ia Positive Macrophage Influx in the Genetic Resistance of Inbred Mice to Rickettsia tsutsugamushi. Inf. Immun. In Press.

6. Jerrells, Thomas R.,B.A. Palmer, and J.G. MacMillan. 1983. Cellular Mechanisms of Innate and Acquired Immunity to Rickettsia tsutsugamushi. Microbiology 1983. In Press.

7. Palmer, B.A., F.M. Hetrick, and T.R. Jerrells. 1983. Production of Immune (γ) Interferon in Mice Immune to Rickettsia tsutsugamushi. Inf. Immun. In Press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6535	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	SUMMARY SCTY	6. DOCK SECURITY	7. REGRADING	HDL MASTER	8a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		CX	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
A. PRIMARY	62770A	3M162770A870	AF	045 WWM5
B. CONTRIBUTING	62 DA	3M162770A871		
C. [illegible]	[illegible]	[illegible]		

11. TITLE (Procede with Security Classification Code)

Development of Anti-Parasitic Disease Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

0615 Pharmacology 0603 Biology 0703 Organic Chemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: [ ] EXPIRATION: [ ]
b. NUMBER:
c. TYPE: [ ] d. AMOUNT:
e. KIND OF AWARD: [ ] f. CUM. AMT.:

18. RESOURCES ESTIMATE

	FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
PRECEDING	83	15.0	442
CURRENT	84	9.0	1,074

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
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ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign [illegible] considered

POC: DA

22. KEY WORDS (Provide Navy & Interim Code) (U) Malaria; (U) Leishmaniasis; (U) Trypanosomiasis; (U) Schistosomiasis; (U) Drug Development; (U) Chemistry; (U) Pharmacology; (U) Biology.

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Precede each with appropriate Security Classification Code)

23. (U) Design, test and develop new drugs with chemoprophylactic or chemotherapeutic activity against diseases of military importance.

24. (U) Chemical samples obtained from a rational synthesis program, industry and academia are tested for activity against the diseases. Information is used in guiding new synthesis and selecting candidates for clinical trials. New test systems are developed. Candidates undergo preliminary pharmacological testing.

25. (U) 82 10-83 09 Approximately 2300 new samples were obtained for testing. Artemisinine has been isolated from a locally growing Artemisia annua. Synthesis has been terminated in the acridine area, but continues in others. Testing, both contract and in-house is continuing. New test systems have been set up in malaria and trypanosomiasis. Method improvement in methemoglobin has been done, and methemoglobinogenic potential of new candidate drugs determined. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82-30 Sep 83.

Project 3M162770A870 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 045 Development of Anti-parasitic Disease Drugs

Investigators:

Principal: COL Craig J. Canfield, MC
Associates: LTC Willis A. Reid, MS; LTC Jonathan D. Berman, MC; MAJ John P. Scovill, MS; CPT P.B. McGreevy, MS; CPT Chris Lambros, MS; CPT Robert F. Miller, MS; Nancy A. Roth, Ph.D, Bing T. Poon, Ph.D.; Ho Chung, Ph.D.

PROBLEM AND OBJECTIVES:

In many parts of the world where U.S. military personnel may be deployed, diseases such as malaria, leishmaniasis, schistosomiasis and trypanosomiasis are endemic. Prevalence of both falciparum and vivax malarias is increasing because of failing control and eradication efforts in many countries. In many of these areas, falciparum malaria has become resistant to currently available drugs. Current chemotherapy of leishmaniasis, schistosomiasis and trypanosomiasis is inadequate. There are no drugs available for prophylaxis, and those that are available for therapy have limited efficacy and dangerous side-effects. The objective of this work unit is the discovery and development of new drugs for prophylaxis and treatment of these diseases in military personnel. In-house research is complemented by and coordinated with contractor laboratory drug testing and research.

PROGRESS:

1. The Research Contract Synthesis Program

During this period, active contractual programs devoted to the synthesis of potential antiparasitic agents were divided as follows: malaria 3, leishmaniasis 1 1/2, trypanosomiasis 2 1/2. Two preparations laboratories and a radiolabel synthesis contract also supported the program. A portion of these contracts also supported the Chemical Defense Program.

The 8-aminoquinolines continue to show good blood as well as tissue schizontocidal activity. Efforts continue in two

principal areas: (a) investigate variation in the 5-OR series; and (b) investigate the effect of substitution in the 2- and 3-positions instead of, and, in addition to, a CH_3 group in position-4.

Synthetic efforts in aminonaphthalenes, amodiaquine analogs, pyrimethamine and acridineoneimines have been discontinued, although testing continues in the last. Toxicity studies (Periarteritis-nodosa) are especially needed on these compounds.

In leishmaniasis and trypanosomiasis, pyridine bis-amidines and oxime precursors continue to show activity. In the area of purine metabolism, efforts have shifted from the sulfamoylated compounds to variations in inosine analogs.

2. Data Processing

Data processing on the CDC 3500 was discontinued in April 1983. This was prior to completion of the conversion to the VAX 11/780. At the end of the fiscal year, biology retrieval was functional, but input/update still needed work. In the inventory subsystem, the shipping cycle was operational, while overdue processing was not. These two subsystems are expected to be operational and ready for enhancements by the end of the 1st quarter of FY84. The chemistry subsystem is not operational and data processing/retrieval is now being done manually. Testing of the subsystem is planned for December 1983. Portions of the integrated report generator are operational, but full implementation awaits completion of the other subsystems.

3. Acquisition of Compounds

Approximately 2300 new samples were obtained for testing from the rational synthesis program, industry, and academia.

4. Organic Synthesis Section

About 93 compounds were submitted for biological testing over the past 12 months, few of which had been described in the chemical literature.

Of special interest is the isolation from the locally growing plant, Artemisia annua, of the sesquiterpene lactone

peroxide, artemisinine (qinghaosu). This compound first obtained in China ca. 13 years ago is reported to have excellent activity against chloroquine-resistant Plasmodium falciparum and cerebral malaria. The synthesis of derivatives of artemisinine and a study of the stability of their pharmaceutical dosage forms will be performed when sufficient artemisinine is obtained.

A licensing agreement, based on the work of this research group, has been signed by the Army and the University of Michigan to enable the commercial development of some of the 2-acetylpyridine thiosemicarbazones in the treatment of herpes simplex 1 and 2.

The synthesis and partial evaluation of some acyl-α-N-heterocyclic thiosemicarbazones containing the ring systems, thiazole, pyrazine, and pyrimidine, has been accomplished. Also, a new series of 2-(α-hydroxyacetyl) pyridine thiosemicarbazones was prepared so as to improve the water-solubility of the most biologically active members of this class of compounds. The increase in hydrophilicity lowered murine toxicity of the thiosemicarbazones but also reduced their antimalarial efficacy.

5. Biological Testing/Studies

(Malaria) Approximately 260 compounds were tested for activity against P. falciparum in vitro. Of these, 7 compounds were found to have antimalarial activities equal to or greater than mefloquine against chloroquine-resistant parasites. Procedures for assessing the antimalarial activities of sulfonamide drugs alone, and in combination with inhibitors of DHFR were implemented to differentiate known clinically Fansidar resistant and susceptible geographic isolates. The elucidation of the mechanisms of resistance (and/or cross-resistance) to these classes of drugs was also pursued. WR 171669 maintained in vitro anti-malarial activity against Mefloquine-resistance strains. Studies in collaboration with the Department of Medicine, WRAIR, were implemented to elucidate purine metabolism of malaria parasites. It became apparent that parasite adenosine deaminase differs from the host enzyme relative to drug inhibition and enzyme kinetics. The primary extramural screen identified approximately 200 compounds with activity out of approximately 3000 candidate compounds tested. Secondary studies were done on

approximately 100 of the active compounds against resistant strains of P. berghei in mice, human P. falciparum in Aotus monkeys and/or vivax-like P. cynomolgi in rhesus monkeys.

(Trypanosomiasis) Approximately 2000 compounds were screened against T. rhodesiense in mice in the extramural screen. Exceptional activity was found in novel imidazopyridinium compounds. Approximately 230 compounds were tested for activity against Trypanosoma rhodesiense in vitro. Of these, 42 compounds were found to have antitrypanosome activities equal to or greater than ethidium bromide. In an in vitro mouse system developed to assess the ability of drugs to cross the blood-brain barrier, 39 different compounds were tested, each at 5 different concentrations, for activity against T. rhodesiense. Eight of these were found to be active against CNS involvement in mice with chronic trypanosomiasis. Radiorespirometric studies found T. cruzi to be metabolically very distinct from Leishmania spp.

(Leishmaniasis) Formycin B was found to be an active antileishmanial agent both in vitro and in vivo; its efficacy was demonstrated to be due to its metabolism into nucleotides and into RNA. The activity of the new antileishmanial agent is probably due to in vivo metabolism, whereas the activity of ketoconazole is due to inhibition of leishmanial sterol biosynthesis. Encapsulation of drugs within red cells augments in vivo activity. About 350 compounds were screened in the extramural screen against visceral leishmania in hamsters. WR 6026, an oral drug undergoing clinical trials, was more efficacious when given as a single dose compared to multiple doses. WR 238605, an oral drug being considered for clinical trials as an antimalarial, was 25 times more efficacious than glucantime, the drug currently used in humans. About 250 compounds were screened against cutaneous leishmaniasis in hamsters and 18 were 1 to 15 times more efficacious than glucantime, the reference compound. Unfortunately, the best three compounds failed to heal cutaneous lesions on Aotus monkeys.

Mucocutaneous isolates from South America (formerly thought to be a single species, L. braziliensis braziliensis) are heterogeneous with radiorespirometric test variation associated with the geographic origin of the parasite. Mucocutaneous leishmaniasis may, in fact, be caused by more than one species/strain of parasite.

Studies on the topical application of antileishmanial drugs continue and seven compounds were found to heal cutaneous lesions on the Mystromys model. These lesions relapsed after treatment with four of the compounds and the remaining three continue to be evaluated.

In an effort to develop a drug screen to detect prophylactic compounds, studies were conducted on the infectivity of leishmanial promastigotes, the developmental stage which passes from sand fly to man in nature. In vitro derived promastigotes in the stationary phase of growth are more infective than log phase promastigotes. Stationary phase promastigotes may be the infective form of Leishmania and should be used to challenge animals in a prophylactic screen.

Cutaneous leishmaniasis was diagnosed in 5 U.S. soldiers. About 30 leishmanial isolates from soldiers infected in Panama were typed by isoenzyme analysis. The soldiers were infected with 6 subspecies of Leishmania. Leishmania braziliensis panamensis and Leishmania mexicana mexicana were the most frequent. Only 20% of the soldiers with L.b. panamensis healed after one course of treatment with Pentostam while 90% of the soldiers with L. mexicana mexicana healed. In vitro cultivation of Leishmania on agar plates is proving to be the most sensitive method for the primary isolation and culture of parasites. A simple kit to type Leishmanial isolates by isozyme analysis was developed and will be field-tested at USAMRU-Brasilia in 1984. Skin test antigen for the diagnosis of Leishmaniasis has been prepared and potency studies are being conducted in rodent systems.

(Schistosomiasis) Over 40 compounds were screened as possible topical prophylactic agents against Schistosoma mansoni infections in mice. Eleven of the compounds tested provided 90% or greater protection for 24 hrs after application. Two of the eleven compounds, WR 46234 and WR 34912, were identified as very effective antipenetrants (100% protection) and were studied in greater detail in the mouse model. WR 46234 provides complete protection against infection for 6 days after treatment at a concentration of 0.1 percent (W/V) in methanol. WR 34912 completely protected mice for 24 hrs against infection at concentration below 0.07 percent (W/V) in methanol. These two compounds have low oral toxicities (greater than 1000 mg/kg in rats) and are the most effective schistosome antipenetrants, with only a single application ever reported.

Preliminary studies also were undertaken to evaluate the anti-penetration effect of military clothing fabrics against S. mansoni.

Approximately 900 compounds (representing 2000 potential test groups) were sent to USAMRU-Brasilia for prophylactic and/or curative testing. Greater emphasis was placed on true prophylactic testing (treatment prior to cercarial exposure). Computerization of submission data was initiated and completed during this time period. Computerization of test data was initiated and is near completion.

6. Preliminary Pharmacology

A modified assay for methemoglobinemia was developed to screen for this side-effect. The methemoglobinogenic potential of several new candidate drugs was then studied.

Analytical methods were developed for the antimalarial drugs WR 142,490 (mefloquine), WR 180,409, WR 194,965, WR 171,669 (halofantrine), chloroquine, and the antileishmanial drug WR 6026 by work in-house or on contract. These methods, using primarily HPLC techniques, are being used to support bioavailability and pharmacokinetic studies in experimental animals or in humans in clinical trials. A number of these studies have been completed while others are still in progress. Results of this work have shown that WR 171,669 (halofantrine) is poorly absorbed and has a slow half-life of elimination of 2.7 days in the dog. Chloroquine has also been found to have a prolonged half-life. A new formulation of halofantrine with improved absorption is currently undergoing study.

FUTURE PLANS

Rational synthesis, other compound acquisition, screening and testing will continue in all areas. Conversion of the chemistry data retrieval system to the VAX 11/780 will continue, with transfer of other modules of the CIRS being completed during FY 84. Studies on metabolism, pharmacokinetics, improved formulation, and improved test system design will continue.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6765	83 10 01	DD-DR&E(AR)636

PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. PROGRAMS	MAJ'R PROTOCOL	9A. SPECIFIC DATA - CONTRACTOR ACCESS	9B. LEVEL OF SUM
10 01	D. Change	U	U		CX	☒ YES ☐ NO	A. WORK UNIT

CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
ADV	62770A	3M162770A870	AF	046 WWGE
XXXXX	62770A	3M162770A871	AF	
XXXXX	STOG 52/83-6.2/3			

(Program and Security Classification Codes)

Exploratory Vaccine Development Against Parasitic Diseases.

SCIENTIFIC AND TECHNOLOGICAL AREAS

.3 Microbiology 0603 Biology

START	11. ESTIMATED COMPLETION DATE	12. FUNDING AGENCY			13. PERFORMANCE METHOD
10	CONT	DA			C. In-House

CONTRACT/GRANT

EFFECTIVE:

EXPIRATION:

AMT:

OF AWARD:

CUM. AMT.

14. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	B. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	2.0	237
	CURRENT 84	2.0	234

15A. PERFORMING ORGANIZATION

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RAM USE

4 Foreign Intelligence Considered

(Keywords Each with Security Classification Code)

Antigens; (U) Immunoassays; (U) Parasitic Diseases; (U) Immunity; (U) Vaccines

) Lab Animals (U) Mice (U) RAM ;

(Technical Objective, 16. Approach, 17. Progress (Furnish milestones accomplished by months projected and actual with Security Classification Code)

J) The objective of this work unit is to isolate and characterize antigens particu- / those of malaria, trypanosomiasis and leishmaniasis, and the evaluation of them otential immunogens in experimental animals for the development of a safe and effec- vaccine. These diseases impede military performance whenever troops are deployed idemic areas emphasizing the need for a suitable vaccine to facilitate military ations.

J) The approaches used in these studies are to develop techniques for the isolation arasitic antigens; to use standard biochemical and immunochemical procedures to acterize and purify these antigens; to develop quantitative in vitro immunoassay to tor the purity of these isolated antigens; and to determine the effectiveness as ines of these antigens in experimental animals.

J) 82 10 - 83 09 Genetic relationships among Leishmania isolates are being rmined by kinetoplast DNA (kDNA) analysis. Rapid methods for isolation of kDNA, and electrophoretic separation of restriction enzyme-generated kDNA fragments were devel . kDNA fragment separation is an extremely sensitive method for differentiating hmania species and strains. Identical Leishmania were found in patients with similar ase who live within a limited geographic area. The stability of the kDNA fragment erns is not affected by the time parasites reside in man or by multiple exposure to -leishmania drugs. DNA hybridization is being used to detect as few as 100 Leishmani sites in man, animals and insect vectors. To facilitate hybridization, kDNA fragment cloned into E. coli plasmids to construct species and/or disease-specific DNA hybri tion probes. Non-radioisotopic procedures are being tested to detect DNA hybridiza- s so a field technique can be developed. For technical report see Walter Reed Army itute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

(Do not continue upon contractor's request)

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NOV 82 AND 1498-1, MAR 83 FOR ARMY USE ARE OBSOLETE.

PROJECT 3M162770A870 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 046 Exploratory Vaccine Development Against Parasitic Diseases

Investigators:

Principals: Peter R. Jackson, Ph.D.
LTC Wayne T. Hockmeyer, MSC

Associates: John A. Wohlhieter, Ph.D.
Mr. John M. Stiteler

roblems and Objectives:

The problem under study is the development of vaccines against human parasites that cause leishmaniasis, trypanosomiasis, or malaria. These diseases impede the military performance of troops deployed in endemic areas, necessitating effective vaccination procedures. Parasite identification is crucial to vaccine production and the current goal is to develop Leishmania identification methods based on kinetoplast DNA (kDNA) analysis. In addition to objectives detailed in the last annual report, new objectives are to determine if: (1) kinetoplast DNA of Leishmania isolated from patients remains stable despite the duration of the patient's disease, (2) chemotherapy of Leishmania alters kinetoplast DNA stability, (3) kDNA from visceralizing or cutaneous Leishmania from the Old World can be used as DNA hybridization probes for diagnosing infections, (4) kDNA hybridization can detect Leishmania parasites in sandflies, (5) sequences of Leishmania kDNA can be cloned into plasmids of E. coli to prduce species or disease-specific hybridization probes, (6) non-radioactive procedures can be used to detect specific kDNA hybridizations, (7) in support of the Leishmania hybridization work, a rapid test for parasite viability can be developed.

Progress:

Analysis of Leishmania by gel electrophoretic separation of restriction enzyme generated kDNA fragments was used to determine that the same parasite is responsible for visceral disease in at least 6 humans in northeast Brazil. Another visceralizing Leishmania is causing disease in at least 8 Kenyans. We determined that kDNA fragments analysis is reliable since results do not change using kDNA from parasites maintained for over 1 year in

culture, or from Leishmania amastigotes sequentially isolated from a patient with chronic cutaneous disease, or from Leishmania sequentially taken from a patient after multiple chemotherapy failures. Through kDNA hybridization procedures we have detected as few as 100 Leishmania using promastigotes from culture or within sandflies or amastigotes from tissue blots. Hybridization of kDNA is therefore one of the most sensitive ways to detect Leishmania infections in man, animals or insects. We have determined that cross-hybridization of kDNA sequences occurs between Leishmania species causing cutaneous (L. tropica, L. major) and visceral disease (L. donovani, L. infantum, L. chagasi). Through a collaboration with a molecular genetics company, Codon, fragments of kDNA, specific for these species, are being cloned into E. coli plasmids. These plasmids will serve as species specific hybridization probes for research on leishmaniasis and the detection of disease. Non-radioactive methods for detecting kDNA hybridization using biotin-labelled kDNA probes and strepavidin linked with a chromogenic enzyme or fluorochrome, are being studied. These procedures will lead to a field test for detecting Leishmania in man animals and vectors. To support the kDNA hybridization program, which requires the use of healthy parasites, we have developed a rapid (30 second) and sensitive method for detecting viable intracellular and extracellular Leishmania, using the fluorogenic esterase substrate, fluorescein diacetate.

Recommendations:

1. Restriction endonuclease digestion of kDNA should continue so that additional populations of Leishmania can be detected. New restriction enzymes should be evaluated in this procedure. To simplify the procedure, flat-bed, agarose gels should be used.

2. The cloning of kDNA sequences specific for Leishmania causing New World and Old World cutaneous and visceral diseases should be continued. Hybridization of cloned material should be performed to determine the specificity of the DNA probes and their sensitivity in detecting parasites in tissue preparations from man or animals, or within sandflies.

3. Non-radioactive procedures for detecting DNA hybridization should be aggressively pursued. The sensitivity of the procedure should be compared with radioactive methods. Enzymatic and fluorescence detection methods should be evaluated with the aim of producing a field test for Leishmania parasites in man, animals and vectors.

4. Alternatives to nitrocellulose for use in hybridization of kDNA probes should be evaluated. These may be paper or nylon material supports.

Formal Presentations:

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'G: AND TECHNOLOGY WORK UNIT SUMMARY | DA OG 6759 | 83 09 30 | DD-DR&E(AR)636

	M. Termination	U	U		CX		

PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
62770A	3M162770A871	AH	047 WR
62770A	3M162770A871	AH	

S100 82/83 6.2/3

ntion and Treatment of Military Important Diseases in the Tropics

inical Medicine 0613 Microbiology

	CONT	DA	C. In-House

	FISCAL YEAR	RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	CUMULATIVE OBLIGATIONS
	PREVIOUS 83	83	6.0	592
	CURRENT 84	84	0.0	0

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TAYLOR,D N.

POC: DA

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nimals (U) Monkeys (U) Malaria; (U) Dengue; (U) Dengue Hemorrhagic Fever; robe; (U) Flavivirus (U) RAM I (U) Mice (U) Dogs

he technical objective is to develop new approaches to the prevention and treat ropical diseases of military importance. Malaria and dengue are emphasized f severity and for propensity to cause high attack rates shortly after the on- litary operations.

pproaches include characterization of the cellular immune response in patients with malaria, epidemiologic/ecologic studies to determine vectors and hosts in develop control methods, and studies to determine the etiologic factors of den- rhagic fever, and the etiology of hepatitis.

2 10-83 09 Studies were initiated on arachadonic acid and its metabolites and sible role in the mediation of the vascular permeability in DHF. An outbreak of ver associated with signs and symptoms of clinical neuropathy was investigated. n risk factors associated with the transmission of flavivirus was done during ason in a rural area of Thailand. Studies on immune dysfunction in malaria were . Diagnostic studies of dengue including in vitro dengue antibody synthesis of l blood mononuclear leukocytes and the development of dengue type-specific sero ssays using mouse monoclonal antibodies were continued. Studies on the in vitro synthesis in leukocytes of the blood and CSF of patients with JE were also con- ther dengue studies were continued on the development of a primate model for ies on the use of the antibody capture technique for diagnosis and definition velopment of IgG and IgM in the CSF of dogs with rabies were continued. The DNA dies of toxigenic E. coli were continued and expanded. A study on the role of the transmission of enteric disease was completed. For technical report see the ed Army Institute of Research Annual Progress Report 1 Oct 82-30 Sep 83. d due to changes in AMS. See USAMRDC Supplement to AR 37-.00-84.

198 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS [illegible] AND [illegible] MAR 84 (FOR ARMY USE) ARE OBSOLETE.

Project Number:	3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS
Title:	Prevention and Treatment of Military Important Diseases in the Tropics
Work Unit Number:	047
Investigators:	COL Michael W. Benenson, MC; LTC Donald S. Burke, MC; LTC George S. Ward, VC; LTC Charles H. Hoke, MC; LTC Peter E. Echeverria, MC; MAJ Michael R. Elwell, VC; MAJ Horace K. Webster, MSC; MAJ George S. Childs, MSC; MAJ David N. Taylor, MC; MAJ Richard C. Andre, MSC; CPT Ronald M. Rosenberg, MSC; Katchrinnee Pavanand, M.D.; Ananda Misalak, M.D.; Rapin Snitbhan, M.D.; Markpol Tingpalapong, DVM, Chiraphun Duangmani, M.D.

1. Dengue hemorrhagic fever at Bangkok Children's Hospital, 1983

PROBLEM: To determine the incidence of dengue hemorrhagic fever (DHF) and the serotype of dengue virus causing DHF in patients at Bangkok Children's Hospital (BCH).

BACKGROUND: For 21 years this laboratory has maintained a collaboration with the clinicians at BCH. During this time the laboratory has developed its capability for isolation of dengue viruses, while the clinicians at BCH have refined their diagnostic and therapeutic skills. At the present time, serologic evidence for dengue infection is found in about 90% of the cases diagnosed as DHF by the BCH clinicians.

A total of 4064 patients have been diagnosed as having DHF (Table 1). About 20% of these have yielded a dengue virus isolate. Overall, dengue 2 was the most frequent isolate, and dengue 4 was a relatively rare isolate. Dengue 1 and 3 were isolated with intermediate frequency.

During the period of surveillance, annual cycles have occurred, with the highest mean number of cases occurring in August, in which an average number of 40 DHF cases were admitted. The largest single month was July, 1980, in which 131 admissions occurred. In that year, 788 cases occurred, from which 240 dengue isolates were obtained. Since then DHF activity has been less.

Current Data: In 1983, 252 cases have occurred (Table 1), marking the year as the third mild one in a row for DHF. As usual, August was the largest month, with 63 cases occurring. Twenty-four dengue isolates have been isolated thus far from the 68 specimens which have been processed thus far, for an isolation rate of 35%. Of these isolates, the largest number were dengue 2.

Table 1. Dengue isolates from DHF patients, 1962-October, 1983 (Excluding 1967-72).

YEAR	TOTAL CASES	DEN ISOL	D1	D2	D3	D4	% D1	% D2	% D3	% D4	MAJOR TYPE
1962	148	50	17	23	9	1	34	46	18	2	D 2
1963	156	35	8	17	17	0	23	29	49	0	D 3
1964	332	135	39	53	39	3	28	50	19	3	D 2
1965	88	12	0	8	3	1	0	67	25	8	D 2
1966	55	10	7	1	0	2	70	10	0	20	D 1
1973	135	22	5	13	4	0	23	59	18	0	D 2
1974	151	21	8	7	6	0	38	33	29	0	D 1
1975	399	14	1	8	5	0	7	57	36	0	D 2
1976	176	9	0	6	1	2	0	67	11	22	D 2
1977	495	66	0	37	10	19	0	56	15	29	D 2
1978	185	33	0	28	1	4	0	85	3	12	D 2
1979	301	61	2	58	0	1	3	95	0	2	D 2
1980	788	240	50	174	14	2	21	73	6	1	D 2
1981	196	36	11	21	3	1	31	58	8	3	D 2
1982	206	25	4	17	1	3	16	68	4	12	D 2

* 68 Specimens processed through October

CONCLUSIONS:

1. Dengue 2 continues to be isolated most frequently from DHF patients.

2. An increase in DHF activity in the next year or two is expected, as it is unusual to have 4 consecutive low years.

RECOMMENDATIONS:

1. This extremely valuable study of the etiology of DHF in Bangkok represents the most extensive longitudinal study of the etiology of DHF in existence.

2. This study should be continued, with modifications appropriate for the expected increase in DHF activity.

2. Epidemic Peripheral Neuropathy Associated with Dengue

PROBLEM: In January 1983 the epidemiolgy service at the refugee holding center at Panar Nikom, Thailand, reported an epidemic of peripheral neuropathy. During the preceding two months 25 patients had presented to the camp health service with signs of severe combined motor and sensory neuropathy. Many of the patients related a history that the illness had begun abruptly with a fever and rash followed in a few days by the rapid onset of neuropathic symptoms. Because dengue was considered among the possible causes of the epidemic, AFRIMS was consulted.

PROGRESS: Baseline rates of peripheral neuropathy at the camp were estimated to be 1 or 2 cases per month, presumably due to nutritional deficiency. Thirty cases were identified with a history of neuropathic symptoms within the past two months, 17 of whom also gave a history that the illness began with a fever. Males outnumbered females 22 to 8. Twenty-four cases were between the ages of 15 and 44. Twenty-five cases were examined by a neurologist in February and 17 of those examined had persistent objective evidence of neurological involvement. At that time six of the cases were admitted to Bangkok General Hospital for intensive evaluation. All had normal physical examinations and routine laboratory studies except for neuropathy. Nerve conduction studies showed impairment in five of the six cases studied. Sural nerve biopsies in these five cases were non-diagnostic, and no evidence of inflamation was found. CSF was normal in all six cases. Screening for toxic metals in blood and hair was negative. Serum specimens were obtained from 21 neuropathy cases, 91 healthy young adult household cohabitants of neuropathy cases, and 92 randomly selected young adult camp residents. Sera were tested for elevated dengue antibodies by HAI (titer >160) and IgG antibody capture immunoassay (absorbance >.90). Sera from 11 of 13 cases of neuropathy with fever had evidence of recent flavivirus infection, whereas only 1 of 8 cases of neuropathy without fever ($p=.002$) and 11 of 81 random controls ($p< 10^{-6}$) did. Household contacts of febrile cases had a higher prevalence of high flavivirus antibodies than did contacts of afebrile cases or random controls ($p<.001$ for both). Isolations were not attempted from case sera as no specimens were available from acute cases. Isolation attempts from all five sural nerve specimens were negative. Two viruses were isolated from Aedes aegypti collected in case houses; both were dengue type 1. The pathogenetic relationship between the dengue infections and neuropathy in these cases is not clear.

FUTURE OBJECTIVES:

(1) Dengue should be considered among the possible causes of acute peripheral neuropathy with fever.

3. In Vitro Dengue Antibody Synthesis by Patient Peripheral Blood Mononuclear Leukocytes

PROBLEM: There are no currently available methods for the rapid diagnosis of dengue virus infections. In our previous attempts to develop a suitable method, we have taken two conventional approaches: first, to detect virus specific antigens in blood or other clinical specimens, and second, to detect virus-specific early antibody (IgM) in serum. Neither of these approaches has proven fully satisfactory. Relatively low levels of virus and presumably virus-specific antigen circulate in the blood during acute dengue (as compared to some other virus diseases such as hepatitis B), and of what little there is, it is often blocked by the patient's own antibodies, especially in secondary infections. Our efforts at detection of virus-specific early antibody in patient sera were partially successful in that a rapid and specific solid phase antibody capture immunoassay was devised. However, the diagnostic sensitivity of the assay was entirely dependent upon the time after the onset of illness that the test serum sample was obtained. In most patients with primary dengue, IgM anti-dengue activity could not be detected in serum obtained at the time of admission, not until a few days later. In our preliminary studies with the antibody capture immunoassay, we noted that this type of assay is especially powerful in the detection of virus-specific antibodies in biological fluids with low total antibody content but in which the specific activity of that antibody is high. We reasoned that one such biological fluid with a low total Ig content but a high specific activity was recently synthesized antibody by leukocytes from acutely infected dengue patients.

PROGRESS: During the last half of 1982 and early in 1983, we obtained peripheral blood mononuclear leukocytes (PBML) from 108 patients hospitalized with a provisional clinical diagnosis of DHF at Childrens Hospital. Clinical, serological, and virological evalutation of the patients was according to standard AFRIMS-BCH protocol (see DHF-PUO 1982). Seven of these cases were subsequently proven to not be due to dengue, while 101 were dengue related. Eleven of the 101 showed a primary seroresponse pattern, and 90 a secondary seroresponse pattern. Of the secondary cases, 68 had diagnostically high levels of dengue HAI antibodies (> 1:1280 to at least one antigen) in the acute serum sample, while 22 had relatively low (non-diagnostic) levels of HAI antibodies in the acute serum. Ficol - hypaque purified PBML were washed extensively and the cells were dispensed into wells of a 96 well polystyrene "U" bottom plate in which specific wells had been previously sensitized with goat anti-mu or anti-gamma human immunoglobulin heavy chain specific anti-sera. One hundred microliters of cells were added to sensitized wells

at concentrations of 10^{-6}, 10^{-5}, and 10^{-4} cells per milliliter. No specific antigens or mitogens were added. Equal aliquots were cultured as intact cells without inhibitors, intact cells with cycloheximide (2.5 micrograms per milliliter), or as cells which were disrupted by one cycle of rapid freezing and thawing. Patient sera and control sera containing known dengue antibodies were tested on each plate. Plates were incubated overnight at 37 C. in 5% CO2, then washed. "Captured" IgG or IgM was detected by stepwise addition of dengue type two suckling mouse brain antigen, hyperimmune antiflavirus IgG conjugated to horse-radish peroxidsase, and substrate, as would normally be done in our standard antibody capture immunoassay procedure for serum. A positive result was defined as an absorbance value of >2 times the absorbance value obtained using the known negative control serum at a 1:100 dilution. Only in the last 66 cases studied was the activity of the disrupted PBML studied. Activity detected in the disrupted cell aliquot was taken to be due to preformed intracellular immunoglobulin. Results are summarized in the following tables.

PATIENT GROUP	# TESTED	M SYNTH	G SYNTH	M OR G SYNTH
NOT DENGUE	7	0	0	0
PRIMARY	11	7	1	7
SECONDARY (LOW)	22	3	17	17
SECONDARY (HIGH)	68	22	68	68

PATIENT GROUP	# TESTED	INTRACELL M	INTRACELL G	INTRACELL M or G
NOT DENGUE	4	0	0	0
PRIMARY	6	5	1	5
SECONDARY (LOW)	11	1	8	8
SECONDARY (HIGH)	45	7	45	45

These results demonstrate that during acute dengue infections there are cells circulating in the peripheral blood that are actively synthesizing dengue antibodies.

FUTURE OBJECTIVES:

1. This unique approach for the rapid diagnosis of acute dengue infections should be developed further. Attempts should be made to demonstrate specific antibody containing cells by staining of washed viable cells in suspension and staining intact but fixed cells. Ideally these intact and viable or fixed cells could be precisely quantitated through the use of a fluorescent activated cell sorter.

2. PBML obtained from the acute blood of DHF patients should be used as fusion partners for the development of human anti-dengue monoclonal antibodies.

3. The general applicability of this diagnostic approach for other infectious diseases should be evaluated.

4. Development of Dengue Type-Specific Serologic Assays Using Mouse Monoclonal Antibodies

PROBLEM: Existing serological methods for determining the infecting virus type in dengue virus infections are based on the neutralization of virus growth in cell cultures or in inoculated animals. These methods are time consuming, relatively expensive, and require expertly trained technical staff for their performance. We hypothesized that a new assay for sero-type specific antibodies in human sera could be developed using type-specific murine monoclonal antibodies. We envisioned testing human sera for their ability to block the attachment of a labelled mouse monoclonal antibody to its epitope on the homologous dengue virus surface glycoprotein (V3).

PROGRESS: One hundred and forty-two dengue antigen-reactive but otherwise largely uncharacterized monoclonal antibodies in the form of cell culture supernatant fluids (CCF's) were shipped from WRAIR to AFRIMS. CCF's were screened by antibody capture radioimmunoassay or enzyme-linked immunoassay (ACRIA or ACELISA) as follows: (1) the plastic solid phase was sensitized with goat anti-mouse IgG (2) CCF at a 1:10 dilution was added to saturate all binding sites on the solid phase (3) dengue antigen in the form of supernatant fluid of cultures of infected C6/36 cells was added (4) bound antigen was detected with iodine-125 or peroxidase labelled human convalescent DHF IgG. Binding of an antigen to the fixed CCF was quantitated as the "relative antigen binding activity"

(RABA). The monoclonal which is a flavivirus group one which reacted well with all four dengue serotypes as well as with [illegible] viruses, and which, by definition the RABA will not react with [illegible] viruses. An initial trial was performed comparing the binding spectrum of monoclonal CCF's by ACRIA at ARBRI to that obtained by IFA at WRAIR using a panel of 21 CCF's of various specificities. The concordance was excellent, except that one type specific monoclonal antibody (AD3-1583) as determined by IFA was unreactive by ACRIA. This monoclone has subsequently been shown to react with a non-structural protein (not with E). Based on an initial series of tests using the 1, 2, 3, 4, E, and uninfected control antigens, the 142 monoclonal CCF's were characterized as follows: flavivirus group, 27; dengue complex, 27; dengue subcomplex (more than one type but not all four), 13; type one, 14; type two, 9; type three, 4; type four, 1; non-reactive, 73. Next the bank of CCF's was screened against other flaviviruses known to be found in Thailand, Kesselsron, Langat, and Tembusu. All of the 27 CCF's previously designated as flavivirus group reactive also reacted with all three of these virus antigens. Surprisingly, some of the previously designated "complex reactive," "subcomplex reactive," and "type specific" CCF's also reacted with some of these antigens. For example, the " dengue complex reactive" monoclone AD3-187 was found to bind both Tembusu and Kesselsron, but not Langat.

Next, a series of blocking experiments was conducted to determine if those monoclones that shared a similar binding spectrum cross blocked one another. Based on the results of the binding experiments, mouse ascitic fluids (MAF's) were prepared at WRAIR for certain clones. In the first series of blocking experiments using labelled AD2-4G2, most flavivirus group reactive CCF's blocked, but some did not, suggesting the presence of at least two flavivirus group specific epitopes on V3. A surprise finding was that some of the CCF's caused <u>increased</u> binding of the labelled AD2-4G2 to antigen. Two monoclonal antibodies were found that were especially strong "promoters" of AD2-4G2 binding: the "dengue complex" reactive AD3-187 and the "dengue two specific" AD2-2F3. Five to 10 fold more labelled 4G2 is bound to Dengue 2 in the presence of either of these monoclones. The discovery of the phenomenon of "promotion" of binding of one monoclone by another greatly complicates the prospects for developing a type specific serologic assay based on the blocking of monoclonal antibody attachment to antigen by test human sera, for it suggests that the dengue V3 protein is not a fixed structure. Instead, binding of non-specific

antibodies at a remote site may alter the structure of a type specific epitope. Additional cross-blocking experiments are underway to develop a more complete epitopic map of the dengue V3 and to define more precisely the nature of the "promotion" phenomenon.

FUTURE OBJECTIVES:

(1). Development of type-specific serologic assays should continue as a high priority effort. Alternative approaches using monoclonal antibodies should be explored, such as the definition of those clones that react with "linear structure defined" epitopes and the preparation of oligopeptide antigens.

(2). Monoclonal antibodies should be raised against virus strains native to Thailand, in an effort to develop strain, rather than type specific assays, for use in epidemiological studies.

5. Arachadonic Acid Metabolites as Mediators of the Increased Vascular Permeability of Dengue Hemorrhagic Fever

PROBLEM: Dengue hemorrhagic fever is characterized by fever, thrombocytopenia, increased vascular permeability (as evidenced by an elevated hematocrit), and variable degrees of bleeding ranging from a positive tourniquet test to overt major gastrointestinal hemorrhage. In most patients the major life-threatening pathophysiological event is an abrupt increase in the vascular permeability at the time of the onset of the shock. No systematic study of the poential mediators of this increase in vascular permeability have been made. Since the macrophage and other phagocytic cells of the reticuloendothelial system are the main cells infected during DHF, and since at least some macrophage lysosomal enzymes such as acid phosphatase are released into the plasma during acute DHF, a reasonable hypothesis is that the vasoactive mediators of shock in DHF are synthesized and released by macrophages. Recently Austen and colleagues developed a radioimmunoassay for the detection and quantitation of leukotrienes. Leukotrienes, like prostaglandins, are metabolites of arachidonic acid, and they have been shown to be potent mediators of immediate type hypersensitivity reactions, especially vascular permeability. We therefore set out to measure plasma and leukocyte levels of leukotrienes and other vasoactive arachidonic acid metabolites in DHF.

PROGRESS: Ten milliliters of acid-citrate-dextrose anticoagulated blood were obtained on hospital day 1, 2, and day 14 from six patients with clinical diagnoses of DHF with shock at Childrens Hospital. Plasma was precipitated with cold ethanol and the supernatant immediately frozen. Peripheral blood mononuclear cells were obtained by Ficol-hypaque fractionation, washed, divided into four aliquots, and two aliquots each incubated for 2 minutes or 2 hours. The entire cultures were then snap frozen in liquid nitrogen. Plasma and cell cultures were transported to Boston for testing. To date all plasmas have been tested by RIA for leukotrienes C4 and B4, with negative results. RIAs of the leukocyte samples do not yet exist. Selected samples will be fractionated by high-pressure liquid chromatography to test for immuno-unreactive leukotrienes.

FUTURE OBJECTIVES:

1. A systematic and integrated search for possible mediators of the increased vascular permeability in DHF should be undertaken. All potential mediators should be sought and carefully measured, such as histamine, kinins, and complement factors, as well as arachidonic acid metabolites.

2. Clinical specimens obtained directly from patients should be inoculated directly into appropriate bioassay systems (such as the hamster cheek pouch) in an effort to detect circulating mediators.

6. Development of a Sub-human Primate Model for Dengue Hemorrhagic Fever

PROBLEM: Many of the fundamental problems concerning the pathogenesis of dengue hemorrhagic fever, such as the role of enhancing antibodies, the requirement for specific sequences of serotypes, the types of cells ingested in vivo, and the nature and source of the mediator(s) of increased vascular permeability, could be resolved if a reliable animal model of DHF were available. Attempts to develop such a model in the past have been totally unsuccessful. Our recent epidemiologic studies of DHF in Cuban infants under one year of age, taken together with supporting laboratory data, have suggested a critical role for antibody-dependent enhancement of virus growth in cells with Fc receptors. We therefore sought to develop such a model for infant DHF based on this current

epidemiological and laboratory information.

PROGRESS: Over a series of seven experiments, total of 44 primates were tested: 17 were control animals which received either no virus inoculation or no pre- treatment with dengue immune serum, while 27 received some combination of serum and virus that might be expected to produce enhanced virus growth in vivo. The virus strain Den 2/D80-616 was used throughout. This virus was selected because it had caused a fatal illness in a human infant and showed good "enhancability" in vitro. Five different antibody preparations were tested in the model: (1) Serum 2734/80, obtained from the mother of case D80-616 at the time the infant was admitted to the hospital, (2) serum 1799/81, obtained from the same woman approximately 18 months later, (3) serum 0653/82, obtained from the mother of another infant DHF case about two years after the death of the child, (4) mouse monoclonal antibody AD2-4g2, which reacts with a flavivirus group determinant on the surface glycoprotein (V3) of the dengue virion, and (5) Rhesus monkey monotypic Dengue 1 immune serum E-87. In all series the test primates were inoculated with a dose of serum which was calculated to give a dilution of antibody in the extracellular fluid space which would give maximum enhancement of growth of strain D80-616. Of the 27 test animals only one animal died: infant Rhesus monkey DA-9 died with profound thrombocytopenia, diffuse internal and external hemorrhages, and sustained viremia. At necropsy this animal was found to have a single large tuberculous mediastinal node, with absolutely no evidence of disseminated disease elsewhere. Other than diffuse hemorrhages, little patholgy was found. Dengue virus was isolated from liver, spleen, lymph nodes, and thymus. Another animal in the same trial (DA-4) also developed sustained thrombocytpenia but did not die. Both DA-9 and DA-4 had been inoculated with serum 1799/81. Attempts to reproduce this experiment using the same serum in tuberculin skin test positive animals were unsucessful. None of the other experimental animals in the other trials developed signs of DHF-like disease, although occasional low platelet counts were seen.

FUTURE OBJECTIVES:

(1) Attempts to produce a model of DHF should continue.

(2) Possible strategies include duplication of the human situation as closely as possible by immunizing female primates before pregnancy so that the infants are rendered passively immune through transplacental antibodies, or by

inoculating primates with carefully selected monoclonal antibodies with especially strong enhancing properties.

(3) In future models the state of activation of the reticuloendothelial system must be careful controlled.

7. In Vitro Antibody Synthesis by Leukocytes Obtained from the Blood and Cerebrospinal Fluid of Patients with Acute Japanese Encephalitis

PROBLEM: In the course of development of the antibody capture assay for the rapid diagnosis of Japanese encephalitis virus infections through the detection of virus specific antibodies in the cerebrospinal fluid, we noted that the activity of anti-JE IgM and IgG in CSF was greater than could be accounted for by passive diffusion alone. The assumption has previously been made that antibodies are synthesized in the human CNS during encephalitis, but this has never been directly proven. Because we have been able to detect antibody synthesis by peripheral blood mononuclear leukocytes (PBML) obtained from the blood of patients with acute infections, we set out to test the hypothesis that circulating mononuclear leukocytes actively synthesize JE antibodies in the peripheral blood and in the CNS during acute JE infections of humans.

PROGRESS: All patients admitted to the Kampangphet, Thailand Provincial Hospital during the 1982 JE season with a clinical suspicion of infection of the CNS were studied. PBML and CSF leukocytes were extensively washed and cultured for 72 hours without specific antigenic or mitogenic stimulation, and the culture supernatant fluids tested for JE IgM and IgG by antibody capture immunoassay. Sixteen cases were subsequently proven to be due to JE by conventional serologic techniques and 12 were proven to be not due to JE. None of the cultures of cells from the blood or CSF of control cases (not JE) showed any synthesis of JE antibodies. Admission PBML from JE cases showed IgM and IgG synthesis in 9 and 14 cases, respectively. CSF cells from 4 cases showed in vitro synthesis of IgG and one also showed synthesis of IgM. Cases in which JE antibody synthesis by CSF leukocytes could be demonstrated tended to be older males with more mental status impairment, higher CSF leukocyte counts, and greater and broader serum seroresponse patterns than did JE cases in which synthesis by CSF leukocytes could not be demonstrated.

FUTURE OBJECTIVES:

(1) Techniques should be developed for the histochemical and/or immunofluorescent identification and quantitation of JE specific antibody producing cells in brain and CSF.

(2) Circulating PBIL and CSF leukocytes from acutely ill JE patients may be suitable "fusion partners" for the development of JE specific human monoclonal antibodies.

8. Isolations of Japanese Encephalitis Virus from Kampangphet, Thailand, 1982

PROBLEM: To isolate strains of Japanese encephalitis virus from a number of human, pig, and mosquito sources in an epidemic area for comparisons of molecular characteristics.

PROGRESS: During the rainy season of 1982, a prospective surveillance of encephalitis in a province about 200 kilometers north of Bangkok was established. All cases of encephalitis seen at the hospital were examined and tested using the MAC ELISA for evidence of IgM anti-JE virus antibody in the CSF. Homes of selected confirmed cases were visited, and mosquito traps put out. JE seronegative sentinal pigs were placed in the vicinity of several of these homes. Mosquito collections and pig bleedings were performed every third night, on the average, during the period of greatest transmission of JE virus. Pig sera were tested for the appearance of JE antibody and virus sought in specimens collected 4 to 7 days before the seroconversion, since pigs would have been most likely to be viremic at that time. Mosquito collections were sorted and speciated. Pools of 200 mosquitoes were analyzed by using an ELISA for the presence of JE antigen and cultured by inoculation of C6/36 aedes albopictus cells. Initially, ELISA and culture results were compared, but because of the large number of pools, ELISA was subsequently relied upon for screening.

RESULTS: Five hundred and six mosquito collections were performed. One hundred and eleven of these, containing 1,759 pools, (16 pools per trap) with approximately 350,000 mosquitoes have been sorted. Virus isolation from 70 of the 111 counted traps has been completed. 54 Viruses have been isolated (Table 1), almost exclusively from dry ice baited light traps.

Table 1. JE Virus Isolations, Kampangphet, 1982. (As of 20 Oct 83)

Number	Virus	Method of Confirmation	Source
22	JEV	Neutralization	Mosquito
5	JEV	IFA (neut pending)	Mosquito
13	Tembusu	Neutralization	Mosquito
6	Flavivirus	IFA (neut pending)	Mosquito
7	Other	6 sent to YARU for identification	Mosquito
4	JEV	IFA	Pig serum
1	JEV	IFA	Human Brain

Of 26 JE virus isolates analyzed thus far, 19 were identified by ELISA antigen detection on the mosquito pool (Sensitivity = 73%), while 7 strains came from ELISA negative pools. The mean ELISA OD for ELISA positive, culture positive pools (defined as: OD > 2 x (Control negative mosquitoes)) was 0.137, as compared to a mean of 0.025 for control pools. The mean of culture positive, ELISA negative pools was 0.05, as compared with a mean of 0.04 for the control negatives, confirming that the ELISA method does not distinguish all positive pools.

Of 21 sentinal pigs bled serially, HAI rises indicated infection in 10. JE virus was isolated from 4 of these. One isolate was obtained from a human brain.

WORK REMAINING: Many ELISA positive pools remain to culture on tissue culture. Three of the JE strains have been sent to USAMRIID for fingerprinting. Mosquito sorting should be completed within 2 months, so ecologic variables can be analyzed.

CONCLUSION:

1. Mosquito and pig collections in epidemic areas are productive sources of JE virus.

2. The ELISA for screening pools of mosquitoes is somewhat insensitive, but considerably less time consuming than the culture method. Its use is appropriate where large numbers of specimens require screening.

3. Large amounts of JE virus circulate among the vectors and amplifier hosts in the northern part of Thailand during epidemics of encephalitis.

FUTURE OBJECTIVES: Analysis of the mosquito and pig collections has proved fruitful in that all goals for

isolation of virus strains were met. However, the unanticiapted large number of mosquitoes has resulted in an extremely large work load for the laboratory. Analysis of a sample of the pools may be necessary. After analysis of the dara, this study will be complete.

9. Development of IgM Anti-Rabies Antibody In the CSF of Humans with Rabies

PROBLEM: To test the hypothesis that formation of antiviral IgM antibody can be detected in the CSF of patients with rabies by applying principals learned in the study of Japanese encephalitis.

BACKGROUND: This laboratory has reported the development of an IgM capture method of detecting virus specific antibody in the CSF of patients with encephalitis due to Japanese encephalitis virus (Burke, DS., et al. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J Clin Microbiol, pp 1034-1042, (1982).). A similar assay, using anti human mu chain, test CSF or serum, inactivated rabies virus in the form of Merieux rabies vaccine, and I125 labeled rabbit anti rabies antibody was developed. We previously reported that this test allowed detection of IgM antibody in the serum of many individuals receiving pre-exposure rabies prophylaxis. More recently, we have evaluated the test in several patients with confirmed rabies encephalitis.

PROGRESS: To date, three patients with rabies encephalitis have been tested. Serial CSF and Serum specimens were collected and tested in the rabies MAC RIA and neutralization assays.

The first patients developed no detectable antibody of any type in their CSF prior to death. However, the third patient demonstrated a dramatic rise in the CSF and serum IgM just prior to death. The specimens which contained the IgM antibody were the only ones in the three patients in which specific neutralizing antibody was detected (Table 1).

Table 1. Anti-rabies antibody in serial specimens of a patient with confirmed rabies.

Date	Days to Death	CSF p/n	Serum p/n	Rabies neut	IgM peak
3/12	12	.9	1.4	-	
3/13	11	1.2	1.1	-	
3/15	9	.9	1.0	-	-
3/17	7	1.0	1.4	-	
3/19	5	.9	1.5	-	
3/20	4	1.4	1.5	-	
3/21	3	1.0	1.6	-	
3/22	2	1.3	2.1	+	
3/23	1	1.9	2.5	+	+
3/24	0	1.9	3.1	+	

Sucrose density gradient fractionation of the serum and CSF samples from this patient demonstrated the appearance of a 19 s peak in the antibody in the late serum and CSF specimens, confirming the presence of IgM antibody in these specimens.

In contrast to the regular appearance of virus specific anti JE antibody in Japanese encephalitis, the occurrance of IgM anti-rabies antibody in the serum and CSF of patients with rabies was seen in only one of three patients studied. The test results were slightly more positive in the serum specimens, suggesting the possibility that diffusion of antibody could account for the CSF positivity. The rise in the counts in this test near the time of the patient's death suggests that the antibody was not effective in halting the progress of the disease.

CONCLUSIONS: IgM antibody may appear in the CSF of rabies patients, but does not seem to do so in all cases.

FUTURE OBJECTIVES: This test provides an assay that may facilitate an understanding of the pathogenesis and treatment of rabies. However, the test gives low levels of positive results even when it is positive. Although it has limited promise as a diagnostic test, further evaluations of clinical specimens should be done.

10. Detection of Rabies Immunoglobulin in Serum and Cerebrospinal Fluid of Quarantined Dogs by IgM Antibody Capture RIA

PROBLEM: Rabies is an important zoonotic disease in Thailand. Presumptive diagnosis may be made on clinical signs but confirmation is not made until the dog dies or is

euthanized and specific fluorescent antibody staining of brain tissue for rabies virus antigen is completed. An antemortum diagnostic technique would be valuable to permit initiation of prophylactic therapy of the patient without euthanizing the dog or waiting until death occurs.

OBJECTIVES: To determine if rabies infection can be diagnosed in the living animal by detecting rabies specific IgM in the serum or cerebrospinal fluid.

PROGRESS: Serum & CSF samples were obtained from thirty-seven dogs that were held in rabies quarantine at the Thai Red Cross Society. Twenty-four of these dogs developed typical clinical signs of rabies and died from one to three days after entering quarantine. Mouse inoculation of hippocampus from the brains of these dogs resulted in death in 21 of 24 of the injected litters and brain smears of these mice were all positive by FA for rabies. Six of the rabid dogs had rabies virus in their CSF, which was demonstrated by mouse inoculation.

Thirteen of the quarantined dogs did not have rabies and all but one survived. This dog died of a canine distemper-like illness but the specific cause of death was not determined. An immunoglobulin M-antibody capture radioimmunoassay (MACRIA) was performed on each serum and CSF sample. Significantly higher counts per minute (cpm) were found in the CSF & serum of the 21 dogs with proven rabies when they were compared with the 13 non rabid dogs. The three dogs that died following a clinical rabies illness had negative mouse inoculation tests but significantly higher CSF and serum MACRIA counts than the non-rabid dogs. The ratio of the CSF: serum cpm in known rabid and clinically rabid dogs was 1.19-1.20 while this ratio in the nonrabid dogs was 0.71. When sucrose density gradient separation of the rabid dog serum was tested the major count peak was in the early fractions where IgM would be expected to appear.

FUTURE OBJECTIVES:

1. Test sequential samples of experimentally infected dogs to determine how early after infection rabies IgM can be detected.

2. Determine effects of vaccination with live virus on serum and CSF IgM.

11. Identification of Enterotoxigenic Escherichia coli in Patients with Diarrhea in Asia with Three Enterotoxin Gene Probes

OBJECTIVE: To apply the DNA hybridization assay in identifying ETEC in Asia.

PROGRESS: Nine hundred and eighty-four enterotoxigenic Escherichia coli (ETEC) and 733 non-ETEC isolated from patients with diarrhea in Asia (one isolate/patient) were examined for homology with radiolabelled fragments of DNA encoding heat-labile toxin (LT), or heat-stable toxin of porcine origin (ST-P) or of human origin (ST-H). Two hundred and forty-six ETEC that produced LT and ST as determined by the Y-1 adrenal and suckling mouse assays were homologous with the LT probe. Of these 246 LTST ETEC 156 (63%) were homologous with the ST-H, 46 (19%) with the ST-P, and 44 (18%) with both probes. Four hundred and one LT ETEC were homologous with the LT probe. Of 337 ST ETEC identified by the suckling mouse assay, 244 (72%) were homologous with the ST-H, 84 (25%) with the ST-P, and nine (3%) with both probes. None of the 733 isolates that were non-enterotoxigenic as determined by the Y-1 adrenal and suckling mouse assays were homologous with genes coding for enterotoxin.

Four isolates (not included among the 984 ETEC examined) that were initially considered to produce LT because sterile culture supernatants produced rounding of Y-1 adrenal cells were not homologous with the LT probe. Sterile culture supernatants of these four isolates caused rounding after eight hours and subsequent destruction after 24 hours of Y-1 adrenal tissue cultures. This effect was not inhibited by convalescent human cholera antiserum, Swiss Serum Institute cholera antitoxin, or antiserum to purified LT. These isolates were also negative in the Biken test previously used to identify LT producing E. coli . The DNA hybridization technique with three enterotoxin gene probes is a specific technique to identify ETEC in a large number of specimens in Asia.

FUTURE OBJECTIVE: The DNA hybridization assay will be used to examine E. coli for enterotoxigenicity from Africa, the Near East, the Philippines, Indonesia and Peru. Improvements in the test to examine specimens directly without testing individual E. coli isolates is planned.

12. Absence of Nucleotide Sequence Homology Between Genes for Vibrio cholerae Toxin and Vibrio fluvalis

PROBLEM: Vibrio fluvalis have been isolated from patients with diarrhea and from a variety of environmental sources in various parts of Asia. The enteropathogenicity of this organism has not, however, been clearly defined.

PROGRESS: V. fluvalis from different sources in Asia were examined in the DNA hybridization assay (1) to determine whether these organisms shared DNA sequence homology with cloned genes for V. cholerae toxin (2).

Ninety-two isolates of V. fluvalis isolated in Asia (Legend) were examined. None hybridized with radiolabelled genes coding for cholera toxin under stringent or more relaxed hybridization conditions (50% formamide at 65 C, or 25% formamide at 54.5 C) (3). The enteropathogenicity of this organism does not appear to be due to an enterotoxin whose structural genes show nucleotide sequence homology with V. cholerae toxin.

FUTURE OBJECTIVES: Study completed

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Legend: Source of 92 Vibrio fluvalis examined for DNA homology with cloned Vibrio cholerae toxin genes

Source of Isolates	Country of Origin	No. of Isolates
Patients with diarrhea	Japan+	17+
	Thailand	14
	Bangladesh	6
	Singapore	3
	India	2
Patients without diarrhea	Japan	6
	Thailand	4
Water	Thailand	12
Pigs	Thailand	7
Food	Thailand	6
Flies	Thailand	5
Fish	Thailand	3
Shellfish	Thailand	3
	Japan	1
Cows	Thailand	3
Total		92

+ Isolates from travelers from Southeast Asia on arrival at Osaka International airport. Isolates from Thailand were isolated during longitudinal studies of enteric disease in Soongnern, Thailand.

13. Flies as a Source of Enteric Pathogens in a Rural Village in Thailand

OBJECTIVE: To determine if flies are important disseminators of enteric pathogens in a rural village in Thailand.

PROGRESS: The village of Ban Pong in northeastern Thailand was studied from January through December 1981 to determine the importance of flies as sources of enteric pathogens. The number of flies, that were predominantly Musca domestica, increased in kitchens and animal pens in the hot dry season at a time of the year when the incidence of diarrhea was highest in the village. Enterotoxigenic Escherichia coli (ETEC), Shigella, non-01 Vibrio cholerae and Vibrio fluvalis were isolated from 40 percent of fly pools from yards, 38

percent from animal pens, 35 percent from bathrooms, and 8 percent from kitchens. ETEC were isolated from one fly pool in May and another in June when the incidence of ETEC infections was highest in the village. Flies often carry and presumably disseminate enteric pathogens in rural Thailand.

FUTURE OBJECTIVE: Study completed.

14. Immune Dysfunction in Malaria: A Biochemical Approach

PROBLEM: Hereditary deficiency of the purine enzyme, adenosine deaminase is associated with severe combined immunodeficiency disease (SCID) - a condition in which both T- and B-lymphocyte function is impaired. Partial restoration of lymphocyte function can be achieved in SCID patients by enzyme replacement therapy involving whole blood or packed RBC transfusion. It thus appears that the ADA in normal RBC is sufficient to correct in part the purinogenic defect in ADA deficiency lymphocytes. The precise mechanism for this effect is not understood although it may involve the role of the red cell mass in systemic adenosine metabolism in a way that influences purine metabolism in lymphocytes. Acute human malaria infection is characterized by immune suppression. In particular there is a decreased functional responsiveness of mononuclear cells - especially T-lymphocytes. It is also known that the intraerythrocytic malaria parasite produces major changes in the purine metabolism of the host red cell mass.

PROGRESS: Adenosine deaminase (ADA) was shown to be greatly increased in acute P. falciparum malaria. The parasite ADA enzyme was isolated and characterized. A high level of ADA activity in the peripheral blood would be predicted to perturb purine homeostasis with consequences for host lymphocytes. A defect in cyclic nucleotide (cAMP) metabolism has been identified in mononuclear cells from patients with acute malaria. The defect involves reduced levels of endogenous cAMP and decreased adenylate cyclase activity. An adenosine receptor has been shown to be associated with adenylate cyclase on the surface of lymphocytes. When adenosine combines with the surface receptor adenylate cyclase is activiated and cAMP is produced. In malaria there appears to be a defect in the coupling mechanism such that cAMP production is depressed. Work is currently underway to investigate the mechanism for the cAMP defect. Additionally work is being done to test whether the immunopotentiator,

isoprinosine, can reverse the cAMP defect. Studies involving monoclonal antibodies are also underway to identify which specific lymphocyte subsets are associated with the biochemical defect.

RECOMMENDATION: These studies suggest what may be a biochemical correlate of immune dysfunction in human malaria infection. The perturbation in erythrocyte adenosine metabolism caused by the malaria parasite may produce a defect in lymphocyte purine metabolism which renders this cell functionally defective. Work is currently underway to confirm these preliminary observations in a larger population study and to elucidate specific lymphocyte subsets. Specific emphasis is being given to studies on malaria lymphocyte adenosine metabolism, adenosine receptors and cyclic nucleotides.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OB 6526	2. DATE OF SUMMARY: 83 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 82 10 01	4. KIND OF SUMMARY: D.Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. MRN INSTR'N: CX	8a. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT

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11. TITLE: (U) Field Studies of Rickettsioses and Other Tropical Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 0613 Microbiology 0603 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 07	CONT	DA	C. In-House

17. CONTRACT/GRANT
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c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
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21. GENERAL USE
H Foreign Intelligence Considered

22. KEY WORDS: (U) Scrub typhus; (U) Rickettsia tsutsugamushi; (U) volunteers; (U) Leptotrombidium; (U) Mites; (U) Chiggers; (U) Malaysia

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS:

23.(U) This work unit has as its ultimate goal the elimination of scrub typhus, a disease endemic in the Asiatic-Pacific region, as a military medical problem. Specific problems relating to the epidemiology, pathogenesis, diagnosis, treatment, and prevention of this disease are being studied.

24.(U) 1. Develop and evaluate reliable assays that measure and predict immunity to scrub typhus. 2. Test in human volunteer field studies the efficacy and operational practicality of doxycycline for the prevention of scrub typhus. 3. Conduct in the field sero-epidemiological and entomological studies to determine the incidence, etiology and threat of febrile illnesses of military medical importance occurring in Peninsular and Eastern (Sabah and Sarawak) Malaysia. 4. Determine the geographic distribution and characterize isolates of R. tsutsugamushi, the etiologic agent of scrub typhus.

25.(U) 82 10 - 83 09 1. The USAMRU-M was redirected to conduct field studies which seek applied solutions to operational military medical problems. 2. Studies were initiated to determine the etiology and distribution of febrile illnesses, particularly those of rickettsial etiology and of military importance, in central Peninsular Malaysia and Sabah. 3. The efficacy of doxycycline as a prophylaxis for scrub typhus was evaluated in a company of soldiers participating in a military field exercise. 4. The onset and longevity of the human immune response, both humoral and cellular, following infection with R. tsutsugamushi was determined and correlated with the protection and absence/presence of clinical scrub typhus. 5. Cultured human endothelial cells were described as an in vitro model for the study of R. tsutsugamushi induced cytopathology. 6. Five manuscripts were published, five are in press, and five were submitted for consideratic For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

A self-Mo is contingent upon authorization approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

Work Unit 048 Field Studies of Rickettsioses and Other Tropical Diseases

Investigators:
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Associates: Dr. Ng Kee Peng, Ph.D.
Miss Elsie Gan, B.S.

CMI RESPONSE OF MICE TO INFECTION WITH *RICKETTSIA TSUTSUGAMUSHI*

Problem: To develop and evaluate a reliable assay for the measurement of cellular immune responses to *R. tsutsugamushi* infection. Cell mediated immunity (CMI) has been demonstrated to be a major factor in acquired resistance to *R. tsutsugamushi* infection in mice. The need for meaningful CMI assays for both the mouse and the monkey, as well as the transfer and application of this technology to human scrub typhus studies, for which there are no published data, requires the development of lymphocyte transformation (LT) and macrophage inhibition factor (MIF) assays. These assays could be used to measure the onset and longevity of cellular immune responses to infection with *R. tsutsugamushi*. In addition, the relevance of each assay in predicting immunity to reinfection with homologous or heterologous strains of *R. tsutsugamushi* could be determined.

Progress: The membrane and soluble factions of French pressure cell treated, tissue culture grown strains Karp and Gilliam of *R. tsutsugamushi* were prepared and used in this study. Both Karp and Gilliam infected Balb/c and C3H/He mice were solidly immune to back-challenge with Karp throughout a 1 year study period. Transfer of spleen cells from Karp and Gilliam infected mice to their respective syngeneic normal recipients resulted in protection against a subsequent potentially lethal Karp challenge of the recipients. These CMI transfer experiments proved that spleen cells from infected mice were able to transfer protection against a lethal Karp challenge in naive recipients for at least one year.

Mitogenic responses to PHA were suppressed in Karp infected Balb/c and C3H/He mice from day 11-14 to day 28. Positive LT responses to homologous and/or heterologous antigens were detected in 80% of the Karp infected mice on day 7 and then were suppressed until day 28. At 365 days post-infection, positive lymphocyte responses were still detected in all groups of mice. Between 7 to 44 days post-infection, supernatants derived from spleen cell cultures from Karp and Gilliam infected mice demonstrated an ability to inhibit in vitro macrophage migration. Furthermore, at the time of peak infection (day 14-21) supernatants from cells incubated without antigen were also found to have MIF activity. This activity however, was not enhanced when antigen was added to the cell cultures.

Delayed-type hypersensitivity (DTH) was detected at 7 days post-infection (PI) in 40% of Balb/c mice infected with either Karp or Gilliam. However, DTH was not detected in Karp-infected C3H/He mice until 21 days PI. By day 60 PI, less than 40% of all mice tested have positive DTH response. The MIF assay is clearly superior to the footpad swelling test for the detection of DTH.

Recommendation: The mouse study has been completed and a manuscript is in preparation, however, the phenomenon of transient suppression during acute infection warrants further study.

CMI RESPONSE OF MONKEYS TO INFECTION WITH R. TSUTSUGAMUSHI

Problem: To define and study the CMI responses of cynomolgus monkeys following primary infection and subsequent challenge with R. tsutsugamushi.

Progress: The onset of CMI responses in cynomolgus monkeys (10/group) having their first exposure to R. tsutsugamushi were determined and correlated with the clinical responses after reinfection. Lymphocyte transformation was detected as early as 3 days PI in 50% of the monkeys previously infected with Gilliam. None of the monkeys previously infected with Karp exhibited positive LT response at day 3 PI. A transient period of suppression of lymphocyte responsiveness to rickettsial antigens and PHA mitogen was seen in all monkeys. This period of suppression occurs between day 7 and 21 PI. Clinical signs of disease in these monkeys were mild.

The monkey study is completed and a paper is currently being prepared for publication. The study indicated an earlier response to heterologous antigen than homologous antigen in the LT assay. The transient suppression also occurred 1 week earlier than those observed in the mouse study.

Recommendations: The LT responses of infected monkeys were in general inconclusive. The poor response may be attributable to the use of comparatively crude antigens in our CMI assays. The preparation of purified antigens should be pursued. Additional studies on the cellular immune responses of R. tsutsugamushi infected monkeys should be conducted if this animal is to be used as a model for the study of scrub typhus.

CMI RESPONSE OF VOLUNTEERS TO PRIMARY INFECTION WITH R. TSUTSUGAMUSHI

Problem: To study the onset and longevity of CMI in humans previously exposed to R. tsutsugamushi, while taking doxycycline prophylaxis, and subsequently challenged 21 months later via R. tsutsugamushi infected chiggers.

Progress: Rickettsial antigen concentrations of 100 and 400 µg/ml were optimal for routine use in human LT assays. The use of antigen concentration as great as 400 µg was necessary to obtain a response in some subjects with previous infection(s). Lymphocytes from normal volunteers did not respond to rickettsial antigens.

Peripheral blood lymphocytes were isolated every six days from the eight volunteers, participating in a laboratory based challenge study, who had taken doxycycline prophylaxis during their initial R. tsutsugamushi infection 21 months earlier. Two of the eight volunteers did not have positive LT responses to rickettsial antigens prior to challenge via R. tsutsugamushi infected chiggers. One volunteer was treated for scrub typhus whereas the other did not completely fulfill the criteria established for a diagnosis of scrub typhus, was not treated, and subsequently recovered after experiencing a week of low grade fever (≥38°C). Suppression of lymphocyte responsiveness to rickettsial antigens and PHA was observed in all volunteers between 6 and 18 days after challenge. Peak LT responses were seen at day 60. The presence of MIF production was pronounced in all lymphocyte cultures from volunteers between 6 to 60 days post-infection. However, the peak time of MIF activity varied from donor to donor.

Recommendations: Since this study strongly suggests that the LT assay can be used for predicting the susceptibility of humans to scrub typhus infection and as a diagnostic research tool for early detection of infection, additional work is encouraged such that: Future studies include larger sample sizes in order to properly assess individual variation in healthy and infected subjects. The correlation of the LT assay as an index of cellular immunity relative to indexes of humoral immunity is examined within individuals and also evaluated relative to population averages thus permitting a basis for rational comparison. Purified R. tsutsugamushi strain specific antigens and culture conditions are characterized and optimized for the LT assay in order that the strain specificity of the assay can be properly evaluated. The LT assay is incorporated in the list of immune parameters measured in future scrub typhus human trials especially clinical field trials involving doxycycline prophylaxis.

HUMORAL RESPONSE OF VOLUNTEERS TO PRIMARY INFECTION WITH R. TSUTSUGAMUSHI

Problem: The longevity of human R. tsutsugamushi specific antibody, as measured by the IFA test, has been contrastingly reported to be 12 years and one year. Thus, the clinical significance of a R. tsutsugamushi specific antibody titer for a single serum collected from a febrile patient living within a scrub typhus endemic area is difficult to interpret. The Weil-Felix OXK test for R. tsutsugamushi induced agglutinins has been reported to be negative in up to 50% of scrub typhus cases; is frequently negative in secondary infections; and may be falsely positive in cases of leptospirosis or louse-borne relapsing fever.

Background: Clinical details of the study have been previously reported. Briefly, two groups of 10 volunteers were deliberately infected under laboratory conditions by allowing Leptotrombidium fletcheri chiggers known to be infected with multiple strains of R. tsutsugamushi to feed on each volunteer. One group of volunteers received once weekly a 200 mg dose of doxycycline and the other received placebo, both groups starting two days before infection and continuing for six weeks thereafter. Nine placebo volunteers and one doxycycline volunteer developed scrub typhus and were treated on the third day of their illness.

Progress: R. tsutsugamushi specific antibody (IFA) persisted for more than 15 months in 18 of 19 volunteers assessed throughout the study. Fifteen months after exposure IFA (IgG) titers were 50-200

and there had been little alteration for nearly a year. Equal peak antibody titer to more than one strain of R. tsutsugamushi developed in all placebo volunteers. Our volunteers appeared to have been infected with as many as six rickettsial strains. This multiple response was not unanticipated in that other studies have shown that individual chiggers in our L. fletcheri colony are infected with and transmit multiple strains of R. tsutsugamushi. Eight of the nine placebo volunteers that developed scrub typhus each had four-fold rises (IFA) from baseline antibody titers by the eighth day of disease (10-16 days post-infection). In all but one volunteer a significant specific IgM response did not precede an IgG response. Weil-Felix OXK agglutinins were detected only in those volunteers not having R. tsutsugamushi specific antibody at the time of infection. The OXK agglutinins consistently appeared later than the specific antibody detected by IFA.

A significant OXK response was most often detected in placebo volunteers (treated on the third day of disease) by the fourteenth day after onset of disease. The longevity of OXK agglutinins was variable. Fifteen months after infection, four of the placebo volunteers that had developed a significant OXK response still had a serum titer of 2200, and in two of these volunteers, the titer remained at 800. The decline in titer to below 200 in the remaining four volunteers still under observation occurred three to ten months after infection.

Only one doxycycline-prophylaxis volunteer developed a significant OXK titer, which was delayed. The volunteers that remained well while receiving doxycycline prophylaxis developed significant IFA titers in the fourth week after exposure to infection. These titers ranged from 50 to 200 and were declining in most subjects by the end of the prophylaxis period. After prophylaxis was stopped, eight of these nine volunteers developed symptoms and/or signs that could be attributed to the multiplication of rickettsiae. Three of these eight volunteers experienced a subsequent second rise in R. tsutsugamushi specific IgG titer.

Recommendation: Eight of the placebo volunteers have been challenged with R. tsutsugamushi 21 months after the primary infection. The clinical, humoral and CMI responses of these volunteers are being measured and should be analyzed and compared with those induced by the primary infection.

OPERATIONAL EVALUATION OF THE EFFICACY OF DOXYCYCLINE PROPHYLAXIS FOR SCRUB TYPHUS

Problem: To establish in a military operational setting the efficacy and practicality of once weekly oral doxycycline for the prevention of scrub typhus. Recently, in a laboratory based study described earlier in this report oral once weekly doxycycline was shown to prevent the development of clinical scrub typhus in the majority of volunteers deliberately infected with R. tsutsugamushi (9). The drug has been once trialed in the field (2), but interpretation of the results were hampered by the low incidence of scrub typhus among the control group and poor compliance of volunteers with the regimen, in which control measures to assure compliance were found insufficient.

Progress: A company size group of British Infantry conducted an 8 week joint training exercise in the central mountainous area of Peninsular Malaysia. Eighty three volunteers from the group participated in a double-blind placebo controlled field study, of these, 64 spent most of the period in primary or secondary jungle, while the remaining 19 supported the operation from 2 base camps. Whole blood was collected from each volunteer before, at the end of, and at one and four weeks after the risk period in the field, as well as from volunteers upon their presentation with a febrile illness, and two weeks thereafter. Samples were subjected to serological analysis for rickettsiae (OXK, OX19, and IFA tests), typhoid fever, leptospirosis, melioidosis, and flavivirus (yet to be completed); whole blood for rickettsial isolation and culture of salmonella and leptospira; and blood films were examined for malaria. All clinical signs and symptoms were monitored and recorded. All medication, times of ingestion of the trial drugs, the side effects experienced and movements in the field were recorded by each volunteer in a log, and each volunteer was questioned according to a standard questionnaire at the end of the risk period, and at the end of the trial, about these aspects.

The packaging of the prophylactic regimen was designed to facilitate in the field, convenience of transport, integrity of medication and maximal compliance. Each once weekly doxycycline dose was packed with one once weekly dose of antimalarial medication (Maloprim) in one heat sealed pocket of a 4 pocket soft plastic pouch. Each pocket was labelled with the volunteer's name and the date that the dosage was to be ingested. The pouches, containing medication for a 28 day period could be folded to pocket size. This type of packaging did not interfere with the soldiers' activities, (i.e. low crawl, repelling, fording rivers, traumatic contact with hard surfaces), nor was it uncomfortable to carry, bulky or compromisingly noisy when manipulated. The soft pouches did present a slight drawback in that occasionally a capsule was crushed. Nine volunteers reported painstakingly ingesting the powder contents of crushed doxycycline capsules.

Forty eight of 83 volunteers kept complete and up to date records on their personal logs, which comprised small proforma sheets kept in a protective plastic container. Thirty three volunteers recollected or recorded at least one deviation from strict compliance with their trial medication regimen. Twenty five of the 33 deviations were minor, being corrected within only 72 hours of the scheduled dosage period. Eight (9.6%) volunteers reported greater deviations, being either late by 5 days, or not ingesting one or more doses.

Fifteen (18.1%) volunteers reported or recorded adverse symptoms following ingestion of the doxycycline capsules and antimalarial tablets. Of seven volunteers reporting nausea on at least one occasion, only one attributed the symptom to ingestion of the medication prior to a meal, and only one reported experiencing nausea after every dosage. Other symptoms reported could not be attributed to the ingestion of medication.

Only three volunteers presented to the medical monitor with febrile illness during the course of the field exercise. In only one of these cases was a diagnosis of scrub typhus considered. Collection, analysis and completion of clinical, serological, culture and isolation data continues and when completed the trial medication versus placebo code will be broken and the data correlated. Unfortunately, a review of the available data suggest that only one clearly defined case of scrub typhus may have occurred during the unseasonably dry operational period.

An integral part of this study was implementation of the concept of a study site risk assessment survey. An entomology team (3 men for 10 days) was deployed to the study site immediately after the volunteers evacuated each operational area. Sixteen sites representative of most elevations and habitats occupied by troops during the study were selected as a basis for risk assessment. The survey involved the trapping, identification, and bleeding of rodents. Potential vector mites were identified.

Rattus whiteheadi (50), Rattus exulans (31), and Rattus sabanus (17) constituted 69.4 percent of the total 141 rodents collected. Thirteen R. whiteheadi, 28 R. exulans, and 11 R sabanus were seropositive (21/50, IFA) for R. tsutsugamushi. Seropositive rodents collected in fewer numbers included Rattus raja raja (1/2), Rattus raja surifer (1/9), and Rattus tiomanicus jalorensis (3/7). A total of 4,792 engorged larval chiggers were collected from 141 rodent hosts (3 genera, 12 species). Of these, 3,213 were identified, representing 8 genera and 20 species. Leptotrombidium deliense and L. arenicola constituted only 4.1 percent of the total number collected (137 and 60, respectively). The majority of engorged chiggers identified were represented by five species of Gahrliepia (88.6 percent). A total of 6,800 black plates were placed during survey of the 16 areas. Only 149

larvae were collected by black plate during the survey (an average of 1 chigger per 45 black plates). The majority, 112, of the larvae (50 L. deliense and 62 Schoengastia vieta) were collected from two small areas and the 37 remaining specimens were collected randomly from many other sites.

Although IFA test results of the rodent survey indicated a very high (40.4%) incidence of rats having R. tsutsugamushi specific antibody, and thus establishing the presence of the etiologic agent of scrub typhus in the area, the overall risk of contracting scrub typhus posed to volunteers operating in the study site was considered to be minimal due to the very low density of mite vectors in the area during the extremely dry period of this study.

Recommendations: A second and considerably larger field trial, as is currently scheduled for the first quarter FY84 in a known scrub typhus endemic area, should be encouraged. The concept of infectious disease risk assessment should be incorporated wherever possible in military operation plans as well as in medical research field studies.

FEBRILE ILLNESS IN CENTRAL PENINSULAR MALAYSIA (PERAK)

Problem: Seroepidemiological surveys conducted during the mid-1970s in the Mentekab, Bukit Mendi and Jengka Triangle areas of Peninsular Malaysia established the importance of scrub typhus in these specific areas of Malaysia (1). However, this information was drawn from a limited area having a relatively homogeneous ecology. We have only sparse epidemiological data on febrile illness, in particular scrub typhus, occurring in other more ecologically diverse areas of Malaysia. During FY83 field studies were undertaken to broaden our knowledge of the etiology and distribution of febrile illness, particularly those of rickettsial etiology, in other areas of Malaysia.

Progress: Computer data programs were adjusted to allow for the storage and correlation of more demographic and serologic data, and to allow for greater flexibility and circumspection in interpretation of statements on clinical findings. Collection of new epidemiological data began with a detailed survey of febrile illness presenting to the major hospital in Perak state, and another of febrile illness presenting to the health center of an operational military base. In addition, a limited survey of the incidence of R. tsutsugamushi specific antibody among febrile

patients presenting to 6 rural Sabah health centers, selected for the ecological diversity of the areas they serve, was begun late in the yea:

Of 141 cases studied (major hospital, Perak) over a period of 5 months, 7 cases were serologically confirmed as scrub typhus, 13 as murine typhus, 20 as typhoid, 13 as leptospirosis, 14 as melioidosis, and 15 as malaria (2 Plasmodium falciparum, 13 P. vivax). Unfortunately, PUO cases that presented to the hospital staff as "obviously malaria or leptospirosis" were often not admitted to the study. Thus, our figures most likely under report these two diseases. The incidence of melioidosis is striking in an area where there is low physician awareness of the disease, and equally of note is the incidence of scrub typhus during an unusually dry period. Five of the seven scrub typhus patients were putatively exposed in the urban or urban fringe area in and around Ipoh.

PUO patients presenting to the military base (3,000 military personnel, 7000 dependents and civilian personnel) were entered into a more comprehensive study. Of the 298 febrile cases studied over a 5 month period, 9 were diagnosed as scrub typhus, 6 as typhoid fever, 5 as leptospirosis, 5 as melioidosis and 19 as malaria (all P. vivax). Diagnosis of scrub typhus was made, apart from clinical grounds, by the IFA test alone in 2 cases, by IFA and OXK agglutination in 3 cases, by isolation of the organism from mice, and the IFA test (with or without OXK confirmation) in 3 cases, and by isolation alone in one case. Five of the nine cases presented during a three week period in June-July when there was a noted increase in rainfall in central Peninsular Malaysia, immediately following a comparatively very dry period. Seven out of the 9 cases appear to have been exposed in the area where the Perak-Kelantan border meets that of Thailand, an area consisting mainly of primary mountainous forest. Since comparatively few of the soldiers were deployed in this area it would appear to be an area of high endemicity for scrub typhus. Of the 6 typhoid cases all but one could be ascribed to exposure either within the main study base or in an auxillary training base on the west coast of Peninsular Malaysia. Of the malaria cases, 13 were contracted in and around the main base, and only one originated from areas of operations within the mountainous jungle.

Recommendations: Field oriented studies, in particular those capable of providing both baseline and risk assessment data, should continue to be an important part of this unit's mission.

CHARACTERIZATION OF R. TSUTSUGAMUSHI ISOLATES

Problem: To obtain, determine the geographic distribution, and characterize strains of R. tsutsugamushi within the endemic Asiatic-Pacific region. Five of the eight prototype strains of R. tsutsugamushi are predominant in isolates from Peninsular Malaysia, Thailand, Taiwan, the Philippines, Hong Kong, Australia and the islands of the Northern New Hebrides and Santa Cruz groups. While these areas represent a large segment of the endemic region, isolates from a number of the areas on the periphery of the region have as yet to be obtained and characterized.

Progress: Isolates of R. tsutsugamushi have been obtained from both China and Pakistan. Those from China were originally isolated from chiggers and rodents in 3 different Chinese provinces. Three isolates were found to be virulent when characterized subsequent to inoculation of outbred mice. Four of the 5 isolates were shown to be similar to the Thai animal 716 prototype strain while the fifth isolate was shown to be similar to the Gilliam prototype strain of R. tsutsugamushi when characterized by the direct FA procedure. The recently obtained isolates from West Pakistan are presently being propagated and characterized.

Recommendations: Efforts should continue to acquire isolates from Japan and other countries in the endemic area for which we have little or no reliable information. Isolates should be fully characterized upon acquisition and propagation.

IN PURSUIT OF A R. SENNETSU-LIKE AGENT IN MALAYSIA

Problem: Recent collaborative studies between the USAMRU-M and the University of Illinois (Dr. Miodrag Ristic) have identified the frequent presence of what appears to be R. sennetsu specific IgG in the sera of a substantial number of Malaysians presenting to rural Malaysian health clinics and subsequently diagnosed as having a pyrexia of unknown origin (PUO). However, R. sennetsu, the etiologic agent of a well defined clinical entity (infectious mononucleosis like; fever, malaise, headache, anorexia, lymphadenopathy) sennetsu rickettsiosis, is known to occur only in western Japan.

Progress: During FY83 this international collaborative study was expanded to delineate the presence of R. sennetsu specific antibody activity in, (A) sera collected during 1981-82 from patients thought to possibly have infectious mononucleosis, (B) sera collected during the mid-1970s from PUO patients, and the sera of PUO patients reporting during 1983-84 to (C) the Ipoh General Hospital, Perak and (D) to a military health clinic located in the mountainous heart of central Malaysia. A collection of demographic data was begun so that the epidemiology of R. sennetsu or a R. sennetsu-like agent, if present in Malaysia, can be described.

This year, 191 human sera, consisting of 2 dissimilar groups, A and B were examined (A by Ristic, A and B by Ristic and USAMRU-M) by the IFA test for R. sennetsu specific IgG. For this purpose Dr. Ristic provided our laboratory with over 500 R. sennetsu antigen slides and subsequently the IFA test was standardized in both laboratories against a common pool of R. sennetsu positive sera. Isolation of R. sennetsu was attempted from whole blood specimens corresponding to several of the IFA positive (≥1:10) sera in groups A and B. Two as yet not fully characterized R. sennetsu-like agents have been isolated by Dr. Ristic's group.

Thirty two of 89 group A sera collected (routine submissions to the Virology Division, IMR) from patients suspected of having infectious mononucleosis were shown to have a titer of ≥1:10 for R. sennetsu. All but one of the patients having a titer to R. sennetsu had at the time of sample collection complained of fever and of lymphadenopathy. Insufficient information was available on these patients to prepare a demographic analysis. However, data from 102 group B PUO cases investigated by the USAMRU-M in the mid-1970s are more comprehensive. The sera were collected from patients presenting with any two of the following signs and or symptoms; fever, lymphadenopathy, headache and rash, and for whom, after extensive investigation, no definitive diagnosis was made. Patients positive for R. sennetsu antibody ranged in age from 6 to 77 years. Sera from 60 of 82 males, but from only 11 of 20 females, were positive (≥1:10). Sera from 51 of 68 Malays, compared with 9 of 15 Indians, and only 6 of 19 Chinese were positive at ≥1:10. A wide range of occupations, both rural and urban, were represented among those patients having positive titers: they included students, office and factory workers, plantation and padi workers, teachers and odd-job men. Their abodes likewise were quite diverse. Fever and lymphadenopathy were the most frequent signs among patients from both groups A and B, whose sera contained what appears to be IgG specific for R. sennetsu.

Most recently the demographic data and sera of over 300 PUO patients reporting during 1983 to central Malaysia health centers (military and civilian) have been entered into this study.

Recommendations: It would appear that in Malaysia the presence of antibody specific for R. sennetsu may be associated with a definable clinically entity. The entity should be further defined and documented to allow possible cases of sennetsu rickettsiosis to be more readily recognized. The significance of R. sennetsu specific IFA titers of ≤1:10 and ≤1:20 needs further clarification. Recently obtained stock cultures of R. sennetsu should be plaqued using the same method presently employed for R. tsutsugamushi, modified as necessary. Plaque purified stocks should be propagated for the preparation of antigen required for serological testing. Plaquing should also permit us to characterize and titrate both stock cultures and field human isolates. This collaborative effort with the University of Illinois should continue.

CULTURED HUMAN ENDOTHELIAL CELLS AS IN VITRO MODELS FOR THE STUDY OF R. TSUTSUGAMUSHI INDUCED CYTOPATHOLOGY

Problem: R. tsutsugamushi has been studied only in established cell lines, thus an in vitro/in vivo comparison of the cytopathology of infection has been hampered by several factors. Established cell lines represented cells that have "dedifferentiated" both structurally and functionally from their tissue of origin. In addition, previous work has frequently focused on the irradiation or chemical treatment of cultured cells to inhibit their rate of multiplication. Human endothelial (HE) cells, however, maintain their differentiated structural and functional attributes when cultured in vivo and their growth need not be inhibited.

Progress: R. tsutsugamushi was propagated in umbilical cord derived HE cells and titrated on days 0, 4, 8, 12 and 16 post-infection. Procedures were developed for the preparation of ultra-thin sections for transmission electron microscopic (TEM) studies and TEM photomicrographs of R. tsutsugamushi infected and uninfected human endothelial, L-929, and MRC-5 cells have been taken. A manuscript incorporating the results and interpretations of the photomicrographs is in preparation.

Recommendations: This project which employs what appears to be a key cell type in the scrub typhus disease process should be continued and expanded. SEM/TEM studies have, thus far,

concentrated on growth and release phases of R. tsutsugamushi in HE cells. The study should be expanded to include an examination of the penetration and exit phase, intracellular replication and cytopathology of R. tsutsugamushi in human endothelial cells.

ENTOMOLOGICAL STUDIES OF R. TSUTSUGAMUSHI VECTORS

Problems: (a) Previous studies of several successive generations of R. tsutsugamushi infected Leptotrombidium arenicola and L. fletcheri have demonstrated very high filial and transovarial infection rates approaching 100 percent (4,5). Leptotrombidium arenicola, L. fletcheri and L. deliense, each derived from R. tsutsugamushi free colonies, are capable of acquiring R. tsutsugamushi by feeding on R. tsutsugamushi infected mice and the infection in subsequently passed transtadially to the adult mites, however, transovarial passage is not known to occur (8,10). The question of uptake, destiny, location and fate of R. tsutsugamushi is both infected and uninfected chigger lines has not been studied by transmission electron microscopy. (b) Multiple R. tsutsugamushi strains can occur simultaneously in chiggers, however, the infected mites in our L. arenicola colony have not been shown to harbor the Gilliam strain of R. tsutsugamushi (6). This phenomenon may provide a marker for an evaluation of the genetic or physiologic ability of infected vs uninfected chigger lines to acquire R. tsutsugamushi (i.e. Gilliam strain) and pass it not only transtadially, but more importantly, transovarially. (c) Leptotrombidium deliense has the broadest distribution of all R. tsutsugamushi vectors. It is ubiquitous throughout its range, and is considered to be the most significant vector of scrub typhus in Southeast Asia (7). Unfortunately, only one limited study, has been published on infected laboratory colonies of L. deliense (3), and this laboratory does not have a colony of R. tsutsugamushi infected L. deliense.

Progress: (a) Transmission electron microscope procedures have been developed for the deutonymph and tritonymph (adult) stadia. (b) Unfortunately, the L. arenicola controls previously thought to be naturally infected with only Karp, Kato, TA686, TA716, and TA763 were shown to be positive for the Gilliam strain by the DFA technique, therefore, the acquisition and subsequent transmittal of the Gilliam strain to successive generations could not be substantiated. There appears to be a factor in Gilliam fed mites which effects egg laying which was not present in the controls. The Gilliam strain was not consistently detected in successive

generations, but the Gilliam strain may have been present in such low numbers as to be undetectable by DFA. However, the results of two recent feeding trials suggest that it might be possible to select a line in which the Gilliam strain is completely absent. DFA results from the larvae of three L. arenicola lines revealed the absence of the Gilliam strain in the F1 and F2 generations and Gilliam was not demonstrated by DFA in larvae from the same progenitor lines for the two previous generations. (c) Efforts to collect, propagate and establish an infected L. deliense line are ongoing.

Recommendations: Modifications of standard TEM techniques of fixation, embedding, staining, and sectioning, which are peculiar to larval, protonymph and teliophane stadia should be completed, thereafter a sequential examination by TEM should be conducted on all stadia, and on the larval stages both during and after engorgement. The study to determine the effect of the Gilliam strain on fecundity should not be continued. Efforts will be accelerated to obtain and establish an infected colony of L. deliense.

ISOLATION CHARACTERIZATION AND EVALUATION OF R. TSUTSUGAMUSHI MARKER ANTIGEN

Problem: Our LT and MIF assays utilized both membrane (Frac. 4) and soluble (Frac. 3) antigens which were prepared by a modification of the French pressure cell procedure described by Dasch et al. However, due to the crude nature of these antigens, an undesirable degree of serotype cross reactivity often occurs in both LT and MIF assays. Thus, if we are to have a better understanding of the duration of both homologous and heterologous immunity to R. tsutsugamushi, it is necessary to (a) identify and isolate specific antigen(s) that could be used in the LT assay which would differentiate between initial and subsequent homologous and heterologous infections, (b) isolate a specific immunogen that could be used to obtain high affinity antibody in animals for use in an early antigen detection assay and (c) isolate rickettsial protein fraction(s) that would elicit effective CMI responses in selected laboratory animal models.

Progress: Crude membrane and soluble fraction of rickettsia antigens were analyzed with polyacrylamide gel electrophoresis. Six bands of protein were separated from the membrane antigen and designated from top to bottom as M1 to M6. Three protein bands

were separated from the soluble antigen and were designated as S1 to S3 (top to bottom of gel). These bands were cut out from the gel and pooled together respectively so that a sufficient amount of the protein band may be accumulated for animal inoculation as well as for LT assay.

Recommendations: The antigenicity of each protein band should be studied in the LT and MIF assays. The appropriate fraction(s) should be qualitatively recovered and characterized.

CLONING OF R. TSUTSUGAMUSHI AND ISOLATION OF AVIRULENT STRAINS

Problem: Antigenic heterogeneity among R. tsutsugamushi strains is a well established characteristic. Many isolates from humans, rodents, and chiggers have been shown by DFA to react with as many as 6 strain specific antisera. The determination of whether this multiple reactivity is the result of a mixture of strains within a single isolate or a single strain expressing a mosaic of strain antigens is important to our understanding of the induction of actively acquired immunity to scrub typhus. The predominant strains identified in R. tsutsugamushi isolates from countries in the endemic region are Karp or Karp-related. The Karp-related strains are TA716, TA763 and TA686, two of which (TA716 and TA686) are avirulent for mice. The majority of isolates that react with the Karp-related strain specific antisera appear to be mixtures of two or more strains.

Progress: Several human R. tsutsugamushi isolates were cloned. Multiple clones of one of the isolates are presently being propagated for direct FA characterization and determination of virulent/avirulent qualities.

Recommendation: Efforts to plaque and clone multiple reactive isolates and to identify avirulent clones should continue.

RAPID FIELD DETECTION OF R. TSUTSUGAMUSHI ANTIGENS

Problem: To develop a field/laboratory test that will allow for a more rapid definitive diagnosis of scrub typhus. The latex agglutination procedure, which focuses on antigen detection, is under investigation. Our experience has shown that the recognition of scrub typhus is often difficult in highly endemic areas because classical signs of scrub typhus (such as eschar and rash) are seldom observed. Isolation of R. tsutsugamushi

using mouse inoculation often requires two or more months before a definitive diagnosis is made. Rapid, sensitive, specific and field adapted serological methods and/or a more rapid means of isolating and identifying the organisms are needed.

Progress: Recently prepared high titer rabbit anti-R. tsutsugamushi Gilliam strain specific antibody, partially purified by ammonium sulfate precipitation and DEAE cellulose extraction, was absorbed to latex particles. Consistent agglutination occurred when antibody coated latex particles were mixed with L-929 propagated Gilliam strain R. tsutsugamushi which had been subjected to French pressure cell treatment. This agglutination was titrated to an endpoint against antigen dissolved in normal mouse serum and normal human urine. The latex agglutination procedure is currently being evaluated for its ability to detect the presence of antigen in the sera and urine of R. tsutsugamushi infected mice.

Recommendations: Sera and urine of acutely ill PUO patients should be tested for the presence of agglutinating antigen. Specific antibody to additional strains of R. tsutsugamushi should be prepared for absorption to latex particles.

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5. Twartz, J.C., A. Shirai, E. Gan, and M.G. Groves. Serologic responses in human volunteers infected with Rickettsia tsutsugamushi. J. Infect. Dis.

Presentations:

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2. Lewis, G.E., Jr. Arbovirus activity in Malaysia. Presented at the U.S. Military Medical Research Laboratories, Southeast Asia, Review of Arbovirus and Malaria, Baguio, Philippines, 7-12 May 1983.

3. Lewis, G.E., Jr. Overview of the malaria situation in Malaysia. Presented at the U.S. Military Medical Research Laboratories, Southeast Asia, Review of Arbovirus and Malaria, Baguio, Philippines, 7-12 May 1983.

4. Lewis, G.E., Jr. Scrub typhus. Presented to the Diploma of Applied Parasitology and Entomology Course 1983 (SEAMEO-TROPMED PROJECT), Institute for Medical Research, Kuala Lumpur, 15 August 1983.

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*Underlining indicates the individual who presented the paper.

RESEARCH AND TECHNOL WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 08 6525	83 10 01	DD-DRAE(AR)635
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GRP MRKS	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
82 10 01	D. Change	U	U		CX	☒ YES ☐ NO	A. SUM SUM

11. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AN	049 WWC2
b. [illegible]	62770A	3M162770A871	AH	
c. [illegible]	STOG 62783-6.273			

12. (U) Schistosomiasis, Malaria and Leishmaniasis Studies in Brazil

13. SCIENTIFIC AND TECHNOLOGICAL AREAS
0615 Pharmacology 0603 Biology 0613 Microbiology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
73 07	CONT	DA	In-House

18. CONTRACT/GRANT			RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousand)
a. DATE/EFFECTIVE	b. EXPIRATION	PREVIOUS FISCAL YEAR			
c. NUMBER		FISCAL YEAR	83	2.5	108
d. TYPE	e. AMOUNT	CURRENT FISCAL YEAR			
f. KIND OF AGENCY	g. CUM. AMT.		84	3.0	100.

19. RESPONSIBLE DOD ORGANIZATION
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272-4548

23. MILITARY USE
H Foreign Intelligence Considered

24. ASSOCIATE INVESTIGATORS
Bosworth, A B
Prata, A R

POC: DA

25. (U) Rats; (U) Mice; (U)Brazil; (U)Malaria; (U)Schistosomiasis; (U)Leishmaniasis; (U)Chemotherapy; (U)Immunology; (U)Epidemiology; (U)Drug Resistance; (U)Entomol.

26. RAH I

3.(U) Study biology of malaria vectors in Brazil; Study strain specificity of immunity falciparum malaria; Determine reservoir hosts for mucocutaneous leishmaniasis; nduct primary screening of chemicals for anti-schistosomiasis prophylactic and erapeutic activity. These diseases are of primary military medical importance and uld be acquired by DOD personnel deployed in numerous areas of the world.

.(U) Breeding sites, population dynamics and flight range of Anopheles darlingi are udied at a remote site in the southwestern Amazon. Falciparum malaria parasites and mune sera are collected at various locations in the Amazon and growth inhibition says will be used to study immune phenomena. Wild mammals are trapped in an endemic ea for mucocutaneous leishmaniasis and examined for infections. Standard protocols e used to screen for anti-schistosomal activity of candidate chemical compounds exposed white mice.

82 10 - 83 09

.(U) Unique breeding site found for An. darlingi. Patterns of dispersal and flight nge documented. Population size and fluctuation measured. Control strategy proposed d will be tested. Plasmodium falciparum and immune sera collected from three locations the Amazon. 201 wild mammals of 20 species were trapped and examined for leishmamial fections. Parasites isolated recently from Proechimys inheringi are being studied confirm identity. 2305 chemicals were screened for anti-schistosomal activity and gnificant activity was detected in 21. For technical report see Walter Reed my Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

27. [illegible]

PROJECT: 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

Work Unit 049 Schistosomiasis, Malaria and Leishmaniasis Studies in Brazil

Investigators: LTC Stephen C. Hembree, Ph.D.; MAJ(P) Anthony B. Bosworth, Ph.D.; Dr. Aluizio R. Prata, M.D.

PROBLEMS AND OBJECTIVES

Malaria, leishmaniasis and schistosomiasis continue to pose a threat to American military personnel who are or who might have to be stationed in the Middle East, Africa, the Far East, the Caribbean or Latin America. These diseases also inhibit development and cause great human misery, a potential source of political unrest, in many of the developing countries where they are found.

Malaria is a major and growing public health problem in Brazil. Since 1975, the number of reportes cases has more than doubled. More than 220,000 cases were reported in 1982, the vast majority from the Amazon region. In this region the population is low and highly dispersed, and medical care (and, thus, case reporting) is only available in population centers where occurrence of malaria is low, relative to that in remote areas. Drug resistance has reduced the effectiveness of available medicines, even where the population has access to medical care. Attempts to control malaria in rural areas by use of residual insecticides has had only limited success because of behavior patterns of the people, because of the structural characteristics of their dwellings and because DDT has been shown to have a repellent effect on the primary vector. The situation is degenerating, as indicated by Ministery of Health statistics, and practicable alternative control measures are desparately needed. These must be constrained to the behavioral and economic realities of the region. Possibilities include the development and proper use of new drugs, a malaria vaccine, and/or new vector control strategies. Optimum implementation of any of these possibilities requires a detailed understanding of the transmission biology and dynamics of malaria under the extant conditions. Because of the remoteness of the region and the paucity of trained field epidemiological specialists in Brazil, only the gross aspects of malaria epidemiology in the Amazon are known. The objective of our field malaria studies is to provide basic information on

vector biology and transmission dynamics upon which alternative malaria control strategies can be based.

Technology for the production by recombinant DNA methods of a vaccine against falciparum malaria will soon be available. Strong evidence indicates that immunity to malaria is strain specific. This raises questions about the number of antigens that must be represented in a vaccine and the degree of cross protection provided for one strain by antigens from another. We know practically nothing about strains of malaria from an immunological stand-point. Fundamental questions, such as whether or not one or more than one strain exists in a given area, and what is the range of distribution of any given strain, have not been answered. It may be possible to use growth inhibition assays in microcultures to study strain specificity of immunity. The objective of our malaria immunology studies is to use growth inhibition assays to address fundamental questions about malaria strains such as those above.

Cutaneous and mucocutaneous leishmaniasis are zoonoses with wild mammal reservoirs. Leishmaniasis is widespread in Brazil. This vector borne disease is difficult to diagnose in its early stages. Culture of some strains of the eticlogic agent can not be done reliably. Treatment is extended, involves the use of toxic drugs and often has to be repeated because of ineffectiveness and/or relapse. It is difficult to confirm cure. The disease is potentially hideously disfiguring and may have a fatal outcome. The many days required for therapy and the detailed follow-up required to confirm cure would constitute an extreme burden on medical facilities. The grossly disfiguring effects of advanced mucocutaneous leishmaniasis would horrify and have a negative psychological affect on troops, unless they could be given genuine assurances. We are presently unable to prevent this disease, and the reservoir hosts and vector of Leishmania braziliensis braziliensis are inadequately known for control strategies to be implemented against them. It is highly relevant to the development of locally effective transmission control methods that the animal reservoirs for the disease be identified. Leishmaniasis research at USAMRU/Brasilia has the objective of determining the reservoirs for mucocutaneous leishmaniasis, caused by L. b. braziliensis, at a study site where the scope of transmission appears to have been extended to women and children, concomitant with habitat modification in government encouraged agricultural development.

There currently is no single drug that is a totally satisfactory treatment for schistosomiasis. It also is highly desirable that, in addition to developing better therapeutic agents,

we also develop prophylactic methods, either drugs, treatments for exposed skin or treatments for the uniform, which will reduce casualties resulting from exposure to schistosomiasis. The mode of transmission of this disease is such that troops moving through or stationed within an endemic area could be expected to experience a high level of exposure and infection. This would result in unaffordable and unnecessary loss of combat strength and would result in a burden on medical facilities. It should be possible, through sustained research effort, to avoid these problems. Research in schistosomiasis at this laboratory has the objective of primary screening of chemical compounds for prophylactic or therapeutic activity. Standardized screening procedures in a mouse - Schistosoma mansoni - Biomphalaria glabrata system are being used.

PROGRESS

Malaria: Modified Lincoln Index and Barley Triple Catch methods were used to estimate population sizes of Anopheles darlingi at a study site on the Ituxi River, State of Amazonas. Considerable variation was noted in the results acquired by different indexing methods as well as from season to season and from year to year. Physiological age, mating success and ovipositioning history of the population were assessed by dissecting and examining adult females. Over 94 percent of the population had ovaries in the pre-resting or resting stage of development. Ovaries of over 95 percent demonstrated no evidence of previous ovipositioning. Examination of recaptured mosquitoes, known to have had a blood meal before release, indicated a high rate of ovipositioning. The insemination rate of wild An. darlingi was 76.6 percent. Infected adult females were found, and malaria was diagnosed by slide examination amongst people living at the study site. Concentration of An. darlingi in the vicinity of human dwellings was confirmed by simultaneous biting collections at the study site and at other locations up to 2 km away. Biting collections produced over 8,000 adult females, 73.5 percent of which were collected in the early evening peak feeding period and the remainder in the dawn peak feeding period. Sampling with directional intercept traps indicated that fed mosquitoes were leaving the partially cleared study area during or shortly after the dawn feeding period.

Culminating a four year search for the larval breeding site of An. darlingi in the area, larvae were found along with those of An. nuneztovari, in a unique, previously unreported, breeding habitat within a few hundred meters of the study area. Small numbers of highly dispersed larvae were found in grassy areas

recently flooded by the rising river. Larvae were often found between floating leaf litter and the surface film. They were very difficult to see on cursory observation and were difficult to capture. The habitat is being more completely studied and described. The observations have been repeated at other locations in the study area and at different seasons. Internally consistent characteristics of this species in this area are highly dispersed breeding in low density, strong flight capability, very strong host seeking behavior and a tendency to concentrate in areas occupied by people. It seems to approach human habitations at dusk, to have a strong dusk and weaker dawn feeding peak, to remain in the vicinity of habitations at night, and probably to return to the jungle to find suitable resting microhabitats and ovipositioning sites at dawn. Further study of the behavior and biology of this species are important to the development of strategies for its control.

Related progress includes the initiation of seasonal prevalence and spatial distribution studies of known and potential malaria vectors in the vicinity of the city of Labrea, Amazonas. Taxonomic specimens and eggs for colonization attempts have been sent to the US. Colonization attempts there and in Brazil have not yet been successful. Entomological assistance has been provided the Institute for Tropical Diseases in Manaus, Amazonas, in initiating a malaria epidemiology project in the State of Rondonia.

Malaria parasites (Plasmodium falciparum) and associated sera have been collected on the Ituxi River, from the vicinity of Labrea and from the vicinity of Manaus. These are being held in liquid nitrogen until a license in granted by the Brazilian government to import the radioactive tracer materials needed to do growth inhibition assays in the malaria immunology study. Verbal confirmation has been received that the license would be granted, but the number has not yet been received.

Leishmaniasis: Sixty-seven trap nights at the Três Braços, Bahia, study area yielded 201 wild mammals of 21 species, and two specimens of an additional species were caught by hand. Four distinct habitats were sampled: tall forest, secondary scrub, plantation (banana and cacao trees), and tall grassland. Both species diversity and trapping success varied by habitat: tall forest - 17 species, 2.3 percent trapping success; secondary scrub - 9 species, 25.0 percent trapping success; plantation - 6 species, 2.0 percent; tall grassland - 7 species, 21.3 percent trapping success. Most species showed strong habitat preference. Twelve of 22 species were found in only 1 of the 4 habitats represented and an additional 6 species were found in only 2 habitats. Four

species were found in all 4 habitats sampled.

All mammals collected were necropsied in the Três Braços laboratory and tissue specimens (skin, spleen and liver) from all animals captured were inoculated intraperiotoneally and into the feet of hamsters. Two isolates have been recovered from the rodent Proechmys inheringi, 22 of which were trapped from the tall forest habitat, only. These isolates are currently being studied to confirm their identity.

Additional progress includes the near completion of a new health post at Três Braços that will include laboratory and animal rooms for our use. Arthropod ectoparasites were collected from all specimens and have been sent to specialists. Fourty-six species of fungi have been isolated from the fur of these mammals in a cooperative study with the University of Brasilia. We are cooperating with Division of Mammals, Smithsonian Institution, in a study of the mammals from the Brazilian coastal forest region, an area poorly represented in their collections, by providing them study material.

Schistosomiasis: In the Primary Mortality Test (PMT) system, designed to detect potential prophylactic activity in candidate compounds, 1058 compounds were tested. 245 compounds were toxic and 804 compounds were non-toxic but inactive. Significant activity, by the definitions of the protocol, was detected in 9 compounds. An additional 140 compounds were retested in this system to validate previous results. In the Primary Curative Test (PCT) designed to detect potential therapeutic activity in candidate compounds, 1247 compounds were tested. 113 compounds were toxic and 1122 were non-toxic but inactive. Significant activity, by the definitions of the protocol, was detected in 12 compounds. An additional 44 compounds were retested in this system to validate previous results. A total of 2489 compounds were tested or retested, and 21 active compounds were found. Many of the compounds tested are proprietary. Data were provided the Division of Experimental Therapeutics, WRAIR, for further processing and to use in the selection of other compounds for testing.

The mouse colony supporting this study has been returned to the University of Brasilia bioterio, where it will be under the supervision of a veterinarian. The snail colony remains capable of supporting the project.

Preliminary studies in an area being considered for a field epidemiology schistosomiasis study indicate high endemicity exists

in the human population. Additionally, what appears to be Schistosoma mansoni was found in wild mammals frequenting aquatic habitats: Didelphis albiventris (2 of 6 specimens); Oryzomys eliurus (1 of 4); Zygodontomys lasiurus (3 of 36); and Cavia aperea (29 of 44 specimens).

RECOMENDATIONS

1. Increase the scope of the malaria, leishmaniasis and schistosomiasis projects to include epidemiology in human populations.

2. Vigorously continue all aspects of the entomological part of the malaria project.

3. Initiate entomological studies to determine the vector of L. b. braziliensis in the Três Braços study area.

4. All studies should have as their final objective the development of practicable control strategies for the relevant diseases in Brazilian populations.

PRESENTATIONS

Bosworth, A., 1983. Aspects of the directional flight of Anopheles darlingi mosquitoes at Floresta, Amazonas. Ann. Mtg. Am. Mosq. Control Assoc., Lake Buena Vista, Florida. 27 Feb - 3 Mar 1983.

Bosworth, A. 1983. Entomological hazards in tropical medicine. VII Curso de Aperfeiçoamento em Medicina. Faculty of Medicine, University of Brasilia. 1 Sept - 29 Oct 1983.

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e primatas. Acta Amazonica, 12(2): 465-482.

Travassos da Rosa, A.P.A., R.B. Tesh, F.P. Pinheiro, J.F.S. Travassos da Rosa and N.E. Peterson. Characterization of eight new phlebotomus fever serogroup arboviruses (Bunyaviridae: Phlebovirus) from the Amazon Region of Brazil. Am. J. Trop. Med. Hyg. (Submitted for Publication).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA 08 6530	2. DATE OF SUMMARY: 83 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE ENTRY SUMMARY: 82 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. WORK SECTY: CA	8a. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AN	050 WW06
b. SECONDARY	62770A	3M162770A871	AH	
c. [illegible]	STOG 82/83-6.2/3			

11. TITLE: (U) Vaccine Development in Trypanosomiasis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 0603 Biology 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 09	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	7.0	191
	CURRENT 84	8.0	319

19a. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
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20. GENERAL USE
H
Foreign Intelligence Considered

20a. PERFORMING ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: Muriithi, I
NAME: Wellde, B T POC:DA

21. KEY WORDS: (U) LSU Animals (U) RAM T (U) T. brucei; (U) Volunteer; (U) Kenya; (U) Monkey (U) Trypanosomiasis; (U) Vaccine; (U) Africa; (U) Cattle; (U) Goat (U) Immunity

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide brief individual paragraphs identified by number, precede text of each with security classification code.)

23. (U) The objective of this program is to develop an effective, practical vaccine against African trypanosomiasis, useful to both military and civilian agencies. Related benefits include acquisition of knowledge pertaining to trypanosome immunity, host response and pathology of infection. There is a requirement for these studies which should provide a basis for rational development of a vaccine for this disease which would constitute a serious hazard for military personnel operating in the endemic area. 24. (U) Experiments conducted at WRAIR and in Kenya have demonstrated that experimental animals can be successfully immunized with irradiated trypanosomes. Rodents, cattle and monkeys can be rendered completely resistant to a challenging infection of T. rhodesiense 25. (U) 82 10 - 8309 During this period the investigators continued to monitor the antigenic stability of parasites from western Kenya. Current evidence indicates that there was a significant antigenic shift in the 1980-81 outbreak. Epidemiology and treatment record analysis studies continued. A treatment center was opened in western Kenya situated north of the Lambwe Valley endemic area and to the east of the Ugandan epidemic area. This center will serve as a routine treatment facility and research facility for the evaluation of standard drugs available and USMRDC developed drugs effective in screens against human African trypanosomiasis. An experimental compound WR 163577 is being evaluated in the goat model against T. brucei infection. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 October 1982-30 September 1983.

*Available to contractors upon authorization's approval.

DD FORM 1498 MAR 88 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1/55A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

WORK UNIT 050 VACCINE DEVELOPMENT IN TRYPANOSOMIASIS

INVESTIGATORS:

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ASSOCIATES: LTC J.D. CHULAY, MC

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MAJ A. WOZNIAK, MSC

CPT R.F. BEACH, MSC

CPT K.J. LINTHICUM, MSC

PROGRESS IN TRYPANOSOMIASIS RESEARCH

Epidemiology Survey

Data collected during the 1981-82 survey is being collated and processed. Medical records of sleeping sickness patients have been screened for treatment regimen, relapses, etc. Followup interviews and examinations have been performed on confirmed cases. Over seven hundred individuals have been identified as having sleeping sickness between 1961 and 1983. The deaths are being examined separately in an attempt to determine cause and relationship to sleeping sickness and/or treatment. Likewise various treatment regimens followed over the years are being examined for patterns of cure, relapse, drug reaction and fatalities. These data will be used to formulate protocols for treatment of patients at the Alupe treatment center.

Alupe Treatment Center

A physician was assigned to head the center and began organizing the staff in late September. Equipping the facility and training the staff is progressing on schedule. It is anticipated that initial studies will center around the documentation of WHO approved treatment regimens in an effort to compile reliable data to support future protocols.

Lambwe Valley Trypanosome Studies

Studies conducted jointly with the Kenya Trypanosomiasis Research Institute have indicated that the parasites involved in the 1980-81 epidemic were different than those identified in the valley between 1973 and 1979. Isoenzyme analysis and serum neutralization studies are in agreement. Recent cases identified from outside the valley have been caused by parasites identified as being of the valley types of 1973-79 indicating that the dissemination occurred before the recent epidemic. These studies are continuing at the present time. To date there have been 46 confirmed cases in calendar 1983.

PROGRESS IN LEISHMANIA RESEARCH

Biochemical Characterization of Kenyan Leishmania Isolates

Cellulose acetate electrophoresis (CAE) is now being used to characterize Leishmania isolates. Unknowns are electrophoretically separated alongside already characterized control isolates then stained for specific enzymes to produce zymograms. The location of enzyme bands on such zymograms (migration distance from the origin) can be compared and the similarity of the unknown and one of the controls determined visually. At present, characterization is based on such comparisons using 5 different enzymes: PGI - phosphoglucoisomerase, G6PDH - glucose-6-phosphate dehydrogenase, ME - malic enzyme, PGM - phosphoglucomutase and ASAT - aspartate aminotransferase. Our controls include the following:

NLB #	Taxonomic identity	origin/characterized at
005	L. adleri	Kenya/Liverpool School Trop Medicine
070	L. major	Jericho Valley/Hadassah Med School
061	L. donovani	Ethiopia/Hadassah Med. School

Nine additional enzymes, for a total of 15, will be screened in an effort to identify which systems most clearly differentiate our control strains. It is hoped that as few as 4 well chosen enzymes will differentiate all Leishmania occurring in Kenya. This estimate is based on the observations of Dr. Richard Kreutzer, who has indicated that 4 enzymes are sufficient to separate all New World species of Leishmania he has examined - this includes much of the WRAIR Leishmania Bank.

A CAE project, already underway involves characterization of 6 isolates from 5 different species of rodents captured during a leishmaniasis reservoir study. Preliminary results are shown below.

Leishmaniasis Reservoir Study: Characterization of Isolates from Small Rodents

ISOLATE	RODENT				
NLB#	GENUS	PGI	G6PDH	PGM	ASAT
095	Arvicanthis	III*	IV	VIII	XI
089	Tatera	III	IV	VIII	XI
098	Mastromys	III	IV	VIII	XI
088	Aethiomys	III	IV	VIII	XI
057	Taterillus	III	IV	VIII	XI
	CONTROLS				
005	L. adleri	I	IV**	VI	IX
061	L. donovani	II	V	VIII	X
070	L. major	III	IV**	VIII	XI

* for each enzyme a different Roman numeral is assigned to each different banding pattern.

** same band pattern for G6PDH

A similar analysis is also being applied to sandfly isolates. All projects remain in the pilot study catagory until more enzymes are screened and in some cases better replicates of those enzymes already examined are produced.

Band migration will eventually be quantified, all bands being assigned a relative migration value (R_f) which indicates migration distance as a proportion of the distance migrated by a reference band.

Localization of Leishmania Donovani In Experimental Infected Sandflies: An Indicator of Vector Competence

Only three of the more than forty sandfly species occurring in Kenya are thought to transmit Leishmania donovani. These are Phlebotomus martini, Phlebotomus celiae and Phlebotomus vansomeranae. The vector competence of these species reflects their anthropophilic biting behavior and their ability to sustain L. donovani in the foregut of the alimentary system, from where the promastigote forms of the parasite are probably transmitted during bloodfeeding. The present study compared the fate of L. donovani in P. martini and Sergentomyia schwetzi, a nonvector sandfly. At regular intervals, following infection, both species were dissected to determine if and when promastigotes moved from the mid-gut to the head of the insect. Prior to dissection flies were also allowed to feed on hamsters thereby correlating parasite localization in the alimentary system with transmission of L. donovani. The results suggest that anterior migration is a prerequisite for transmission of L. donovani and that the physiological conditions which promote such transmission-favoring movement do not occur in the nonvector species.

Leishmania major in Kenya (East Africa): Transmission to a Human by Bite of a Naturally Infected Phlebotomus duboscqi Sandfly

We isolated Leishmania from a Phlebotomus duboscqi female captured in Baringo District, Kenya, and from papular lesions that developed at sites where this sandfly had fed on a human. When characterized by cellulose acetate electrophoresis (8 enzymes examined) these isolates proved to be identical to known Leishmania major strains from a human and a rodent (Arvicanthis sp.) and different from Leishmania donovani and Leishmania adleri which also occur in Baringo. This is the first case of human cutaneous leishmaniasis caused by L. major reported from Kenya.

Comparison of Three Culture Media for Isolating Leishmania donovani from Splenic Aspirates in Kenyan Visceral Leishmaniasis

Three culture media were compared for their sensitivity in isolating Leishmania donovani from splenic aspirates from patients with visceral leishmaniasis. A total of 151 splenic aspirates were obtained from 18 patients before, during and after chemotherapy. Aspirates were cultured in Schneider's Drosophila medium supplemented with 20% fetal bovine serum (SCH), a rabbit blood-agar diphasic medium (NNN) overlayed with normal saline (NS), and NNN overlayed with SCH. Giemsa stained aspirate smears were microscopically

examined. Of the 77 aspirates that were positive by any method, 88% were positive on smears, 57% were positive in NNN/SCH, 29% were positive in NNN/NS, and 25% were positive in SCH. Microscopy plus culture gave complementary results. We suggest that for optimal diagnosis and evaluation of response to treatment of visceral leishmaniasis in Kenya, splenic aspirates should be examined by microscopy and cultured in NNN/SCH.

High-Dose Sodium Stibogluconate Treatment of Cutaneous Leishmaniasis In Kenya

Cutaneous leishmaniasis caused by Leishmania aethiopica usually responds poorly to conventional doses of pentavalent antimonial drugs. We treated three patients with cutaneous leishmaniasis acquired in Kenya, presumed or documented to be caused by L. aethiopica with intravenous sodium stibogluconate, 18-20 mg Sb/kg body weight twice daily for 30 days. All patients had a good response to treatment, with disappearance of parasites from skin smears and cultures after 14 to 27 days, clinical healing of the lesions, and no recurrence during a 3 to 18 month follow-up. Side effects of treatment were minor. We conclude that this high dose sodium stibogluconate regimen is safe and effective for treating cutaneous leishmaniasis caused by L. aethiopica in Kenya.

PROGRESS IN RIFT VALLEY FEVER RESEARCH

Mosquito Species Succession In A Dambo In An East African Forest

The mosquito larval and pupal fauna of a dambo in a primary forest in Nairobi Area, Kenya was monitored during the short rainy season. The relative density of the immature stages of 6 species was recorded daily for a 3 month period. Aedes (Aedimorphus) cumminsii mediopunctatus (Theobald), Ae. (Neomelaniconion) lineatopennis (Ludlow), and Ae. (Mucidus) sudanensis (Theobald) were the first 3 species collected following flooding. Culex (Culex) quasiguiarti (Theobald), Anopheles (Anopheles) coustani (Laveran) and Cx. (Cux.) theileri (Theobabld), were collected beginning 16, 17 and 33 days respectively following flooding. Each of the 3 Aedes spp. disappeared after one generation. All populations decreased to zero, after day 48.

Transovarial Maintenance of Rift Valley Fever Virus In Kenya By Aedes Lineatopennis mosquitoes

Rift Valley fever virus was isolated from reared adult male and female Aedes lineatopennis collected as larvae and pupae on a ranch in Kenya during November and December 1982. This suggests transovarial transmission of the virus and supports an hypothesis that the virus is maintained during the interepizootic periods by transovarial transmission in Aedes lineatopennis.

Blood Feeding Activity Of Mosquitoes At A Flooded Grassland Dambo In Kenya

The biting activity of mosquitoes encountered after flooding of a grassland dambo in Kenya was examined using human and calf bait. A total of 2,319 female mosquitoes, representing 9 species, were collected during a 96 hr period at human bait and a 48 hr period at calf bait. Aedes lineatopennis was the most commonly captured species. It represented 85% of the specimens collected at human bait and 96% of the specimens collected at calf bait. Diel biting activity was established for Ae. lineatopennis, Ae. cumminsii mediopunctatus and Ae. dentatus.

RECOMMENDATIONS

African Trypanosomiasis

It is recommended that human treatment studies and Lambwe Valley monitoring continue. The use of the goat model should be expanded and refined.

Leishmaniasis

Drug efficacy and pharmacokinetic studies should continue on currently available compounds until such time as new compounds or new formulations are available for field trials. Vector-reservoir field studies should be expanded. Controlled biochemical typing, morphologic taxonomy and transmission studies should be implemented as colony raised sandfly become available.

Rift Valley Fever

Efforts should continue along present lines of investigation since present data is preliminary.

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10. Spencer, H.C., Masaba, S.C., Chulay, J.D. and Nguyen-Dinh, P.: Field Evaluation in Kenya of the 48-Hour In Vitro Test for Plasmodium falciparum Sensitivity of Chloroquine. Am. J. Trop. Med. Hyg. 32(5), 916-921.

11. Wellde, B.T. and Chumo, D.A.: Persistence of Berenil in cattle Trop. An. Hlth. Prod. 15, 95-102.

12. Wellde, B.T., Chumo, D.A., Adoyo, M., Kovatch, R.M., Mwongela, G.N. and Opiyo, E.A.: Haemorrhagic Syndrome in Cattle Associated with Trypanosoma vivax Infection. Trop. An. Hlth. Prod.

Manuscripts In Press

1. Linthicum, K.J., Davies, F.C., Bailey, C.L. and Kairo, A.: Mosquito Species Succession in a Dambo in an East African Forest. Mosq. News.

Manuscripts Submitted

1. Beach, R.F., Kiilu, G., Hendricks, L.D. and Leeuwenburg, J.: Leishmania major in Kenya (East Africa): Transmission to a Human by Bite of a Naturally Infected Phlebotomus duboscqi Sandfly.

2. Githure, J.I. and Chulay, J.D.: Comparison of Three Culture Media for Isolating Leishmania donovani from Splenic Aspirates in Kenyan Visceral Leishmaniasis.

3. Linthicum, K.J., Davies, F.G. and Kairo, A.: Blood Feeding Activity of Mosquitoes at a Flooded Grassland Dambo in Kenya.

4. Roberts L.W., Wellde, B.T., Reardon, M.J. and Onyango, F.K.: Mechanical Transmission of Trypanosoma brucei rhodesiense by Glossina morsitans (Diptera: Glossinidae).

5. Sixsmith, D., Watkins, W., Chulay, J.D. and Spencer, H.C: Antimalarial Activity of Dihydrofolate Reductase Inhibitors In Vitro.

6. Spencer, H.C., Kipingor, T., Agere, R., Koech, D.K. and Chulay J.D.: Plasmodium falciparum in Kisumu, Kenya: Differences in sensitivity to amodiaquine and chloroquine.

7. Spencer, H.C., Watkins, W.M., Sixsmith, D.G., Koech, D.K. and Chulay, J.D.: Plasmodium falciparum Malaria in Kenya with In Vivo and In Vitro Resistance to Pyrimethamine/Sulfadoxine and Cross Resistance to Chloroquine.

8. Watkins, W.M., Sixsmith, D.G. and Chulay, J.D.: The Activity of Proguanil and Its Metabolites, Cycloguanil and p-Chlorophenylbiguanide, Against Plasmodium falciparum In Vitro.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

AGENCY ACCESSION	3. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6500	83 10 01	DD-DRA(AR)636

4. DATE PREV SUMRY	5. KIND OF SUMMARY	6. SUMRY SCTY	7. WORK SECURITY	8. REGRADE	9a. JOINT INTEREST	9b. SPECIFIC DATA: CONTRACTOR ACCT	9c. LEVEL OF SUM
2 10 01	DA Change	U	U		CX	XX YES ☐ NO	WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	62770A	3M162770A87Q	AD	051 WU15
CONTRIBUTING	62770A	3M162770A871		
XXXXXXXX	USTOG 32/83-6123			

11. TITLE (Procede with Security Classification Code)

(U) Gastrointestinal Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

0613 Microbiology 0611 Life Support 0603 Biology

13a. START DATE	13b. ESTIMATED COMPLETION DATE	14. FUNDING AGENCY	15. PERFORMING METHOD
73 07	CONTINUED	DA	C. In House

16. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

17. RESOURCES ESTIMATE	a. PROFESSIONAL MAN-YRS	b. FUNDS ($ in thousands)
PREVIOUS FISCAL YEAR 83	8.0	839
CURRENT FISCAL YEAR 84	8.0	820

18. RESPONSIBLE DOD ORGANIZATION

a. NAME: Walter Reed Army Institute of Research

b. ADDRESS: Washington, DC 20307

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20. GENERAL USE

H

Foreign Intelligence Considered

ASSOCIATE INVESTIGATORS

a. NAME: AXELROD, D A

b. NAME: CHENEY, C P POC: DA

21. KEYWORDS (Provide Security Classification Code)

Activity (U) Bacterial Mucosal Adherence; (U) Pili; (U) Pathogenic E.coli; (U) Gut Associated Lymphoid Tissue; (U) Intestinal Epithelial Transport; (U) Myoelectric Activity

22. (C) TAT P

23 (U) Research efforts in this department continue to be directed toward Gastrointestinal diseases of military importance. Focus is on enteropathogenic bacterial diarrheal disease caused by pathogenic E.coli, but also Salmonellosis, Shigellosis and Cholera. These have critical military relevance because of their influence on troop mobility, particularly following deployment of units to new areas.
24 (U) Studies of bacterial diarrhea are being conducted in 4 general areas 1) Mucosal Adherence as a determinant of bacterial colonization. 2) Intestinal immune response to bacterial infection. 3) Pharmacologic modification fo effects of infections on intestinal transport and 4) Motility. Studies utilized preparations of intestinal membrane fractions, bacterial adherence factors (pili), isolated and functionally characterized intestinal mononuclear cells, in vivo is isolated and in vivo acute and chronic recording of intestinal myoelectric activity.
25 (U) 82 10- 8309 Mucosal Adherence: Colonization factor antigen (CFA/II) adherence pili have been prepared in quantity and tested in humans as an oral vaccine against adherent, toxigenic E.coli which cause Traveler's diarrhea. Immunology: A synthetic octapeptide, the antigenic site for a large immunogenic peptide, has been prepared and utilized as an in vitro immunogen of the mucosal immune system. Transport: Membrane vessicle studies confirmed the enhancement of D-glucose uptake by cholera toxin previously seen in Ussing chambers. Motility: Migrating action potential complexes are a peristaltic response induced by a variety of stimuli including enterotoxins, mucosal damage and luminal distention. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83

24. SUPPLEMENTARY INFORMATION (Publications)

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND MAY NOT BE USED FOR ANY SUBMISSIONS AFTER [illegible]

Project: 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

Work Unit 051 Gastrointestinal Diseases of Military Importance

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PROBLEMS AND OBJECTIVES

i. Role of Mucosal Adherence in Bacterial Colonization

Colonization of the small intestine is a prerequisite for the production of clinical diarrhea by many enteropathogens, including the enteropathogenic (EPEC) and enterotoxigenic (ETEC) groups of E.coli. The latter group (ETEC) is responsible for a majority of cases of Traveller's diarrhea. One important mechanism promoting small bowel colonization is the adherence of bacteria to the intestinal mucosal surface. In order to develop effective means of preventing and treating bacterial diarrhea we have been attempting to answer the following questions: What are the structures (adhesins) on the surface of bacteria which enable them to specifically attach to the host's mucosal cells? What are the receptors, or binding sites, for bacteria on the host's intestinal cells? What immunologic or pharmacologic means can be used to prevent or reverse the adherence of pathogenic bacteria to the intestine? Can an adherence antigen be used as an effective oral vaccine against enteric infection with pathogenic E.coli?

ii. Role of Host Immune Mechanisms

The mechanisms of local immunization and the regulation of local immune responses at the level of the intestinal mucosa are being studied to develop better oral vaccines. Synthetic peptide immunogens are being developed as oral vaccines to give protective intestinal mucosal immunity. The goal is to have vaccines giving long term T-lymphocyte immunity without interfering secretory

antibody production thus allowing subsequent booster oral immunization for both specific T-lymphocyte and local secretory antibody immunity. Peptide antigenic site modification is the approach being taken leading towards cross-reactive T-lymphocyte immunization without cross-reactive antibody formation as a prototype for initial intestinal immunization allowing for booster immunization with the modified peptide antigen. Lymphocytes from the gut associated lymphoid tissue (GALT) are being used for in vitro immunization and challenge with synthetic peptide antigens.

iii. Alterations of Intestinal Transport

The objective is to obtain a better understanding of certain functional properties of the intestine. We are particularly interested in the secretion of fluid and ions into the intestinal lumen. We hope to obtain further information on the nature of the secretory process (processes), including it's anatomical location, mechanism of operation and its sensitivity to various reagents, both stimulatory and inhibitory. These secretory processes are of particular interest with respect to bacterial diarrhea because they may be involved in the relevant pathophysiology. Attempts to answer the following questions may aid the development of more effective or simpler therapies for bacterial diarrhea. What are the mechanisms for salt and water transport under normal conditions and in the secretory state induced by bacterial toxins and other secretory stimuli? Can pharmacologic agents reverse the salt and water secretion induced by bacterial toxins and other secretogogues?

iv. Alterations of Intestinal Motility

In what ways is gastrointestinal motility altered in enteric infection and do these alterations contribute to the genesis, symptomatology, pathophysiology and resolution of enteric disease? What are the mechanisms and neurohumoral pathways by which enteric infection and enterotoxins mediate alterations of gastrointestinal motility and what is the effect of diet and therapeutic agents on these pathways? Do luminal toxins produce changes in gastrointestinal motility similar to those produced by native disease? Are diet, mucosal transport processes, mucosal damage, mucosal inflammation, luminal contents and distension, and gastrointestinal motility responses related? Do motility and therapeutic intervention affect enteric microbial colonization, infection, immunity and host responses? How do motiltiy patterns correlate with peristalsis and transit time? Do alterations in one intestinal area affect motility in another area? How do small intestinal and colonic motility relate to produce a net clinical pattern?

PROGRESS

1. Role of Mucosal Adherence in Bacterial Colonization

Traveler's diarrhea (TD) is of great military concern because of the high likelihood that it will have a debilitating effect on troops shortly after their deployment to endemic areas. TD is most commonly caused by enterotoxigenic E.coli (ETEC). ETEC strains from TD patients produce surface pili or fimbria which function as colonization factor antigens (CFAs) which promote association of organisms with the intestinal mucosa. Two antigenic types (CFA/I and CFA/II) were initially recognized in human ETEC isolates, but additional CFA's have subsequently been demonstrated and more remain to be discovered. Studies of analagous animal infections show that susceptible hosts can be protected from intestinal E.coli colonization by passive administration of IgA antipilus antibody. In theory, an effective vaccine against ETEC-induced TD should stimulate local secretion of IgA against CFA and oral administration of purified CFA should induce a maximal response.

Purified preparations of CFAs (pili) from enterotoxigenic E.coli ETEC) are candidates as oral vaccines against TD since native preparations stimulate a mucosal secretory IgA response (anuual report 1982) which could prevent intestinal colonization by organisms experessing such CFAs. Since CFAs are prepared by harvest from cultures of human ETEC, no viable parent organisms must remain in the final oral vaccine preparations. Filtration sterilization has been impractical because CFAs are particulate. Other methods of sterilization, such as radiation or formalin, might alter antigenicity. To compare the immunogenicity of CFA preparations sterilized by gamma radiation (100 kilorads) and formalin (0.1%), we harvested CFA/II pili from an O6:H16 ETEC strain of E.coli (M424C1), documented purity by electrophoresis, sterilized them by radiation or formalin and compared their abilities to stimulate specific anti-CFA/II IgA following inoculation into rabbit ileal (Thiry-Vella) loops. Loops were inoculated with 8, 1mg doses of radiated or formalin treated CFA/II preparations over 4 weeks. Loop secretions were collected daily and specific anti-CFA/II IgA measured with a double sandwich ELISA with native CFA/II immobilized on the microtiter wells. Formalin and radiation sterilized preparations gave parallel responses at a 1/20 dilution of loop secretions and reached peak and plateau responses during the week following the final immunization. 3 of 4 rabbits immunized with the formalin treated CFA/II preparation had peak and plateau responses indistinguishable from each other and from the responses of rabbits inoculated with radiation-sterilized pili. Mean plateau responses in the two groups were

indistinguishable. These results indicate that preparations of CFAs from ETEC can be sterilized either by radiation or formalin, yet retain their mucosal immunogenicity, inducing an IgA response which recognizes native CFA. These results support development of CFA preparations as oral vaccines for TD.

In collaboration with Dr. S. Berman at the Forest Glen facility we have prepared a lot of CFA/II pilus antigen which is sterile, immunogenic in animals, and safe for oral administration. An IND application was approved to permit testing of the vaccine product for immunogenicity and efficacy in human volunteers under the supervision of Dr. M.M. Levine and colleagues at the Center for Vaccine Development, University of Maryland, and initial studies have been performed.

The CFA/II pilus (fimbrial) preparation developed in our laboratory from the 06:H16 E.coli strain M424C1 and prepared in quantity by Dr. Sandy Berman at Forest Glenn was tested for immunogenicity after oral administration and efficacy in protecting against challenge with E.coli strain E24377A, an (0139:H28) CFA/II bearing strain, at the Center for Vaccine Development of the University of Maryland. 10 healthy volunteers received Eight 1.7mg doses of oral CFA/II (2 doses/week for four weeks) . Each dose was given with 2gms of sodium bicarbonate and 90 minutes following 300mg of cimetadine intended to prevent the adverse effects of gastric acidity on the protein preparation. No adverse reaction were noted. Sera were collected on day 10, 21, and 28 and jejunum fluid on day 14 and 29 following the end of vaccination. Only 2 of 10 vaccinees developed significant serum antibody rises against the CFA/II vaccine as measured by ELISA. These 2 vaccinees also developed a secretory IgA antibody response in jejunal fluid. 8 of the vaccinees were challenged by 5 x 10^8 organism of strain E24377. 6 of 8 controls developed diarrhea (67%) and 3 of 8 vaccinees (38%). Only one of the 2 vaccinees who develops serum and jejunum antibody to CFA/II vaccines was challenged. This volunteer did not experience diarrhea. These studies suggest that although immunization with CFA/II might be protective, the CFA/II preparation administered orally was poorly immunogenic.

Additionally characterization of the CFA/II vaccine was obtained in light of recent information on the antigenic heterogeniety of CFA/II bearing strains. "CFA/II" is not a single antigen but comprises three separate antigens, a common surface antigen termed coli surface antigen 3 (CS3) produced on virtually all CFA/II positive strains and coli surface (CS) 1 and 2 which are alternatively produced on CFA/II positive strains. Best morphologic evidence indicates that CS 1 and CS2 are pilus (or fimbrial) structures and that CS3 is a finer (fibrillar)

structure. Our vaccine strain possessed CS1 and CS3 and the vaccine preparations contained predominately the CS3 component (95%). To quantify antibody responses in volunteers to the separate CFA/II components, purified CS1 and CS3 antigens were prepared at the Center for Vaccine Development. Only one volunteer developed antibody rises (in serum) to CS3 (the major vaccine components) and no volunteers developed antibody rises to CS1. In contrast, half of the volunteers who received this challenge strain (E24377A) bearing both CS1 and CS3 developed serum antibodies to both CS1 and CS3. Orally administered CFA/II vaccine containing predominately the CS3 component did not promote a serum antibody response comparable to the natural infection with E.coli bearing these components.

A major focus of the department has been the elucidation of the mechanisms whereby a naturally occuring, enteroadherent O15:K-:NM E.coli strain induces diarrheal disease in rabbits with specific emphasis on defining the determinants of enteroadhernce both on the bacterial cell and the intestinal mucosa. Over the last year, this rabbit infection with E.coli strain RDEC-1 has emerged as a model highly analalgous to naturally occuring human disease caused by the enteropathogenic E.coli (EPEC) strains or serogroups. The relevance of RDEC-1 infection of rabbits as a model for the pathogenesis of EPEC disease in human infants is suggested by: 1) close morphological similarity of the adherence process in the two diseases (close adherence with pedastal formation and loss of microvillar cytoskeletal architecture in areas of adherence) and 2) similar age of susceptibility (weaned infants in both instances). In investigating the determinants of RDEC-1 adherence to rabbit intestinal epithelial cell apical (brush border) membranes, we have developed evidence that the close enteroadherence of RDEC-1 to rabbit intestine is conferred by the expression of specific adherence pili which are distinct from the Type 1 or common pili but are expressed only under certain growth conditions. These specific adherence pili on RDEC-1 have been termed AF/R1 since they are the first attachment factor described specifically for rabbit intestine. Three lines of evidence have been developed. First, when the expression of pili by RDEC-1 is phenotypically suppressed by growth in enriched (Brain Heart Infusion) media, the organisms lose their capacity for in vitro adherence to isolated rabbit brush borders. In contrast, when grown in Penassay broth, RDEC-1 organisms are densely piliated and adhere avidly to isolated rabbit brush borders. These pili are distinct from Type I pili by criteria of hemagglutination and immnoprecipitation. Second, the property of in vitro adherence to rabbit brush borders can be genetically transferred along with the transfer of RDEC-1 pili to other bacterial species. These properties appear to be transferred along with a plasmid of

approximately 80,000 molecular weight. Third, isolated preparations of RDEC-1 (AF/R1) pili adhere directly to the intestinal surface of the rabbit, but not of the rat, guinea pig or human ileum. The adherence of isolated pili AF/R1 to frozen sections of rabbit intestine is linear along the mucosal surface as demonstrated by immunofluorescence, and corresponds to the pattern of adherence seen with the intact, piliated, RDEC-1 organisms. Taken together, these result suggest that this unique type of pilus, (AF/R1) expressed under certain growth conditions, is responsible for the species-specific adherence of RDEC-1 organisms to the rabbit intestine. If the analogy of RDEC-1 enteroadherence to EPEC enteroadherence is correct, EPEC strains may also elaborate specific adherence pili under appropriate growth conditions in vivo. This suggests an area for further investigations of EPEC strains.

Work on the elucidation of the intestinal mucosal receptors for RDEC-1 E.coli has progressed. Previous studies showed that receptors for the adherent pathogen, RDEC, on rabbit intestine are first detectable at the onset of weaning. In adults, enteroadherence is mediated by specific receptors on host ileal brush border membranes (BBMs). In vitro experiments showed that BBMs of rabbits younger than 21 days lack RDEC-1 receptors, suggesting that infants may be resistant to enteroadherence and colonization by RDEC-1. To test this hypothesis, we fed litters of rabbits aged 12 and 16 days 10^7 RDEC-1 and examined them on the fifth day (aged 16 and 20 days) for mucosal adherence (light microscopy) and colonization (CFU/gm) in jejunum, ileum and cecum. A nonadherent, nonpathogenic E.coli (640) served as control. Adherence was examined in relation to epithelial maturity and the presence/absence of goblet cell mucus. Enteroadherence was first observed in 20 day rabbits where it occured uniformly in the cecum (dense, 6/9; patchy, 3/9) but only rarely in the ileum (sparse, 3/9), where RDEC-1 adhered only to mature appearing ileal cells. Adherence correlated with depletion of segments, although sparse adherence was seen in one 16 day cecum and two 16 day ilea. Adherence of 640 did not occur. At sacrifice all segments in all rabbits were colonized and no differences in colonization were observed in 16 day rabbits between RDEC-1 and 640. In contrast, in 20 day rabbits, ileum and cecum were more heavily colonized by RDEC-1 than by 640. Thus, consistent with in vitro studies, 16 day rabbits do not express mucosal receptors for RDEC-1. 20 day rabbits express receptors densely in the cecum and have limited receptors on mature ileal cells. The limited colonization seen in 16 day infants does not seem to depend on enteroadherence. In 20 day infants, the presence of RDEC-1 receptors appears to favor RDEC-1 colonization of cecum and ileum. Thus weanling infants may be susceptible to enteric E.coli

infections not only because of withdrawal of maternal immune protection, but also because of the development of specific mucosal receptors for enteropathogens.

In vitro adherence of the rabbit enteropathogenic E.coli strain RDEC-1 to rabbit intestinal brush borders is mediated by a specific pilus attachment factor (AF/R1); but evidence that AF/R1 is expressed during in vivo infection is lacking. A specific anti-AF/R1 response during infection with RDEC-1 would indicate that AF/R1 was present and immunogenic. We therefore prepared rabbits with isolated Thiry-Vella (T-V) intestinal loops; inoculated them per os with RDEC-1 in feces. Special care was taken to keep the T-V loops free of RDEC-1 infection. All animals had increases in specific anti-AF/R1 IgA secretion by two weeks which peaked in 40 days. These responses paralleled the IgA response to LPS, but the anti-AF/R1 assay was not inhibited by LPS. These results indicate that AF/R1 pili, which are expressed in the course of RDEC-1 infection, are immunogenic and elicit a specific local secretory IgA response in the intestine.

As outlined above enteric infection with RDEC-1 E.coli depends on colonization mediated by the interaction of bacterial adherence factors (AF/R1 pili) with host mucosal receptors. Infection might be preventable by interfering with the AF/R1 pilus/receptor interaction by inducing an active local immune response to AF/R1 antigen. In vitro, specific anti RDEC-1 IgA blocks adherence of organisms to gut membranes. In vivo, passive adminstration of antibody via the colostrum prevents neonatal E.coli infection of pigs. To determine whether active oral immunization with colonization factor antigens can protect against infection, we orally immunized adult rabbits with pilus adhesins, challenged them with an infectious dose of the adherent rabbit enteropathogen RDEC-1, and determined the degree of colonization of intestinal segments. AF/R1 pili were prepared from RDEC-1. 2 kg rabbits were orgastrically immunized with 5, 1mg doses of pili at 4 day intervals and challenged with 10^7 viable Nalidixic acid (Nal) resistant RDEC-1 organisms in bicarbonate 1 day prior to the last pilus dose. Rabbits were sacrificed at 5 days and RDEC-1 counts/gm determined from dilutions of jejunal, ileal and cecal homogenates after overnight growth on MacConkey agar containing Nal. RDEC-1 challenge is known to cause diarrheal disease in 70-80% of animals in 7-10 days with heavy cecal, lesser ileal, and least jejunal colonization. 5 days following challenge, colony counts in control animals (N=7) expressed as log (+/-SEM) of the geometric mean counts were: jejunum 3.9 (+/-0.9), ileum 4.8 (+/-1.1), cecum 5.8 (+/-1.1). Counts in animals who received pili (N=5) were: jejunum 2.2 (+/-1.0)($p < .05$), ileum 2.1 (+/-1.0), ($p < .05$), cecum 2.3 (+/-1.0)($p < .05$). p values by Wilcoxon rank sum and one-tailed t-tests

indicate decreased geometric mean counts in the inoculated animals. In summary, oral inoculation with AF/R1 pili from RDEC-1 limited colonization in the ceca of animals challenged iwth an infectious dose of RDEC-1. These results suggest that oral immunization with adherence factors may prevent enteric disease by limiting intestinal colonization.

Although many tissue and host specific, non-mannose sensitive (NMS) adhesins have been described for enteroadherent E.coli, the role of Type-1, mannose sensitive (MS) pili in intestinal adherence is uncertain. We tested the ability of several E.coli strains, grown to promote Type 1 (MS) or NMS pilus expression, to interact in vitro with intestinal brush border membranes (BBM). E.coli strains tested were RDEC-1 (rabbit enteropathogen), 640 (rabbit fecal commensal), HS (human fetal commensal), H10407 (human enteropathogen) and B_{AM} (Type 1 prototype). Type 1 pilus expression was demonstrated by MS guinea pig hemagglutination, colonial morphology on minimal medium and agglutination with anti-Type 1 antisera. Bacterial adherence to BBM was shown by aggregometry and by phase microscopy. Type 1 pili on RDEC-1 mediated MS adherence to rabbit, rat and guinea pig BBM. In contrast, AF/R1 pili on RDEC-1 mediated species-specific (rabbit, not rat or guinea pig) NMS adherence to BBM. Expression of Type 1 pili on other E.coli strains tested did not mediate BBM adherence. These results indicate that Type 1 pili are a functionally heterogeneous group which, in some intestinal E.coli, might contribute to in vivo enteroadherence.

ii. Role of Host Immune Mechanisms

The isolated rabbit ileal lamina propria mononuclear cells contain uncommitted lymphocytes, T lymphocytes and monocytes as reported in the 1981 and 1982 Annual Progress Reports. Further characterization of this mononuclear cell population has been carried out and reveals the cells to be 50-80% viable by trypan blue dye exclusion; 45% monocytes, macrophages, or epithelial cells by positive non-specific esterase stain· 55% lymphocyte by negative non-specific esterase stain 20% epithelial cells and 80% mononuclear cells by Wright-Giemsa stain; less than 2% B lymphocytes by immunofluorescent stain for surface immunoglobulin; and 20% T lymphocytes by indirect immunofluorescent stain using 9AE10 monoclonal antibody specific for rabbit T lymphocytes. These results clearly indicate that 35% of the lymphocytes are neither T lymphocytes nor B lymphocytes. Since these non-T non-B lymphocytes comprise the largest percentage of the lymphocyte population at the level of the lamina propria, their properties and function (possible T lymphocyte precursors) such as capability of being sensitized for effector function and/or regulatory function remain

to be determined. The study of both T lymphocytes and the non-T non-B lymphocytes will be greatly enhanced by using the fluorescence activated cell sorter (FACS) in collaboration with T. Jerrell, Dept. of Rickettsiology. Fixed T lymphocytes with indirect immunofluorescent stain using the specific monoclonal antibody are currently being separated using the FACS. The separated cells are being studied with the electron microscope to determine the morphology and if the unusually small mononuclear cells 25-50 u^3 seen by electron microscopy and cell sizing are either the T lymphocytes or the non-T nonB lymphocytes or both. Separation of viable T lymphocytes is expected by this approach which will greatly enhance the in vitro immunization and challenge experiments testing synthetic peptide antigens leading towards the development of more effective synthetic peptide vaccines against enteric pathogens.

The synthetic peptide (GLY-ASN-THR-ILE-VAL-ALA-VAL-GLU) is the antigenic site on a 13AA peptide found to be immunogenic for rabbit ileal lamina propria lymphocytes in vitro. Preliminary results have indicated that the 13AA peptide immunogenicity is quite dose dependent and that at different doses it can also function as a toleragan These results indicate that this in vitro system with the addition of T lymphocyte separation and subsequent T lymphocyte/monocyte ratio adjustment might provide insight into dose requirements and mechanisms of immunization and tolerance induction of the immune system at the level of the intestinal lamina propria is possible.

Conformational restriction of the synthetic octapeptide in physiological solution has been found by on-going 600 MHz high resolution protein NMR study in collaboration with Axel Bothner-By at Carniege-Mellon University as reported in the 1981 and 1982 Annual Progress Reports. Ambiguity regarding the proton spectral assignments of VAL_5 vs VAL_7 still remained. In further experiments, changing the phosphate buffered saline pH from 7.4 to 3.0 protonated the C-terminal GLU carboxyl group. Due to the greater proximity of VAL_7 to GLU, a greater chemical shift is expected for the VAL_7 alpha hydrogen doublet then for the VAL_5 in the NMR spectrum. The experimental results are seen below:

Amino acid	THR	ILE	VAL_2	VAL_1
Chemical shift pH 7.4	4.361	4.215	4.156	4.125
Chemical shift pH 3.0	4.337	4.204	4.118	4.109
pH 7.4 and pH 3.0 Differences	0.024	0.011	0.038	0.016

The mean control difference (THR, ILE, VAL_1) ± SD is 0.017 ± 0.007. Therefore with a 99% confidence limit assignment, VAL_2

corresponds to VAL_7 and VAL_1, corresponds to VAL_5. Also noted were no changes in the rotameric side chain population at pH 3.0. This is important since the NH amide proton spectrum requires pH 3.0 to be seen. Further experiments are on-going to complete the NH amide proton spectral assignments.

The complete synthesis of the octapeptide GLY-ASN-THR-ILE-VAL-ALA-VAL-GLU has been accomplished by solid-phase synthesis as reported in the 1982 Annual Progress Report. Monitoring the solid-phase peptide synthesis deprotection and amino acid coupling efficiency has been significantly improved by developing a new rapid microanalytical chloride determination. Pyridine hydrochloride added to peptide containing resin suspended in CH_2Cl_2 form a reversible hydrochloride salt of any free N-terminal amino groups. An automated peptide synthesizer carried out the amino-HCl formation and removes the excess pyridine hydrochloride. Modifications developed for rapid monitoring include 1) an alternate preparation of pyridine hydrochloride, 2) use of a chloridometer which measures 0.01 to 10.0 umole Cl, 3) weighing 2-3 mg of dried resin with peptide attached (0.5 mmole amino acid substitution/g resin) to indicate when greater than 99.8% deprotection or coupling has occurred, and 4) a single tube preparation of the resin for chloride measurement.

In collaboration with J. Baker currently in the Clinical Investigation Service, WRAMC, a sensitive assay for detection of antibodies against the octapeptide has been developed using enzyme-linked technology (ELISA). This assay is sensitive to a dilution of 10^9 using a monoclonal antibody developed with K. Esser, Dept of Immunology. This assay clearly demonstrated the monoclonal antibody to be IgM isotype since blocking inhibition studies would be precluded with this isotype antibody further collaborative studies were carried out with H. Kaprowski, Wistar Institute. Several IgG isotype monoclonal antibodies were found which should be ideal for blocking inhibition studies using analogs of the octapeptide.

To ultimately enhance the intestinal secretory immune response, a rabbit model has been developed. Human breast milk supernatant has been reported to induce isotype specific B-lymphocyte activation of human peripheral blood lymphocytes. A similiar substance in the rabbit and what effect it may have upon the gut-associated lymphocytes was determined. Lymphoid cells from Peyer's patch, mesenteric lymph node, laminia propria, spleen, and peripheral blood were exposed to 7 day supernatants of rabbit milk cells. After 3 days of culture, supernatants were tested for total IgA, IgG, and IgM using a newly developed ELISA assay. No immunoglobulin responses were noted from the laminia propria or

mesenteric lymph node. Positive lymphocyte responses to the milk cell supernatants were seen in 5/6 rabbits giving elevated IgA in Peyer's patch and spleen; in 3/5 rabbits giving elevated IgA in peripheral blood lymphocytes; in 5/6 rabbits giving elevated IgG in peripheral blood lymphocytes; in 4/6 rabbits giving elevated IgG in Peyer's patch and in 3/6 rabbits giving elevated IgG in spleen. Autologous and isologous milk cell supernatants gave greater than one log fold IgG lymphocyte responses in Peyer's patch, and peripheral blood lymphocytes and greater than one log fold IgA responses in the spleen, Peyer's patch, and peripheral blood lymphocytes. Rabbit milk cells in culture spontaneously release a substance into the media which functions as a polyclonal B-cell activator upon rabbit Peyer's patch, spleen and peripheral blood lymphocytes. This activation is probably not an allogenic effect since autologous substance is as effective as isologous. These results indicate the possibility of purifying this substance and its use both in in vitro and in vivo experiments leading towards enhancement of secretory immune responses in the intestine.

iii. Alterations of Intestinal Transport

D-glucose uptake rate was studied in mucosal brush border membrane vesicles prepared from normal and cholera toxin-treated rat small intestine. The initial rate of D-glucose uptake, as measured by exposing the vesicles to the bathing solution for only 3 sec., was significantly higher in the vesicles pretreated with cholera toxin than in the untreated vesicles. The initial uptake rate was significantly decreased when chloride in the bathing solution is replaced by either isothionate or sulfate. These results are consistent with those obtained from Ussing Chamber-voltage clamp experiments.

The effect of external magnesium concentration on sodium and chloride transport across rat small intestine was studied in the Ussing Chamber under short-circuit conditions. The normal magnesium concentration in the Ringers solution is 1.2 mM. When magnesium was completely removed from the bathing solution on both sides of the tissue, the short-circuit current, potential difference and sodium absorption were increased. When magnesium was removed separately from either mucosal or serosal side, mucosal magnesium removal produced the same changes as those of removing magnesium from both sides and serosal magnesium removal had no effect on the short-circuit current and net sodium and chloride flux but some change in potential difference and conductance. When magnesium concentration was increased from 1.2 to 6.0 mM on both sides, the short-circuit current was decreased but the net sodium absorption was again increased. Either an increase in serosal or mucosal magnesium to 6.0 mM resulted in increase in net sodium

absorption, but only 6.0 mM magnesium in serosal solution decreased the short-circuit current. The mechanism of the changes in ion transport produced by varying magnesium concentration is still unclear. We will continue to study effects of magnesium on sodium transport across brush border and/or basolateral membranes in vesicle preparations.

Avascular segments of fetal rat small intestine transplanted to the subcutaneous tissues of syngeneic rats will become vascularized and develop certain properties of normal rat intestine. Conceivably, perfusion of these "neogut" segments with nutrients, or their anastamosis to native intestine may offer the potential for enteral nutrition in the subject with short gut syndrome. To further characterize this tissue, neogut was grown from segments of fetal small intestine transplanted to the subcutaneous tissues of host adult Sprague-Dawley rats. Approximately 10% of the intestines developed adequately for study. We examined the electrophysiologic parameters of short circuit current (Isc), potential difference (PD), and tissue conductance (G) of full thickness pieces of neogut (n=8) and compared them with adult rat ileum (n=15) mounted in Ussing chambers. Transport of d-glucose ($uM/cm^2 \cdot hr$) from mucosal to serosal surface and the reverse was assessed by measuring flux (J) of C^{14}-glucose across the tissue in the chamber (neogut n=4, ileum n=8). Anatomy was studied with light microscopy, scanning and transmission electron microscopy (EM). Electrophysiologic and flux data expressed as mean ± standard error of mean:

	PD (mv)	Isc ($\mu eq/cm^2 \cdot hr$)	G ($mmho/cm^2$)	Glu J M S	Glu J S M
NEOGUT	.9 ± .2	.7 ± .1	19 ± 3	.85 ± .54	.07 ± 0.1
ILEUM	3.1 ± .3	3.2 ± .4	29 ± 2	.76 ± .17	.25 ± .06

Light microscopy demonstrated neogut to be similar to normal ileum having a mucosa composed of mixed epithelial cell types; submucosa and a bilayer muscularis were intact. However, scanning EM showed the neogut epithelium has a cobblestone texture with flattened villi relative to normal ileum. Transmission EM demonstrated neogut columnar epithelial cells to have prominent basal nuclei and abundant rough endoplasmic reticulum and mitochondria in the apical cytoplasm similar to the absorptive epithelial cells of normal ileum. Tight junctions between adjacent cells and short, even microvilli were present. These data show that neogut has structural and physiological characteristics similar to normal intestine. Refinement of techniques to maximize these properties offers hope that this tissue may provide a nutritionally useful accessory gut.

The level of total rat small intestine brush border protein phosphorylation is decreased by the addition of trifluoperazine (100 uM) in the presence of Ca^{2+} (1mM). Addition of exogenous calmodulin (1 uM) significantly increased the total phosphorylation of intestinal brush borders and this increase was eliminated in the presence of trifluoperazine. Specific brush border protein phosphorylation in the presence of trifluoperazine, demonstrated a decreased incorporation of ^{32}P into a single brush border protein (98,000 m.w.). The specific phosphorylation of this same protein was increased by the addition of exogenous calmodulin. The addition of cGMP (5 uM) did not affect phosphorylation of the trifluoperazine sensitive protein, (98,000 m.w.) but did increase the phosphorylation of another brush border protein (86,000 m.w.). Trifluoperazine did not affect the cGMP-induced increase in the phosphorylation of the lower molecular weight protein. Purified microvillus membrane proteins did not contain the 98,000 molecular weight protein which was present in brush borders and whose phosphorylation was inhibited by trifluoperazine.

Techniques are currently available to assess intestinal transport in humans quantitatively. A triple human intestinal perfusion system has been developed and successfully used in eight volunteers. Results obtained from perfusion of standard solutions agree with values reported by other laboratories.

Intestinal transport may be effected by systemic factors such as intravascular volume depletion. As volume-depletion is a frequent concommitant of diarrheal illness, hormonal factors may ameliorate viral or bacterial-mediated intestinal secretion. Preliminary results document increased absorption of water, sodium, and chloride under conditions of short-term salt depletion in normal volunteers.

As preliminary results suggest effective sodium and water conservation by the ileum in sodium deprived normal human volunteers, the effect of salt deprivation on cholera-toxin-mediated intestinal secretion will be assessed using the rat in vivo perfused intestinal loop model.

iv. Alterations in Intestinal Motility

We have previously shown (Annual Progress Report 1982) that in rabbit ileal loops bacterial enterotoxins produce a diarrheogenic motility pattern, the migrating action potential complex (MAPC) and that this motility pattern is responsible for peristaltic movement of luminal contents. This abnormal motility pattern may contribute to the diarrhea and abdominal cramps of enteric infection. We also showed that MAPC's were evoked by binding of a specific mucosal

receptor by the B subunit of cholera toxin and raised the possibility that potential B subunit vaccines might have untoward effects on intestinal motility.

In investigating the MAPC further, we have currently shown that in addition to enterotoxins, certain lectins, mucosal damage and luminal distension may evoke this response. The lectins ricin and wheat germ agglutinin (WGA) induce MAPC's in rabbit ileal loops, raising the possibility that certain ingested foods may cause abnormal motility patterns. These lectins bind different mucosal receptors than does cholera toxin (CT). Unlike CT and WGA, ricin also produces mucosal damage and inflammation, and induces a second abnormal motility pattern, repetitive bursts of action potentials (RBAP). MAPC's and RBAP's were produced in the absence of inflammation and lectins when mucosal damage was induced by intraluminal instillation of hypertonic sodium sulfate. MAPC's were also induced by stretch of the intestinal wall by luminal fluid distension. Stretch receptors, presumably located in the submucosa, stimulate different afferent neurons than the mucosal enterotoxin receptors. Quantitatively, luminal distension was the strongest stimulus and enterotoxin the weakest stimulus for MAPC generation. When the effects of CT and luminal distension were combined, the resulting MAPC frequency was greater than CT alone and similar to luminal distension alone. We therefore suggest the MAPC is a peristaltic intestinal response to a variety of sensory stimuli that occur in the setting of enteric infection: enterotoxins, dietary lectins, mucosal damage and luminal distension. Although the pathways for these responses are unknown, we suggest that they may represent multiple sensory afferents converging on a few intramural interneurons or/and ganglion cells that utilize a single efferent motor pathway. Therapeutic modulation of intestinal motility by selective pharmacologic intervention of afferent pathways or by non-selective intervention of efferent pathways awaits more specific definition of the receptors and pathways involved.

Initial attempts to define intrinsic pathways utilizing an <u>in vitro</u> system for suspension of extrinsically denervated loops of bowel in an oxygenated, nutrient bath (described in the Annual Progress Report 1982) have continued to present problems in maintenance of tissue viability. Therefore, a different model employing transplantation of extrinsically denervated and devascularized fetal rat intestine to back and abdominal subcutaneous tissue of host syngeneic rats has been developed in collaboration with CPT. B.L. Bass and LTC, J.W. Harmon, Division of Surgery, WRAIR. The transplanted intestine has been shown to become vascularized, to grow to adult proportions and to exhibit what visually appears to be peristalsis. The anatomic and

transport characteristics of this transplanted tissue are similar to normal ileum. Electrophysiologic studies of the smooth muscle in the transplanted intestine show that it spontaneously generates both slowly fluctuating membrane potentials (slow waves) and action potentials (spikes). Some of the action potentials conform to MAPC's providing electrical confirmation that the muscle is capable of contraction and peristalsis.

Little additional work has been done on simultaneous measurements of fluid transport and myoelectric activity utilizing in vivo perfusion of intestinal loops with C^{14} PEG. Delay in this important work resulted from our previously mentioned observation that luminal distension is a strong stimulus for spike activity. Careful monitoring of degree of distension induced by the perfusion will be required.

Continued development of a model for recording chronic intestinal myoelectric activity from unanesthesized rabbits as being more physiologic than the acute in vivo ileal loop model (Annual Report 1982) has resulted in improvements in surgical technique, electrode design and placement resulting in significant improvement in the quality of electrical signals. However repeated restraint of rabbits for serial recordings resulted in airway trauma and respiratory embarassment. Therefore, studies were halted and an electrical swivett assembly developed to allow recording from unrestrained rabbits. This swivett assembly is presently undergoing testing. In addition, extension of the chronic rabbit model to incorporate a surgically created jejuneal blind loop has significantly enhanced the pathogenicity of RDEC infection. We suspect that the mechanism may be altered motility allowing enhanced bacterial growth and mucosal adherence.

A non-human primate model for recording chronic intestinal myoelectric activity from unanesthesized, chair adapted monkeys has been developed. Our initial work has been in validation of the model by comparing fasted and fed changes of a normal motility pattern, the migrating myoelectric complex (MMC), as monitored by computer analysis of spike burst activity. Differences in these patterns have been shown to be partially mediated by alterations in blood glucose concentrations.

Computer programs that allow separate analysis of slow wave frequency and spike activity (MAPC, MMC and unpatterned spike activity) have been validated and reported. As a result of this enhanced capability, a new motility pattern, the stationary action potential complex (SAPC), has been recognized and reported. Additional development of computer analysis techniques has been recognized and new hardware is in the process of acquisition.

FUTURE PLANS AND RECOMMENDATIONS

1. Role of Mucosal Adherence in Bacterial Colonization

Studies of the use of purified E.coli adherence antigens as mucosal immunogens to protect aganist enteric infection with adherent enteropathogenic organisms will continue both in animals and in human studies. The decrease in colonization of intestinal segments of rabbits with the pathogen RDEC-1 following oral inoculation with AF/R1 pili is encouraging. These results may have been the result either or stimulation of a local antibody response or the blocking of the mucosal receptor sites by binding of the purified attachment factors to the intestine. Experiments will be continued to differentiate between these effects in the rabbit model and to determine the optimal timing, dose, and route of adminstration of pilus antigen to induce a local secretory response. This approach will be expedited by the use of the Thiry-Vella loop model. Results obtained in the past year indicating that antibody secretion in the intestine is represented by antibody secretion in the isolated loop will be utilized to study sequential local antibody responses following various immunization regimens.

Additional studies with CFA/II (CS3/CS1) will be undertaken to differentiate whether the poor immunogenicity observed with the oral preparation was the result of failure of the delivery of the antigen to the mucosal immune system (ie the Peyer's patch epithelium). Several plausible reasons for failure of delivery of the orally administered antigens exist. The antigens may have been altered by gastric acidity or more likely by pancreatic proteases despite attempts to protect with antacid and cimetidine. Alternatively, the antigen may have been taken up by the absorptive epithelial cells for which it has a demonstrated affinity and thereby effectively prevented from reaching the mucosal immune systems. Alternatively the antigen may have been adversely effected by the method used for sterilization of the oral preparation ie gamma radiation. Additional experiments will be performed to measure local immune response following direct administration of the antigen preparation into the small intestine via small intestinal tube. Antigens will be prepared following alternate methods of sterilization in particular by low dose formalin to determine whether gamma radiation and the deleterious effect on the antiger. Ultimately encapsulated methods of antigen delivery may have to be utilized to delivery an orally administered protein antigen to the mucosal immune system. In vitro studies will also be undertaken to determine the influence of acid environment strongly basic environment and proteases on the antigenicity of the antigen preparations as measured ELISA system.

The rabbit model of RDEC-1 will be used to explore these possibilities since it provides a model for pilus (fimbria) mediated enteroadherence. Studies in the present year confirmed the expression of AF/R1 pili in RDEC-1 infection in vivo since antibody to AF/R1 developed in the course of infection and oral administration of AF/R1 decreased RDEC-1 colonization. Studies will be undertaken in the rabbit Thiry-Vella loop model to determine whether pili with high affinity for the rabbit intestinal mucosal cells ie AF/R1 have greater or less immunogenicity than those with low affinity for the rabbit mucosal cells ie CFA/II in which significant local immunogenicity has been demonstrated in the rabbit. These studies will directly explore the hypothesis that binding of an adherence antigen to the mucosal cells inhibits antigen exposure to the mucosal immune system.

Recent demonstration that type 1 pili on RDEC-1 confer in vitro mucosal adhesiveness to enterocyte membranes have reopened the question of the role of type 1 pili in intestinal colonization and the possible role of type 1 pilus as a protective immunogen for a broad range of enteric E.coli infections. The antigenic and functional heterogeneity of pili classified as type 1 is currently being investigated. It may be possible to provide protection if attention is paid to the common antigenic determinants of type 1 pili rather than to their variable regions.

Monoclonal antibody to AF/R1 pili is being developed in collaboration with the Dept. of Bacterial Diseases. These antibodies will have multiple applications including purification of AF/R1 components for large scale preparation of pilus antigens, examination of pilus binding and antigenic sites, evaluation of the relation of AF/R1 pilus to other adherence antigens, localization of AF/R1 pilus by immunofluorescence during in vivo infection, and quantitation of pilus expression on E.coli using inhibition of ELISA. This last application will have particular value in investigating the optimal growth conditions for production of E.coli pilus antigens.

ii. Role of Host Immune Mechanisms

The synthetic peptide (GLY-ASN-THR-ILE-VAL-ALA-VAL-GLU) is an antigenic site for rabbit ileal lamina propria T-lymphocytes and contains a smaller antigenic site for antibody binding. The larger peptide antigenic site for the T-lymphocyte suggests that conformation of the antigenic site may be more important for the T-lymphocyte antigen receptor then for antibody binding. Since the conformation of the octapeptide is now known, the development of two analog series may be possible with the use of the already

established computer assisted molecular modeling utilizing energy minimization strategies. One series would have basically very similiar over-all conformations but maximally different primary structures and the other series would have very dissimilar conformations with minimal changes in primary structure. These analogs would then be synthesized using the already established peptide synthesis capability. The peptides conformations would actually be determined using the 600 or 750 MH_2 high resolution proton NMR facility at Carniege-Mellon University. The analog peptides would be tested for the presence or lack of cross-antigenicity with the original octapeptide using rabbit ileal lamina propria T-lymphocytes in vitro and antibody binding. This study will be testing the hypothesis that the conformation of a peptide antigenic site is more critical then the amino acid sequence for recogniton by sensitized T-lymphocytes in contrast to antibody binding where the opposite is true. Several peptide analogs may be found which give good T-lymphocyte cross reactivity but no cross reactive antibody binding. These peptide analogs would then be prototypes for further development of oral vaccines. The analogs would be conjugated to synthetic polypeptides having specific receptors on enterocytes and possibly further conjugated to a synthetic adjuvant such as muramyl dipeptide. The conjugates will also be tested in vitro for maintenence of original analog antigenicity. The immunogenic conjugates will also be tested for mucosal absorption and ability to sensitize a chronic ileal looped rabbit. Ultimately it will be determined if rabbits can be protected from enteric pathogens following oral immunization with the apppropriate synthetic peptide vaccines.

iii. Alterations of Intestinal Transport

Substances to be absorbed or secreted by the intestine must cross at least two barriers in series, the membranes at the two sides of the epithelial cell layer. In order to have net active transport across this series array, the membranes at the two sides must have different functional properties. For the past several years, much of our research has been directed toward obtaining information on the transport properties across the whole intestine. Over the next year we intend to add to our research the experimental approach designed to obtain information on the transport properties of these individual barriers and to understand how the barriers contribute to the overall properties of the intestine. Influx studies of ions and non-electrolytes across mucosal membrane using influx chambers and vesicle preparations are planned. The initial experiments will be to estimate the glucose: Na^+ coupling coefficient in the brush border membrane.

Further studies involving the anti-secretory agents berberine should include 1) the effect of berberine on the glucose-induced absorption of water and electrolytes and 2) establishing a correlation between berberine-induced changes in the phosphorylated protein intermediate and berberine-induced changes in cyclic nucleotide-induced electrolyte transport.

Drugs of potential use in diarrheal diseases including potential absorption stimulators, such as dopamine, bromocriptine etc. and potential secretion inhibitors, such as trifluoroperazine, chlorpromazine, fluphenazine etc., will be evaluated in terms of their effects on the basal and bacterial toxin-altered intestinal water and electrolyte transport. These drugs will also be evaluated on the basis of the alterations which they cause in cyclic nucleotide concentration and membrane phosphorylation levels; as well as the possible involvement of calcium and calcium binding proteins.

Finally, transport studies in rat small intestinal vesicle preparations and human subjects will receive special attention in the next year.

iv. Alterations in Intestinal Motility

Future studies on the effects of enteric infection, enterotoxins, dietary lectins, mucosal injury, inflammation and luminal distension on intestinal motility will utilize three approaches: (1) acute myoelectric recordings from in vivo intestinal loops; (2) in vitro micropuncture of single cells and tissue strips; and (3) chronic recordings in vivo from animals with implanted electrodes.

Pharmacologic study of acute in vivo intestinal loops in rabbits and in transplanted fetal rat intestine will examine the neurohumoral pathways (particularly those intrinsic to the gut) for slow waves, MAPC and RBAP generation. Additional lectins will be surveyed to assess the frequency of motility altering lectins in diets. Loop perfusion with C^{14} PEG under careful monitoring of luminal distension (probably by maintaining a perfusion pressure that is subthreshold for MAPC generation) will allow investigation of the relationship between motility and transport.

Development of single cell isolation and micropuncture techniques for in vitro isolation of mucosal, ganglionic and smooth muscle cells and tissue strips will allow direct investigation of cell membrane receptors and intracellular mediators (such as calcium and cyclic nucleotides) and their effects on transmembrane electrical potentials, transport and tension development. In

addition, functional characterization of isolated cells from various muscular layers as well as their interactions with each other and with the myenteric nervous plexus and mucosal cells could be determined. Elucidation of neurohumoral mechanisms and intercellular communications at a cellular level will be extremely important in analyzing the net responses of the organism to enteric infection and in assessing potential areas for therapeutic intervention.

Utilizing an electrical swivett assembly, in vivo studies of unrestrained rabbits with chronically implanted electrodes will resume. Normal fasting and fed motility patterns will be established. Studies performed in acute in vivo intestinal loops will be expanded in this more physiologic model. The effects of pharmacologic agents and native infection (RDEC) on motility, morphology and flora will also be studied. Extension of this model to create a jejuneal blind loop may enhance the effects of RDEC and allow induction of infection pathogenic for humans (Shigella, Salmonella, Cholera, Invasive E.coli). Subcutaneous tunnelling of a loop of bowel to allow sterile cutaneous puncture would enable periodic quantitative culture of luminal contents. Implantation of electrodes on Thiry villa loops would enable direct instillation of microorganisms, toxins and pharmacologic agents into the study loop, permit studies of motility effects in non-contiguous intestine and allow simultaneous transport studies using C^{14} PEG perfusion to be serially performed on unanesthesized animals. Implantation of electrodes on transplanted fetal rat intestine will permit chronic study of motility from extrinsically denervated intestine in unanesthesized animals. Studies from these isolated in vivo intestinal segments should greatly clarify the role of extrinsic and intrinsic factors in the control of motility.

Future in vivo studies using the non-human primate model will assess the effects of enteric infection pathogenic for humans (Shigella, Salmonella, Invasive E.coli, hepatitis A), enterotoxins, dietary lectins, mucosal damage and luminal distension on motility, intestinal flora, transport and morphology. Using data gained from rabbit and rat studies, the neurohumoral pathways involved in these responses and potential sites for pharmacologic intervention will be assessed. Development of an implantable radiotelemetry apparatus to broadcast signals from intestinal electrodes thereby enabling recording of motility from unrestrained monkeys could be a significant improvement in experimental design.

Continuing development of computer software will interface with recent hardware acquisitions which allow greatly expanded memory, faster processing and continuous Fourier analysis of myoelectric signals. These advances will allow simpler and more

accurate and complete capture of myoelectric signals translating into better frequency analysis, noise reduction, timing and spike quantitation. These improvements in signal processing may permit capture of signals from smaller and less invasive probes and will allow direct correlation of slow wave and spike potentials. A longstanding tenet in motility is that slow waves are a basic control mechanism for spikes and hence intestinal contraction. Development of these programs should permit direct measurement and testing of the relationships and control mechanisms of myoelectric events. In addition, second generation computer programs will permit simultaneous sampling from a greater number of electrodes, expand pattern recognition to RBAP and minute rhythm, and allow assessment of event propogation between electrodes. Utilizing this program analysis of colonic motility patterns, which are much more complex than those of the small intestine, may become possible. Meaningful assessment of colonic, as well as small bowel, motility and transport is essential before definitive conclusions about the net effect of motility in clinical diarrheal states and therapeutic responses can be addressed (ie. are observed small bowel events compensated or exaggerated by colonic responses).

With the development of less invasive oral pressure, myoelectric and radiotelemetry probes and more accurate computer programs for data collection and interpretation, extension of these studies to patient populations should be possible. Eventually studies correlating myoelectric, pressure, transit, transport and microbiologic events should be performed.

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PROJECT 3M162770A871

PREVENTION OF MILITARY DISEASE HAZARDS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6531	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORD'S SUMRY	8a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AI	154 WWGH
b. CONTRIBUTING				
XXXXXXXXX	STOG 82/83-6	2/3		

11. TITLE (Include with Security Classification Code)

(U) Prevention and Treatment of Plague

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PREVIOUS 83	1.0	338
	CURRENT 84	1.0	318

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

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TELEPHONE: 301-427-5176 or 5110

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: HARRISON, D N POC: DA

NAME:

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Provide each with Security Classification Code) (U) Yersinia pestis; (U) Plague; (U) Diagnosis; (U) ELISA; (U) Serology; (U) Antigenemia; (U) Vaccines; (U) Immunization

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code)

23. (U) Develop capabilities to diagnose, prevent, treat and control plague to protect troops against pneumonic and bubonic disease.

24. (U) Rapid diagnostic tests are developed and evaluated using clinical specimens. New plague vaccines and therapeutic drugs are assayed for efficacy in animal models. Strains of Y. pestis are examined for virulence and antibiotic susceptibilities.

25. (U) 82 10 - 83 09 Sensitivity of the ELISA for specific Fraction 1 (F1) antigen of the plague bacillus was improved, and it will now detect 50 picograms of F1 (i.e., 1 nanogram/ml. This test and similar ELISA procedures that measure titers of IgG and IgM antibodies were incorporated into a kit. Shelf-life studies have shown that reagents in the kit are stable for at least a year if stored in the refrigerator and for three months at 20 degrees Centigrade. The kit was field-tested in South Africa using specimens from both bacteriologically confirmed cases of plague and clinically suspect cases that occurred during 1982-1983 in northern Namibia. ELISA proved to be much more sensitive than the passive hemagglutination procedure used previously in that region. ELISA for F1 antigen also was examined for its ability to detect antigenemia in laboratory rats with acute disease. High levels of F1 antigen were detected. A procedure was developed that employs enzyme-labelled staphylococcal protein A for tests of rodent sera which otherwise could not be tested by ELISA. A study was completed to compare lots of F1 antigen produced in the United States, South Africa and the USSR using high-performance liquid chromatography. All lots contained contamination with other proteins. A micro-test plate was constructed to quickly determine biochemical reactions for isolates suspected of being Y. pestis. Seven isolates could be tested for 11 biochemical characteristics in each 96-well plate. The prototype was field-tested in South Africa. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

* Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

WORK UNIT 154 PREVENTION AND TREATMENT OF PLAGUE

Investigators:

Principal: MAJ James E. Williams, MSC

Associates: Daniel N. Harrison, Ph.D.
SSG Carol A. Braden
SSG Guy L. Tyndal
William L. Roelke, Jr., MS
PFC Vincent D. Palmer

Problems and Objectives

Plague remains a threat to unvaccinated and possibly to vaccinated military and civilian populations in many parts of the world, especially in catastrophic or wartime situations that preclude application of modern sanitary practices. In addition, there exist potentials for plague from variant bacilli that are resistant to therapeutic drugs and from nonencapsulated strains that are not detected by the serological tests now used for diagnosis. The objective is to improve capabilities for protecting troops against pneumonic and bubonic plague. The current research program focuses on three requirements for reducing the impact of plague on soldiers in combat: i) rapid and sensitive procedures for use in the field to diagnose plague infections, to evaluate drug susceptibilities for effective therapy, and to characterize new strains of the pathogen for military intelligence, ii) improved techniques for the production and assay of plague vaccine to insure that sufficient supplies of efficacious vaccines could be prepared quickly during emergencies, and iii) a forecasting system to facilitate determinations of where and when plague surveillance or control activities may be necessary.

Progress

The research emphasis in FY83 was on the development of field-portable tests for the rapid diagnosis of plague. Sensitivity of the ELISA for specific Fraction 1 (F1) antigen of the plague bacillus was improved, and it will now detect 50 picograms of F1 (i.e., 1 nanogram/ml). This test and similar ELISA procedures that measure titers of IgG and IgM antibodies were incorporated into a kit. Shelf-life studies have shown that reagents in the kit are stable for at least a year if stored in the refrigerator and for three months at 20°C. The kit was field-tested in

South Africa using specimens from both bacteriologically confirmed cases of plague and clinically suspect cases that occurred during 1982-1983 in northern Namibia. ELISA proved to be much more sensitive than the passive hemagglutination procedure used previously in that region for the diagnosis of plague. The ELISA for F1 antigen also was examined for its ability to detect antigenemia in laboratory rats with acute disease. High levels of F1 antigen were detected in the blood and in other body fluids and tissues at 3 days post infection with virulent plague bacilli. These results indicate that the ELISA for F1 antigen will be valuable for rodent plague surveillance and control efforts. In addition, a procedure was developed that employs enzyme-labelled staphylococcal protein A for tests of rodent sera which otherwise could not be tested by ELISA, as species-specific conjugates are not available. The ELISA with protein A was evaluated using sera of laboratory infected California ground squirrels (Spermophilus beecheyi). ELISA procedures and all other kinds of serological tests currently in use for plague diagnosis are based on the F1 antigen of Y. pestis. A study was completed to compare lots of F1 antigen produced in the United States, South Africa and the USSR using high-performance liquid chromatography (HPLC). All lots of F1 examined by HPLC contained contamination with other proteins, but the USSR preparations of F1 antigen were the cleanest. Efforts to develop monoclonal antibodies to specific Yersinia pestis proteins other than F1 for use in new diagnostic tests have continued. A prototype of a micro-test plate was constructed to quickly determine biochemical reactions for isolates suspected of being Y. pestis. Seven isolates could be tested for 11 biochemical characteristics in each 96-well plate. The prototype was field-tested in South Africa and found to be deficient in some respects. Several improvements were achieved subsequently, but the requirement for this customized test plate has been reduced somewhat. In ongoing evaluations of commercially available systems, we have now identified a product that is simple to use, portable, and can be used efficiently to identify Y. pestis in 4 hours if care is taken in the interpretation of certain reactions that may vary from the expected. The product was evaluated in tests of 100 strains of Y. pestis from various regions of the world. A similar search among commercial products for an effective test system to rapidly determine antibiotic susceptibilities for Y. pestis isolates has not been successful, as yet, so development of such a capability has been given added emphasis.

Recommendations

Rapid tests to diagnose plague based on specific Y. pestis proteins other than F1 antigen are needed to recognize infections

with variant plague bacilli. Additional work is required on the established ELISA tests for measuring antibodies to F1 antigen to reduce the time required for testing, improve field portability and remove residual "background" coloration for increased sensitivity. The development of a portable kit to rapidly determine drug susceptibilities should be emphasized. Evaluations of new anti-bacterial drugs for chemotherapeutic value against plague should be undertaken. Work on computerized techniques to improve forecasting capabilities should be continued.

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PROJECT 3S162772A874

METHODS AND TECHNIQUES FOR COMBAT CASUALTY MANAGEMENT

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OG 6770	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORD'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AG	181 WWL4
b. CONTRIBUTING				
c. ~~XXXXXXXXXX~~	STOG 87/83-6.2/4			

11. TITLE (Provide with Security Classification Code):
(U) Management of Military Blast Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	2.0	169
	CURRENT 84	2.0	239

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE
FINA

ASSOCIATE INVESTIGATORS
NAME: SAMPSON, J POC: DA
NAME:

22. KEYWORDS (Provide EACH with Security Classification Code): (U) Blast injury; (U) Pulmonary dysfunction; (U) Gastrointestinal hemorrhaging; (U) Pulmonary hemorrhaging; (U) Medical/Surgical Treatment

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23(U) This project proposes to respond to the threat of potential blast related problems which may be experienced by the Army in the field. Our ultimate goal is to optimize the treatment of blast-injured casualties. Toward this goal we are evaluating techniques which will allow documentation of the natural history of gastrointestinal and pulmonary injuries. The threat of exposure of American soldiers to blast waves from enemy weapon systems which may exceed established thresholds is increasing.

24(U) Fuel-air explosives will be used to create injuries in two different species to determine if the distribution of injuries are similar. The advantages and disadvantages of utilizing these two species of animals will be enumerated and evaluated. The rapid technique for the in vivo estimation of lung water content using a double-dilution technique will be assessed in the laboratory to determine the reliability, repeatability and accuracy of this technique so that the natural history of blast injuries can be followed.

25(U) 82 10-83 09 A review of the world experience with gastrointestinal effects of blast injury was prepared and published in Military Medicine. Plans for a Laboratory Blasting Device were obtained from Great Britain and the device is being built. Liaison was developed with the BRL at Aberdeen Proving Grounds, and it is expected that we will participate in blast testing programs at APG. For technical reports see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

* Available to contractors upon originator's approval

Project 3S162772A874 METHODS AND TECHNIQUES FOR COMBAT CASUALTY MANAGEMENT

Work Unit 181 Management of Military Blast Injury

Investigators:
Principal: John W. Harmon, LTC, MC
Co-investigator: John A. Sampson, CPT, MC

Background and Objectives:

The human body cannot tolerate blastwave environments for overpressures greater than a few psi without sustaining severe-to-lethal damage. Blastwaves with significant overpressure can be created either accidentally or in a hostile battlefield environment. Very little is known about the diagnosis and care of blast injured casualties primarily because, in general, the magnitude of the blast overpressure is unknown and the injuries, which could range from mild to severe, are primarily internal. The object of the current effort is to evaluate battlefield airblast threats. The battlefield airblast threat is quite significant due to the number and variety of weapons, both conventional and nuclear, for which blast overpressure is a primary kill mechanism. Such weapons can range from small hand grenades containing one pound of condensed high explosives to 8,000 pound bombs, 2,000 pound fuel-air explosive weapons (8,000 pound HE equivalent), small tactical nuclear weapons in the few kiloton yield range or multi-megaton nuclear devices. The blastwave profile to which an unprotected soldier is subjected, of course, depends on both the yield or energy release of the weapon as well as the standoff or range at which the soldier is located from the center of the explosion. These factors are accounted for in the threat definition.

In addition to defining the airblast threat, a series of airblast environment exposure tests were performed in which the S-Cubed fuel-air explosive (FAE) blast source was used to subject animals to lethal doses (LD) of airblast overpressures that ranged between 30 and 90 percent (LD_{80} - LD_{90}). The WRAIR team of pathologists, veterinarians and technicians examined in detail the damage sustained by various vital organs to determine the degree and nature of the damage. The static and stagnation pressures as functions of time were measured at the ranges at which the animals were placed. The ultimate objective of this part of the program is to correlate airblast overpressure profiles with tissue damage and eventually develop techniques to care for and cure blast injured casualties.

Progress:

1. Threat Definition. During this fiscal year an Air Blast Threat Definition was prepared under contract by S-Cubed for the WRAIR COBIC program. A major aspect of this definition was a nomogram for estimating lethality from fuel-air explosives. The nomogram is included as Figure 1. In order to illustrate how the nomograph is used, a dashed line has been placed on Figure 1. Assume a 100-pound bomb has exploded subjecting a person to an overpressure of 50 psi. The dashed line starts at 50 psi on the vertical scale and moves horizontally to the overpressure curve. From that curve, the dashed line goes vertically downward crossing the scaled range at a value of $r/w^{1/3}$ of 4 and continuing downward to the yield curve of 100 pounds in the lower right-hand quadrant. The line then goes to the left crossing the range scale at 20 feet and continues to the left into the lower left-hand quadrant which provides an estimate of the degree of damage sustained. The dashed line continues in that quadrant until it is directly beneath the 100 pound yield scale. It is seen that for this case, the injuries would be in the region of severe-to-lethal. If, on the other hand, it was known that a person was 20 feet from a 100-pound bomb that was detonated, the point in the damage curve could be found immediately by starting at the lower vertical range curve and moving to the left. If it were of interest to know the pressure and impulse to which the person had been subjected. the dashed line could start at the lower vertical range scale and move to the right until it intersects the 100-pound yield line, then up to the pressure and scaled impulse curves.

2. Fuel Air Field Study. During this year, the data from the 1982 FAE field study in San Diego was collated and evaluated and the reports completed. This preliminary study identified the difficulties in obtaining a pure blast injury in the FAE environment. In the 1982 study, secondary and tertiary injury undoubtedly contributed to the injury.

During 1983, a new field study was planned. It incorporates a new animal sling which should minimize secondary and teritary injury. It utilizes the pig as an experimental animal as this animal seems to be as well suited as any for both pulmonary and gastrointestinal evaluation in an animal scaled similarly to man. The thrust of the new field study will be to correlate pathology and serum enzyme markers of injury.

3. Blast Behind Armor. The Ballistics Research Laboratory of the Aberdeen Proving Grounds has been asked to evaluate the environment within a US Armored Personnel Carrier which is penetrated by a high explosive. They have requested that the Division of Surgery, WRAIR, evaluate the effects of blast in these

circumstances. Planning an approach to this problem and getting support for this new aspect of our mission has been a top priority in the final quarter of fiscal 1983.

Recommendations for the Future:

1. Fuel Air Explosive Testing. At this time, it is still not known whether FAE's represent a unique blast threat, nor is it known how best to manage casualties from FAE's. Operationally, we will have to decide whether to continue to collaborate with S-Cubed which has excellent blast facilities, but has limited support for biologic testing, or whether to utilize the Lovelace facility in Albuquerque which could carry on better biological evaluations.

2. Blast Behind Armor. We expect that this will be our top priority project in 1984 because of intense Army interest in this project. We plan to devote personnel and money to this project to try to assist the BRL in its evaluation.

Publication:

Sedgwick, R.T. and Groethe, M.A. Airblast Threat Definition and Exposure Tests in Support of the Care of Blast Injured Casualties (COBIC) Program, SSS-R-83-5963, January 1983.

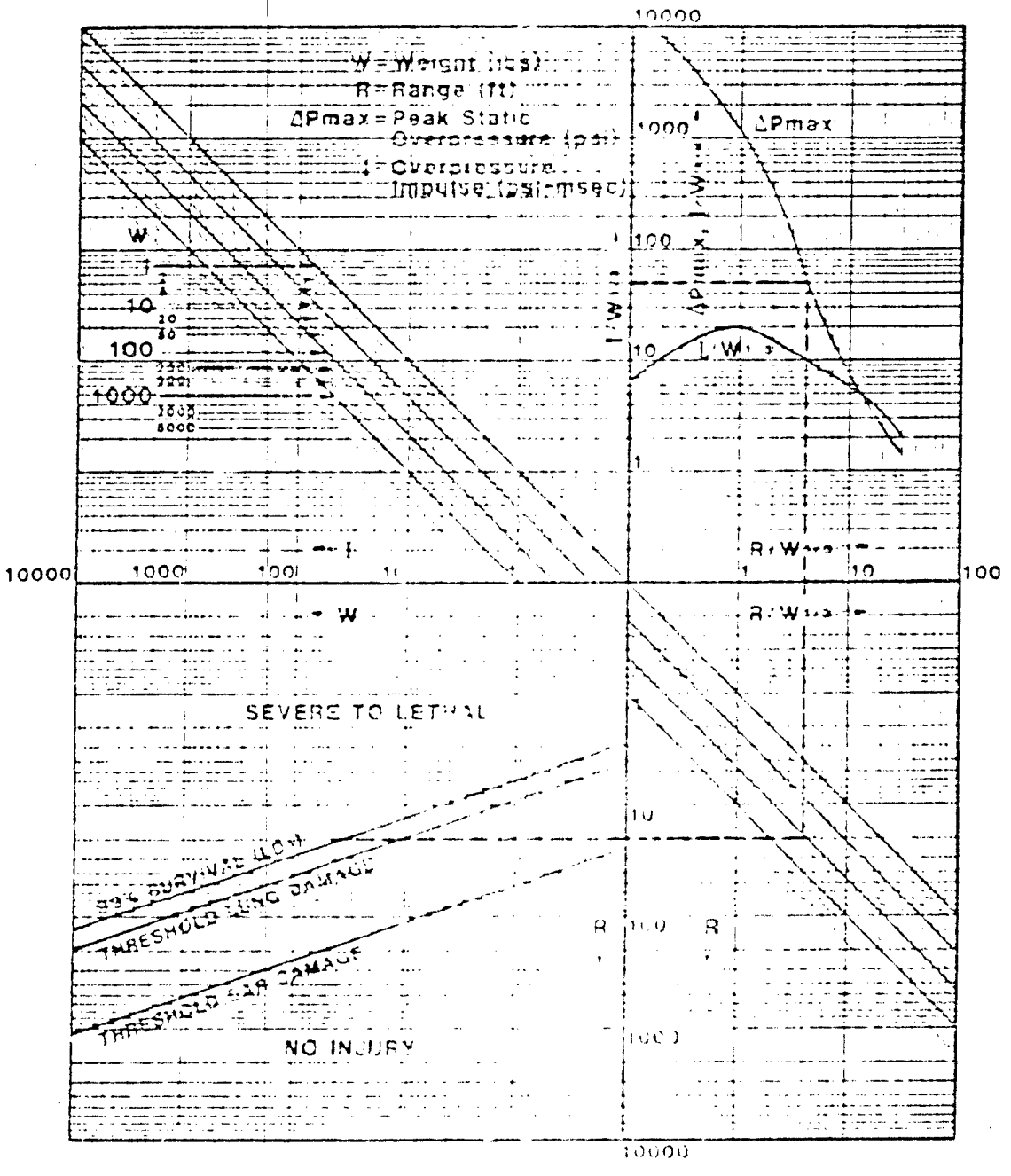

Figure 1. Nomograph for estimating the lethality of condensed high explosive or fuel-air explosive weapons.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6769	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GRP'S INSTR	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	BB	182 WWL5
b. CONTRIBUTING				
c. [redacted]	STOG [redacted]	[redacted]		

11. TITLE (Provide with Security Classification Code):

(U) Biomedical Aspects of Medical Material

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

008800 Life Support 002400 Bioengineering

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY		16. PERFORMANCE METHOD
80 10	CONT	DA		C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 83	1.0	97
	CURRENT 84	1.0	91

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H JR

TELEPHONE: (202) 576-3551

21. GENERAL USE

FINA

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Surgery

ADDRESS: Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic participation)

NAME: HARMON, J W

TELEPHONE: (202) 576-3791

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: SCHWEITZER, E

NAME: POC: DA

22. KEYWORDS (Provide EACH with Security Classification Code): (U) Laboratory models: (U) Medical Material Systems; (U) Biomedical support; (U) Life support systems

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide brief individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) The primary objective is to develop and provide laboratory models for biomedical assessment of medical material systems. Medical material systems currently being developed will continue to undergo operational testing to determine if such systems are usable and useful. Our objective will be to exploit these newly developed materials in the environment that they are designed to be used in. These studies will not only assure the military relevancy of such materials, but they will also assist in the integration of such materials into the armamentarium of the Army Medical Corps.

24 (U) Appropriate animal models and bench models will be developed and utilized to accomplish our objectives. Each medical material system will have an individual evaluation to determine which method of assessment will be used. After completion of each assessment, the data will be analyzed statistically, where possible, and a summary statement issued.

25 (U) 82 09 - 83 10 A simple autotransfusion device for possible Battalion Aid station use has been invented and testing is being initiated in animals with hemothorax. Animal testing for tumorogenicity of cyanoacrylate tissue adhesive was carried out in rodents confirming earlier studies. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

* Available to contractors upon originator's approval

Project 3S162772A874 METHODS AND TECHNIQUES FOR COMBAT CASUALTY MANAGEMENT

Work Unit 182 Biomedical Aspects of Medical Materiel

Investigator:
Principal: Jonn W. Harmon, LTC, MC
Co-Investigator's: Eugene Schweitzer, CPT, MC
Jerry Hauer, CPT, MSC

Background and Objectives:

The primary objectives are to develop and provide laboratory models for biomedical assessment of medical materiel systems. Appropriate animal models and bench models will be developed and utilized to accomplish this goal. Each medical materiel system will have an individual evaluation to determine which method of assessment will be used. After completion of each assessment, the data will be analyzed statistically, where possible, and a summary statement issued. These studies will not only assure the military relevancy of such materiels, but they will also assist in the integration of such materiels into the armanentarium of the Army Medical Corps.

Progress:

Two projects were directed at the evaluation of Medical Materiel, one to evaluate tissue adhesive and the other to evaluate a field autotransfusion device.

A study of the turmourogenic effect of butyl-cyanoacrylate (BC) is being carried out. BC is a tissue glue similar to so-called "Super glue" available in hardware stores. The Army Medical Corps has been interested in cyanoacrylate glues because they may have the potential to control bleeding in combat casualties. BC is a relatively recently developed analog with a butyl side chain. A cyanoacrylate glue with a methyl side chain has been shown by Page[1] to cause sarcomas in rats. We have tested BC in a similar way because of the possibility that it would not cause sarcomas. In fact, sarcomas did occur in rats injected with either 0.1 or 0.4 mg of BC subcutaneously (see Appendix A, letter to FAA describing results of WRAIR studies on BC). It remains unknown whether the sarcomas that developed in these animals were a result of exposure to BC specifically or whether they were a result of a non-specific mechanical effect of an irregularly shaped mass being present in the subcutaneous tissue. A new experiment with appropriate controls for this effect would be required to make this differentiation.

The further responsibility for studying cyanoacrylate glues was transferred this fiscal year from WRAIR to USAIDR by the USAMRDC.

Another project in this work unit has been the early development of field autotransfusion device (FAD) for forward use. Such a device has the potential to allow a medic to transfuse a severely bleeding casualty with his own shed blood. This could save lives especially in patients with hemothorax, a condition for which such a device is particularly well suited.

Such a device was envisioned by Swan and Swan[2]. CPT Eugene Schweitzer, currently an investigator at WRAIR, in collaboration with COL Ken Swan, MC, USAR, have initiated development of such a device. A patent application has been filed. Testing of this device has begun in dogs. A hemothorax is created and 500 cc of blood is autotransfused with the device back into the dog. Studies of the survival of autotransfused red cells using Chromium 51, as well as studies of such transfusion on clotting parameters, are currently underway.

Recommendations for the Future:

Further testing of the FAD will be undertaken in the next fiscal year. Serious consideration will be given to arranging a "hand off" of this device for further development.

The Division of Surgery has been requested by DARCOM to participate in evaluation of the Bradley APC. If this participation is funded, this activity will be a major component of this work unit.

References:

1. Page, Larsen, Siegmund in Proceedings: Symposium in Physiologic Adhesives, p. 11-23, University of Texas Press, 1966.

2. Swan, KG and Swan, RC. Gunshot Wounds, Physiology and Management. PSG Publishing Co., Littleton, Mass., p. 96, 1980.

APPENDIX A

Division of Surgery

Thomas Callahan, PhD
Bureau of Medical Devices
8757 Georgia Avenue
Silver Spring, MD 20910

Dear Doctor Callahan:

In view of your responsibilities for reviewing cyanoacrylate glue, I want to make you aware of some studies done at the Division of Surgery, Walter Reed Army Institute of Research. The use of this agent is controversial because Page reported in the Proceedings of the Symposium on Physiological Adhesives, University of Texas Press, 1966, p. 11-23, that methyl-2-cyanoacrylate was tumorigenic in rats. He found fibrosarcomas in 8 of 59 rats injected with 0.4 ml of this material subcutaneously. As a result of these studies, the Federal Drug Administration (FDA) has refused to allow the marketing of cyanoacrylate glues for surgical use in the United States. As far as we know, no human tumours have been associated with cyanoacrylate use in Europe, or in civilian dental and ophthalmic surgery in the United States where it is widely used. Because the agent has not caused tumours in humans, some people have doubted the validity of Page's study or proposed that the tumorigenicity was related to the use of his particular cyanoacrylate analogue.

To investigate the possibility that the more modern analogues might not be tumorigenic, Colonel Arthur Fleming of the Division of Surgery, obtained some n-butyl cyanoacrylate from Canada. He injected this agent into rats using a protocol very similar to Page's. The injections were carried out 3-4 June 1982. As of our most recent review of this project in May, 1983, tumours have been found in 4 of 23 rats injected with 0.1 ml and in 4 of 24 rats injected with 0.4 ml of the agent.

The WRAIR pathologists have evaluated four of these tumours by light microscopy and one by electron microscopy. There is general agreement that these tumours are poorly differentiated sarcomas that are aligned in most characteristics with the malignant fibrous histiocytoma family of rats. There have been no metastases documented to date in any of the cases, however, these tumours grow rapidly to several centimeters in diameter and contain evidence of blood vessel invasion. From the present study, it is impossible to determine whether the tumours associated with subcutaneous deposition of n-butyl cyanoacrylate in rats represent a genuine carcinogenic effect of this compound or one of its components. Similar tumours have been associated with subcutaneous implantation of impermeable sheets or discs of plastic or metal, representing a non-specific, solid-state carcinogenic effect which is a response to a physical, rather than chemical characteristic of the implanted material. This solid-state carcinogenic principle may also be operational with subcutaneous n-butyl cyanoacrylate and may represent a species-specific response of the rat. Nonetheless, the study

certainly confirms Page's findings and goes further in that the 0.1 ml injection caused tumours in the WRAIR study, but not in Page's. The US Army Institute of Dental Research will be following up these studies to try to determine whether the effect is chemical or solid state.

We are making you aware of these unpublished results in order to assist you in carrying out your responsibility for making regulatory decisions.

Sincerely,

FRANKLIN H. TOP, JR., MD
Colonel, Medical Corps
Director

PROJECT 3M162734A875

MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6479	83 1001	DD-DR&E(AR)436

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORD'R DISTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 1001	D. Change	U	U		NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	AJ	161 WWME
b. CONTRIBUTING				
XXXXXXXXXX	STOG [illegible]	[illegible]		

11. TITLE (Procede with Security Classification Code)

(U) Development of Anti-Chemical Warfare Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology012600 Pharmacology 012100 Organic Chemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING 83	5.0	356
FISCAL YEAR — CURRENT 84	5.0	482

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL

NAME: TOP, F H JR

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

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Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic participation)

NAME: CANFIELD, C J

TELEPHONE: (202) 427-5411

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Provide EACH with Security Classification Code) (U)Drug Development;(U)Chemical Defense;(U)Molecular Modeling;(U)Chemical Poisons;(U)Pharmacodynamics;(U)Nerve Agents;(U)Chemical Synthesis

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with its security Classification Code.)

23.(U) To develop new drugs with protective activity against injury to military personnel in the event of exposure to chemical poisons.

24.(U) Potentially active drugs will be identified and obtained by synthesis of purchase. Candidate drugs will be tested in laboratory model systems to establish protective efficacy, mechanisms of pharmacological effects, effects on physiological response and pharmacokinetic characteristics. Results will be used as input to computer-assisted molecular modeling system for evaluation to guide design of new compounds. Information is used in selection of candidate drugs for clinical trials.

25. (U) 8210-8309 More than 200 compounds were screened for efficacy against toxic doses of cyanide. Hydroxycobalamin was active therapeutically, with a protective ratio of 8.2 in combination with nitrite + thiosulfate. WR 2823 was active prophylactically over five hours' duration with a protective ratio of greater than three. Oral efficacy was not found. Four extramural testing systems are in process of validation. Synthesis of prospective compounds is supported by an extramural program of 22 contracts and compounds have been synthesized as oxime reactivators of acetylcholinesterase, non-oxime reactivators, antimuscarinic and pretreatment drugs. A new and superior route of synthesis of oximes was developed. For technical report, see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

* Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M162734A875 Medical Systems Of Nonconventional Environment

WORK UNIT 161 Development of Anti-Chemical Warfare Drugs

INVESTIGATORS:

Principal: COL Craig J. Canfield, MC
Associate: COL David E. Davidson, Jr., VC
LTC Robert O. Pick, MS
Dr. Melvin H. Heiffer
Dr. David Davis
Ms. Marie M. Grenan
Dr. A.J. Lin
Dr. Howard S. Lowensohn
Dr. Lily C. Tang

PROBLEM AND OBJECTIVES:

Antidotes currently available to protect or treat U.S. military personnel who may be attacked with chemical weapons are inadequate and, for some types of chemicals which are potential threats, antidotes are non-existent or unsuitable for mass administration. The development of suitable antidotes would improve the capacity of military units to perform effectively in the event of use of chemical agents, reduce the number of medical casualties and deter the use of chemical agents by an enemy.

PROGRESS:

Intra-and extramural programs of synthesis and testing of candidate compounds have been conducted.

The extramural synthesis program is supported by 22 contracts. In addition to contractual synthesis of oxime reactivators of acetylcholinesterase, non-oxime reactivators, antimuscarinic compounds and pretreatment drugs, there are contracts for radiolabeling, larger scale preparations, chemical analyses and data handling and inventory services. Compounds have been synthesized in all areas of interest, with the most significant findings in the oxime area. Gram and kilogram quantities of two bis-quaternary pyridinium oximes were sythesized

by a new route which makes available compounds of interest which were previously difficult to synthesize in sufficient amounts. Several new mono-quaternary imidazolium oximes have shown activity against Soman in vitro and in rodent test systems.

The intramural synthetic program has produced four prospective non-hallucinogenic anticonvulsant drugs and establishment of the laboratory for study of muscarinic binding is nearly complete.

The extramural anti-cyanide testing program is supported by four contracts, all in the initial year of funding. Laboratory model systems have been established and are being validated. The systems include rodent models for study of candidate cyanide scavengers and methemoglobin formers, a canine model for advanced study of methemoglobin kinetics and an in vitro culture system with hepatocytes for screening and biochemical studies.

Major areas of emphasis in intramural testing of candidate cyanide antagonists have been a) methemoglobin formers, b) thiols and other sulfur-containing compounds and c) cobalt-containing compounds, including hydroxycobalamin. In combination with nitrite and thiosulfate against cyanide in mice, hydroxycobalamin afforded a protective ratio of up to 8.2. It is very well tolerated, but the necessity for intravenous administration, the very short duration of effectiveness and the large dose required for protection appear to limit its potential utility. Although it would not appear to have potential for prophylactic application, it may be a useful adjunct to existing therapy in medical management of cyanide casualties.

More than 200 candidate compounds in a variety of classes of cyanide antagonists have been examined for prophylactic activity and ten thiols or thiol analogs have been discovered which have protective ratios of two or better. The most promising agent in this group is a phosphorothioate, WR 2823. This compound had a maximum protective ratio of three in mice when administered alone and five when administered in combination with nitrite and thiosulfate by injection. Protection of five hours' duration suggests potential prophylactic applicability. Because the compound was not effective orally, it is being microencapsulated for protection against acid hydrolysis in an effort to achieve oral effectiveness.

Pharmacologic studies of the effects of atropine on the muscarinic controls of the cardiovascular system required a prior extensive literature survey of the physiology, pharmacology and chemistry of atropine and its effects. The completed survey has been written as a review article and submitted for publication.

FUTURE OBJECTIVES:

Directed synthesis of compounds in chemical classes with known or suspected activity as protectants against chemical agents will continue. New synthetic routes will be developed to allow greater recovery of compounds or preparation of novel chemicals of interest. Laboratory models currently in use or under development will be employed in testing the efficacy of candidate compounds and in the investigation of modes of activity. Pharmacologic studies of effects of cholinergic compounds and their effects on muscarinic controls are to commence and formulation studies, such as microencapsulation of WR 2823, will continue in an effort to develop orally efficacious preparations.

PUBLICATIONS:

1. Lowensohn, H.S. Atropine's Effects Upon the Muscarine Controls: The Heart and Its Systemic Output. Submitted to Physiological Reviews.

2. Tang, L.C., Schoomaker, E., and Weissman, W. Cholinergic Agonists Stimulate Calcium Uptake and cGMP Formation. Submitted to Biochem. Biophys. Acta.

3. Tang, L.C. A Personal and Scientific Biography of Dr. George C. Cotzias. Accepted by Neurotoxicology.

PRESENTATIONS:

1. Tang, L.C. 1982. Brain Enzyme and Tumor Incidence. 13th International Cancer Congress.

2. Tang, L.C. 1982. Manganese and L-dopa. First International Neurotoxicity Meetings of Selected Chemicals.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 8600	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	AL	164 WWJF
b. CONTRIBUTING				
XXXXXXXX STOG		82/83-6.2/1		

11. TITLE (Procede with Security C. classification Code)

(U) Behavioral Toxicology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

013400 Psychology 012900 Physiology 012600 Pharmacology 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY		16. PERFORMANCE METHOD
80 10	CONT	DA		C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	B. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	2.0	366
	CURRENT 84	2.0	402

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20307

RESPONSIBLE INDIVIDUAL

NAME: Top, F H JR

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Division of Neuropsychiatry
Washington, D.C. 20307

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Elsmore, T F

TELEPHONE: (202) 576-2483

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Hursh, S R

NAME: Raslear, T G

POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Provide KWCM with Security Classification Code)

(U) Chemical Defense; (U) Behavior; (U) Neuropsychiatry; (U) Toxicology; (U) Chronopharmacology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Methods for assessing the impact of chemical defense-related compounds upon behavior will be evaluated. The overall objective will be development of testing protocols with maximum sensitivity to behavioral effects of chemical defense agents for use in the Army's effort to develop and field better antidotes to chemical agent exposure.

24. (U) The techniques of operant and respondent conditioning will be used to generate behavioral baselines which will be sensitive to the effects of chemical defense-related compounds. Dose-effect and time course functions will be determined in rodents and primates on procedures spanning a range of behavioral functions. Chronopharmacological effects will be evaluated. Methods of curve fitting and time series analysis will be used to evaluate drug effects.

25. (U) 82 10 - 83 09 Major findings: No significant interactions were found between the time of day at which the cholinergic drugs atropine, atropine methyl nitrate, or physostigmine were administered and their effects upon learned behavior of rats. Atropine was shown to affect the short-term memory of mice, but not long-term memory. This effect was due to the effects of the drug upon the central nervous system. Effects of drugs upon a monkey's ability to learn a sequence of responses were similarly found to be centrally mediated. Disturbances in time perception and disruptions in circadian rhythm patterns were found in rats following single injections of the organophosphate DFP, providing models for testing efficacy of CW therapeutic and pretreatment agents against long-term psychiatric sequelae of exposure to CW agents. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

Project: 3M162734A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

Work Unit: 164 Behavioral Toxicology

Investigators:

Principal:	Elsmore, T.F., Ph.D.
Associate:	Hursh, MAJ, S.R.; Raslear, CPT, T.G.; Leu, CPT, J.R.

Objectives:

The overall objective of this work unit is the development of testing methods to evaluate behavioral effects of compounds used for pretreatement or therapy of chemical warfare agent exposure. This work unit will evaluate both the inherent toxicity of these compounds and their efficacy in preventing possible long-term behavioral and neurological deficits produced by these agents.

Progress:

There have been two major efforts in this area in the past year, studies on the behavioral toxicology of atropine and other cholinergic compounds, and studies on long-term sequelae of organophosphate (OP) poisoning.

An extensive experiment was conducted to determine whether the behavioral decrements produced by these compounds depended on the time of day when they are administered. This study was a follow-up of an earlier study which showed that the toxicity of the OP soman varied with time of day. Rats were trained to press a lever on a fixed-interval 60" schedule to earn food pellets. These food sessions were conducted every four hours around the clock. When performance had stabilized the animals were treated with either saline, atropine sulfate, atropine methyl nitrate, or physostigmine before and during the sessions. No significant interactions were seen between the effects of the drugs and the time of day at which they were administered.

A major goal of this work unit is the development of rapid, inexpensive assays for behavioral toxicity. Further, it is desirable that these assays provide some information regarding the specific behavioral functions affected. To these ends, mice were trained to find food in a six-arm radial maze in which only three of the arms contained food. This task provides information on both short- and long-term memory functions, as well as on locomotor performance. With this procedure it was demonstrated that both atropine sulfate and atropine methyl nitrate produced increases in the time to complete the task. Only atropine sulfate, however, resulted in any decrements in memory function, producing significant dose-related increases in short-term memory errors. These data suggest that the

effects of atropine on locomotion are peripheral in nature, but that the effects on memory are probably effects on the central nervous system.

Another goal of this work unit is the demonstration of generality of results across species. This goal was addressed in a study in which a similar finding to the one with mice, described above, was made with rhesus monkeys who were trained to repeatedly learn new sequences of behavior to obtain food. In this procedure, both atropine and atropine methyl nitrate produced decreases in the rate at which animals solved the problems, but only atropine sulfate produced decreases in overall accuracy.

We have previously reported that the CW agent soman produces brain lesions in rats following as single dose. (WU 221,DAOG6753,FY82 WRAIR annual report for more information). In addition, there are numerous reports in the literature of long-term psychiatric and neurological changes in humans following accidental exposure to OP compounds. Several studies are being performed in rats to define the behavioral deficits that may be expected following organophosphate poisoning, and to determine the effects of several standard therapeutic drugs in preventing these long-term effects.

Long-term effects on cognitive functions are of particular interest. Rats were trained to distinguish between tones of different durations. They were then treated with various doses of DFP, an organophosphate, and retested. High doses tend to compress the time sense. That is, the rats tend to judge short tones longer than prior to treatment. These effects persisted at least 6 months following drug administration.

Chronobiological methods are being applied to this problem. Mammals normally exhibit changes in their activity patterns across the day. It was shown in several studies that these normal patterns are disrupted by single doses of DFP such that the animals show more than the usual amount of activity during the light portion of the day-night cycle (their normally inactive period), and less activity than normal during the dark. These effects persist for at least 30 days following drug administration. We have tested several "therapeutic" drugs for prevention of these long-term effects. To date, atropine sulfate, 2-PAM chloride, and diazepam have been tested, and found to have no beneficial effect.

Future objectives:

Continue chronobiological studies with different drugs and combinations, extend studies to different species, including squirrel monkeys and rabbits, extend studies to include non-food types of motivation, examine different "cognitive" functions in both primates and non-primate species, and extend findings to soman, rather than DFP.

Publications

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300534	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	AX	166 WUHA
b. CONTRIBUTING				
c. OCH PROGRAM ELEMENT XXXX	STOG 82/83-6.2/1			

11. TITLE (Provide with Security Classification Code)

(U) Antiradiation Drug Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012100 Organic Chemistry 014100 Radiobiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 83	2.0	150
FISCAL YEAR CURRENT 84	2.0	171

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Biochemistry

ADDRESS: Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic participation)

NAME: Alving, C

TELEPHONE: (202) 576-3248

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Richardson, E

NAME: Wassef, N POC: DA

21. GENERAL USE

FINA

22. KEYWORDS (Provide KeyCit with Security Classification Code)

(U) Drug Carriers; (U) Liposomes; (U) Antiradiation Drugs

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide individual paragraphs identified by number. Provide text of each with Security Classification Code.)

23. (U) The objective is to develop liposomes as carriers of drugs and agents for protection against radiation effects involves cells of the reticuloendothelial system. Macrophages are key cells that may play a pivotal role in protection against radiation. The objective will be to determine the effectiveness of liposomes for delivery both of conventional antiradiation drugs and also of agents that stimulate macrophages to produce increased hematopoietic activity. There is military relevance in this research.

24. (U) The approach will be to use inbred mice that have been lethally or sublethally irradiated as a model to determine the effectiveness liposome-encapsulated drugs. Effectiveness will be judged both by increased survival of the animals and by increased hematopoietic activity in the spleen. A standard test of hematopoietic activity that was originally developed at WRAIR and is now widely used is the appearance of nodules that signify hematopoietic activity in the spleen. These nodules presumably are induced by the secretion of colony stimulating factor by macrophages. 25. (U) 82 10 - 83 09

This work unit has been in operation for one year. During this time specialized facilities for working with appropriate mouse models has been developed. Because of the inadequate animal facilities available at WRAIR for this specialized work, special cages were set up in a clean laboratory, and the dose-response parameters for radiation lethality and for determining hematopoietic activity were established. The system for measuring hematopoietic activity in the spleen was standardized. For technical report see WRAIR Annual Progress Report 1 Oct 82 - 30 Sept 83.

*Available in limited quantities upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3M162734A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

WORK UNIT: 166 Antiradiation Drug Development

INVESTIGATORS:

Principal:	Carl R. Alving, M.D., COL, MC
Associates:	Nabila M. Wassef, Ph.D., DAC; Earl C. Richardson, DAC
Assistant:	PFC Denton Hargis

DESCRIPTION:

The approach will be to use inbredmice that have been lethally or sublethally irradiated as a model to determine the effectiveness liposome-encapsulated drugs. Effectiveness will be judged both by increased survival of the animals and by increased hematogpoietic activity in the spleen. A standard test of hematopoietic activity that was originally developed at WRAIR is is now widely used is the appearance of nodules that signify hematopoietic activity in the spleen. These nodules presumably are induced by the secretion of colony stimulating factor by macrophages. One agent that is known to be one of the most effective stimulators of hematopoietic activity (lipid A from endotoxin) was tested in both alone and in liposomes. Using this substance enhanced hematopoietic activity was demonstrated with liposomes. In addition the liposomes alone had some enhanced hematopoietic activity, and the possibility that this was due to endotoxin contamination is being investigated.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300535	83 10 01	DD-DR&E(AR)636

DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GROUP MARKING	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	62734A	3M162734A875	AJ	167 WWP6
CONTRIBUTING				
********	STOG 82/83-6 2/1			

TITLE (Procede with Security Classification Code):

agnosis and Monitoring of Nerve Agent Intoxication by Clinical Chemistry

SCIENTIFIC AND TECHNOLOGICAL AREAS:

6800 Toxicology 012100 Organic Chemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 10	Continuous	DA	C. In-House

CONTRACT/GRANT

DATES/EFFECTIVE: EXPIRATION:

NUMBER:

TYPE: a. AMOUNT:

KIND OF AWARD: b. CUM. AMT.

17. RESOURCES ESTIMATE	PRECEDING/CURRENT	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	83	2.0	70
	84	2.0	72

18. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20307

SPONSORING INDIVIDUAL

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TELEPHONE: 202-576-3551

19. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div. of Pathology & Neuropsychiatry

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PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)

NAME: Andersen, G L

TELEPHONE: 202-576-2183

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Tyner, F C

NAME: Moe, J B POC: DA

20. GENERAL USE

INA

KEYWORDS (Provide Each with Security Classification Code): (U) Intoxication; (U) Nerve Agent; (U) Cerebrospinal luid; (U' Enzyme Changes; (U) Specific Proteins; (U) Clinical Monitoring; (U) Diagnosis

TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code):

3(U) To develop methods for reliably and quickly establishing a diagnosis of nerve gent intoxication. Determine effective means of monitoring nerve agent casualties and ssessing clinical progress. Establish a profile of clinical chemical changes which ill serve as a reference for expedient medical diagnosis, treatment, and dispositior of erve agent casualties in the field. Correlate clinical chemical changes with stru. ural changes in experimental animal models of nerve agent intoxication. Investigate elationships between clinical, chemical, and biochemical changes in serum and cerebrospinal fluid in various experimental models of nerve agent intoxication.

4(U) Serum and cerebrospinal fluid of carnivores, rodents, and nonhuman primates will e examined following nerve agent exposure to determine whether there are characteristic hanges in the enzyme (acetylcholinesterase, CPK, LDH, SGOT, and others as indicated) ontent. Additionally these fluids will be tested for presence of central nervous ystem-specific proteins which may be released following damage to the brain or spinal ord. The central nervous system will be evaluated using standard neuropathological ethods. The results of these enzymatic, biochemical and structural studies will be orrelated temporally with clinical, survival and dose data to establish correlative rofiles of nerve agent - induced damage to the central nervous system.
82 10 - 83 09

5(U) A highly reliable model for intoxication of awake cats by slow intravenous dministration of Soman was developed. In these intoxicated cats, classical signs of cute organophosphate intoxication were produced. The LD50 for Soman in this model, was n the range of 11-15 ug/kg. Significant changes in electrolyte, enzyme and biochemical values were observed in intoxicated cats. For technical report see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Project 3M162734A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

Work Unit 167 Diagnosis and Monitoring of Nerve Agent Intoxication by Clinical Chemistry

Investigators:
Principal: Gary L. Andersen, MAJ, VC
Associate: James B. Moe, LTC, VC; Isaac J. Hayward, CPT, VC; C. Fred Tyner, COL, MC

Description:

Technical methods for quickly and reliably establishing diagnosis of nerve agent intoxication are developed and investigated. Efforts are aimed at deriving effective means of monitoring nerve agent casualties and assessing clinical progress in these casualties. Experimentally exposed animals are studied in an attempt to establish a profile of clinical chemical changes which might serve as a reference for expedient medical diagnosis, treatment, and disposition of human nerve agent casualties in the field. Structural changes in tissues of experimentally exposed animals are correlated with clinical chemical changes in blood and cerebrospinal fluid. Studies are designed to determine and define acute toxicological parameters of nerve agent poisoning, as well as delayed neuropathological effects in survivors of acute intoxication.

Progress:

The initial investigation in this project was conducted during FY 83, using exposure facilities at the US Army Medical Research Institute of Chemical Defense, (USAMRICD), with collaborative assistance from the staff of USAMRICD. This was the first experience of this group of investigators with intoxication with Soman by slow intravenous infusion in awake cats. Briefly, cats were physically restrained, a catheter placed through the skin into one of the femoral veins. Following collection of baseline blood samples, cats were placed in a restraint stand and infused with a dilute Soman solution at a rate of 1 ug Soman/kg body wgt/minute until clinical signs of intoxication developed or until a calculated LD_{50} (15ug/kg body wgt) was infused; the infusion was stopped when either of the endpoints was reached. The cats were then removed from the restraint stand and placed in a cage for observation. All 21 of the cats which were intoxicated with Soman under this regimen developed classical signs of acute organophosphate toxicity within 10 minutes of termination of the infusion. These signs either developed during the later stages of infusion or upon removal from the restraint stand and placement in a holding cage. Typically, there was licking, nystagmus, and muscle fasciculation followed by tremors, salivation, chewing, loss

of equilibrium, and, finally, seizures. None of the cats were treated with specific antedotes for organophosphate toxicity, but were closely observed and supported with nursing care and parenteral fluids as judged clinically prudent. Nine of the cats died, or were euthanized when judged to be moribund, within 48 hours of intoxication. One additional animal died, after an apparent complete recovery, on postintoxication day 4; therefore, the dosage regimen used resulted in a mortality rate of 48 per cent. Recovery to a relatively normal state occurred in as little as 6 hours in some cats, while others were incoordinated or depressed as long as 48 hours after intoxication. Parenteral fluids containing 5% dextrose appeared to result in improved general strength and survival in comatose and recumbant cats. Recovery was complete in 10 of the animals which survived the first 4 days and one otherwise normal survivor was permanently blind. The dose range to achieve these effects was quite narrow, varying from 11-15 ug/kg body weight. Survivors were selected for euthansia at roughly weekly intervals for up to 7 weeks after intoxication. Prior to euthanasia, cerebrospinal fluid and blood samples were collected for enzymatic, chemical and hematologic examination.

In blood samples collected at 30 minutes after termination of intoxication, significant increases were seen in the following, parameters: glucose, SGOT, SGPT, LDH, CPK, K^+; and a significant decrease in acetylcholinesterase (ACHE). Values for these parameters in cerebrospinal fluid generally paralleled those in blood, however, technical problems in collection diminished the quantity and quality of cerebrospinal fluid specimens and raised questions about individual results. In animals which were sampled in a moribund state within 48 hours of intoxication, most of the above mentioned changes were still evident except for ACHE which was still decreased, but higher than the 30 minute samples, suggesting a gradual return to normal values. At 9 days after intoxication SGOT and CPK were elevated in both cats examined, glucose increased in one cat and K^+ was increased in the other. At 14 days, and at subsequent intervals, all serum parameters were essentially normal. In one cat there was a markedly elevated CPK in the cerebrospinal fluid on day 14. Pathologically, the CNS lesions identified were suggestive of a hypoxic pathogenesis, whereas subtle lesions in the peripheral nervous system of cats examined 30 or more days postexposure suggested a direct toxic effect of Soman. The findings from this original experiment indicate that there were significant chemical and enzymatic changes in blood and cerebrospinal fluid following experiental Soman intoxication of the awake cat. Furthermore, these results suggest that this model is a highly reliable, reproducible one for the study of organophosphate toxicity in mammals. With these results in hand, it is evident that there are two aspects which require more complete investigation: 1) the sequence and amplitude of biochemical and enzymatic changes in blood and cerebrospinal fluid during the first

48 hours of intoxication; and 2) the question of whether a peripheral neurophathy develops following a single, acute, intoxication with Soman. Additionally, with improved collection techniques it will be possible to improve the quality of cerebrospinal fluid samples and determine whether there is a pattern of biochemical and enzymatic changes in this medium, separate from those seen in serum. The issue of a more effective diagnostic and therapeutic regimen will be more easily addressed following resolution of these points.

PROJECT 3E162777A878

HEALTH HAZARDS OF MILITARY MATERIEL

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6484	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISPN INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A878	BB	041 WWJD
b. CONTRIBUTING				
c. ~~CONTRIBUTING~~	STCG 82/83-6,2/2			

11. TITLE (Precede with Security Classification Code)

(U) Biological Interactions with and Hazards of Microwave Radiation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

014100 Radiobiol 012900 Physiol 014000 Rad Chem 017000 Wave Prog 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	c. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	4.0	993
	CURRENT 84	3.0	814

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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RESPONSIBLE INDIVIDUAL

NAME: Top, F H JR

TELEPHONE: (202) 576-3551

21. GENERAL USE

Foreign Intelligence Considered

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Dept of Microwave Research
Div of Neuropsychiatry
Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic institution)

NAME: LARSEN, L E

TELEPHONE: (202) 576-3615

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: JACOBI, J H

NAME: HUNT, E L POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Microwave Hazards; (U) Bioeffects; (U) Dosimetry; (U) Biophysics; (U) Military Medicine; (U) Psychology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief technical paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To provide technical and medical information to the Surgeon General, system developers and agencies responsible for safety standards in order to protect the health and effectiveness of military units and affected civilian populations in microwave and RF environments. This requires analysis of the biophysics and bioeffects attributable to non-ionizing radiation under laboratory conditions which reasonably simulate and/or predict operational exposures.

24. (U) To perform basic and applied research on the problem of microwave and RF interactions with biosystems at all levels of analysis from the cellular and molecular to metazoan physiology, pathophysiology and behavior. This requires development of measurement systems for dosimetric analysis, in vitro and in situ; the evaluation of frequency, power level, polarization and modulation as important parameters of the radiation; and the use of low level energy to assess the functional state of cells and tissues.

25. (U) 82 10 - 83 09 The previous findings of increased ocular lens pathology in vitro with pulsed exposures as compared to continuous wave exposures of equal average power have been confirmed & extended. Furthermore, a distinctive energy-per-pulse dependance was shown in the damage threshold for pulse-modulated exposures. The dosimetric studies have continued with completion of a new three dimensional scanner and image display/processing systems. A new program for correction of refractive errors in dosimetric images has begun to compare ray based (linear as well as non linear) & diffraction methods. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sept 83.

Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Project 3E162777A878 HEALTH HAZARDS OF MILITARY MATERIAL

Work Unit 041: Biological Interactions With and Hazards of Microwave Radiation

Investigators.
Principal: LTC(P) Lawrence E. Larsen, MD
Associate: John H. Jacobi, M.S.; Edward L. Hunt, B.A.; Peter V.K. Brown, M.S.; Charles N. Rafferty, Ph.D.

Introduction

The major features of the microwave program in the subject fiscal year have been the continuation of building problems at the Forest Glen Microwave Exposure Facility and the continuation of engineering staff attrition. The latter has reached crisis proportions inasmuch as no engineering staff remains. In the last four years, three engineers have left the department for higher paying positions in industry. The most recent departure among these was the most significant.

The facility problems at Forest Glen represent a continuation of a MCA begun in 1977 which is still not complete. The major deficiencies with respect to the MCA are failure of environmental control in the four microwave exposure chambers. Additional facility problems fall into the domain of the post Engineer. These consist of architectural, mechanical, and electrical deficiencies which have been the subject of work and discussion since the time of beneficial occupancy in 1979. They remain unresolved in spite of attempts to have the deficiencies corrected by the DFEA. A decision was made that the scope of work exceeded the capacity of DFEA resources. This led to the formulation of a plan for contract maintenance. In spite of a germinal period of nearly two years, that plan has yet to be implemented. Lastly, the eight animal modules at Forest Glen remain inoperative. This has been the case since 1980/1981. The plan administered by DFEA to correct the installation errors and to provide subsequently a maintenance contract has not been implemented in spite of the fact that funds were made available from our budget in early 1982.

Dosimetry

The major areas of progress in this program element consist of the further development of DART (Dosimetric Analysis by Radio-frequency Tomography) data acquisition system, and numerical analysis of ray based as well as diffraction based methods for recovery of resolution in the direction of propagation. The objective is to provide a map of the spatial variations in microwave energy dissipation and energy storage in specific target organs as functions of frequency and polarization.

The DART data acquisition system now includes a scanner with three linear axes and one rotary axis. The position encoders and servomotor drives as well as their respective digital interfaces have been completed. In addition, the microwave network analyzer has been modified to accommodate a faster processor/controller and the microwave program library has been modified to take advantage of the new processor/controller.

The numerical analysis program has demonstrated the unsuitability of ray based tomographic methods due to an inability to accomplish ray linkage in media where the relative dielectric constant varies by more than a very few percent. The diffraction based methods have been selected for further development. The major difficulties remaining are related to the numerical accuracy of the results under the normal assumption that the target is weakly scattering. The limitations of the first Born and first Rytov approximations were investigated. It was found that the Rytov case was only slightly better than the Born case (contrary to what one might be led to expect from the literature); and that higher order approximations are necessary. The condition of multiple scattering was also studied.

Pulse Power

The objective in this technical area is to investigate the limitations of the present safety standard wherein pulse modulation is ignored. The major activities in this program area were continuation of the studies of ocular lens in vitro and the development of a high peak power exposure system for use with in situ preparations. Portions of the millimeter wave Annual Report are pertinent to this work area, and the reader is referred to DAOG 6755, Project 3M161102BS10, Work Unit 214.

The ocular lens studies have expanded and confirmed the earlier findings that pulse modulated microwave power with appropriate selection of pulse parameters does produce histopathology with qualitative and quantitative distinctions from continuous wave (CW) exposure of the same average power. Recent work has expanded these findings to biochemical endpoints, but data analysis is not yet complete enough to present any conclusion.

The high peak power transmitter has a design goal of 1,500 kilowatts of RF power output over a pulse width range from 1 to 100 microseconds. The major problem is the design and construction of a hard tube modulator to switch the required 4 million watt video pulses needed to accomplish this goal. Progress in this area has been slow due to a series of high voltage failures which have destroyed substantial portions of the equipment, thereby introducing serious procurement delays. In addition, the aforementioned staff shortages have delayed progress. Nevertheless, substantial progress has been made. We have successfully operated the redesigned modulator (the Mark III version) to switch two million watt video pulses over the required range of pulse widths and duty factors. RF

testing has not yet taken place with the Mark III modulator, but testing earlier i this fiscal year with the Mark II design produced ca 150 kilowatts of RF power with pulses up to 15 microseconds in length under seriously reduced video pulse (ca. 0.4 megawatts) conditions. This represents the highest reliably obtained RF power output yet produced by the transmitter. Other aspects of the exposure system are completed as detailed in previous reports.

Behavior

This program area is essentially indolent. Much of the reason is the aforementioned facility problems at Forest Glen which have denied the program any free field exposure facility for six years.

Dielectric Relaxation

The major areas of progress in this program element have been the extension and confirmation of continuing work with measurements of complex permittivity in situ; and the exploration of physiologic influences upon complex permittivity. The former studies have demonstrated that complex permittivity in situ differs from tabled values (often derived from autopsy specimens) by as much as 25%. It has also demonstrated that regional blood flow alterations under normal physiologic conditions may also change the complex permittivity in the radar S band by 10 to 20%. These factors also apply to regional variations within various target organs (e.g. brain and kidney).

This program has also explored methods for the determination of bound water content from dispersion measurements in the radar S band. The bound water studies are nearly finished with respect to data acquisition, but data analysis is not far enough along to allow presentation of conclusions at this time.

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2. S.X. Pan and A.C. Kak, "A Computational Study of Reconstruction Algorithms for Diffraction Tomography: Interpolation vs. Filtered Back Propagation", IEEE Trans Acoustics, Systems and Signal Processing, in press, Oct 1983.

3. T.C. Guo, W.W. Guo, L.E. Larsen and J.H. Jacobi, "On Heat Transfer in Surface Cooled Objects Exposed to Microwave Heating", IEEE Trans Microwave Theory and Tech., MTT-31,783-785, 1983.

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6. C. Gabriel, R.J. Sheppard, and E.H. Grant, "Dielectric Properties of Ocular Tissue at 37 C", Phys. Med. Biol. 28:43-49, 1983.

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9. P.J. Stewart-DeHann, L.E. Larsen, J.H. Jacobi, M.O. Creighton, M. Sanwall, J.R. Trevithick, "In Vitro Studies of Biochemical and Morphalogical Changes in Rat Lenses Irradiated by Microwaves", Proceedings of the Canadian Federation of Biological Societies, Vol 26, PA161, pp 81, 1983.

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12. L.E. Larsen and J.H. Jacobi, "Microwaves Offer Promise as Imaging Modality", Diagnostic Imaging, pp 44-127, Nov 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6472	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPN INSTR	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
82 10 01	D change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

11. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A878	AB	042 WWIA
b. CONTRIBUTING				
c. [illegible]	STO; 82/83-0.2/2			

12. TITLE: (U) Non-auditory effects of blast overpressure

13. SCIENTIFIC AND TECHNOLOGICAL AREAS: 017100 Weapons Effects 013300 Protective Equipment 016200 Stress Physiology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
78 03	CONT	DA	C. In-house

18. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

19. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS ($ thousands)
FISCAL YEAR PREVIOUS 83	6.0	422
FISCAL YEAR CURRENT 84	7.0	312

20. RESPONSIBLE DOD ORGANIZATION

a. NAME: Walter Reed Army Institute of Research

b. ADDRESS: Washington, D.C. 20307

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d. GENERAL USE

Foreign Intelligence Considered

21. PERFORMING ORGANIZATION

a. NAME: Walter Reed Army Institute of Research

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SOCIAL SECURITY ACCOUNT NUMBER:

d. ASSOCIATE INVESTIGATORS

NAME: HOYT, R F

NAME: JAEGER, J J

POC: DA

22. KEYWORDS: (U) Human Volunteers; (U) Impulse Noise; (U) Blast Overpressure; (U) Pulmonary [illegible] Physiology; (U) Gastrointestinal Physiology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS:

23. (U) To define the physiologic effects of blast overpressure (BOP) exposure upon the human. To develop a laboratory model of blast injury. To assist in special studies of weapon specific BOP at the direction of HQ, USAMRDC.

24. (U) High velocity water jet of discrete impulse will model blast effects on large animals. Assays of lung water and parenchymal function will be used to assess injury. Pathologic comparison of water jet and blast-injured specimen will be made. Chronic effects of repeated BOP exposure will be assessed in man and animals. Pathophysiologic events of blast injury will be monitored by implantable transducers. 210 83-09 Ten human volunteers were exposed to artillery blast waveforms in order to determine the effects of clothing ensemble on intrathoracic pressure (ITP). The Kevlar vest raised ITP as compared to fatiques alone. Performed initial validation study of water jet impactor, designed additional test equipment needed for water jet validation studies. Showed no effect of lung volume on injury potential at high level of blast. Developed capability for chronic measurement of transthoracic electrical impedance (TTI) in sheep using subcutaneous electrodes. Field study showed TTI to be insensitive to relatively large changes in lung weight caused by blast. Developed capability for chronic lung lymph cannulation in sheep and conducted field study of lung lymph flow at "threshold" of lung injury for single blast. Contractor developed a chamber for impulsive loading of gastrointestinal tract which will be used to investigate correlates of bowel injury. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

(Reverse of this form may be classified when filled in)

DD FORM 1498 1 MAR 86 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 86 (FOR ARMY USE) ARE OBSOLETE.

579

Project 3E162777A878 HEALTH HAZARDS OF MILITARY MATERIAL

Work Unit 042: Non-auditory Effects of Blast Overpressure

Principal Investigator: Yancy Y. Phillips, M.D., MAJ, MC

Associate Investigators: James J. Jaeger, Ph.D., MAJ, MSC
Robert F. Hoyt, Jr., D.V.M., MS, MAJ, VC
Andrew J. Young, Ph.D., CPT, MSC
Patrick E. Lorenz, Ph.D., CPT(P), MSC
Thomas G. Mundie, Ph.D., CPT, MSC
Kenneth T. Dodd, Ph.D., GS-12

Problem Statement and Objectives

Certain Army weapon systems currently in use and others still in development produce levels of blast overpressure which exceed the limits defined in MIL STD 14748. The research objective of the Department of Respiratory Research is to define the risk of non-auditory injury to crewmembers from the blast overpressure produced by these weapon systems. Toward this end experiments are conducted to obtain various types of physiological and biophysical data which can be related to injury to the larynx, trachea, lungs or gastrointestinal tract. The overall goal of the WRAIR program is the development of generalizable criteria for the assessment of the health hazard of impulse noise and complex wave environments.

Progress and Accomplishments in FY 83

a. A field study was conducted at the Lovelace Research Facility in Albuquerque, NM, to assess the utility of using transthoracic electrical impedance (TTI) as a means of detecting low level lung injury in sheep caused by blast overpressure. Results indicated that although there was a small transient decrease in TTI within 30 seconds of blast exposure, there was no direct quantifiable relationship related to lung injury. Therefore, the TTI technique was not found suitable for detection of low level blast injury to the lung.

b. Mechanical tests of a water jet impactor intended to be a laboratory simulator of air blast indicated that significant valve timing errors are responsible for its unsatisfactory performance. Corrective measures are currently being taken.

c. In order to assess human risk of non auditory injury from repeated exposure to blast from the British 1-81mm mortar, an animal study was conducted using a mortar blast simulator. There was no evidence of injury to the larynx, lungs or gastrointestinal tract of 36 sheep exposed to 300 consecutive mortar blast waves of the worst case blast exposure condition.

d. The second phase of human exposures to artillery-level blast waves was conducted at the Lovelace testing facility in Albuquerque, NM to assess the effect of body clothing ensemble on intrathoracic pressure. It was previously found that orientation and arm position did not significantly affect intrathoracic pressure during exposure to blast waves with peak pressures of up to 3 psi. Some uniform ensembles,however, affected intrathoracic pressures. Significantly higher intrathoracic pressures resulted when the men wore KEVLAR vests than when they were exposed to a 3 psi blast in fatigues alone. This increase is not felt to represent a greater risk of nonauditory injury at these low blast levels.

e. Studies designed to assess the magnitude and consequences of epithelial tissue damage to the larynx caused by repeated exposure to low level blast have been initated using rats as subjects.

f. An experiment conducted with sheep failed to demonstrate an effect of lung volume (maximum inflation to maximum deflation) on the severity of lung hemorrhage produced at a relatively high level of blast exposure.

g. We have succeeded in adapting the acute and chronic lung lymph fistula techniques in our laboratory for assessment of lung solute and water exchange as an indicator of pulmonary microvascular function.

h. A field study was conducted at the Lovelace research facility in Albuquerque, NM, utilizing the above mentioned chronic lung lymph preparation in sheep to assess the effect of a single blast exposure at the threshold for grossly detectable lung injury on pulmonary capillary permeability. Results are currently being analyzed.

i. A member of our research group completed a tour of several European blast research facilities (at Meppen, Germany, Porton Down, England, and Saint Louis, France). The principle finding was that previous reports of pulmonary injury to pigs exposed to BOP crew position of the German Carl Gustav 84mm recoilless rifle must be viewed with skepticism due to questionable design and euthanasia methodology.

j. Complex blast wave exposure experiments of large animals were initiated. This work will define the risk of non-auditory injury from complex wave exposure such as that encountered in enclosures or armored vehicles. Preliminary work supports resonance effects and the vulnerability of organs other than the lung.

k. The Blast Overpressure Group at WRAIR hosted the fourth annual meeting of RSG-6 (NATO Panel VIII, Effects of Impulse Noise) in May 1983 and presented data from human volunteer studies and lung inflation data. WRAIR was tasked with drafting a group position on the non-auditory effects of impulse noise.

l. A four month contract for a scoping study of the correlation between gastrointestinal injury and blast loading was completed by the JAYCOR Corporation, San Diego, CA in the third quarter of FY83. The following contract milestones were achieved:

1. The JAYCOR Fluid Dynamics Laboratory was awarded a certificate from the Animal and Plant Health Inspection Service of the Department of Agriculture as a qualified animal study test facility.

2. JAYCOR designed and fabricated an 8-inch diameter horizontal impaction, top-observation window test chamber designed to accomodate an entire GI tract of a rabbit. This will allow high speed photography of pressure wave/GI tract interactions. General experimental animal preparation and design procedures were established. Three general types of GI bowel injury were identified from blast loading in the JAYCOR chamber: contusion, mucosal bleeding, and wall rupture.

3. Key parameters which appear to directly affect the injury threshold in the gastrointestinal tract include the presence of air bubbles, bubble size, material properties of each major section of bowel (i.e. jejunum vs. colon), internal pressure of the individual GI sections (as relating to volume), method of impact loading, and repetition rate of impact loading.

Recommendations and Objectives FY 84

1. It is essential to the overall program that an empiric damage risk criteria be developed for Friedlander waves. This would be a generic tool for non-auditory risk assessment of weapons which generate Friedlander waves, thus assisting in the assessment of the health hazards of existing and future blast overpressure environments. Previous work has shown that with Friedlander waves of relatively short duration that the number of exposures, peak pressure of the waves, and A-impulse interact to determine damage to different organs by systems. We have also observed that laryngeal injury always accompanies or precedes injuries of other organs, specifically, the gastrointestinal tract or lungs. Therefore, the absence of laryngeal injury is a reliable indicator of the absence of other non-auditory damage. We intend to develop in our laboratory fiberoptic laryngoscopy techniques in sheep in order to detect subclinical evidence of blast induced injury. It is important that this generic damage-risk criteria be developed in conjunction with other members of Research Study Group 6.

2. Further work with the double-peak study is planned. This is the simplest of all complex wave circumstances in which two Friedlander waves of equal strength impinge upon a target with the only variable being the time between the peaks.

3. Work contributing to the sheep and human model effort will assume a more important role. Measurement of chest-wall motion will be important in validating the finite element model. Pressure transducers introduced endobronchially are expected to show differences in pressure-time history and different wave shape at different distance from the chest wall. This is a consequence of wave propagation in lung parenchyma which is suggested both by modeling and basic research. Demonstration of this wave phenomenon is critical to model validation and to the elucidation of injury mechanism.

4. A firteen month contract was recently awarded to JAYCOR for further investigation of the mechanism of G.I. injury and to establish the injury thresholds in terms of these parameters.

References Cited

None.

Bibliography

1. Hoyt, R. F., Jr. and Tholen, M. A.: Oral Pathology In Concepts in Veterinary Dentistry, Veterinary Medicine Publishing Company, Edwardsville, KY, 1983.

2. Jaeger, J., Phillips, Y., Young A., and Hoyt, R. F., Jr.: Low Level blast Exposure in Humans. In Technical Proceedings of the Muzzle Blast Overpressure Workshop Held 25-26 May 1982 at U.S. ARRADCOM Dover, New Jersey pp 542-555.

3. Hoyt, R. F., Jr. and Withrow, S. J.: Oral malignancy in the dog. Journal of American Animal Hospital Association. In Press

4. Verma, P.S., Hoyt, R. F., Jr., Jackson, A. J. and Phillips, Y. Y: Pharmacokinetics of intravenously administered desmosine in sheep. In press.

5. Phillips, Y. Y., Copley, J. B., and Stor, R. A.: Thrombocytopenia and low-dose heparin. South. Med. Jour. 76(4):526-528, 1983

Presentations

Hoyt, R. F., Jr.: Cricothyroidotomy, Tracheotomy, and Tracheostomy. Emergency War Surgery Training Program, Symposium on Military Veterinary Medicine, WRAIR, Washington, D. C., March 1983.

Verma, P. S., Hoyt, R. F., Jr., Jackson, A. J., and Phillips, Y. Y: Radioimmunoassay of desmosine and its pharmacokinetics in sheep. Presented at the 67th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Ill., April, 1983 (abstract).

Young, A. J., Evans, W. J., Sharp, R. L., Costill, D. L., and Maher J. T.: Intramuscular pH with excercise at high altitude. Presented at the 67th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Ill., April, 1983.

Jaeger, J., Young, A. J., Phillips, Y. Y and Hoyt, R. F., Jr: Lung inflation effects on blast lung injury. Presented at the fourth annual meeting of RSG-6 (NATO Panel VIII, Effects of Impulse Noise), WRAIR, Washington , D.C., May 1983.

Jaeger, J., Young, A. J., Phillips, Y. Y and Hoyt, R. F., Jr.,: Low level human blast exposures. Presented at the fourth annual meeting of RSG-6 (NATO Panel VIII, Effects of Impulse Noise), WRAIR, Washington, D. C. May 1983

Yelverton, J. T., Richmond, D. R., Flectcher, E. R., Phillips, Y. Y, Jaeger, J., and Young, A.: Bioeffects of simulated muzzel blasts. Presented at the Eight International Symposium on Military Application of Blast Simulation, Spiez, Switzerland, June, 1983.

Phillips, Y. Y: Dry gas eucapnic hyperventilation: a simplified technique for bronchoprovocation. Presented at the thirty-fifth annual Carl W. Tempel Symposium on Pulmonary Disease and Allergy-Immunology, Fitzsimons Army Medical Center, Aurora, Colo., January 1983.

PROJECT 3E162777A879

FACTORS LIMITING SOLDIERS EFFECTIVENESS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6453	83 10 01	DD-DR&E( )636

DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. FORE SECURITY	7. REGRADING	8. DOD'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
2 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. 6.2 UNIT

NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	62777A	3E162777A879	AA	041 WW
CONTRIBUTING				
CONTRIBUTING	STOG 82/83-6	2/2		

TITLE (Procede with Security Classification Code):

J) Military Preventive Psychiatry

SCIENTIFIC AND TECHNOLOGICAL AREAS:

)3500 Clinical Medicine 013400 Psychology 021900 Physiology

START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	C. In-House

CONTRACT/GRANT

DATES/EFFECTIVE: EXPIRATION:

NUMBER:

TYPE: AMOUNT:

KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	B. FUNDS (In Thousands)
PRECEDING FISCAL YEAR	83	7.5	746
CURRENT FISCAL YEAR	84	7.5	838

RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
Washington, DC 20307

ADDRESS:

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GENERAL USE

Foreign Intelligence Considered

20. PERFORMING ORGANIZATION

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Division of Neuropsychiatry

ADDRESS: Washington, DC 20307

PRINCIPAL INVESTIGATOR (Provide Rank If U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Harris, J

NAME: Van Vranken, E POC: DA

KEYWORDS (Provide EACH with Security Classification Code): (U) Psychiatric Illness; (U) Military Adjustment; U) Environmental Factors; (U) Social and Psychological Factors; (U) Stress

TECHNICAL OBJECTIVE, 19A. APPROACH, 19B. PROGRESS (Provide brief abstract paragraphs identified by number, precede each with security classification code):

. (U) This unit examines the dynamics of those specific factors within military or-
nizations and environments that conduce to psychiatric illness, operate to produce
ychiatric casualties and lead to the generation of dysfunctional behaviors and decre-
nts in military performance. These studies have direct relevance for the development
programs of intervention and prevention and the development of effective techniques
r the minimization of psychiatric casualties.

. (U) The methods of clinical psychiatry, social and clinical psychology, social an-
ropology and field epidemiology are used to identify factors that generate psychi-
ric casualties, behavior dysfunction and performance dysfunction and decrement in
der to modify such factors or the relationship between them.

. (U)82 10-83 09 Studies of family stress and its effects upon active duty soldiers in
its deploying to the Sinai MFO continue and data analysis is underway. Studies have
mmenced on the impact of New Manning System/COHORT on military families and the per-
rmance and sustained ability of the active duty soldier as per tasking from TAGO.
estionnaires and interviewing are being carried out in designated COHORT and New
nning System units. Studies of stress in Special Forces units and the interrelation-
ip of A Team structure and family stress continue both at JFK Center and field de-
oyments. Studies of the life cycle of career NCOs are in the process of development
th special focus on areas of health and stress risk. Development of a Manual of mili-
ry and combat psychiatry is presently underway. Studies of stress in military com-
nities and families and data reduction and analysis continue. Studies of the dimen-
ons of morale in cross-cultural perspective have been developed utilizing materials
veloped by the IDF. This work is about to be piloted. For technical report,
e WRAIR Annual Progress Report, 1 Oct 82 - 30 Sep 83.

Available to contractors upon originator's approval

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 041 Military Preventive Psychiatry

Investigators.
Principal: David H. Marlowe, Ph.D.
Associate: COL Franklin D. Jones, MC; COL Norman M. Camp, MC; COL Jesse J. Harris, MS; LTC Edwin W. Van Vranken, MS; LTC Larry H. Ingraham, MS; MAJ Stanley Holgate, MS; MAJ James A. Martin, MS; MAJ Terrence D. Fullerton, MS; CPT Robert H. Stretch, MS; CPT Linda K. Jellen, MS; CPT Kathryn H. Knudson, MS; CPT Darleen M. Vernon, MS; CPT Ronald Smith, MS; Joseph M. Rothberg, Ph.D.; Charlene S. Lewis, Ph.D.; Mary L. Lozano, Ph.D.; David Segal, Ph.D.; Mady Segal, Ph.D.; Reuven Gal, Ph.D.; Caren M. Carney, M.A.; Richard Howard, M.A.; Richard Oldakowski; SFC Thaddeus M. Savage; SSG Edgar N. Marshall; SSG Richard W. Pickle; SSG Emily M. Kukura; SP5 Diana L. Smith; SP5 Daniel J. Helm; SP5 Alvin B. Taylor; SP5 Veronica L. Davis; SP5 Dawn D. Caban; SP5 Stewart Franklin; SP5 Gary Killiebrew; SP4 William Marshall; SP4 Donna M. Ross.

Description

Neuropsychiatric casualties have represented a major source of manpower loss in every armed conflict in which the United States Army has been involved. In times of peace the Army suffers significant personnel losses and costs as a function of behavioral dysfunctions, performance decrements, effectiveness deficits, psychosomatic illnesses, psychogenically based disorders and neuropsychiatric diseases. Many of these losses and costs appear to involve predisposing risk factors that are parts of the general and human ecology of the Army. Unique aspects and demands of military life engender both strains and stresses that further the risk of the individual and the group for dysfunctional and ineffective behavior. The symptomatic and often costly responses to stressful events and factors in the military are in part determined by the health status and coping styles of the individual and in part by the social milieu in which stressful events are experienced. This unit examines the interaction of the individual and group within this special set of ecological settings, ranging from the intense, life-threatening multiple stresses of combat to the daily stresses and strains of garrison and training, examines the dynamics of those specific factors within the military organizations and environments that conduce

to psychiatric illness, operate to produce psychiatric casualties, and lead to ineffectiveness, the generation of dysfunctional behaviors, and decrements in military performance.

Progress

Health Problems of Deployment: In order to gain a better understanding of the impact of both brief and extended periods of separation on military families, two studies have been undertaken. The first examined health and family stress in both deploying and non-deploying soldiers and their families during three short-term deployments (4 weeks or less) and one extended (6 month) deployment. The second study involved family members of three battalion-sized units whose sponsor deployed overseas for 6 months as part of a multinational peace keeping force. Although data collection continues, preliminary findings suggest that for families of both brief and extended deployers, general health ratings remained stable with moderate increase in reported stress-related symptoms. For both groups, the most frequently reported problem was child-centered. Initial findings suggest that deployment separation may create various degrees of psychosocial disruption and stress and that families are most likely to seek assistance from less specialized support elements such as kin, the Rear Detachment, and wives' support groups when these are perceived as concerned and accessible. The data collected during the course of the deployment of units in the 82nd Airborne Division as part of the MFO in the Sinai is presently under analyses. Special areas of study include the relationship of unit cohesion to health, performance, and patterns of leadership. The relationship of soldier stress while deployed to family events and stress is also under analysis as is material on the relationship of patterns of adaptation to soldier health and deployment. Initial analyses have been completed of the relationship between the peace keeping task and psychological and social adaptation to the Sinai.

Supportive Structure for Wives: In conjunction with the studies of health problems of deployment, an investigation of the Sinai Family Support Group (SFSG) was also conducted. This study focused on the structural composition of the support group, including the family members, the Rear Detachment and thirteen Army agencies and offices. The several key elements for success were identified, including Command sponsorship, a group of dedicated "key" wives and the various types of information which Army families need in order to resolve their problems in the most effective manner.

Stress on Special Forces Troops and Their Families: This research examined the stresses of Special Forces resulting from (a) frequent deployments, (b) exposure to danger, (c) inability to share work concerns with families because of classification requirements, and (d) soldier and military demands for highly cohesive work groups. Data collection by means of observations, interviews, and questionnaires is complete. Preliminary analyses of the psychometric properties of the questionnaires are completed. Analysis of the data continues.

COHORT Family Study: In conjunction with a DA study of the New Manning System (NMS), this department is studying the impact of the NMS on the health and general well being of military families. Fourteen units scheduled to become operational during 1983 and 1984 have been systematically chosen for participation in this study. These units represent infantry, armor, and field artillery companies at four major U.S. installations. All but two of these units will deploy overseas (Europe or Korea). Using a combination of self-administered survey instruments, supplemented with direct interviews, data is now being collected from family members at six important nodal points in the units' three year life cycle.

Early Army Experiences of Lieutenants: This research, done in collaboration with scientists at the US Army Military Academy, focuses on the experiences of graduates in their first assignment. The design is longitudinal. Questionnaire data is complete from the first COHORT; preliminary data analysis was presented at professional meetings. Questionnaire administration, interviews, and field observations continue for subsequent COHORTS.

The Wives of Career Enlisted Service Members - Application of a Life Stress Model: A stratified, random sample (N=315) of women married to enlisted personnel at a large FORSCOM installation was used to examine the effects of military related life stress issues on a number of outcome variables. The descriptive information and perceptions of these women concerning life satisfaction issues were informative. Analysis of the study model suggested that assessment of life stress conditions specifically related to military family life can substantially contribute to an understanding of the health and general well being of military wives. Each of the life stress conditions also provide a specific focus for community and command intervention efforts.

Role Stress and Strain on Army Noncommissioned Officers: This research examines the stress and strain inherent in the role of noncommissioned officers by means of career histories told to a professional interviewer. In this reporting period five career histories have been completed, and data reduction and analysis has begun.

Dimensions of Morale from a Cross-Cultural Perspective: This research examines common dimensions of morale across cultures. The method adapts a highly successful questionnaire measure of morale used by the Israeli Defense Force for use with American forces. The object is to determine whether the same dimensions obtain across the two armies, and to search for correlates of morale measures. This work is at the pilot stage.

Golden Acres Study: Data analysis from the study carried out of a military housing area at Ft Bragg, NC, continues. It is anticipated that write up and analysis of this work will be completed in FY85.

Future Recommendations and Objectives

Anticipated progress through FY84 will involve the continuation of ongoing studies and completion of in-stage projects. Further elucidation of the relationship of patterns of individual soldier and family stress to health and performance is anticipated. A better understanding of the dynamics of the relationship of unit cohesion, morale, and soldier support networks to stress tolerance, health outcome and performance, is expected as well as more fine-grained analyses of many of the problems under consideration. Long-term studies such as those of Special Forces, health problems of deployment, COHORT family and units and dimensions of morale will continue in response to both the needs of the Army and the furthering of our knowledge in these critical areas.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 041 Military Preventive Psychiatry

Papers Presented at Scientific Meetings

1. Harris, J.J., COL. Peace keeping in the Sinai: Notes from a Participant Observer. Inter-university Seminar on Armed Forces and Society, Washington, DC, 28 April 1983.

2. Jellen, Linda K., CPT; Van Vranken, E.; Marlowe, D. Coping with Stress of Extended Separation by Family Members of American Soldiers. Paper presented at Third International Conference on Psychological Stress and Adjustment in Times of War and Peace, Tel Aviv, Israel, 26 January 1983.

3. Jones, F.D., COL. Psychiatric Lessons of War. Presented at Third International Conference on Psychological Stress and Adjustment in Time of War and Peace (3ICPSATWP), Tel Aviv, Israel, 3-6 January 1983.

4. Jones, F.D., COL; Harris, P.; Fong, Y.H. Behavioral Responses in Adaptation to Hostage Situations. Presented at 3ICPSATWP, Tel Aviv, Israel, 3-6 January 1983.

5. Belenky, G.L.; Jones, F.D.; Marlowe, D.H.; Harris, P. Behavioral Responses in Adaptation to Combat. Presented at 3ICPSATWP, Tel Aviv, Israel, 3-6 January 1983.

6. Harris, P.; Jones, F.D.; Fong, Y.H.; Belenky, G.L; Marlowe, D. Behavioral Responses in Adaptation to Disasters. Presented at 3ICPSATWP, Tel Aviv, Israel, 3-6 January 1983.

7. Harris, P.; Jones, F.D.; Fong, Y.H.; Belenky, G.L.; Marlowe, D. Behavioral Responses in Adaptation to Refugee Situations. Presented at 3ICPSATWP, Tel Aviv, Israel, 3-6 January 1983.

8. Jones, F.D. Lessons of War for Psychiatry. VII World Congress of Psychiatry, Vienna, Austria, 11-16 July 1983.

9. Jones, F.D. Psychiatric Lessons of War for Future Combat. AMEDD Military Psychiatry Conference entitled William C. Menninger Memorial Conference: Military Psychiatry and Stress, Topeka, KS, 25-29 April 1983.

10. Lewis, C. Family Support Groups. Presentation to meeting of National Guard Generals' wives, Washington, DC, 12-13 October 1983.

11. Van Vranken, E. Work Induced Separation and Family Stress in an Army Population. Paper presented at the National Council on Family Relations Pre-Conference Workshop, Family Life in the Military, Washington, DC, 12-13 October 1982.

Project 3E162777A879 Factors Limiting Effectiveness

Work Unit 041 Military Preventive Psychiatry

Publications

1. Adelaja, O. and Jones, F.D. (Eds) War and its Aftermath. Proceedings of Military Section, World Psychiatric Association Meeting, Lagos, Nigeria, 26-29 November 1979, John West Publisher of Nigeria, 1983.

2. Jones, F.D. Importance of psychiatry in the military world. International Revue of Army, Navy and Air Force Medical Services, 54:21-22, 1981 (France).

3. Hales, R.E. and Jones, F.D. Teaching the principles of combat psychiatry to Army psychiatry residents. Military Medicine 148 (1):24-27, January 1983.

4. Jones, F.D. Combat and Its Aftermath: A Historical View. In War and Its Aftermath, O. Adelaja and F.D. Jones, (Eds), John West Publisher of Nigeria, 1983.

5. Jones, F.D. Combat Psychiatry and Modern Warfare. In War and Its Aftermath, O. Adelaja and F.D. Jones (Eds), John West Publisher of Nigeria, 1983.

6. Jones, F.D.; Crocq, L.; Adelaja, O.; Rahe, R.; Rock, N.L.; Mansour, F.; Collazo, C., and Belenky, G.L. Psychiatric casualties in modern warfare, I, Evolution of treatment. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

7. Jones, F.D.; Harris, P., and Fong, Y.H. Applications of military psychiatry in civilian disturbances: Disasters, terrorism, hostages and refugees. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

8. Jones, F.D. and Belenky, G L. Sanctioned use of drugs in combat. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

9. Cline, W.R.; Jones, F.D., and Howard N. Psychiatric combat readiness in Army and Navy Forces. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

10. Jones, F.D. Lessons of war for psychiatry. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

11. Jones, F.D. and Belenky, G.L. Warfare and the US military family. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

12. Jones, F.D.: Stokes, J.W.; Newhouse, P.A.; Belenky, G.L., and Crocq, L. Neuropsychiatric casualties in chemical, biological, and nuclear warfare. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

13. Hales, R.E.; Jones, F.D., and Holloway, H.C. Training issues in combat psychiatry. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

14. Marlowe, D.H. Violence and Aggression: The Disentanglement of Social and Individual Bases. Robert K. Fullinwider, Ed., Conscripts and Volunteers: Military Requirements, Social Values, and the All-Volunteer Force, Maryland Studies in Public Philosophy (Totowa, NJ: Rowman and Littlefield, 1983).

15. Marlowe, D.H. and Ingraham, L.H. Emphasizing Cohesion, Morale Can Help Prevent Battle Stress. Army, 33, No. 7, July 1983, pp 16-17.

16. Spencer, K. and Knudson, K. Differential Development of Affective Role-taking Ability and Prosocial Behavior. Journal of Genetic Psychology, 1983, 143, 97-102.

17. Martin, J. The Wives of Career Enlisted Service Members: Application of a Life Stress Model. Unpublished Doctoral Dissertation, Univ. of Pittsburg, PA, 1983.

18. Martin, J. Wives in Transition. Military Bulletin. (Forthcoming)

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20. Van Vranken, E.; Jellen, L.; Knudson, K.; Marlowe, D., and Segal, M. The Impact of Deployment Separation on Army Families. Divison of Neuropsychiatry Report Series, Walter Reed Army Institute of Research, Washington, DC, in press.

21. Marlowe, D.H. The AVF and the Draft. In Robert K. Fullenwider, ed., The Morality of Military Service. Maryland Studies in Public Philosophy. Totowa, N.J.: Rowman and Littlefield, 1983.

22. Marlowe, D.H. Women, Men, and the Draft. In Robert K. Fullenwider, ed., The Morality of Military Service. Maryland Studies in Public Philosophy. Totowa, N.J.: Rowman and Littlefield, 1983.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION: DA OC 6454	2. DATE OF SUMMARY: 83 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISPOSITION	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AA	042 WWJ3
b. CONTRIBUTING				
c. CONTRIBUTING	STOG 82/83-6,2/2			

11. TITLE: (U) Military Psychiatric Epidemiology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	5.5	498
FISCAL YEAR CURRENT 84	5.5	522

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: Camp, N
NAME: Rothberg, J POC: DA

21. GENERAL USE
Foreign Intelligence Considered

22. KEYWORDS (Provide EACH with Security Classification Code): (U) Military Adjustment; (U) Psychiatric Illness; (U) Epidemiology; (U) Behavioral Dysfunction; (U) Psychosocial Factors

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief individual paragraphs identified by number, provide text of each with Security Classification Code.)

23. (U) This unit examines military organizational, social, psychological, and environmental factors that create risk for and conduce to psychiatric disease, psychosomatic illness, behavioral dysfunction and physical illness as they affect Army personnel and impact on care giving agencies.

24. (U) The methods of epidemiology, including records surveillance, population and demographic analysis, questionnaire and field and cohort studies as well as methods of the psychological and social sciences are used to delineate environments of risk for psychiatric illness and periods of special risk for such illness at critical points in the career of the soldier.

25. (U)82 10- 8309 Analysis of outpatient data concerning troops and families of the 82nd Airborne Division continue. Analysis of data in the study of stress and perceived stress among drill sergeants presently continues. It is anticipated that the first phase of this project will be complete in FY84. Analysis of data collected on patterns of psychiatric care giving and use of psychotropic medication in the Vietnam conflict is underway and will be completed in FY84. Analysis of data gathered on post-traumatic syndrome among Vietnam veterans who remained on active duty is presently underway and due for completion in the next FY. This study has been extended to include active members of the AMC, at its request. Work continues on the study of well-being of female personnel. Work is almost complete on the development of a normative base for the General Well Being Scale in the Army. Work continues on the epidemiology of suicide and on other selected and special epidemiological topics. A preliminary study of the relationship of unit cohesion to soldier well-being and effectiveness has been completed as have pilot studies of mental health providers and the developmental phases of a study of stress and health problems among Hispanics in the US Army. An evaluation of a Corporate Fitness program being carried out at the Army staff and at a divisional staff is in progress. For technical report, see WRAIR Annual Progress Report, 1 Oct 82 - 30 Sep 83.

* Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 042 Military Psychiatric Epidemiology

Investigators.
Principal: David H. Marlowe, Ph.D.
Associate: COL Franklin D. Jones, MC; COL Norman M. Camp, MC; COL Jesse J. Harris, MS; LTC Edwin W. Van Vranken, MS; LTC Larry H. Ingraham, MS; MAJ Stanley Holgate, MS; MAJ James A. Martin, MS; MAJ Terrence D. Fullerton, MS; CPT Robert H. Stretch, MS; CPT Linda K. Jellen, MS; CPT Kathryn H. Knudson, MS; CPT Darleen M. Vernon, MS; CPT Ronald Smith, MS; Joseph M. Rothberg, Ph.D.; Charlene S. Lewis, Ph.D.; Mary L. Lozano, Ph.D.; David Segal, Ph.D.; Mady Segal, Ph.D.; Reuven Gal, Ph.D.; Caren M. Carney, M.A.; Richard Howard, M.A.; Richard Oldakowski; SFC Thaddeus M. Savage; SSG Edgar N. Marshall; SSG Richard W. Pickle; SSG Emily M. Kukura; SP5 Diana L. Smith; SP5 Daniel J. Helm; SP5 Alvin B. Taylor; SP5 Veronica L. Davis; SP5 Dawn D. Caban; SP5 Stewart Franklin; SP5 Gary Killiebrew; SP4 William Marshall; SP4 Donna M. Ross.

Description

The military environment places demands and strains upon its population that are markedly different from those of civilian environments. The demands and differences in terms of individual and unit effectiveness and performance, mental and physical health, and behavioral disruption and dysfunction have chronic effects in peacetime. In periods of deployment and combat, such stresses may have acute effects on the capability of units and individuals to perform their missions. This unit examines military organizational, social psychological, and environmental factors that create risk for and militate against psychiatric disease, psychosomatic and physical illness, behavioral dysfunction and disruption of performance as they affect Army personnel and impact on care giving agencies. The methods of epidemiology, including records surveillance, population and demographic cohort studies and methods of the psychological and social sciences are used to delineate factors conducing to risk as well as mitigation for such illnesses, disruptions and dysfunctions.

Progress

Vietnam Psychiatry Study: Data collection from 76% of the available psychiatrists who served in the Army in Vietnam was completed. Preliminary analysis reveals dramatic confirmation of the need to consider the human factors regarding the war in Vietnam as phasic with a pronounced degradation in troop morale over time. This finding is linked to the respondents acknowledgment of mounting psychiatrist role conflict, especially in those whose Vietnam service was limited to a tertiary care facility. A notable finding was that only 18% of respondent psychiatrists signified that they had, with any frequency, treated combat psychiatric casualties within 48 hours of onset of symptoms.

Stress and Health: The study of the stress factors in health and illness behavior has developed an individual outpatient data system to provide quantative assessments of the outpatient sick call rate. This technique has characterized the significant predeployment increase in sick call rates during field training and during peace keeping as part of the Multinational Force and Observers in the Sinai. Although the health of the United States soldiers in the Sinai was better than that of the UN forces in that area in 1975-1976, there was a worsening compared to the same battalion 18 months previously in the United States.

The Normative Base for the General Well Being Scale in the Army: The General Well Being Scale has seen extensive use in the work of this department, but it has no norms for military populations. In order to develop adequate norms for the Army, all known Army samples of the GWB have been merged into a single data base. Psychometric scale analyses and between group comparisons are complete, as is a review of the GWB literature. The final report of this work is in preparation.

General Well Being of Army Women: A sample of Army women and Army men is being given a questionnaire packet consisting of Dupuy's General Well Being Scale (1978), the Cornell Medical Index (Brodman & Wolff, 1949), the Zung Depression Scale (Zung, 1965), and the Army Satisfaction Index in order to assess overall levels of general well being among Army women and men in both traditional and non-traditional fields (for women and to relate general well being scores to other measures of health, both psychological and physical). This work extends the research of Knudson and Alva (1980) that found women in both traditional and non-traditional Army units experiencing extremely low measures of psychological well being as measured by Dupuy's Scale.

Post Traumatic Stress Studies: There has been continued data collection, analysis and expansion of the protocol entitled, "Delayed Stress Response Syndrome and Patterns of Psychosocial Support." In January 1983, the protocol was expanded to provide an assessment of the psychosocial adjustment of all active duty Vietnam and Vietnam-era veterans in the Army Nurse Corps. Data collection began in February 1983 and is now complete. Preliminary analysis has been started and is still underway. Following OMB approval in January 1983, the final phase of the protocol was initiated. Questionnaires were sent to 2500 civilian Vietnam and Vietnam-era veterans beginning in March 1983. Data collection has been completed. Data has been keypunched at NIH and initial analysis is expected to begin shortly.

DCSPER Corporate Fitness Evaluation: During the past year, we have been collecting data evaluating a stress management/ corporate fitness program instituted in the office of the DCSPER, US Army. Four waves of data have been collected thus far. In addition, extensive interviews have been carried out with members of the ODCSPER staff. At the present time, preliminary analyses of the data are being completed including multivariate and factor analysis. It is anticipated that data gathering will be complete in the next fiscal year and analysis and write up will proceed apace.

Drill Sergeant Assist: Multivariate analyses have begun exploring well being and distress among drill sergeants and a control group of non-drill sergeant NCOs. Perceived social supports and job satisfaction consistently: enhanced psychological well being and hopefulness; and mitigated general psychological distress, depression, and psychosomatic and paranoid tendencies. Although drill sergeants evidenced lower degrees of psychological health than non-drills, the control group seemed more severely depressed and psychosomatically oriented. Analyses continue.

Health Care Utilization Study: "Factors Contributing to Patterns of Utilization and Medical Care in the Army: - Implementation of this study at Ft George G. Meade as originally conceived has been delayed due to unforeseeable complications at the target post. These include the net loss of several battalions critical to the study, and the rotation of the original approving or sponsoring principals. Currently while another suitable post and proponent is being secured, efforts are underway to critically review the project design so as to take advantage of recent advances in the area of the sociology of medical utilization.

Hispanic Soldiers Study: An exploratory pilot study has begun seeking understanding of patterns of stress and health care utilization among Hispanic soldiers. Through the application of anthropological techniques and survey instruments, we are addressing (1) past and present health problems among Hispanic soldiers, (2) types of stress and stress responses experienced by Hispanic soldiers and their families, and (3) the cultural differences that exist within the Hispanic military community.

Mental Health Providers Study: In concert with a research project within the Department of Psychiatry, WRGH, a pilot survey of over 200 line officers was conducted to ascertain their perceptions of Army Mental Health services and providers. Preliminary analysis suggests that commanders feel they do have adequate resources for seeking advice on troop and family problems and see themselves as the first line of intervention for providing assistance to soldiers under their command.

Cohesion and Well Being: A preliminary study was carried out in support of an exercise studying the performance and reactions of small military units in MOPP gear. This study looked at the relationship between measures of unit cohesion and integration, psychological well being and evaluation of exercise effectiveness. Over the course of the two week field exercise, four measures were taken 5 times when the soldiers were presented with a unique training experience. The measure resulted in a significant difference between means. Attitudes towards platoon members remained stable but well being and attitudes towards the parent organization fluctuated. Soldiers who viewed both task and parent organization positively appeared to have less stress.

Suicide Studies: The study of the epidemiology of suicide in the Army has identified several items which correlate with suicide. The marital status-specific rates show that divorced or separated soldiers have a six times higher suicide rate compared to single soldiers and seven times higher compared to married soldiers. Army and civilians differ in the extent to which exogenous factors influence the suicide rate. The seasonal variation is moderate for the data from the United States (Spring and Fall peaks within 5% of the annual mean) and large for the Army (January and June peaks in excess of 25% above the annual mean). These monthly rates of suicide correlate highly with the monthly PCS moves within the Army ($r=0.89$). Both the marital status-specific and the month-specific data are consistent with the notion that one of the major factors in Army suicide is the actual or anticipated loss of love object. Future analysis of the suicide rates for soldiers experiencing different rotational schedules could be of considerable scientific and/or policy interest.

Future Recommendations and Objectives

Basic epidemiological analyses presently under way will be continued into the future with the immediate goal of developing sets of indicators relevant to troop readiness status, and to individual and unit abilities to perform optimally on the battlefield of the present and the future.

Developments during the course of the next FY will concentrate on the continuation and completion of studies presently under way. Further work is anticipated on the relationship between family health status and stress, unit cohesion and health and performance outcome for the active duty soldier. Work on social supports and other protectors against stress consequences, ill-health and breakdown will continue.

Monitoring of other data will continue in order to develop medical early warning indicators of unit status and potential patterns of disruption. Further work will be developed in the study of the military unit as a social support system protecting against or conducing towards illness and performance disruption and maintenance.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 042 Military Psychiatric Epidemiology

Papers Presented at Scientific Meetings

1. Camp, N.M. Operational Stress Among Army Psychiatrists in Vietnam. William C. Menninger Memorial Conference: Military Psychiatry & Stress (AMEDD Military Psych. Course) 26 Apr 1983.

2. Belenky, G.L. and Jones, F.D. Review of Contemporary Studies in Combat Psychiatry. VII World Congress of Psychiatry, Vienna, Austria, 11-16 July 1983.

3. Jones, F.D. and Belenky, G.L. The Lebanon Experience: Lessons of the Arab-Israeli War. AMEDD Military Psychiatry Conference entitled William C. Menninger Memorial Conference: Military Psychiatry and Stress, Topeka, KS, 25-29 April 1983.

4. Stokes, J.W.; Jones, F.D.; Hales, R.E. and Rock, N. Mental Health Resources in Future Combat. Presented at 3ICPSATWP, Tel Aviv, Israel, 3-6 January 1983.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 042 Military Psychiatric Epidemiology

Publications

1. Belenky, G.L.; Newhouse, P.A., and Jones, F.D. Prevention and Treatment of psychiatric casualties in the event of a war in Europe. International Revue of the Army, Navy and Air Force Medical Service, 55:303-307 (France), 1982.

2. Belenky, G.L.; Noy, S.; Solomon, Z, and Jones, F.D. Psychiatric casualties (Battle Shock) in Israeli Defence Forces in the War in Lebanon, June-September 1982. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

3. Crocq, L.; Jones, F.D.; Adelaja, O.; Rahe, R.; Collazo, C.; Mansour, F., and Belenky, G.L. Psychiatric casualties in modern warfare, II, Future warfare. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

4. Crocq, L.; Crocq, M.A.; Barrois, C.; Belenky, G.L., and Jones, F.D. Low intensity combat psychiatric casualties. In Psychiatry: The State of the Art, Volume VI, Peter Berner, Editor, Plenum Publishers, NY, in press.

5. Rothberg, J.M.; Rock, N.L., and Jones, F.D. Suicide in United States Army personnel 1981-1982. Military Medicine, in press.

6. Stretch, R. and Figley, C. Combat and the Vietnam Veteran: Assessment of Psychosocial Adjustment. Armed Forces and Society (in press, scheduled for Vol 10, No. 2).

7. Rothberg, J.M. and Jellen, L. The Individual Outpatient Data System: An Overview. Abstracts of the 100th Meeting, 76, American Public Health Association (1982).

8. Rothberg, J.M.; Tahmoush, A.J., and Oldakowski, R. The Epidemiology of Causalgia Among Soldiers Wounded in Vietnam. Military Medicine 148:347-350, 1983.

9. Rothberg, J.M.; Jellen, L., and Oldakowski, R. The Health Consequences of Deployment I Data Gathering. Defense Technical Information Center, Alexandria, VA. Document Number ADA124509, 1983.

10. Jellen, L. and Rothberg, J. The Health Consequence of Deployment II. Types and Rates of Outpatient Sickcall Visits of Active Duty Soldiers and Their Family Members, June 1980-May 1981. Defense Technical Information Center, Alexandria, VA. Document Number ADA124510. (1983)

11. Jellen, L. and Rothberg, J. The Health Consequences of Deployment III. Types and Rates of Outpatient Sickcall Visits, June 1980-May 1981 Combat Arms and Support Troops. Defense Technical Information Center, Alexandria, VA. Document Number ADA124436. (1983)

12. Jellen, L. and Rothberg, J. The Health Consequences of Deployment IV. Additional Survey of Injuries of Combat Soldiers. Defense Technical Information Center, Alexandria, VA. Document No. ADA124161. (1983)

13. Jellen, L. and Rothberg, J. The Health Consequences of Deployment V. Impact of Military Activity and Associated Transitional Periods on Combat Arms Outpatient Sickcall Rates. Defense Technical Information Center, Alexandria, VA. Document Number A124496. (1983)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA 0C 6457	2. DATE OF SUMMARY: 83 10 01		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUM'RY: 82 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8A. GRADE METH'D: NL	8B. SPECIFIC DATA - CONTRACTOR ACCESS: ☑ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AB	043 WWJ6
b. CONTRIBUTING				
c. CONTRIBUTING	STOC 62783-0.272			

11. TITLE: (U) Military Stress: Circadian and Ultradian Factors

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 016200 Stress Physiology 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATE/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
FISCAL YEAR: PREVIOUS 83	5.0	537
CURRENT 84	5.0	782

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE: Foreign intelligence considered

22. KEYWORDS: (U) Stress; (U) Biological Rhythms; (U) Chronobiology; (U) Electrophysiology; (U) Psychophysiology; (U) Human Volunteer

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Numbered paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Achievement of an understanding of the temporal organization of biological functions attendent upon sustained exposure to stressors in military environments. Information developed provides indicators of the magnitude and time-course of stressor induced behavioral and physiological disorders that are the precursors of the production of psychiatric and combat casualties.

24. (U) Monitoring techniques are employed in the laboratory and in the field to obtain detailed behavioral, electrophysiological, and biochemical measures of functioning during sustained operations. A variety of time series analysis techniques are applied to these data to assess changes that precede and accompany stress responses.

25. (U) 82 10 - 83 09 Observations of sleep management practices in the field resulted in the development of a formal Sleep Discipline Protocol at Ft. Hood. Analyses of the data from the 72-hour sleep deprivation study continued. Preliminary analysis of the Lexical Decision Task showed performance to be affected by a) amount of sleep loss b) type of stimulus presented c) location of stimulation and d) individual differences in sleep deprivation effects. Self-scored activation decreased irregularly over the first two days and then increased. Self-scored affect decreased continuously showing cyclical variations riding on a strong downward trend. Heart rates, general activity level, and oral temperatures showed circadian variations, with oral temperature also showing a small downward trend. Additional refinements to the complex demodulation program have permitted the characterization of the relationships between a) circadian rhythm of oral temperature and sleep deficit and b) cognitive-perceptual difficulties and circadian rhythm. Studies relating physical fitness to various cognitive, psychological, and physiological measures showed a) that fitness may attenuate decrements in cognitive work capacity for certain tasks as stress levels increase and b) an inverted U-shaped relationship between aerobic fitness and mental performance. For technical report see WRAIR Annual Progress Report 1 Oct 82 - 30 Sep 83.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE

Project 3E.627771A879 FACTORS LIMITING SOLDIERS' EFFECTIVENESS

Work Unit 043: Military Stress: Circadian and Ultradian Factors

Investigators:

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Associates: LTC Daniel P. Redmond, MC;
David Thorne, Ph.D.;
Harvey Babkoff, Ph.D. (Visiting Scientist);
Helen Sing, M.S.
Robert J. Pleban, Ph.D.

Problems and Objectives

The temporal organization of physiologic function and performance in military environments is studied in laboratory and field settings. Investigations seek to determine the magnitude and time-course of stress-induced performance degradations and the progressive psychophysiologic adaption to stressors such as sleep deprivation, continuous combat operations, temporal desynchronization, and life threat. Current efforts focus on the impact of rapid troop deployment over long distances by air, the characterization of the stress related effects of combining occupational life threat with shiftwork, the relationship between cerebral hemispheric laterality and circadian variations in military performance, and the effects of physical fitness across selected cognitive, physiological, and psychological indices.

Progress

Evaluation of the role of sleep loss and biological patterns of sleep and performance in operational settings were continued from observations during Reforger-82 and again during sustained operation CPX exercises in September 1983, by the 1st Cavalry Division. Analysis of the physiological data obtained from the studies mentioned above as well as from the Firefighter (shiftwork) study has been deferred, pending the final evolution and proving of the Biological Signal Analysis system. Observations made from these investigations underscored the important role of cognitive awareness and volitional choice on the quality and quantity of sleep acquired in the field. As a consequence, presentations, literature, and consultations were provided to command staff on tactics designed to conserve soldier resources under high

intensity sustained operation environments. These efforts resulted in the generation of a formal Battalion Sleep Discipline Protocol at Ft. Hood (Gaylor and Plair, 1983).

Analyses of physiological, behavioral and psychological data from the 72-hour sleep deprivation study continued (see Thorne, Genser, Sing, and Hegge, 1983 and the 1982 annual report). Preliminary analysis of the Lexical Decision Task showed that words were identified for a much longer period than were non-words. Subjects with few response lapses showed a) a deficit in identifying non words which was much more pronounced for left hemifield stimulation than for right hemifield stimulation and b) individual differences in sleep deprivation effects.

Self-scored activation decreased irregularly over the first two days of sleep deprivation and then tended to increase. This increase was unexpected and unexplained. Self-scored affect decreased continuously with increasing sleep deprivation and closely resembled the decline in cognitive performance on PVB tasks, showing cyclical variations riding on a strong downward trend.

Heart rate and general activity level showed circadian variations but exhibited no marked change in either mean value or variability. In contrast, oral temperatures showed large circadian variations superimposed upon a small downward trend of perhaps a half-degree Fahrenheit over the 72-hour period.

Additional refinements to the multiple complex demodulation program have thus far permitted the characterization of the relationships between a) circadian rhythm of oral temperature and sleep deficit and b) cognitive-perceptual difficulties and circadian rhythm. Further refinements will include partioning of other (higher frequency) components in the data sets and comparing them among all data sets to cull out the subtle changes as they occur in time which are not otherwise discernible with other analytical techniques.

The relationships among physical fitness, mental performance, mood state, and various physiological measures have been the focus of three recent studies. In the first study, the results obtained indicate that under simulated sustained combat operations a) fitness may attenuate decrements in cognitive work capacity for certain tasks requiring prolonged mental effort, particularly as the cumulative effects of sleep loss and

other stressors begin to mount and b) as overall stress levels increase, fitness may have a beneficial effect in moderating fatigue rate. A cross-sectional study found an apparent inverted U-shaped relationship between cognitive performance and aerobic fitness levels. The third study, currently in progress, is concerned with the effects of an aerobic fitness program (vs no aerobic fitness program) across a variety of cognitive, psychological and physiological indices.

Future Objectives

Accumulation and analysis of data concerning sleep loss, fragmented sleep, sleep discipline, shift changes, jet lag, and similar factors in sustained operations and CBW environments will continue with enhanced facilities for data management and analysis. Active coordination of programs including direct collaboration at the bench level is underway with DCIEM, NHRC, George Washington University and the University of Arkansas sleep laboratory. Analyses of the on-going fitness study should get underway later in the year.

Presentations and Publications

1. Gaylor, A.K., and Plair, D. "2nd BN, 5th CAV Sleep Protocol", 6 September 1983.

2. Hegge, F.W., and Redmond, D.P. Sleep Discipline and Shiftwork in sustained operations. Draft manual and presentation to the 82nd Airborne Division staff, March 1983.

3. Pleban, R.J., Thomas, D.A., and Thompson, H.L. Physical fitness as a moderator of cognitive work capacity and fatigue onset under sustained combat-like operations, in review.

4. Redmond, D.P. Sleep discipline in sustained operations. Presentations and classes for Division and Battalion Staff, 1st CAV DIV, August 1983.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OG 6452	2. DATE OF SUMMARY: 83 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 82 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DOD'N INSTR'N: NL	9a. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	9b. LEVEL OF SUM: b. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AB	044 WWJ7
b. CONTRIBUTING				
XXXXXXXX	STOG 82/83-6.2/2			

11. TITLE (Provide with Security Classification Code): (U) Neuroendocrine Response to Military Stress

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 016200 Stress Physiology; 012600 Pharmacology; 002300 Biochemistry; 013400 Psychology; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	c. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING: 83	3.0	417
FISCAL YEAR CURRENT: 84	4.0	589

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE: Foreign Intelligence Considered

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NAME: Oleshansky, M A
Mougey, E POC: DA

22. KEYWORDS (Provide EACH with Security Classification Code): (U) Stress; (U) Hormones; (U) Neuropeptides; (U) Combat psychiatry; (U) Human volunteers

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number, precede text of each with Security Classification Code.)

23. (U) To examine neuroendocrine correlates of stressors specific to the military environment. Types of stress to be studied will include physical and psychological stressors, including continuous performance and stressful social interaction.

24. (U) In both laboratory and field studies, we will attempt to correlate endocrine and performance data. This will provide a basis for optimization of work/rest schedules and stress management, and consideration of medical prevention/treatment regimens.

25. (U) 82 10 - 83 09. A study of the hormonal effects of 72 hr sleep deprivation in young men revealed marked increases in urea excretion in urine, suggesting that sleep deprivation may increase protein breakdown even when food and water are freely available. A report has been prepared summarizing the experience of foreign military medical personnel in management of battleshock casualties sustained in several conflicts during the last decade. In addition, a comparative analysis has been made of the doctrine of comrade and self aid on the battlefield. Also, a book of papers on combat psychiatry has been assembled and edited for publication. We have shown that psychological stress can elevate plasma levels of beta-lipotrophic hormone as well as beta-endorphin in female rats. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

* Available for corrections upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 66 FOR ARMY USE ARE OBSOLETE

Project 3E162777A879 MEDICAL FACTORS LIMITING SOLDIER EFFECTIVENESS

Work Unit: 044 Neuroendocrine Response to Military Stress

Investigators:

Principal:	Meyerhoff, J.L., M.D.
Associate:	Belenky, G.L., M.D., LTC, MC; Kant, G.J., Ph.D.; Oleshansky, M.A., M.D., MC: Mougey, E.H., M.S.; Pennington, L.L., B.S.

Objectives:

Neuroendocrine responses to stressors typical of combat and the military environment are studied in order to identify conditions and processes leading to physical and/or psychiatric breakdown in combat. Types of stressors studied will include physical and pharmacological as well as psychological stressors. In both laboratory and field studies, we will attempt to correlate endocrine and performance data. This will provide a basis for optimization of work/rest schedules and stress management, and consideration of medical screening, prevention and treatment regimens.

Progress:

We have conducted a comprehensive review of the relative incidence of combat psychiatric casualties in the Israeli Defense Forces (IDF). Psychiatric casualties were a significant source of manpower loss for the IDF in the 1973 Arab-Israeli War and in the 1982 war in Lebanon. In the 4 weeks of the 1973 Arab-Israeli War, the ratio of psychiatric casualties to wounded in action was approximately 30:100. In the 1982 war in Lebanon, from June through December, the ratio of psychiatric casualties to wounded was 23:100. The majority of psychiatric casualties were cases of battle shock (pure emotional reaction to the stress of battle). In both wars, intense battle stress was the primary cause of battle shock, battle shock cases emerged within hours of the beginning of hostilities, and were most prevalent where the battle was most intense. In both wars, symptoms were typically anxiety, depression, fear, and sleep disturbance. In 1973, all battle shock casualties were evacuated to the rear; only a few returned to their units during the war; many became chronically disabled. Following the 1973 war, the IDF adopted the U.S. doctrine of forward treatment. Using forward treatment, the IDF was successful in sending 75% of soldiers back to duty within 72 hours. For administrative reasons some of these soldiers never returned to their units, and a few soldiers relapsed. Overall, 60% of psychiatric casualties were returned to combat duty following forward treatment. In comparison to forward treatment, rearward treatment was significantly less effective, returning only 40% of soldiers to their units.

Prolonged sleep deprivation is characterized by psychological and physiological fatigue as measured by decrements in performance on cognitive or physical tasks. We determined the hormonal and metabolic effects of prolonged sleep deprivation on 6 young men undergoing 72 hrs of sleep deprivation in a supportive laboratory setting where food and water were freely available. Physical stress was absent and psychological stress minimized to that intrinsic to sleep deprivation itself. We measured urinary constituents that would be expected to reflect changes in psychological activation (cortisol) and shifts in metabolism (glucose, urea) as well as electrolyte balance. Cortisol, urea, glucose, electrolytes and other compounds were measured in five consecutive 24 hr urine collections including a 72 hr sleep deprivation period. Urines were collected during a 24 hr pre-deprivation day, 3 days of sleep deprivation and a recovery day. While urinary cortisol decreased only slightly, marked changes in other urinary constituents were observed. Urinary urea rose markedly during sleep deprivation, glucose decreased and urinary electrolytes decreased. These data indicate that sleep deprivation under ad lib food and water conditions can cause disturbances in normal metabolism characterized especially by increased protein catabolism.

In collaboration with investigators at Johns Hopkins University, we have examined the effects on team performance of the social stress of replacing a trained member of an established group with an untrained newcomer. We have measured changes in 24 hr urinary testosterone (TS) in these subjects. Two ten day experiments were conducted, each employing a design in which a three person group competed for several days, at which time the least successful competitor was removed from the group and replaced with an untrained newcomer, who had been kept completely isolated from the group. In each experiment, the original group member who was least competitive had urinary testosterone levels markedly different from the other two members. In the first experiment, the replaced subject had lower TS levels. In the second experiment, the replaced subject began the study with TS levels twice those of the other two: his levels dropped by 50% on the second day, and the subject withdrew himself from the experiment on the third day. An initial study was carried out to assess the effects on group dynamics of using a female with previous experience in the task to replace one member of an all-male three person working group. She succeeded in convincing the group to change their work schedules, and although some hostility was expressed toward her, the group's performance was somewhat improved.

We have initiated studies of the effects on endocrine physiology of conducting extended field exercises in chemical warfare protective suits (MOPP gear), compared to the ordinary field uniform. We collected 24 hr urine volumes in 10 soldiers participating in the exercise who volunteered for our study. Preliminary analysis shows a drop in 24 hr volume and an increase in specific gravity of the urine in soldiers wearing either type of uniform. The urine samples will be assayed for cortisol levels to attempt to infer if wearing the MOPP gear was more stressful.

We are continuing to evaluate the utility of plasma cyclic AMP as a psychoendocrine stress marker. In preliminary studies using armed forces personnel, we have seen increases in plasma cyclic AMP following exercise. We have also found increases following infusion of isoproterenol, an adrenergic agonist. We believe that plasma cyclic AMP will be a useful index of the degree of stimulation of adrenergic receptors in clinical studies of stress. In a preliminary study in rats, we have seen indications that beta-lipotrophic hormone is more responsive to psychological stress than is beta-endorphin. Attempting to develop an assay for plasma thyrotropin releasing hormone (TRH), in collaboration with the University of Indiana, we have measured the activity of pyroglutamic-aminopeptidase, the rate-limiting enzyme that rapidly destroys TRH in serum.

Future Objectives:

We are planning further follow-up studies of IDF psychiatric casualties, to correlate medical care records with battle events. In conjunction with the Dept. of Military Psychophysiology, we plan to continue to study the biochemical correlates of the performance decrements induced by sleep deprivation. In particular, we wish to determine whether additional food intake might prevent the increase in urea excretion seen in the initial study. We are preparing a protocol for the study of psychoendocrine correlates of stress in soldiers appearing before soldier-of-the-month boards. In connection with this and other studies, we are setting up a high-pressure liquid chromatographic method to measure plasma catecholamines. We are also planning psychoendocrine studies in soldiers in the "over forty" cardiovascular program, in conjunction with the Walter Reed Army Medical Center, Department of Cardiology. In collaborations with Duke University and the Massachusetts General Hospital, additional studies are planned on the effects of stress on subjects with "type A" personalities, the stress of public speaking, and stressful interviews. We are also studying patients with "panic disorder", a condition with marked similarities to "Soldier's Heart".

Presentations:

Belenky, G.L. Simulated battles and their implications for the prevention and treatment of battle stress casualties. Paper presented at the Third International Conference on Psychological Stress and Adjustment in Time of War and Peace, Tel Aviv, Israel, 2-6 January 1983.

Belenky, G.L. Behavioral responses in adaption to combat. Paper presented at the Third International Conference on Psychological Stress and Adjustment in Time of War and Peace, Tel Aviv, Israel, 2-6 January 1983.

Belenky, G.L. Combat psychiatry in future wars. Paper presented at the AMEDD Symposium Division and Combat Psychiatry, Seattle, Washington, 14-28 March 1983.

Belenky, G.L., Noy, S., Solomon, Z., and Jones, F.D. Battle shock casualties in Israeli forces in Lebanon. Paper presented at the VII World Congress of Psychiatry, Vienna, Austria, 11-15 July 1983.

Belenky, G.L. and Jones, F.D. Contemporary studies in combat psychiatry. Paper presented at the VII World Congress of Psychiatry, Vienna, Austria, 11-15 July 1983.

Kant, G.J., Neurochemical and neuroendocrine responses to stress. Paper presented at the Defense and Civilian Institute for Environmental Medicine, Toronto, Canada, January 1983.

Meyerhoff, J.L. Neurobiology of affective disorders. Lecture presented at the Conference on Depression and the Life Cycle. Department of Psychiatry, University of South Florida, Tampa, Florida, January 1983.

Publications:

Belenky, G.L. and Kaufman, L.W. Cohesion and rigorous training: observations of the air assault school. Military Review 63:24-34, 1983.

Belenky, G.L., Sodetz, F.J., and Tyner, C.F. Israeli battle shock casualties: 1973 and 1982. Division of Neuropsychiatry, WRAIR Report 83-4, August 1983.

Belenky, G.L., Noy, S., Solomon, Z., and Jones, F.D. Battle shock casualties in Israeli forces in Lebanon. Proceedings of the VII World Congress of Psychiatry (in press).

Kaufman, L.W. and Belenky, G.L. Stayin' alive: knowing what to do until the medic arrives. Military Review (in press).

Emurian, H.H., Brady, J.V., Ray, R.L., Meyerhoff, J.L. and Mougey, E.H. Experimental Analysis of Team Performance Effectiveness. Naval Research Reviews, 1983 (in press).

Emurian, H.H., Brady, J.V., Meyerhoff, J.L. and Mougey, E.H. Small Groups in Programmed Environments: Behavioral and Biological Interactions. Pavlovian Journal of Biological Science. 1983 (in press).

Emurian, H.H., Brady, J.V., Ray, R.L., Meyerhoff, J.L. and Mougey, E.H. Experimental Analysis of Team Performance Effectiveness: Methodological Developments and Research Results. Psychological Documents. Vol. 13, p 15, 1983.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OC 6470	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORGN HISTORY	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AA	046 WWQ1
b. CONTRIBUTING				
XXXXXXXXXXXX	STOG 82/83-6.2/2			

11. TITLE (Include with Security Classification Code)

(U) Medical Factors Limiting Soldier Effectiveness

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016200 Stress Psychology 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77/10	Cont'	DA	C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	2.5	62
FISCAL YEAR CURRENT 84	2.7	65

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: ROCK, S

NAME: KAUFMAN, L

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Provide each with Security Classification Code) (U) Epidemiology; (U) Stress; (U) Psychiatry; (U) Human Volunteer; (U) Soldier Effectiveness

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide brief explanatory paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To identify factors in the military organizational, social, psychological and physiological environment that create or increase risk for psychiatric breakdown, behavioral dysfunction, psychosomatic and physical illness, all of which impact on individual and unit effectiveness and consume health care resources.

24. (U) The methods of epidemiology, including records analysis, population and demographic analysis, questionnaires, field and cohort studies, and various observation methods are employed to develop requisite data.

25. (U) 82 10-83 09 A study of the effects of assimilation of families into a military community has been completed. Major findings: type of housing is one variable which affects the assimilation of families. However, the most important variable affecting assimilation is the degree to which soldiers and their spouses identify with or feel part of the community. Those who do not are less likely to extend or reenlist, and are more likely to report problems with their tour. A study of battle stress casualties in the Wehrmacht has been completed. Major findings: these casualties occur in spite of the best leadership; peacetime planning for them is therefore critical. A study of the role of stress problems in patient admissions during REFORGER is in progress. A study of the socialization and integration of new lieutenants into USAREUR units has been completed. Major findings: although new lieutenants are a highly motivated group of officers, confusion in what is their job and how they fit into their units probably reduces the effectiveness of most. Recommendations to help ensure effective assimilation are included. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83.

*Available in instructions upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3E16:777A879 FACTORS LIMITING SOLDIERS EFFECTIVENESS

Work Unit 046 Medical Factors Limiting Soldier Effectiveness

Investigators

Principal: Schneider, MAJ R.J.
Associates: Rock, CPT S.K., Jr.
Kaufmann, CPT L.W.

Description

This field unit, stationed in West Germany with the U.S. Army Europe and Seventh Army, identifies and investigates physical, psychological, social, and organizational factors bearing on the individual, unit performance, and battle readiness. Current efforts focus on five areas identified by commanders as important concerns within the European theater.

INTEGRATION OF FAMILIES INTO THE MILITARY COMMUNITY

An observational and interview study of a cohort of 102 families has been completed. The research focused on the socialization of the family into the larger military community and the implications of this socialization for illness and adjustment. This socialization was studied as a function of housing groups. Two criterion scales-the General Well Being (GWB) and the Psychological Sense of Community (PSC) were used to help assess adjustment. Contrary to our expectation, we did not find that sick call or illness rates differed significantly among housing groups. Also, GWB and PSC scores showed relatively little variation among housing groups. Considerable differences among respondents were found when comparing individuals who were high and low on the two criterion scales. Of the two criterion scales, PSC relates most strongly to other variables of interest to the military. Thus individuals without this attribute were not only more marginal in a number of areas representing community involvement and integration, but such individuals rated a number of potential problems as more severe, in spite of the fact that their own experience with those problems was essentially the same as those with a strong sense of community. Housing area, health, and a feeling of general adjustment or well being are therefore not as important as the degree to which individual family members are integrated into the community. Our data indicate that the cost of failing to integrate families into the military is measured in terms of lost reenlistment, lost extensions, and decreased support of military and community goals.

A number of recommendations were made to help increase integration

and socialization. These include establishing programs to provide essential integrative services through the small unit rather than through centralized "umbrella" agencies. This is to help make the military the focus of socialization, not only ensuring that socialization does occur, but that it occurs in a manner which builds a psychological sense of community around the military community. It is important to provide the small unit leader with a workshop to explicate the relation between family and other variables which interest leaders (e.g., performance, reenlistment, etc.), since many small unit leaders are neither prepared to handle nor sympathetic to the different problems of the married soldier and his family. In addition, emphasis on other integrative programs, such as the sponsorship program for spouses, increased language and general life coping skills for family members, and using stairwell coordinators to help integrate new families are recommended.

BATTLE STRESS REACTION AND THE WEHRMACHT

The purpose of this paper was to present a historical review of the problem of German soldier breakdown due to battle stress in WW II. Most authorities report that stress breakdown did not occur in the Wehrmacht; good leadership, which built highly cohesive units, is the most common explanation for the few reported cases of stress breakdown. The importance of cohesion (a responsibility of leadership) was emphasized in terms of sustaining the force, rather than in terms of preventing stress casualties. However, this leadership responsibility was well recognized and probably did help prevent many stress casualties. Thus, when a soldier did break down in battle, extensive medical efforts were directed at finding an organic basis (i.e., something physically wrong) for his dysfunction.

Our analysis of original source data from World War II indicates that soldier breakdown was a serious problem for the Wehrmacht, although it was usually not diagnosed as such. In spite of this, even as early as 1942, the Army Chief of Staff indicated concern with the problem of attrition due to psychogenic disturbances. In fact by July 1944, the problem became so severe that the Surgeon General requested a separate report on the numbers of soldiers lost due to stress reactions.

This work challenges a widespread although implicit assumption, about soldier response to the stress of battle - the problem can be eliminated by good leaders, who might occasionally need to "kick'em in the rear." Battle stress reactions cannot be eliminated by fiat, and cannot be totally prevented even by the best of leadership. The Israeli Defense Force (IDF) can be used as an example of a modern army with good leadership, high morale, and victory in the face of overwhelming odds. Although winning, the IDF

suffered more stress casualties than killed in action (KIA) in their recent incursion into Lebanon. Medical personnel must be prepared to treat such casualties, and should be prepared to consult to military commanders in how to prevent them.

We concluded that line cadre of all branches should be prepared to expect stress reactions among their troops. Training in stress management should be a regular part of garrison operations. Inclusion of manpower loss due to stress breakdown should be a standard aspect of field problems. Finally, the positive impact of good leadership and the building of cohesion are vitally important leadership responsibilities to reduce or delay the problem.

REFORGER STRESS CASUALTY STUDY

During the 1982 REFORGER exercise stress casualties may have accounted for 23% of the total hospitalizations. Several hypotheses about stress reactions are therefore being tested during REFORGER 1983: REFORGER is more stressful than "normal" duty, and therefore proportionately more soldiers will be hospitalized for stress during REFORGER than at other times during the year. Soldiers assigned to their units for a short time will be hospitalized proportionately more often for stress than soldiers who have been assigned to their units for a longer time. Soldiers assigned to combat service support units will be stress casualties proportionately more often than those assigned to combat support or combat units. Proportionately more females will be hospitalized for stress than males. Proportionately more younger than older soldiers will be hospitalized for stress. Proportionately more lower ranking soldiers will be hospitalized for stress than higher ranking soldiers.

This research is in progress. Data collectioon is anticipated to be completed by the end of October 1983.

THE CAPTAINS' STUDY

This report is an extension of an earlier study of the socialization of lieutenants, and is based on interviews of company commanders. There were several areas about which the company commanders had expressed some confusion or question concerning their own roles and duties. These areas concerned the importance of lieutenants to the functioning of the company, training leadership in the lieutenant, how the company commander learns to train lieutenants, differences between captains and lieutenants in the officer ethic, and who trains the company commander to do his job.

The captains generally agreed that lieutenants were important or

necessary to the unit. Over two thirds (71%) of the captains said that lieutenants were important, and could not be replaced with experienced NCOs. The reasons captains gave for having lieutenants in their units were: a) to train the lieutenants to be company commanders, b) to help share the workload of the captain and/or the platoon sergeant, c) his ability to make decisions, d) they are more mission-oriented than NCOs.

Most of the captains said they could train leadership in the lieutenants, and they clearly recognize their responsibility in this area. Less clear was their explanation of how to teach leadership and their role in the day-to-day process: a) two thirds of the captains said they used counseling to teach leadership, b) over half (57%) of the captains said they used (on-the-job) experience to train the lieutenants, e.g., putting them in situations where they would face new problems, c) almost one third (29%) of the captains said they used officer professional development classes, but with one exception these were not well defined or organized, d) a small number of captains said they used written guidelines or modeling.

When asked where they had learned to train lieutenants the captains reported that their training had come from: a) their own company commanders when they were lietuenants, or from other senior officers (43%), b) their own experiences as a lieutenant (one third), c) a small number mentioned their peers (other captains) or their officer advanced course.

The captains were asked why lieutenants reported engaging in unethical or dishonest behavior. Over one third (38%) of the captains reported that the pressure on the lieutenant to get a job done or have all his equipment "up" resulted in dishonest behavior. One third of the captains said that the lieutenant had a different perception of honesty or truth than captains. Twenty-nine percent of the captains said that the dishonest behavior was a result of inexperience. Twenty-nine percent of the captains also said that the captain is more honest because he knows his career is on the line if he lies. One quarter said that the captain has to be more honest because of the greater responsibilities put on captains. About one quarter of the captains don't believe there is a difference between lieutenants and captains, i.e., there was pressure on both that resulted in "dishonest" behaviors. When asked about training in the officer ethic the captains responded with negative comments about their officer advanced courses. About one third (29%) of the captains said they teach lieutenants ethical behavior by example, while three felt it came from the command climate and three said it couldn't be taught.

The question of who trains captains to do their job is a logical extension of the same question about lieutenants, and was triggered by captains' comments. There was no clear answer from the captains

about who trains them. All the captains agreed that the battalion commander should train them, but only two captains reported that the battalion commander had a formal training program. Almost half (48%) of the captains said the battalion commander trained them through informal methods including talks, on the spot corrections, "blowing his top," and "ass-chewings."

Several of the captains reported reservations about asking questions or asking for help from their battalion commander. The two principle reasons given by the captains for this reticence were: the captains did not want to bother the battalion commander because he was busy, and did not want the battalion commander to think that "I don't know my job." Promotion to captain does not eliminate one's concerns about "how am I doing my assigned job." Over half (57%) of the captains reported that the battalion executive officer also was a source of help and information on problems. More than half (52%) of the captains said the battalion operations officer was a resource. This help is not part of a formal or organized program. Three quarters (76%) of the captains reported that they asked for help from other captains in command, and almost all the captains reported a cooperative relationship among the company commanders. Although only a few reported competition between company commanders, it is a real force because the company commanders believe they will be evaluated relative to their peers.

The officer advanced course was mentioned by only one captain as useful in preparing him for his job as company commander. He said that the advanced course helped him with supply, maintenance, and administrative problems, but had nothing to do with the leadership problems of running a company. Other captains were generally negative about the course. They referred to it as a vacation, a time to see where you stand with respect to your peers. Leadership classes were referred to as "leadersleep," a chance to "get over," and without impact on captains who were already set in their ways.

Although there are many specific differences, little in general differentiates captains and lieutenants with respect to their training for their jobs and their approach to the ethical problems that face them. The captains' programs to train lieutenants are like the battalions commanders' programs to train the captains: they are informal and not clearly defined. The common theme in these responses is that training in basic leadership skills is not quite adequate. This includes not only what should be taught, but when and how. There is a clear rejection of much of the content and technique employed in their advanced courses.

When it comes to dishonest behavior, the captains in comparison with lieutenants are able to use their judgment which has been refined through experience in the organization. This difference, however, is one of degree rather than kind. The captains have developed

several strategies for coping with the problems they confront, while the lieutenants may have none or only one. Thus the captains have several options open to them in a given problem situation that the lieutenants have not yet learned. Further, the captains have a major advantage over the lieutenants we interviewed earlier: they are more self-confident and believe they can find the answer to a problem, either on their own or by asking someone in their unit.

DETERMINANTS OF TANK CREW PERFORMANCE

The purpose of this research is to determine the relationship of selected demographic and psychosocial factors in crew structure to tank crew performance. These relationships are important to the military because of the need to establish determinants of human behavior under combat conditions, and because factors which contribute to improved performance may also be important in predicting or preventing stress reaction. Furthermore, if such relationships are established they can provide standards against which changes in Army policy and unit practices (e.g., practices designed to improve unit morale and cohesion, training techniques, etc.) can be compared.

The immediate impetus for this research comes from two sources: recent Israeli research indicating the importance of psychosocial factors in combat effectiveness and the controversial "Gideon" study (Wallace, 1982). The studies conducted by the Israeli Defense Force (IDF) since the war in Lebanon have reported that, in addition to intensity of combat, the primary predictors of psychiatric breakdown in combat are low morale, poor unit cohesion, and weak leadership. Other factors identified with psychiatric breakdown are age, poor education, low motivation, and low intelligence (Belenky, 1983). Conversely, high morale correlates with increased combat effectiveness. The Gideon study was designed to determine the relationship between the mental aptitude of individual tank crewmen and tank gunnery performance.

The present research attempts to determine the importance of a variety of demographic and psychosocial variables for tank crew performance, emphasizing those identified by the IDF as important in determining combat effectiveness. Results from this research should give a more complete and realistic picture of how a number of key variables impact on tank crew performance in the Army than is currently available. Data were obtained from 45 tank crews of an armor battalion participating in their annual gunnery qualification at Grafenwoehr. Data analyses are in progress.

PUBLICATIONS THIS YEAR:

Rock, S.K., & Schneider, R.J. Battle stress reactions and the Israeli experience in Lebanon: A brief summary. Medical Bulletin, (in press).

Kaufman, L.W., & Belenky, G. Cohesion and rigorous training: Observations of the air assault school. Military Review, 1983, 24-34.

Schneider, R.J., & Luscomb, R.L. Battle stress reaction and the U.S. army, Military Medicine, (in press).

Rock, S.K. Training new lieutenants: A company commander's responsibility, Infantry, (in press).

Kaufman, L.W. Stayin' alive: Knowing what to do until the medic arrives, Military Review, (in press).

Kaufman, L.W., & Collier, G. Meal-taking by domestic chicks (Gallus gallus), Animal Behavior, 1983, 31, 397-403.

Kaufman, L.W., & Collier, G. Cost and meal patterns in wild-caught rats, Physiology and Behavior, 1983, 30, 445-449.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA 300237	83 10 01		DD-DR&E(AR)636
3. DATE PREV SUMRY: 82 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. DOCM SECURITY: U	7. REGRADING:	8. DOD'D MSTR: NL	8A. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT
10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
A. PRIMARY	62777A	3E162777A879		AB	047	WUJM	
B. CONTRIBUTING							
XXXXXXX STOG 87[illegible]							

11. TITLE (Put one DoD security classification code): (U) Neuropharmacological Management of Military Performance and Casualties

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 012900 Physiology 016200 Stress physiology 013400 Psychology 012699 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING: 83	4.0	422
FISCAL YEAR CURRENT: 84	6.0	311

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE: Foreign Intelligence Considered

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POC:-DA

22. KEYWORDS (Provide EACH with Security Classification Code): (U) Shock; (U) Stress; (U) Pharmacology; (U) Trauma; (U) Stimulants; (U) Nervous System; (U) Behavior

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide brief technical paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop neuropharmacological insights into the etiology and treatment of circulatory shock and trauma, and to evaluate electrophysiological, neuroanatomical, autonomic and endocrine correlates of performance and casualty management. Endogenous mechanisms regulating access of biological substances into the brain will allow for manipulations of endocrine or autonomic function to alter central nervous system activity. Results are anticipated to provide novel pharmacological approaches to the management of military casualties and improve performance. Correlates of behavior and autonomic function with altered immune states will be investigated.

24. (U) Measurements of physiological, pharmacological and behavioral function in animals will be performed using established and novel procedures. Baseline measurements will be contrasted with responses to endogenous or exogenous agents in order to determine their ability to restore homeostatic function in models of disease states or acute injury.

25. (U) 82 10 - 83 09. The characterization of selectively different opioid receptor involvement in shock and pain relief was extended to demonstrate the potential utility of delta antagonists to reverse shock while allowing for mu agonists (e.g. morphine) to relieve traumatic pain. It was found that delta and mu recognition sites for opioid ligands are functionally linked as part of the same macromolecular receptor. Prolactin release by opioid agonists is mediated at mu binding sites, whereas the inhibition of sex hormone release by opioids mediated by delta binding sites. Using sophisticated electrophysiological measurements, the endogenous release of opioid peptides results in an anticonvulsant effect, decreasing the probability of subsequent seizures. The restricted access of drugs and hormones into the brain by the blood brain barrier was demonstrated to be at least partially regulated by steroids released from the adrenal cortex. Nerve growth factor was shown to influence the development of the cholinergic nervous system, and results were extrapolated to Alzheimer's disease and organophosphate toxicity. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 82 - 30 Sep 83.

* Available in restricted form upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project: 3E162777A8 79 MEDICAL FACTORS LIMITING SOLDIER EFFECTIVENESS

Work Unit 047 Neuropharmacological Management of Military Performance and Casualties

Investigators:
Principal: Holaday, J.W., Ph.D.
Associate: Mobley, W.C., MAJ, MC; Long, J.B., CPT, MS; Tortella, F.C., Ph.D.; Ruvio, B.A., B.S.; Robles, L.E., B.S.; Rogers, O., B.S.; Black, L.E., B.S.

Objectives:

To develop research designed to evaluate the therapeutic utility of neuropharmacologic agents in experimental models of shock and trauma; to evaluate the role of endogenous substances in arousal and depressed states using pharmacological, behavioral and physiological models; and to provide pharmacological insights into the mechanisms of adaptation and habituation to environmental and behavioral stressors.

Specifically, further studies have characterized specific opioid receptors involved in shock pathophysiology. Electrophysiological assessment of brain and motor function has provided the opportunity to correlate pharmacological and physiological responses with a index of nervous system function within the brain and periphery. Immunohistochemical and radioreceptor techniques have allowed for determination of potential neuroanatomical sites of action for endogenous and exogenous substances which are involved in behavioral or physiological responses. Additionally, determination of potential mechanisms involved in the transportation of hormones and drugs into the central nervous system has provided new insights into endogenous and exogenous regulation of blood-brain barrier penetration.

It is the goal of these studies to apply basic neuropharmacological insights to problems of clinical and military relevance. The results of these studies are anticipated to provide novel pharmacological approaches to the treatment of disorders involving adaptation to shock and other stressors.

Progress:

I. Management of shock and trauma:

It has been recently demonstrated that more than one opioid receptor subtype may be responsible for the diverse actions of endogenous or exogenous opioid substances. Through the use of newly available, specific opiate-receptor agonists and antagonists, it was demonstrated in the that the various cardiovascular responses to opioids may reflect their selective actions upon different receptor subtypes. Specifically, the hypotensive effects of opioid substances that predominantly bind to δ receptors occurs within the peri-third ventricular area (hypothalamus), resulting in a

decrease in sympathetic outflow and a loss of cardiovascular tone. By contrast, opioid substances with a predominant μ affinity act upon brain structures in proximity to the fourth ventricle to produce bradycardia, probably by activation of vagal-parasympathetic effector systems.

Since general opioid antagonists such as naloxone block the pain-relieving effects of opioids while exerting therapeutic effects in circulatory shock, an selective approach was sought to allow for shock reversal while still enabling pain relief from endogenous opioids or morphine. It was shown that the "opioid" component of shock pathophysiology is mediated by the release of endogenous opioid peptides which act upon δ receptors, whereas the analgesic properties of injected morphine are mediated at μ receptors. This selectivity of action at distinct subpopulations of opioid receptors allows for the potential use of δ opioid antagonists to reverse shock, while the μ-agonist morphine could be co-administered to allow for relief of the traumatic pain which may accompany severe injury.

Perhaps of greater importance to an understanding of fundamental opioid receptor physiology, it was shown that prior occupancy of the μ receptors with irreversible blockers had no effect upon shock severity by itself, however the usual therapeutic actions of naloxone or selective δ-antagonists was blocked. Thus, antagonists acting upon the μ binding site must communicate information to δ-binding sites. The potential pharmacological and physiological relevance of this discovery lies in the interpretation of these interactions between μ and δ receptors. These data suggest that they are not two distinct opioid receptors, but instead they represent different binding sites on the same opioid receptor macromolecule which interact through configurational alterations of a common molecular receptor structure.

II. Endocrine responses to opioid activation and shock:

It was shown in experiments with rats and monkeys that the release of the pituitary hormone prolactin was mediated by opioid receptors of the μ-type. Conversely, the opioid-induced inhibition of release of luteinizing hormone (the pituitary hormone responsible for release of the sex steroid, testosterone) was shown to be mediated via actions upon δ-receptors. The physiological and behavioral importance of prolactin is as yet poorly defined, however decreases in sexual function produced by opioids appear to be related to their effects upon δ-receptors to result in a decreased sex hormone release.

Recent work in the Neuropharmacology Branch has revealed the importance of intact sympathoadrenomedullary function in the therapeutic actions of naloxone and thyrotropin-releasing hormone (TRH) in the treatment of endotoxic shock. Specifically, it was shown during the past year that removal of the adrenal gland prevented the therapeutic actions of naloxone in endotoxic and hemorrhagic shock. These findings, in combination with results from other laboratories, indicated that the integrity of the adrenal gland is essential for naloxone's therapeutic actions in circulatory shock. Furthermore, from these studies with adrenalectomized rats, we can conclude that the very large increase in circulating β-endorphin derived from the pituitary in the absence of usual

adrenal feedback regulation must not be directly involved in the cardiovascular effects of shock or their reversal by naloxone.

Further studies were conducted to evaluate the involvement of sympathoadrenomedullary catecholamine outflow in the therapeutic actions of these agents by measuring plasma epinephrine, norepinephrine, and dopamine along with hemodynamic variables during naloxone and TRH treatment of normal and endotoxemic rats. While endotoxic shock was associated with very large increases in plasma catecholamines, neither naloxone nor TRH altered plasma catecholamines relative to saline-treated controls in endotoxemic rats. These results indicate that the therapeutic cardiovascular actions of naloxone and TRH do not simply arise from enhanced sympathoadrenomedullary secretion of catecholamines, and suggest that the spectrum of adrenomedullary compounds released following sympathetic stimulation (including enkephalins, adenosine triphosphate, chromogranin, magnesium, etc.) may act together with catecholamines to elicit the therapeutic actions of naloxone or TRH.

III. Electrophysiological correlates of performance and injury:

A central nervous system (CNS) neuropharmacology research laboratory was established to measure electrophysiological responses in small animals. Application of these technologies dramatically expands the capabilities of the laboratory for defining biological responses to drugs, toxins, neurotransmitters and hormones which modulate brain activity. Using these facilities, rodent models of experimental seizure activity have implemented to define the role of opioid substances and neuropeptides as anticonvulsants and/or proconvulsants. Behavioral and electrographic responses to endogenous and exogenous opioids were evaluated. Data indicates that two separate opioid systems exist which result in the anticonvulsant actions of opioid systems (both μ and δ). Furthermore, using a rat model of electroconvulsive seizures, it was shown that the interval immediately following seizures, characterized by a general behavioral depression, appeared to result from an activation of endogenous opioid systems. This 'postictal' inhibitory effect of endogenously released opioids acted to decrease the probability of the occurrence of a subsequent seizure. This work has relevance to fundamental biological mechanisms which regulate susceptibility to seizures, and suggests novel therapeutic strategies for the treatment of seizure states.

The seizure-induced activation of endogenous opioid substances and their subsequent action as endogenous anticonvulsants were investigated. In addition to the measurement of behavioral components of seizure activity in these studies, this work has involved the development of quantitative analytical radioimmunoassay procedures for measurement of beta-endorphin-like immunoreactivity in biological tissues, including brain, pituitary, and plasma. Furthermore, it was shown that the analgesic effects of acute electroconvulsive seizures was antagonized by a selective δ-antagonist, not by a μ-antagonist. This provides evidence that endogenous opioid systems, activated by electroconvulsive shock, act upon δ-receptors to produce the analgesic state in rats.

IV. Factors governing brain access of neuropharmacological substances:

Research interests in peripheral communications with the brain prompted the development of techniques for the evaluation of physiological, pharmacological, and pathological influences on the permeability of the blood-brain barrier. The blood-brain barrier (BBB) maintains a constant extracellular environment for the nerve cells of the brain, protecting the central nervous system from changes in plasma composition, including peripherally released hormones as well as injected drugs or toxins. This BBB may be regarded as a dynamic interface, with permeability properties which may be influenced and regulated by environmental, behavioral, and pharmacological manipulations. As such, it is a potential target for experimental and therapeutic intervention to influence peripheral and pharmacological communications with the brain.

Repeated electroconvulsive shock (ECS) was shown to increase permeability of the BBB, indicating the possibility that behavioral and neurochemical changes characteristic of ECS (and perhaps other seizure states as well) may be partially due to novel exposure of the neural substrate to circulating humoral substances ordinarily restricted from passage into the brain by the BBB. It was speculated that the body's own release of corticosteroids from the adrenal gland may participate in the regulation of the integrity of the BBB, both under resting conditions and following ECS. Physiological adrenal influences on undisrupted brain microvasculature were examined by measurement of the permeability of the BBB to ^{125}I-bovine serum albumin, a macromolecule (MW=65,000) which is almost completely excluded from the brain due to its large molecular size. Significant increases in the permeability of the BBB to radioactive albumin were produced by adrenalectomy, and were completely reversed by administration of physiological doses of corticosterone. Selective removal of the adrenal medulla (while keeping the adrenal cortex intact) produced the same effect as total adrenalectomy, indicating that the adrenal cortex is primarily responsible for altered BBB integrity. These results indicate that the pituitary-adrenal axis may permissively regulate the entry of macromolecules into the CNS and may thereby indirectly alter the central actions of diffusion-limited drugs and humoral substances. These observations have significant implications of military relevance with regard to drug and toxin entry into the CNS since these substances, as well as acute stress, result in significant activation of the pituitary-adrenal axis.

V. Neuroanatomical interactions with biological responses:

Studies were conducted to analyze neurotransmitter pathways involved in neuropharmacological responses. Nerve Growth Factor (NGF), a peptide which is known to stimulate the growth of peripheral nerves, was purified from mouse submaxillary glands and characterized by acrylamide gel electrophoresis and biological assay. An iodination procedure was then developed to radiolabel the NGF protein. This radiolabeled, purified NGF was then employed in studies of central nervous system cholinergic neurons in newborn rats. Striking increases in choline acetyltransferase activity were documented in neurons of the septum, basal forebrain, and striatum, as well as nerve fibers of the hippocampus and neocortex. Preliminary retrograde transport studies using 125I-NGF in brain were performed to

evaluate the anatomical localizations of the nerve cells which innervated these diverse brain regions. Further attempts to obtain a tool for characterization of NGF led to the development and growth in culture of a hybridoma line to produce a monoclonal antibody against NGF. This antibody was used for affinity purification of NGF and to perform binding studies to analyze NGF binding to specific receptors on a primary line of Schwann cells.

Another project involved neurobiological studies of Alzheimer's disease, a disease of aging brain which results in the the occurrence of senile dementia. These studies have recently been linked to the experimental results with NGF since it was shown that NGF was associated with an alteration of the development of the nervous pathways whose degeneration is believed to be involved in the etiology of Alzheimer's disease.

To elucidate some of the mechanisms underlying Alzheimer's disease, a series of immunocytochemical studies in rats and normal and aged monkeys were conducted. These studies had two primary goals: 1) the anatomical characterization of the basal forebrain and its projections 2) the elucidation of the neurotransmitter(s) found in the cortical plaques of Alzheimer's disease. Data collected to date have provided an accurate map of cholineacetyltransferase positive cells in monkeys as well as a preliminary characterization of cholinergic neuronal fibers pathways. Additionally, immunocytochemical studies of Alzheimer's disease tissue were performed employing an antibody to myelin basic protein, the "insulative" material which surrounds axonal projections of nerve cells. These anatomical investigations provide an opportunity to define the nervous system pathways involved in cholinergic function and are pertinent to investigations of the mechanisms of neuronal toxicities produced by chemical warfare agents.

Future Directions:

Plans for 1984 include the priority of obtaining functional laboratory and office space to afford the opportunity to pursue research objectives outlined in approved and planned research proposals. These studies within the Neuropharmacology Branch will continue to integrate available talent to provide novel insights into the neuropharmacology of circulatory shock. The recent acquisition of sophisticated electrophysiological instrumentation and techniques provide the opportunity to correlate nervous system function with hormonal and drug responses. Studies will be continued to define the blood brain barrier and its alteration during stress and toxic substance exposure. Morphological studies will be conducted to correlate the localization of various neuroactive transmitters with their receptors and their relationship to stress, toxin exposure and behavior.

Emphasis will be placed upon development of psychoneuroimmunology as an entirely new correlate of military performance and casualty management. This involves the acquisition of knowledge about the fundamentals of cellular and humoral components of immune responses to enable their correlation with shock states as well as stress and behavioral disorders. It is anticipated that this integration of nervous system function with immune mechanisms will provide novel insights into the etiology of many aspects of physiological derangements as well as psychosomatic illnesses.

Presentations made:

John W. Holaday, Ph.D.:

1. "Pharmacological Basis of Anaesthesiology", invitational symposium participant, lecture on "Clinical Implications of Endorphin Research", Milan, Italy, Nov 1982.

2. Departments of Anesthesiology and Intensive Care, invited lecture, Technological U. of Munchen, Munich, West Germany, Nov 1982.

3. Session Chairman, "Opiate Receptors", Society for Neurosciences Annual Meeting, Minneapolis, Minnesota, Nov 1982.

4. Department of Neurology, Cornell University Medical Center, New York, New York - Grand Rounds, Nov 1982.

5. Sloan-Kettering Memorial Cancer Research Institute, New York, New York, - Grand Rounds, Nov 1982.

6. Department of Pharmacology, Albert Einstein College of Medicine, New York, New York - Departmental seminar, Nov 1982.

7. Western Pharmacological Soc., invitational symposium participant, "Endorphin antagonists in shock and spinal trauma: Therapeutic implications" Puerto Vallarta, Mexico, Jan 1983.

8. Winter Brain Conference, invited panel participant, "Molecular mechanisms of spinal cord injury and recovery", Keystone, Colorado, Jan 1983.

9. Departments of Anesthesiology, Pharmacology and Toxicology, "Cardio-respiratory actions of opiate systems" and "Neuropeptide involvement in shock and spinal injury", University of Mississippi Medical Center, Jackson, Mississippi. Departmental seminar, March, 1983.

10. Department of Psychiatry, invited lecturer, "Endogenous opiates in schizophrenia and affective disorders", Walter Reed Army Medical Center, March, 1983.

11. Third Annual Neurosciences Symposium, U. Kentucky, invited lecture on "Endogenous opiate systems in seizure mechanisms: involvement in ictal and post-ictal effects", Lexington, Kentucky, Apr 1983.

12. Allegheny General Hospital, grand rounds, "Neuropeptide involvement in shock and trauma", Pittsburgh, Pennsylvania, Apr., 1983.

13. Shock Society, session chairman "Endogenous opioids in shock pathophysiology", Jackson Hole, Wyoming, June, 1983.

14. Sloan-Kettering Memorial Cancer Research Institute, New York, New York, - Grand Rounds, "Possible physiological function of multiple opioid receptors", July, 1982.

15. Bethesda Navy Hospital, Bethesda, Maryland, "Endogenous opioid systems in shock and trauma" and "Multiple opioid receptors: possible physiological function", Sept., 1983,

16. University of California, San Francisco, invited symposium speaker "Septic shock, newer concepts in pathophysiology and treatment", Sept., 1983.

Frank C. Tortella, Ph.D.:

1. Temple University School of Medicine, Philadelphia, lecture on "Antiepileptic Pharmacology", Feb., 1983.

2. Temple University School of Medicine, Philadelphia, lecture on "Central Nervous System Stimulants", Feb., 1983.

William C. Mobley, M.D., MAJ, MC:

1. Chairman, organizing committee, American Society of Neurological Investigation, Washington, D.C., Oct., 1982.

2. Co-chairman, "Pathogenesis of Alzheimer's Disease, American Society of Neurological Investigation, Washington, D.C., Oct., 1982.

3. Invited lecture, "Progressive Rubella Panencephalitis", Dept. of Neurology, Johns Hopkins University School of Medicine, Nov., 1982.

4. Division of Neuropsychiatry lecture, WRAIR: "Nerve Growth Factor Increases Cholinergic Neurotransmitter Enzymes in the Central Nervous System", Walter Reed Army Institute of Research, Washington, D.C., May, 1983.

5. Invited lecture, "Nerve Growth Factor in Cholinergic Central Neurons", Dept. of Neurology, Johns Hopkins University School of Medicine, Sept., 1983.

Patents pending:

1. "Narcotic antagonists in the therapy of shock" Continuation in part, Serial # 248,622, re: neurogenic shock and spinal injury.

2. "Thyrotropin releasing hormone in therapy of shock and as a central nervous system stimulant" Patent application serial number 252,443.

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2. Holaday, J.W., Black, L.B., and Long, J.B. Neuropeptides in shock and trauma. Clinics in Critical Care Medicine, Chernow, B., ed., in press.

3. Holaday, J.W. Opiate antagonists in shock and trauma. J. Emerg. Med., in press.

4. Holaday, J.W., and D'Amato, R.J. Multiple opioid receptors: evidence for μ-δ binding site interactions in endotoxic shock. Life Sci., in press.

5. Belenky, G.L., Gelinas-Sorell, D., Kenner, J.R., and Holaday, J.W. Evidence for δ-receptor involvement in the post-ictal antinociceptive responses to electroconvulsive shock in rats. Life Sci. in press.

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PROJECT 3M4637510993

MEDICAL DEFENSE AGAINST CHEMICAL WARFARE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OH 0610	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORDER NUMBER	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63751A	3M463751D993	AC	061 WWM3
b. CONTRIBUTING				
c. CONTRIBUTING	CARN			

11. TITLE (Precede with Security Classification Code)

(U) Clinical and Ancillary Studies for Antiradiation Drug Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PRECEDING FISCAL YEAR 83	0.0	00
CURRENT FISCAL YEAR 84	2.0	123

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20307

RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: FLECKENSTEIN, L
NAME: PAMPLIN, C L POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Pharmacology; (U) Antidotes; (U) Toxicity; (U) Pharmacokinetics; (U) Quantitation Methodology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief individual paragraphs identified by number, precede text of each with Security Classification Code.)

23. (U) The goal of this work is to develop clinical information on candidate antiradiation drugs that have progressed successfully through preclinical studies. This information is directed toward further development of these drugs through clinical investigations to demonstrate their efficacy and limitations in human subjects using models where necessary. There is military relevance in this research.

24. (U) The first questions addressed with these studies concern determinations of doses of effectiveness and minimal toxicity in man. This work relies heavily on animal model studies since humans cannot be intentionally irradiated. Pharmacokinetic investigations in animal models may be conducted based on analytical chemical data. The resulting information is then applied to similar clinical studies in human subjects. Finally, experiments are conducted to determine the best dosage forms and route of administration for these drugs for their intended purpose.

25. (U) 82 10-83 09 The focus of this work has been on producing a clinical pharmacokinetic profile of WR 2721. The beagle dog is serving as the animal model. The high pressure liquid chromatography (HPLC) assay developed previously has been validated and utilized in the beagle dog in preliminary pharmacokinetic studies of WR 2721. A similar assay for WR 1065 the dephosphorylated metabolite of WR 2721, is being developed in a related project. These two methods, utilized simultaneously, can provide a more complete picture of the bioavailability of the new microencapsulated formulations of WR 2721 that have been prepared for oral administration. Preparations are underway to generate this information using the beagle dog prior to similar human studies. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 82 - 30 Sep 83. Portion of work continued in Project 3M463764D995, Work Unit 070.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 85 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE.

PROJECT 3M463751D993 MEDICAL DEFENSE AGAINST CHEMICAL WARFARE

Work Unit 011: Clinical and Ancillary Studies for Antiradiation Drug Development

Investigators:

Principal: Melvin H. Heiffer, Ph.D.

Associate: MAJ J. von Bredow, Dr. H. Lowensohn

1. Description.

The development of agents which protect against the effects of radiation injury requires a highly integrated multidisciplinary effort to undertake a broad range of preclinical and clinical pharmacological studies. The ultimate goal of this work is to obtain the necessary information to support granting of a Notice of Claimed Investigational Exemption for a New Drug (IND) by the Food and Drug Administration (FDA) for each candidate radioprotectant.

2. Progress.

WR 2721 is an aminopropyl aminoethyl phosphorothioate compound currently being developed by this division for submission to the FDA as an antiradiation drug. This compound shows good radioprotectant activity after intravenous administration. The drug undergoes rapid hydrolysis under acidic conditions and thus would not be expected to survive the acidic conditions of the stomach without significant degradation. As expected, the drug shows little radioprotection in animal studies following oral administration. To be useful in military settings, under field conditions, it is necessary to develop a dosage form which can be taken orally. To this end, drug microencapsulation has been undertaken to protect the drug from acid hydrolysis and deliver the parent drug intact to the intestine. Micro-encapsulation of WR 2721 in various fatty materials has led to three experimental formulation which demonstrate promising *in vitro* characteristics. All 3 formulations show good protection against acid hydrolysis in a pH 1 test medium. Further, it has been demonstrated that the drug is readily released from the dosage form *in vitro* under conditions designed to simulate the intestinal environment.

Development of specific assays for WR 2721 and WR 1065 the diphorphorylated metabolite of WR 2721, will allow bioavailability testing of the experimental encapsulated formulations. The new high pressure liquid chromatographic (HPLC) assay for WR 2721 for

pharmacokinetics studies, and initial results have been obtained in the beagle dog. This study has provided information on the overal plasma level profile of WR 2721 and analysis of the these data has provided useful information for optimizing blood sampling procedures for future pharmacokinetic work. Development of an HPLC assay for the principal metabolite of WR 2721 has proceeded far enough to demonstrate the feasibility of this technique. An internal standard for the assay has been synthesized under contract. Once this standard material is available in quantity, the method will be finalized with reproducibility and accuracy experiments.

3. Future Work.

The total pharmacokinetic profile of WR 2721 continue to be investigated in the dog model. Bioavailability studies will be continued in animals to test the oral absorption of the experimental micro-encapsulated formulations using the HPLC assays for WR 2721 and WR 1065. Formulation work will continue to provide microencapsulated formulations in quantity to support the above studies.

4. Publications.

E.P. McGovern, D.J. Mangold, J.A. Nino, E.M. Gause and L. Fleckenstein, HPLC Assay for S-2-(3-aminopropylamino)ethyl phosphorothioate (WR 2721) in Plasma. J. Liquid Chromatography 6:1523-1534, 1983

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OH 0609	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DOD'S INTR'S	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	D. Change	U	U		NI	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	6375TA	3M463751D993	AB	062 WWM8
b. CONTRIBUTING				
c. [illegible]	CARDS			

11. TITLE (Procede with Security Classification Code)

(U) Clinical and Ancillary Studies for Anti-Chemical Warfare Drug Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
83 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 83	0.0	00
	CURRENT 84	3.0	239

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: PAMPLIN, C L

NAME: DAVIDSON, D E POC:DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Provide JACS with Security Classification Code) (U) Pharmacology; (U) Antidotes; (U) Toxicity; (U) Pharmacokinetics; (U) Quantitation Methodology

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The purpose of this research is to develop clinical information on candidate chemical warfare agent antidotes that have progressed successfully through preclinical studies. This information is directed toward further development of these antidotes through clinical investigations to demonstrate their efficacy and limitations in human subjects. There is military relevance in this research.

24. (U) The first questions addressed with these studies concern determinations of toxic and effective doses in man. This work is supported by prior studies in animal models. Pharmacokinetic investigations in animal models may be conducted based on analytical chemical data. The resulting information is then applied to similar clinical studies in human subjects. Finally, experiments are conducted to determine the best dosage forms and route of administration for these drugs for their intended purposes.

25. (U) 82 10-83 09 Recent research has focused on the combined formulation of 2-PAM-Cl and atropine prior to clinical pharmacokinetic studies. Analytical procedures utilizing high pressure liquid chromatography (HPLC) and gas chromatography (GC) have been developed to analyze, respectively, 2-PAM-Cl and atropine as well as expected major degradation products from both. These procedures are presently being utilized in stability studies to determine the compatibility of these two drugs in combination preparations as well as their individual shelf life in autoinjectors. Results from one of these studies have uncovered 3 degradation products in samples of 2-PAM-Cl maintained at room temperature for 8 years. Work is presently underway to determine the chemical nature of these products. Other studies are underway to determine the hemodynamic and myocardial energetic effects of atropine administered i.v. to dogs. This work will compare these effects in the dog at rest vs. various states of exercise. In addition, a new Notice of Claimed Investigational Exemption for a New Drug (IND) has been initiated for the short-term prophylactic use of pyridostigmine. For technical report see WRAIR Annual Report 1 Oct 82 30 Sep83.

Portion of work continued in Project 3M463764D995, Work Unit 071.

DD FORM 1498

PROJECT 3M463751D993 MEDICAL DEFENSE AGAINST CHEMICAL WARFARE

Work Unit 062: Clinical and Ancillary Studies for Anti-Chemical Warfare Drug Development

Investigators:

Principal: Melvin H. Heiffer, Ph.D.

Associate: LTC C. Pamplin, COL D. Davidson, Dr. G. McCormick J. Notsch

1. Description.

The development of antidotes against various chemical warfare agents requires a highly integrated, multidisciplinary approach spanning a broad spectrum of preclinical and clinical pharmacological studies. The ultimate goal of these studies is to obtain the necessary information to support granting of a Notice of Claimed Investigational Exemption for a New Drug (IND) by the Food and Drug Administration (FDA) for each candidate antidote.

2. Progress.

Accelerated stability studies continue on 2-PAM-Cl and atropine, alone and in combination. This work utilizes high pressure liquid chromatographic (HPLC) and gas chromatographic (GC) analytical methods developed in this Division. These techniques are being used to measure the loss of these two compounds and the appearance of degradation products at a variety of temperatures in various formulations. Part of this work has been devoted to long-term stability studies which have disclosed a 10% degradation of 2-PAM-Cl over eight years at room temperature. The three principal degradation products of 2-PAM-Cl are presently being studied by HPLC and mass spectrometry for identification. This work will be used to determine the stability of single and combination drug preparations in fielded and future autoinjector systems.

Other studies are underway to investigate the cardiovascular effects of atropine in the dog model. This work will compare the effects of i.v. administered atropine on dogs at rest and under exercise conditions. The focus of this study is on the hemodynamic and myocardial energetic effects of atropine. Pyridostigmine is also being investigated as a short-term prophylactic antichemical agent. A new Notice of Claimed Investigational Exemption for a New Drug (IND) has been initiated for this use of the drug.

3. Future Work.

Efforts will continue along each of these lines of investigation to determine the feasibility of combining 2-PAM-Cl and atropine in a single autoinjector and the shelf-life of the best resultant formulation. Work on the effects of atropine in exercising animals will also continue as will efforts to advance the new use of pyridostigmine through further development.

4. Publications.

Lowensohn, H.S.: Atropine's effects upon the muscarine controls: The heart and its systemic output. Physiological Reviews (manuscript in preparation).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6478	83 1001	DD-DRAE(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GRADE ENTER	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF WORK
82 10 01	D. Change	U	U		CX	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63751A	3M463751D993	AB	063 WWM6
b. CONTRIBUTING	62734A	3M162734A875		
XXXXXXXXXX	CARDS			

11. TITLE (U) Development of Antiradiation Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
0703 Organic Chemistry 0615 Pharmacology 0603 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 10	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PREVIOUS 83	2.0	457
FISCAL YEAR CURRENT 84	2.0	206

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
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20. PERFORMING ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
H
Foreign Intelligence Considered

POC:DA

22. KEYWORDS (Provide EACH with Security Classification Code) (U)Drug Development;(U)Antiradiation Drugs;(U)Radiation Protection;(U)Ionizing Radiation;(U)Pharmacodynamics;(U)Chemical Synthesis (U) RAM I

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief explanatory paragraphs identified by numbers. Precede each with Security Classification Code.)

23. (U) To develop new drugs with protective activity against injury to military personnel in the event of exposure to ionizing radiation.

24.(U) Potentially active drugs will be identified and obtained by synthesis or purchase. Candidate drugs will be tested in laboratory model systems to establish protective efficacy, mechanisms of pharmacological effects, effects on physiological responses and pharmacokinetic characteristics. Information is used in guiding new drug synthesis and in selecting candidate drugs for clinical trials.

25. (U) 8210-8309 Fifty-two compounds, including analogs of WR 2721, amidinium compounds bis sulfinates and dithioacids have been synthesized and submitted as candidate radioprotectant compounds. Significant progress has been made in development of orally effective formulations of WR 2721; three microencapsulated formulations have been produced and characterized in vitro. An analytical methodology which is specific and highly sensitive has been developed for quantitation for WR 2721 and a metabolite in biological fluids and tissues. This will enable the performance of pharmacokinetic studies. WR 2721 and WR 638 have been well tolerated in clinical studies. WR 638 by oral administration and WR 2721 by intravenous administration. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 82 - 30 Sep 83

* Available to contractors upon requestor's approval.

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSO AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE O

ORMS 1498A, 1 NOV 68

3M463751D993 MEDICAL DEFENSE AGAINST CHEMICAL WARFARE

Work Unit 063 Development of Antiradiation Drugs

INVESTIGATORS:

Principal: COL Craig J. Canfield, MC
Associate: COL David E. Davidson, Jr., VC
LTC Robert O. Pick, MS
Dr. Melvin H. Heiffer
MAJ Carl J. Neilsen, MS
Dr. Daniel L. Klayman
Mr. William Y. Ellis

PROBLEMS AND OBJECTIVES:

There are no antidotes to radiation exposure which are available to U.S. military personnel in the event of nuclear warfare. The objective of this program is the development of a radioprotective drug which will provide partial protection against both prompt and fallout radiation exposure. WR 2721, a phosphorothioate compound, is a promising candidate but requires developmental effort to overcome its poor oral effectiveness, undesirable side effects and short duration of protective effect (less than two hours). In addition to these developmental studies with WR 2721, identification and development of other compounds with better characteristics will be pursued.

PROGRESS:

The radioprotector program is conducted by extramural contracts with fourteen laboratories, collaborative studies with other government institutions and by limited intramural research.

The extramural synthesis program is conducted under six contracts. Fifty-two compounds, including analogs of WR 2721, amidinium compounds, bis-sulfinates and dithioacids, have been synthesized and submitted for testing. As an adjunct to the extramural effort, procedures for purification of previously synthesized and stored WR 3689 have been developed intramurally.

Biological and pharmacological studies are performed by eight contractors in the extramural program. Newly synthesized

compounds are tested in mice for activity and further development of WR 2721 is pursued. Significant progress has been made in development of orally effective formulations of WR 2721; three microencapsulated formulations resistant to the acid conditions of the stomach, and which rapidly release unchanged drug in the alkaline environment of the intestine have been produced and characterized in vitro. Storage stabilities have been established. Specific and highly sensitive analytical methodology using high performance liquid chromatography has been developed for quantitation of WR 2721 and its thiol metabolite in biological fluids and tissues.

Research into effects of ionizing radiation and of radioprotective drugs on prostaglandin metabolism has recently been initiated by a contract and by a newly-created intramural project. Changes in prostaglandin metabolism after radiation have been reported recently but the medical significance is not well understood. Results of initial studies suggest a relationship between the thiol metabolite of WR 2721 and glucocorticoid control of prostaglandin production.

Two candidate radioprotective drugs are under active clinical investigation under the auspices of the National Institutes of Health. WR638 appears to be well tolerated in oral long term administration (daily for up to four years) in children for treatment of cystinosis. WR 2721 is being administered intravenously to cancer patients in conjunction with radiotherapy (and chemotherapy) in Phase I Trials of human tolerance. At single doses of 1100 and 1300 mg/M^2 (approximately 2 gm), no irreversible or dose-limiting toxicity has been encountered.

FUTURE OBJECTIVES:

Efforts will continue to develop formulations to achieve oral effectiveness of WR 2721 so that a radioprotectant will be available for soldiers on the battlefield. Alternatives to WR 2721 will be sought through synthesis and animal testing. WR 3689, an analog of WR 2721 with superior oral efficacy and tolerance in rodents, will be studied in detail to determine the desirability of proceeding to formal pre-clinical trials. Mechanisms of action of radioprotectant drugs will be investigated, specifically the relationship with prostaglandins and glucocorticoids. The development of the analytical procedure for WR 2721 allows definitive pharmacokinetic studies to be performed in animals and man which will support the logical

development of product formulations and expansion of clinical trials. Studies are in progress to characterize the ability of WR 2721 to protect the lungs of rats against short-and long-term effects of ionizing radiation. The factors responsible for the failure of WR 2721 and most other experimental radioprotectors to effect neurological protection will be investigated.

PROJECT 3M463764D995

NON-SYSTEM ADVANCED DEVELOPMENT OF ANTIDOTES

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303 187	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	A. New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63764A	3M463764D995	AB	070 WWML
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code)

(U) Preclinical Studies of Anti-Chemical Warfare Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology 016800 Toxicology 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	2.0	336
FISCAL YEAR CURRENT 84	4.0	450

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Pharmacodynamics; (U) Antidotes; (U) Toxicity; (U) Pharmacokinetics; (U) Quantitation Methodology; (U) Formulation; (U) Metabolism

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The technical objectives of this work unit are to obtain the necessary information for chemical warfare agent antidotes to support a Notice of Claimed Investigational Exemption for a New Drug (IND). The antidotes will be developed for the defense of military personnel in an integrated chemical/nuclear/conventional battlefield.

24. (U) A highly integrated, multidisciplinary effort is required to coordinate the extramural and intramural studies necessary to develop candidate chemical warfare agent antidotes. The actual studies performed are dictated by scientific rationale and existing federal regulations to include the completion of efficacy and toxicity studies, formulation development, pharmacokinetic and metabolism studies.

25. (U) 82 10 - 83 09 Two candidate nerve agent antidotes, WR 249,943 (MMB4) and WR 249,655 (HI-6), are presently undergoing accelerated stability studies. These studies rely on high pressure liquid chromatographic (HPLC) analysis techniques applied to the various chemical and physical experiments being conducted. Initial results indicate that buffer pH values of 3.0 to 3.5 provide the greatest stability for MMB4. The phosphorothioate WR 2823 and the antileishmanial WR 6026 have shown activity as possible anticyanide drugs. WR 2823 is presently being microencapsulated to protect it from acid cleavage in the stomach. The metabolism of WR 6026 is being studied in isolated hepatocytes and microsomes. Other antiparasitic drugs with similar properties are being studied as candidates for anticyanide activity. For technical report, see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

* Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

3M463764D995 NON-SYSTEM ADVANCED DEVELOPMENT OF ANTIDOTES

Work Unit: 070 Preclinical Studies of Anti-Chemical Warfare Drugs

Investigators:

Principal: Melvin H. Heiffer, Ph.D.

Associate: CPT A. Schroeder, CPT J. Anders, CPT A. Theoharides, SP4 V. Melendez, SP4 H. Velazquez, J. DiGiovanni, J. Bartosevich

1. Description.

The development of agents which protect against the effects of radiation injury requires a highly integrated, multidisciplinary effort to undertake a broad range of preclinical and clinical pharmacological studies. The ultimate goal of this work is to obtain the necessary information to support granting of a Notice of Claimed Investigational Exemption for a New Drug (IND) by the Food and Drug Administration (FDA) for each candidate radioprotectant.

2. Progress.

Accelerated stability studies of two potential nerve agent antidotes, WR 249,943 (MMB4) and WR 249,655 (HI-6) are currently underway. High pressure liquid chromatographic (HPLC) assays have been developed to permit this work which is an examination of the stability of these two compounds under a variety of conditions such as changing temperature, pH, formulation buffer, etc. Results thus far indicate that buffer pH values of 3.0 to 3.5 provide the greatest stability of MMB4.

Possible anticyanide activity has been shown by WR 2823 and WR 6026. Since WR 2823 is a phosphorothioate compound, and therefore acid-labile, it is presently being microencapsulated to protect it from acid cleavage in the stomach after oral administration. The metabolism of WR 6026 is being studied in isolated hepatocytes and microsomes to determine the active component of the drug. Other antiparasitic drugs with structures and properties similar to WR 6026 are being studied as potential anticyanide drugs.

3. Future Work.

Formulation work will continue on WR 249,943 (MMB4) and WR 249,655(HI-6) until the optimum conditions have been determined for further development. After microencapsulation of WR 2823 has

been completed, studies on bioavailability, pharmacokinetics, and metabolism will be conducted. Studies on WR 6026 and related antiparasitic drugs will continue to determine if suitable anticyanide drugs can be developed from this class of compounds.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 303 186	83 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 10 01	A New	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63764A	3M463764D995	AB	071 WWMK
b. CONTRIBUTING				
c. CONTRIBUTING	CARDS			

11. TITLE (Provide with Security Classification Code)

(U) Preclinical Studies of Antiradiation Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology 014100 Radiobiology 016800 Toxicology 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 83	2.0	353
FISCAL YEAR CURRENT 84	3.0	371

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. academic institution)

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TELEPHONE: (301) 427-5393

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: FLECKENSTEIN, L

NAME: POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Pharmacodynamics; (U) Radioprotectants; (U) Toxicity; (U) Pharmacokinetics; (U) Quantitation Methodology; (U) Formulation; (U) Metabolism

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Provide text of each with Security Classification Code.)

23. (U) The technical objective of this work unit is to obtain the necessary information to support a Notice of Claimed Investigational Exemption for a New Drug (IND) for anti-radiation agents being developed for defense of military personnel in an integrated chemical/nuclear/conventional battlefield.

24. (U) A highly integrated, multidisciplinary effort is required to coordinate the extramural and intramural studies necessary to develop candidate antiradiation agents. The actual studies performed are dictated by scientific rationale and existing federal regulations to include the completion of efficacy and toxicity studies, formulation, development, as well as pharmacokinetic and metabolic studies.

25. (U) 82 10 - 83 09 Analytical procedures were developed for the HPLC measurement of the radioprotectant drug WR 2721. Work under contract is now underway to extend these procedures to analyze WR 1065, the major metabolite of WR 2721. This work will support the overall effort to develop an oral formulation of WR 2721. New studies have recently been instituted to examine the comparative effectiveness of WR 2721, the dephosphorylated metabolite WR 1065, and several congeners. This work is being conducted using a variety of subcellular and isolated cell models. Some newer aspects of the latter work will be conducted in FY 84 under 61110191CLA (APC WWMN). For technical report, see Walter Reed Army Institute of Research Annual Report, 1 Oct 82 - 30 Sep 83.

* Available to contractors upon originator's approval

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE.

3M463764D995 NON-SYSTEM ADVANCED DEVELOPMENT OF ANTIDOTES

Work Unit: 071 Preclinical Studies of Antiradiation Drugs

Investigators:

Principal: Melvin H. Heiffer, Ph.D

Associate: Dr. L. Fleckenstein

1. Description.

The development of agents which protect against the effects of radiaton injury requires a highly integrated, multidisciplinary effort to undertake a broad range of preclinical and clinical pharmacological studies. The ultimate goal of this work is to obtain the necessary information to support granting of a Notice of Claimed Investigational Exemption for a New Drug (IND) by the Food and Drug Adminstration (FDA) for each candidate radioprotectant.

2. Progress.

WR 2721 is an aminopropyl aminoethyl phosphorothioate compound currently being developed by the department for submission to the FDA as an antiradiation drug. The drug shows good radioprotectant activity after intravenous administration. The drug undergoes rapid hydrolysis under acidic conditions and thus would not be expected to survive the acidic conditions of the stomach without significant degradation. As expected, the drug shows little radioprotection in animal studies following oral administration. To be useful in military settings, under field conditions, it is necessary to develop a dosage form which can be taken orally. In order to support studies on such an oral form, work is underway to develop a sensitive and specific assay for WR 1065, the dephosphorylated metabolite of WR 2721. This will allow more accurate assessment of the actual amount of drug reaching the blood stream when comparing various oral preparations. This will also allow for the development of more accurate pharmacokinetic data to support the IND.

Initial studies have been conducted to compare the effects of WR 2721 and WR 1065 on glucocorticoid receptors for the purpose of enhancing the radioprotective effect of WR 2721. These methods are being developed in both subcellular fractions and whole cell preparations of rat liver.

3. Future Work.

The HPLC method for analyzing WR 1065 will be used in conjunction with that developed for WR 2721 to determine the best feasible oral preparation for radioprotection by WR 2721. In combination these two analytical methods will also allow for the development of extensive pharmacokinetic and metabolic information on this drug.

The work on the effects of WR 2721 and WR 1065 on glucocorticoid receptors will be extended to animals that have received whole body irradiation.

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