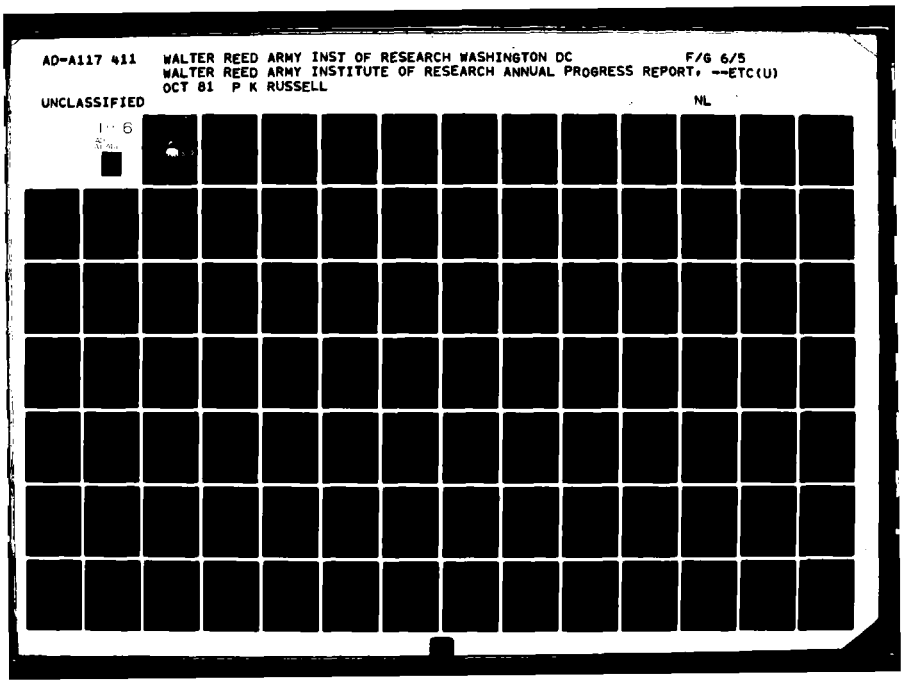
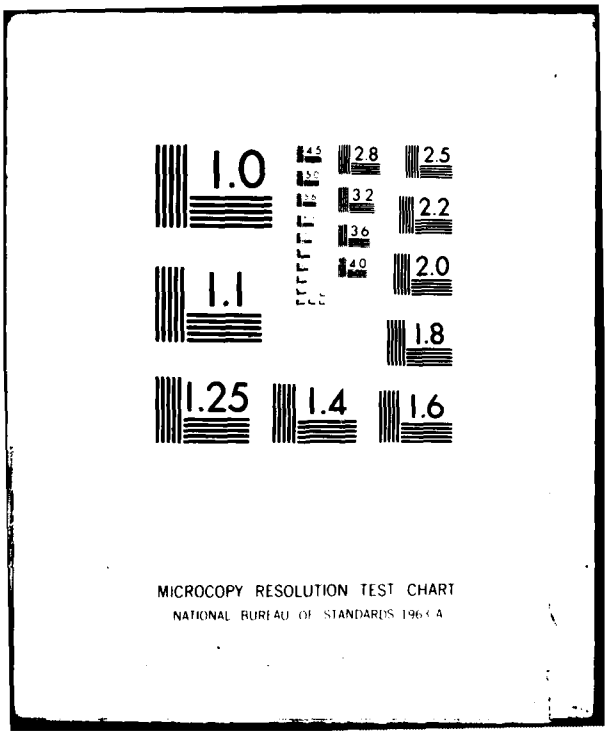

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Medical Sciences	Internal Medicine	Veterinary Medicine
Biochemistry	Psychology	
Communicable Diseases	Psychiatry	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

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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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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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.

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PROJECT 3A161101A91C

IN-HOUSE LABORATORY INDEPENDENT RESEARCH

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

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10. MC CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	106
b. SECONDARY				
c. CONTRIBUTING				

11. TITLE (Include Security Classification Code): (U) Intermediary Metabolism of the Malaria-Infected Erythrocyte (old title: Computer Simulation of Red Blood Cell Metabolism)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002600 Biology 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 10	81 09	DA	C. In-house

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19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
Washington, DC 20012
ADDRESS:

RESPONSIBLE INDIVIDUAL
NAME: Philip K. Russell, COL, MC
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Division of Medicine
ADDRESS: Washington, DC 20012

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)
NAME: Daniel G. Wright, MAJ, MC
TELEPHONE: (202) 576-3358
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: CPT H. Kyle Webster
NAME: LTC June M. Whaun

21. GENERAL USE
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22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Malaria; (U) Metabolism; (U) Computer Simulation; (U) Blood Preservation

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide individual paragraphs identified by number. Provide text of each 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. Compounds such as B12a may prove to have very important uses for protection of military troops against cyanide toxicity in the event of chemical warfare.

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 polyamine metabolism.

25.(U) 80 10-81 09 The metabolic pathways for purine salvage and interconversion have been determined for P.falciparum infected RBC in vitro and P.knowlesi infected RBC in vivo. A pathway involving the purine intermediate, adenylosuccinate, has been found unique to malaria infected RBC. Studies of malaria infected RBC in vitro have identified pathways of guanylate and hypoxanthine metabolism that are specific for the intraerythrocyte plasmodia organisms. These findings have led to the identification of certain blockers of purine metabolism (e.g. Bredinin) as potential antimalarial agents active against both sensitive and resistant strains of falciparum malaria. Studies with inhibitors of polyamine metabolism have shown that in vitro malaria culture techniques can be used to explore the link between polyamine and parasite growth. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

*Available to contractors upon official requester's approval

DD FORM 1498 (1 MAR 80) 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: 106 Intermediary Metabolism of the Malaria-Infected Erythrocyte

Investigators

CPT H. Kyle Webster, MSC; LTC June Whaun, MC; MAJ Daniel Wright, MC; Dr. Brian Hansen, GS-13 (Div of Biochemistry); Dr. Nesbitt Brown, GS-13 (Div of Biochemistry); MAJ Joshua Berman, MC (DCD&I)

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 of 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 in the malarious red cell.

Progress

A. The techniques of continuous erythrocyte culture were adapted for biochemical studies (both flask cultures and microtiter assays were used). Both a chloroquine-sensitive strain of P.falciparum (FCR-3) and a chloroquine-resistant form (Vietnam Smith strain) have been maintained in continuous erythrocyte culture.

B. HPLC methods were developed and refined for the systematic quantitative analysis of all major purine and pyrimidine nucleotides, nucleosides and bases. A dual detector system was established which permits the simultaneous measurement of both concentration and radioactivity for a given purine or pyrimidine compound separated by HPLC techniques. Through use of radiolabelled precursors and appropriate sampling techniques the above methods were used to map purine and pyrimidine metabolic pathways in intact host-RBC/parasite systems. Likewise, it was possible to evaluate the effect of inhibitors, antimetabolites (or candidate antimalarial drugs) on parasite purine and pyrimidine metabolism.

C. The major pathways of purine metabolism were determined for P.falciparum infected human erythrocytes grown in continuous culture (both drug-resistant/sensitive strains) and for rhesus monkey erythrocytes infected with P.knowlesi in vivo.

1. The specific pathways for purine metabolism were identified. The metabolic features of purine salvage and interconversion were characterized.

2. Evidence for de novo purine synthesis was not found.

3. The metabolism of adenosine and the conversion enzyme adenosine deaminase were studied in detail.

D. A pathway of purine metabolism was identified that is specific to the malaria infected RBC (HYP → IMP → AMPS → AMP → ADP → ATP - - → N.A.). [This is the

adenylosuccinate (AMPS) pathway]. The aspartate analogue, hadacidin, was found to inhibit this pathway in PRBC.

The parasitized RBC was also found to utilize Hypoxanthine for synthesis of guanosine nucleotides (HYP → IMP → XMP → GMP → GDP - - → N.A.). This pathway is present in uninfected RBC although there is no known role for guanylates in mature human erythrocytes.

Guanylates, however, are essential to the malaria parasite (e.g., for synthesis of nucleic acids, proteins and folates; and as a co-factor for the synthesis of adenylosuccinate (AMPS)).

E. Bredinin, an imidizole nucleoside, was found to have antimalarial properties. Bredinin at low concentrations (5×10^{-5}M) appears to inhibit IMP-dehydrogenase and thus blocks the synthesis of guanylates from hypoxanthine via IMP in both drug-sensitive and drug-resistant strains of P.falciparum in vitro.

These studies represent one of the first examples of the use of biochemical data gained from the study of human malaria in continuous culture to predict a metabolic target for antimalarial chemotherapy. Our studies identified the importance of guanylate metabolism to the parasite--the only apparent pathway is: HYP → IMP → XMP → GMP. Selection of bredinin was based on a search for inhibitors of this pathway as found in other studies, e.g. cancer chemotherapy screens; and other important selection criteria, e.g., low/minimal toxicity, solubility and stability in aqeous solutions.

This work establishes both an important new metabolic target for directed chemotherapy (guanylate synthesis) and a new class of potential antimalarial drug (imidizole nucleosides/bredinin-like compounds).

F. A dramatic increase in RBC adenosine deaminase (ADA) activity was observed in blood from P.knowlesi infected rhesus monkeys. This increase in ADA activity was directly proportional to the level of parsasitemia but apparently independent of the stage of parasite maturation.

G. Deoxycoformycin, DCF (a specific tight-binding inhibitor of ADA) when administered to rhesus monkeys with fulminant P.knowlesi infection arrested parasite growth. The precise role of ADA in malaria parasite growth and development has not been defined; however, the mechanism appears to envolve disruption of host adenosine metabolism such that production of hypoxanthine required by the parasite in significantly reduced.

H. The increase in RBC ADA activity in acute malaria infection was found to correlate directly with a decreased responsiveness of host lymphocytes to mitogen stimulation.

This work identifies adenosine deaminase as a potential parasite specific target for development of new antimalarial chemotherapy. It also suggests that the increased ADA levels in malaria infection may be related to immune cell dysfunction.

I. The pathways for pyrimidine metabolism were determined for P.falciparum infected human erythrocytes grown in continuous culture.

1. Orotic acid and uridine were actively incorporated by PRBC and converted into all pyrimidine classes (uridine, cytidine and thymidine nucleotides).

2. Exogenous thymine and thymidine were not found to be incorporated into nucleotide pools or nucleic acids of PRBC.

J. Intense thorough preliminary studies were conducted in collaboration with the Leishmania laboratory Division of Experimental Therapeutics, to define the pathways of purine and pyrimidine metabolism in Leishmania parasites. The basic purine pathways were identified for both promastigote and amastigote forms of cutaneous leishmania species.

K. In studies of polyamine metabolism of cultured human RBC infected with P.falciparum, it was found that polyamine levels--putrescine, spermidine and spermine--are correlated with malaria parasite growth. The mature RBC does not produce significant amounts of these metabolites. Polyamine levels were decreased and

parasite growth was arrested in the presence of inhibitors of polyamine synthesis.

Future Plans

We plan to continue the study of purine metabolism inhibitors as potential antimalarial agents, to extend these biochemical studies to the analysis of pyrimidine metabolism in malaria infected human RBC, to develop techniques for establishing synchronous cultures of P.falciparum in vitro using metabolic blockers, to study the biochemistry of drug resistance using the in vitro culture methods, to study the biochemical consequences of malaria infection for immunocompetent cells and host defense in vivo, and to extend our biochemical studies on leishmaniasis infections in humans.

Abstracts and Presentations

1. Hansen BD, Webster HK: Purine metabolism in cultured promastigotes and axenic amastigotes of Leishmania mexicana. Federation Proceedings 40:776.

2. Goodman AM, Webster HK: Alterations in bone marrow uridine nucleotides in B_{12} deficient rhesus monkeys. Clinical Research 29:334.

3. Levine RF, Webster HK: Purine metabolism in megakaryocytes and platelets. Thrombosis and Haemostasis (8th International Congress, Toronto), p. 156.

4. Webster HK, Hansen BD, Berman JD, Hendricks LD: Purine metabolism in Leishmania: effect of mycophenolic acid on the synthesis of guanosine nucleotides. Thirtieth Meeting of American Society of Tropical Medicine and Hygiene (San Juan, PR).

5. Webster HK, Whaun JM, Bean TL, Walker MD, Kark JA: Relation of host red cell vitamin B_6 metabolism to human malaria (P.falciparum) in vitro. Clinical Research 29:352.

6. Whaun JM, Webster HK: Altered purine metabolism in citrate-adenine stored blood: implications for continuous in vitro P.falciparum culture. Clinical Research 29:352.7. metabolism in cultured parasites.

7. Whaun JM, Webster HK: Purine metabolism in cultured parasites. Malaria Immunology and Vaccination Research Workshop, APA/USUHS/USAID Workshop, Bethesda, 1981.

8. Whaun JM, Brown ND, Bean TL: Polyamine metabolism in human parasitized erythrocytes. Fifth Gordon Research Conference on Polyamines, Meridien, New Hampshire, 1981.

9. Weismann WP and Webster HK: Abnormal deoxyadenosine metabolism in uremic erythrocytes. American Society of Nephrology (14th Annual Meeting).

Articles Published, In Press or In Review

1. Webster HK, Haut MJ, Martin LK, Hildebrandt PK: Profiles of purine and pyrimidine nucleotides during synchronous malaria infection (P.knowlesi) in rhesus monkeys. International Journal for Parasitology (in press).

2. Webster H, Whaun JM: Biochemistry of purines during continuous erythrocyte culture of P.falciparum: identification of differences in host-parasite nucleotide pathways. Fifth International Red Cell Conference (Ann Arbor), p. 36.

3. Webster HK, Whaun JM: Purine metabolism during continuous erythrocyte culture of human malaria parasites (P.falciparum). In "The Red Cell" (Brewer GJ, ed.), Liss, New York, pp. 557-570.

4. Webster HK, Whaun JM: Application of simultaneous UV-radioactivity high performance liquid chromatography to the study of intermediary metabolism: I. Purine nucleotides, nucleosides and bases. Journal of Chromatography 209:283-292.

5. Whaun JM, Lin CC, Biederman B, Cornish SJ and Dundas JB: Myeloproliferative disorder with unusual marrow chromosome constitution. Cancer 48:104-109, 1981.

6. Berman J, Webster H: Anti-leishmanial activity of mycophenolic acid and allopurinol against Leishmania tropica-infected human macrophages in vitro. J. Infection and Immunity.

7. Webster HK, Whaun JM: Anti-malarial properties of Bredinin: prediction based on identification of differences in host-parasite purine metabolism. J. Clin. Invest. (in press).

8. Webster HK, Whaun JM, Walker MD, Bean TL: Synthesis of adenosine nucleotides from hypoxanthine by human malaria parasites (P.falciparum) in vitro: identification of a parasite unique adenylosuccinate pathway. J. Biol. Chem. (in review).

9. Berman JD and Webster HK: Effects of mycophenolic acid and allopurinol against Leishmania tropica in human macrophages in vitro. Biochemical Pharmacology (in review).

10. Webster HK, Wiesmann WP: Erythrocyte adenosine deaminase abnormality in malaria infection: implications for immune dysfunction. Nature (in review).

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. GNSI'S INSTR'S	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
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10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	107
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Neural and Behavioral Response to Sensory Stimulation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

013400 Psychology 012900 Physiology 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 10	81 09	DA	C.In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: N/A EXPIRATION:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
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FISCAL YEAR CURRENT 81	1.0	60

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
Washington, D.C. 20012

ADDRESS:

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Neuropsychiatry

ADDRESS: Washington, D.C. 20012

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

NAME: Tyner, C.F., LTC

TELEPHONE: (202) 576-3006

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Raslear, T.G., CPT

NAME:

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Sensory Stimulation; (U) Nervous System; (U) Behavior; (U) Electrophysiology; (U) Attention; (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) This exploratory project will establish and evaluate an animal model to measure the interrelationship between sensory stimulation, nervous system responses (e.g., evoked potentials), and behavioral manifestations of stimulus control, attention and vigilance. Emphasis will be on methods requiring minimal physical and chemical restraint in order to permit realistic stimulations of military environments demanding prolonged vigilance and cognitive performance, and of the disruptors (such as altered sleep and feeding patterns, and noxious stimuli) which impinge upon task performance in those situations. The data base generated will be part of our program to examine the maintenance and decrement of satisfactory military performance in stressful situations.

24. (U) Using the methods of animal psychophysics and electrophysiology, methods will be developed to permit measurement of neural electrical potentials from awake subjects responding to environmental stimuli. Interrelationships between environmental signals and neural and behavioral responses will be established and then studied under conditions which interfere with optimal performance such as circadian desynchronization, sleep loss, increased noise, decreased signal resolution, or emotional disrupters.

25.(U)8010-8109Effects of noise in suppressing behavior were shown to be dependent upon the frequency with which the behavior is rewarded. The context in which rats are required to bisect temporal intervals was shown to influence the bisection point. It was discovered that the organophosphate, DFP, can alter the properties of cortical cells in the same way as other non-organophosphate seizure-producing drugs. For technical report see Walter Reed Army Institute of Research Progress Report, 1 Oct 80-30 Sep 81.

*Available to contractors upon originator's approval

DD FORM 1 MAR 80 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 80 (FOR ARMY USE) ARE OBSOLETE.

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 107: Neural and Behavioral Response to Sensory Stimulation

Investigators:

Principal: Tyner, C.F., LTC, MC

Associate: Raslear, T.G., CPT, ,MSC

Problem:

To establish experimental animal models allowing exploration of the relations among sensory stimuli, brain function and behavior. To increase our understanding of how an individual's sensory environment controls performance capacity. To seek explanations in the vocabulary of brain function for behavioral processes such as "perception," "attention," and "vigilance."

Importance:

The prospect of continuous operations warfare involving sophisticated hardware requires an increase in or knowledge of the processes by which sensory information is acquired, analyzed and used to guide decision-making behavior. Such knowledge must include not only an understanding of the normal mechanisms involved, but also of the effects imposed by fatigue, sleep loss, chemical and other components of extremely stressful environments.

Approach:

This work has used two models. The first involves examining animal behavior and sensory discrimination performance in small laboratory animals exposed to controlled auditory stimuli. The second involves studying the electrical characteristics of the brain regions concerned with the control of skilled movement, in laboratory animals exposed to controlled skin stimuli.

Results:

In a continuation of earlier studies, it was found that the efficacy of pulsed noise, below the pain threshold, in suppressing behavior depends upon the precise quantitative and qualitative nature of the behavior being studied, as well as the variables motivating the behavior. Studies with the duration of stimuli established that psychophysical phenomena previously observed with physical dimensions of stimuli may also be observed with the

temporal dimension. These studies will provide a set of procedures for assessing the effects of additional variables including stress, drugs, and concurrent task demands, upon behavior requiring varying degrees of judgment.

Previous studies have demonstrated characteristic modifications of the responses of sensory cells in the brain, known to be involved in the control of movement, by drugs that induce seizures. Similar effects have been found as a result of the administration of DFP, an organophosphate whose mechanism of action may be related to the anticholinesterase "nerve" agents. This technique, therefore, may have considerable utility in investigating the mechanism of action of these agents, and possible pretreatment or therapeutic drugs.

This program has fulfilled the goal of establishing behavioral and physiological models for assessing the effects of a variety of environmental and pharmacological manipulations upon sensory systems. Upon the termination of this exploratory work unit, these models will be integrated into ongoing basic and applied research projects within this Department.

Publications

Raslear, T.G. On the use of bisection procedures in animal psychophysics. Psychometrika, in press.

Raslear, T.G. Context effects in the bisection of a temporal interval by rats. Paper presented at the 52nd annual meeting of the Eastern Psychological Association, 1981.

Raslear, T.G. Pierrel-Sorrectino, R., and Rudnick, F. Loudness and masking in rats. Submitted to the Journal of Mathematical Psychology.

Raslear, T.G. Stimulus intensity dynamism: A reconsideration. Submitted to The Journal of the Experimental Analysis of Behavior.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6475	81 09 30	DD DRAF(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
80 10 01	H Termination	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
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Prevention of Post-Traumatic Epilepsy

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 012900 Physiology 002300 Biochemistry 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY		16. PERFORMANCE METHOD
78 10	81 09	DA		C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: N/A 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 80	1.0	60
FISCAL YEAR CURRENT 81	1.0	60

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute or Research
Washington, D.C. 20012

ADDRESS:

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Neuropsychiatry

ADDRESS: Washington, D.C. 20012

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

NAME: Meyerhoff, J.L., MD

TELEPHONE: (202) 576-3559

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Bates, V., MAJ

NAME: Kant, G.J., Ph.D.

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) post-traumatic epilepsy; (U) kindling; (U) cyclic nucleotides; (U) neurotransmitters; (U) preventive pharmacotherapy

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

23. (U) Post-traumatic epilepsy occurs in over 40 percent of soldiers subjected to dura-penetrating head injury. Neither improved neurosurgical care of head injuries nor prophylaxis with standard anticonvulsant medications has resulted in a decrease in the incidence of post-traumatic epilepsy. The onset of the seizure disorder usually occurs within two months of the injury, but may not occur for up to 2 years. Understanding of the biochemical factors at work during this latent period could lead to effective preventive therapy that could be initiated immediately following the injury.

24. (U) Animal models such as the kindling procedure also provide a latent period between initial procedures and subsequent development of observable seizures. Other different animal models including rats given subpial injections of ferric chloride and rats sensitive to audiogenic seizures will be developed. Both in vivo and in vitro methods to investigate the role of cyclic nucleotides and neurotransmitters in specific brain regions on the development of epilepsy are planned in each of these models. This information will be used to design preventive measures.

25.(U)8010-8109 Kindling didn't reproducibly change brain cyclic nucleotides. Challenge with isoproterenol elevated cyclic AMP levels in the amygdala of both kindled and control rats to the same degree. Daily treatment with a combination of atropine and mecamylamine reliably and significantly slowed the development of kindled seizures by 61%, neuther drug was effective alone. This work unit is being terminated consequent to the departure of MAJ Bates. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 31.

*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 108: Prevention of Post-Traumatic Epilepsy

Investigators:

Principal: Meyerhoff, J.L., M.D.

Associates: Bates, V., MAJ, MC; Kant, G.J., Ph.D.

Problem:

More than 40% of soldiers receiving penetrating missile injuries of the brain will subsequently develop post-traumatic epilepsy.[1,2]

Importance

Despite improvements in surgical and aseptic technique, the incidence of post-traumatic epilepsy has not decreased since World War I.[3] Understanding the biochemical mechanisms occurring during the latent period between the injury and development of clinical manifestations of seizures is essential to developing rational preventive measures which might be initiated immediately post-injury.

Approach:

We have succeeded in setting up the "kindling" technique for producing epileptiform seizures. Kindling consists of repetitive, intermittent low intensity electrical stimulation of the amygdala. This results in progressive changes in electrical activity and behavior over several weeks, and culminates in a generalized seizure in response to an electrical stimulus which initially had produced no effect. Kindling seems a particularly good model of post-traumatic epilepsy because it permits 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 reminiscent of the delay of seizure onset seen in post-traumatic epilepsy.

Results:

Several reports suggest that noradrenergic neurons inhibit and cholinergic neurons facilitate the development of kindling. A decrease in beta-adrenergic receptors has been found in kindled brain. Because cyclic AMP formation may be stimulated via a beta-adrenergic receptor, we measured cyclic AMP in brains of kindled

rats. There were no consistent differences in brain regional cyclic AMP levels between kindled versus control rats.

Several studies have indirectly implicated cholinergic neurons in kindling. Because nicotinic as well as muscarinic cholinergic receptors are found in brain, we have assessed the efficacy of the cholinergic blockers atropine and mecamylamine on the rate of development of kindled seizures. The group receiving both drugs developed kindled seizures significantly slower than the control group receiving saline (25 vs 15 days), a result confirmed in a second study. Neither drug alone had any effect on kindling. These data suggest that further studies be carried out on the potential of combined anticholinergic therapy in post-traumatic epilepsy, and have implications for seizure management in organophosphate poisoning as well. Because thyrotropin releasing hormone (TRH) potentiates cholinergic activity in brain, we have also initiated studies on the role of TRH in kindling.

REFERENCES CITED

1. Caviness, V.S. Epilepsy: a late effect of head injury. In: The late effects of head injury, (eds. Walker, A.E., Caviness, W.F., and Critchley, M.), pp. 193-201, Charles C. Thomas.

2. Russell, W.R. The development of grand mal after missile wounds of th brain, Johns Hopkins Med. J. 122:250 (1968).

3. Caveness, W.T., Walker, A.E., and Ascroft, P.B. Incidence of post-traumatic epilepsy in Korean veterans as compared with those from World War I and World War II. J. Neurosurg. 19:122-129 (1962).

Presentations

Society for Neuroscience, Cincinnati, Ohio, November 1980. Bates, V.E.,. Meyerhoff, J.L., Kant, G.J., and Lenox, R.H. "In vivo cyclic AMP levels in rat brain regions following kindling."

Publications

Kant, G.J., Meyerhoff, J.L., and Corcoran, M.E. Release of (NE) and (DA) from brain regions of amygdaloid kindled rats. Exper. Neurol. 70(3):701-705 (1980).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C6476	81 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
80 10 01	H. Termination	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	109
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code)

(U) Biochemical Studies on Trypanosomiasis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 10	81 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER: N/A

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 80	4.0	110
CURRENT FISCAL YEAR 81	2.0	246

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Biochemistry

ADDRESS: Washington, D.C. 20012

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

NAME: Olenick, J.G., Ph.D.

TELEPHONE: (202) 576-3017

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Doctor, B.P., Ph.D.

NAME: Sleeman, H.K., Ph.D.

21. GENERAL USE

Foreign Intelligence not considered

22. KEYWORDS (Provide each with Security Classification Code)

(U) Trypanosomes; (U) Antigenic Variation; (U) Surface Coat; (U) Antigen Synthesis

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 of this work unit is the development of immunological and chemotherapeutic protection for military personnel against parasitic and tropical diseases through studies emphasizing the biochemical and genetic mechanisms controlling variable surface antigen biosynthesis in salivarian trypanosomes.

24 (U) The feasibility of immunoprophylaxis is assessed by determining the extent of sequence variation in immunologically unique variant-specific surface coat glycoproteins. Peptide mapping of tryptic digests of glycoproteins is the technique employed.

25 (U) 80 10-81 09 A high performance liquid chromatography separation system was developed and used to map tryptic digests of 4 cloned variant antigenic types of the Wellcome strain of Trypanosoma rhodesiense. The number of peaks resolved for the different variant-specific glycoproteins ranged from 30 to 40. None of the peptide peaks were shared by all four variants. Moreover, the patterns of retention times were totally different. To demonstrate reproducibility, three different preparations of each variant glycoprotein were digested and each digest was chromatographed in triplicate. The within-day peptide fragment retention times of these runs varied by no more than ± 0.04 min, while day-to-day variation was within a range of ± 0.14 min. It is concluded that the variant glycoproteins under present investigation do not contain a region of significant homology and that variation in sequence of amino acids occurs throughout the polypeptide chains. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 80-30 Sept 81.

* Available to unrestricted users 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 MAY 88 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 109 Biochemical Studies on Trypanosomiasis

INVESTIGATORS:

Principal: John G. Olenick, Ph.D.

Associate: M. Patricia Strickler, Ph.D.; SP5 Richard W. Travis, Ph.D.; Seymour Garson, Ph.D.; John A. Kintzios, M.S.; SP4 Roberto Ayala; Bhupendra P. Doctor, Ph.D.

DESCRIPTION:

The objective of this work unit is the development of immunological and/or chemotherapeutic protection of military personnel against infection by parasitic protozoa. An understanding of the biochemistry and molecular biology of parasitic protozoa and of host-parasite relationships is essential in order to effectively develop more efficient means of control, prevention and clinical treatment. The studies reported here are concerned with the phenomenon of antigenic variation in salivarian trypanosomes.

A. Antigenic Variation in Salivarian Trypanosomes

Peptide Mapping of Variant Glycoproteins from Trypanosoma rhodesiense by Reverse Phase Liquid Chromatography

Since conventional peptice fingerprinting techniques to reveal differences in the primary structure of proteins are time consuming and frequently produce ambiguous maps, reverse phase high performance liquid chromatography was employed to map tryptic peptices of variant-specific glycoproteins. All separations were performed on a μ Bondapak C_{18} reverse phase column and elution of peptides was achieved by use of a linear gradient of 12% to 45% acetonitrile-water mixtures containing 0.1% trifluoroacetic acid. Peptides in the effluent fractions were detected by dual wavelength monitoring at 215 and 280 nm. Excellent resolution was accomplished for each digest and peptide map chromatograms were obtained in 1 h. The number of peptide peaks resolved for the different digests ranged from 30 to 40. Based on the hydrolytic peptide specificity ascribed to trypsin, this number of fragment peaks or cleavage sites is consistent with the quantities of lysine and arginine previously found in each of these variant glycoproteins. None of the peptide peaks were shared by all four cloned variant antigenic types. Moreover, the patterns of retention times were totally different. It is concluded from these findings that the variant glycoproteins under present investigation do not contain a region of significant homology and that variation in the sequence of amino acids occurs throughout the polypeptide chains.

The present findings suggest that this technique may have significant applicaiton in the immunochemical characterization of variant-specific glycoproteins. Coupled with the use of monoclonal antibodies directed against variant-specific glycoproteins or any glycoprotein antigens,

the technique should permit the recovery of peptides that can be employed as possible inhibitory haptens, thus facilitating the location or definition of immunogenic determinant sites.

Publications

1. Lyon, J.A., J.M. Pratt, R.W. Travis, B.P. Doctor, and J.G. Olenick. 1981. Use of monoclonal antibody to immunochemically characterize variant-specific surface coat glycoprotein from Trypanosoma rhodesiense. J. Immunol. 126:134-137.

2. Olenick, J.G., R.W. Travis, and S. Garson. 1981. Trypanosoma rhodesiense: Chemical and immunological characterization of variant-specific surface coat glycoproteins. Mol. Biochem. Parasitol. 3:227-238.

Abstracts and Presentations

1. Strickler, M.P., R.W. Travis, and J.G. Olenick. 1981. Mapping of tryptic peptides of variant surface glycoproteins from Trypanosoma rhodesiense by high performance liquid chromatography. Abst. Am. Soc. Parasitol. Paper No. 8, p. 28.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 2531	81 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. GRP'G MOD'S	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM	10. WORK UNIT
80 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A	

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

12. TITLE (Precede with Security Classification Code)

(U) Genetic Basis of Virulence of Bacterial Pathogens

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
80-10	CONT	DA	C. In House

18. CONTRACT/GRANT

a. DATES/EFFECTIVE: N/A 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 81	2.0	150
FISCAL YEAR CURRENT 82	2.0	84

20. RESPONSIBLE DOD ORGANIZATION

NAME: WRAIR

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, Philip K. COL

TELEPHONE: (202) 576-3551

21. GENERAL USE

Foreign Intelligence not considered

22. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Researc

ADDRESS: Division of Biochemistry
Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Provide Rank if U.S. Academic position)

NAME: P. Gemski

TELEPHONE: (202) 576-2594

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: B.P. Doctor

NAME: J.R. Lazere

23. KEYWORDS (Precede each with Security Classification Code)

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

24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Precede 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) 80 10-81 09. Studies of the genetic control and characterization of virulence properties of invasive enterics have continued. The Vwa plasmids of Y. enterocolitica serotype O:8 and O:3, which are associated with the pathogenicity and calcium dependency of this species, have been compared with respect to their DNA fragmentation patterns. After digestion by restriction endonucleases, Vwa plasmids from each serotype yielded different banding patterns. Our studies of three naturally occurring K1-positive rough E. coli strains have demonstrated that the presence of K1 antigen can provide resistance to the bactericidal action of serum for at least some strains of rough E. coli. For technical report see Walter Reed Army Institute Annual Progress report, 1 Oct 80 - 30 Sep 81.

* Available to contractors upon originator's approval

DD FORM 1498 1 MAR 80 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 80 (FOR ARMY USE) ARE OBSOLETE.

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 110 Genetic Basis of Virulence of Bacterical Pathogens

INVESTIGATORS:
Principle: Peter Gemski, Ph.D.
Associate: Janet Lazere, B.S.
B. P. Doctor, Ph.D.

In collaboration with: Baron, L.S., Ph.D. (DCE&I), Collins, H. (DCD&I), Cross, A, MD, (DCD&I), Keren, D. MD, (Univ. of Michigan), Wohlhieter, J. A., Ph.D. (DCD&I) and Yamamoto, N., Ph.D., (Hannemann Medical College, Phila. PA)

DESCRIPTION:

Studies on the pathogenesis of enteric infections have established that some organisms evoke diarrheal disease by an invasive mechanism in which the pathogen penetrates and replicates within gastrointestinal tissue. Without doubt, several bacterial attributes must function in concert to allow expression of invasive events. The polygenic control of invasive virulence remains unelucidated at the present time. Our objective is to study that genetic control of invasive properties of enteric pathogens. An understanding of chromosomal and plasmid genes associated with invasive properties of enterics will provide basic formation 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. Interspecific phage hybrids are also being developed.

A. Studies of the Virulence of Yersinia enterocolitica

In our study of the virulence and plasmid properties of Y. enterocolitica, we have found that a plasmid is associated with the invasiveness of strains. Furthermore, we have revealed that strains harboring this plasmid are calcium dependent when grown in vitro at 37C. Such a temperature dependent calcium deficiency in Y. pestis has been correlated correlated to the production of the V and W antigen complex, an important virulence determinant of plague bacilli. We have continued to characterize the virulence properties of invasive enterics. Vwa

plasmids, previously shown by us to be associated with the pathogenicity and calcium dependency of Yersiniae, have now been compared in two serotypes (O:8 and O:3) of Y. enterocolitica. After treatment with restriction endonucleases Hind III or Eco RI, Vwa plasmids from each serotype yielded different banding patterns, indicating a divergence in the microevolution of Vwa.

B. K1 Antigen-Associated Resistance to the Bactericidal Activity of Serum

It is well established that normal human serum can posses bactericidal activity against some Gram-negative bacteria. Present evidence indicates that antibodies, which can be directed against several structures of the bacterial outer membrane complex, interact with components of the complement system and initiate a series of events at the bacterial membrane that are lethal to the bacterial cell. Activation of the complement system can occur via the classical or alternate pathways.

Among enteric bacteria, it has been shown that several components of the cell wall complex function as determinants of sensitivity to the bactericidal effects of serum. Rough phenotypes, altered in the synthesis of O-antigen, are in general more sensitive to serum killing than their smooth parent strains. Variations in the amounts of capsular antigens have also been related to serum sensitivity. It has been shown that certain plasmids can incrase the resistance of Escherichia coli to the bactericidal effects of normal serum, presumably via a mechanism that causes a modification in the bacterial surface.

We have studied three naturally occurring K1-positive rough E. coli from adult human bvacteremia. Our results indicate that K1 antigen can function as an important determinant of protection from serum bactericidal systems, particularly with rough strains. It is thus conceivable that certain K antigens posses such antibactericidal functions. Further investigations of this point are needed. This is emphasized by our recent studies of the role of K1 antigen in adult bacteremia. Over 60% of rough, serum resistant bacteremic E. coli isolates were found to produce K1 antigen.

C. Role of Antigen Form in Development of Mucosal Immunoglobulin A Response to S. flexneri Antigens

One major stumbling block in the development of an effective means to immunize against shigellosis and other enteric diseases has been the lack of a means to assess sequential mucosal immune responses to different potential immunogens. In the present study, we compared the abilities of live invasive organsims, noninvasive organisms, and nonviable antigen preparations of Shigella to elicit mucosal immune responses. Whereas previous studies have found that effective immunity

was produced best by vaccination with live invasive strains of Shigella, in the present study, live noninvasive strains that did not produce any histopathological damage were consistently able to produce local (immunoglobulin A) immune responses as vigorous as those of the invasive strains. Further, acetone killed shigella antigen was also an effective mucosal immunogen, whereas hot phenol-water-extracted shigella lipopolysaccharide was ineffective, possibly due to the method of preparation. A single oral or parenteral priming was ineffective in enhancing the mucosal immune response when restimulated 1 month later with the same antigen. However, a mucosal memory response was found to be present several months after a triple mucosal stimulation with a locally invasive vaccine strain.

D. Studies of Interspecific Hybrid Phages

Hybrid ϕ80-P22 phages, which retain the protein coat of ϕ80, have been divided into two types with respect to the extent of homology with P22. One hybrid type has a large P22 early gene segment containing the att-erf-c-h21 region. The second type, ϕ80immP22dis, has a larger P22 segment which includes both immunity (c and immI) regions of P22, i.e., immI-att-erf-c-h21. CsCl density centrifigation analyses revealed that the total genome size of these hybrids increases as the size of the inherited P22 segment increased. The hybrids express the P22 att region and insert at the P22 site near the pro chromosomal genes of the host. Some of the hybrid phages recovered from lysogens were found to contain reductions in the size of the P22 DNA segment. In some cases, the total genome length increased despite a reduction in the size of the P22 segment. This increase could represent replacement of a portion of the P22 DNA segment either by host chromosomal genes or a duplication of phage genes.

Hybrid phages of the λ-P22 class have the λ protein coat and contain at least the c region of P22. Such hybrids are isolated from P22 lysates, previously grown on a λ lysogen of a smooth Escherichia coli-Salmonella typhimurium hybrid, WR4028(λ). In phage crosses between the clear plaque mutant P22c2 and wild-type λc+, a new hybrid λ-P22 type which forms turbid (c+) plaques on λc+ lysogens of WR4207 was isolated. P22-λc+ hybrids, composed of the P22 coat with at least the c region of λ, are able to form plaques on the smooth host WR4028(λ) because they also have the second immunity (immI) region of P22. Thus, any λ-P22 hybrid with the λc+ region must also contain the immI region of P22 to be able to form plaques on λ lysogens. Lysogens of WR4028 carrying the new hybrid phage λc+P22immI are immune to both λ and P22. This new phage hybrid contains the erf through immI region of P22 and inserts at the P22 att site near the pro region of the bacterial chromosome. In addition, it confers the ability to produce Salmonella O-1 somatic antigen on appropriate host bacteria. During its replication, the hybrid phage also produces free P22 tails.

A coli mutator Mu-1 phage is unable to grow in a smooth E. coli-S.

typhimurium hybrid strain WR4028 but able to grow in a rough strain WR4027. However P22 phage can not infect this rough strain and this Mu-1 lysogenic derivative. When a mixture of Salmonella rough specific phage (designated as R phage) was plated on WR4027(Mu-1), smooth derivatives which are resistant to R phage were isolated. Thus P22 is now able to grow in this new lysogen, Wr4027(Mu-1)/R, and give rise to Mu-P22 hybrids carrying the protein coat on Mu-1 phage and the early region at least c genes of P22. Genetic analysis revealed that the hybrid carries a small P22 segment including att-erf-c regions. Therefore the Mu-P22 hybrid readily forms its lysogens while frequency of Mu-1 lysogeny is very low. Since Mu-P22 carries P22att region, its prophage is located in bacterial proline gene. Thus Mu-P22 lacks mutator activity.

Publications

1. Gemski, P. and D.E. Griffin. 1980. Isolation and Characterization of Minicell-Producing Mutants of Shigella. Infect. Immiun., 30:297-302.

2. Gemski, P., A. Cross and J. Sadoff. 1980. K1 Antigen-Associated Resistance to the Bactericidal Activity of Serum. FEMS Microbiology Letters. 9:193-197.

3. Keren, D.F., H.H. Collins, P. Gemski, P.S. Holt and S.B. Formal. 1981. Role of Antigen Form in Development of Mucosal Immunoglobulin A response to S. flexneri Antigens. Infect. Immun. 31:1193-1202.

Abstracts

1. Yamamoto, N., M.L. Droffner, S. Yamamoto, J. Konzelman, P. Gemski and L.S. Baron. 1981. Characterization of Hybrids Between Coliphage 80 and Salmonella phage P22. Am. Soc. Microbiol. Abst H6.

2. Yamamoto, N., S. Yamamoto, P. Gemski and L.S. Baron. 1981. Unusual C+ Region and the imm I Region of P22. Am. Soc. Microbiol. Abst. H7.

3. Yamamoto, N., P. Gemski and L.S. Baron. 1981. Isolation of a Hybrid Between Salmonella phage P22 and E. coli mutatator phage Mu-1. Am. Soc. Microbiol. Abst. H8.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2526	81 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'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	111
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with security classification code) (U) Kinetics of immunoglobulin producing cells in peripheral blood during dengue virus infections.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79-10	80-09	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 80	0.2	10
FISCAL YEAR CURRENT 81	0.2	10

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012
RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K. COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: US Army Medical Component, AFRIMS
ADDRESS: Bangkok, Thailand
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic participating)
NAME: Burke, D.S. LTC
TELEPHONE: Bangkok, 282-8141 x 291
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign Intelligence not considered

22. KEYWORDS (Precede EACH with security classification code)
(U) Viruses;(U) Dengue Fever;(U) Arbovirus Infections;(U) 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 technical objective was to determine the time course of specific anti-dengue IgG and IgM immunoglobulin producing cells in the peripheral blood of patients with acute dengue infections, in order to provide basic data on the immune response to dengue. This information will be used in the design and preparation of vaccines to prevent dengue fever, a disease of significant military importance in tropical areas.

24(U) Acute and convalescent blood specimens were obtained from patients with dengue hemorrhagic fever (DHF) or other fevers of unknown origin (FUO), the peripheral blood mononuclear leukocytes (PBML) were obtained by density flotation, PBML were washed then incubated at 10^5 or 10^6 cells per milliliter at 37^0C, and supernatant fluids of PBML were assayed by antibody capture radioimmunoassay for IgM and IgG dengue antibodies. Similar experiments were done with PBML from rhesus monkeys experimentally infected with dengue viruses.

25(U) 80 10 - 81 09 A burst of antibody producing cells appeared in the peripheral blood of experimentally infected monkeys 6 days after virus innoculation; IgM anti-dengue production by PBML lasted 3-5 days and IgG anti-dengue production 10-14 days. On hospital admission 100% of DHF patients (19/19) had IgG anti-dengue producing PBML and 80% (12/15) had IgM anti-dengue producing PBML. In vitro anti-dengue synthesis was not detectable in cultures of human cells obtained after day 14 after admission, at the time of peak plasma antibody levels. Antibody synthesis was blocked by sodium azide or cycloheximide. B cells, but not T cells or macrophages, were identified as the antibody producing cells.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 111: Kinetics of Immunoglobulin Producing Cells in Peripheral Blood During Dengue Virus Infections

PRINCIPAL INVESTIGATOR: Donald S. Burke, LTC

1. Kinetics of Immunoglobulin Producing Cells in Peripheral Blood During Dengue Virus Infections

PROBLEM: A military requirement exists for an immunogenic but non-reactogenic dengue vaccine to protect US troops deployed in endemic areas. In nature some dengue infections run a mild or asymptomic course, while others result in hemorrhagic fever, shock, and death. Usually the more severe disease manifestations are found in persons with serologic evidence of prior infection with a dengue virus type different from the current infecting type; this suggests that perturbations in the immune response may be important in determining disease severity. Safe field testing of dengue virus vaccines will require a deeper understanding of the host immune response during naturally occurring mild and severe dengue virus illness.

IMPORTANCE: Dengue virus infections were a major cause of illness, hospitalization and resultant combat ineffectiveness in virtually all endemic areas in tropical theaters in WWI, WWII, and Vietnam; a more precise understanding of the immunopathologic mechanism involved in severe dengue infections would lead to a more rigorous definition of desired vaccine properties and to safer and more meaningful vaccine field trials.

APPROACH: Acute and convalescent blood specimens were obtained from patients with dengue hemorrhagic fever (DHF) or other fevers of unknown origin (FUO), the peripheral blood mononuclear leukocytes (PBML) were obtained by density flotation, PBML were washed then incubated at 10^5 or 10^6 cells per milliliter at 37°C., and supernatant fluids of PBML were assayed by antibody capture radioimmunoassay for IgM and IgG dengue antibodies. Similar experiments were done with PBML from rhesus monkeys experimentally infected with dengue viruses.

RESULTS: A burst of antibody producing cells appeared in the peripheral blood of experimentally infected monkeys six days after virus innoculation; IgM anti-dengue production by PBML lasted 3-5 days and IgG anti-dengue production 10-14 days. On hospital admission 100% of DHF patients (19/19) had IgG anti-dengue producing PBML and 80% (12/15) had IgM anti-dengue producing PBML. In vitro anti-dengue synthesis was not detectable in cultures of human cells obtained after day 14 after admission, at the time of peak plasma antibody levels. Antibody synthesis was blocked by sodium azide or cycloheximide. B. cells, but not T cells or macrophages, were identified as the antibody producing cells.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 2532	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. CLASSIFICATION	6. DOWN GRADING	7. GRADING	8a. GRP/SUBGRP	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	113
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(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 01	Cont	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 81	4.0	108
FISCAL YEAR CURRENT 82	2.0	167

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K. COL

TELEPHONE: (202) 576-3551

19b. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Division CD and I

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

NAME: Hockmeyer, W.T., MAJ (P), MSC

TELEPHONE: (202) 576-3544

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Nacy, C., Ph.D.

NAME:

20. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Immunity; (U) Leishmaniasis; (U) Tropical Medicine; (U) Macrophages

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 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 artificial immunization against the disease agents and will provide methods for assessing immunity in immunized hosts. Leishmaniasis extends throughout the tropics on every continent except Australia and is thus a threat to military operations in all these areas. U.S. troops are currently contracting disease during training operations in Panama.

24 (U) The approach will be to examine the capacity of macrophages from infected animals to kill intracellular leishmania and to determine whether or not specifically immunized lymphocytes yield products which can influence macrophage killing of the organisms.

25 (U) 80 10-81 09 Resident peritoneal macrophages from 15 inbred mouse strains were assessed for capacity to support intracellular replication of L. tropica and abilty to respond to lymphokines that induce microbicidal activity against the parasite. Macrophages from three strains (A/J, C3H/HeJ, C57BL/10ScCR) failed to respond to lymphokines that induceincreased resistance of activated macrophages to infection, and macrophages from P/J, BALB/cN, C57L/J, and NZW/N mice could not kill intracellular leishmania. Analysis of L. tropica infection of footpads of the 15 mouse strains suggested that susceptibility correlated with inability of macrophages to respond to lymphokines that induce intracellular destruction of the parasite. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 80-30 Sept 81.

* Available for contractors 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 MAY 68 (FOR ARMY USE) ARE OBSOLETE

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 113: Immune Mechanisms in Leishmaniasis

Investigators:

Principals: Carol A. Nacy, Ph.D.
MAJ Wayne T. Hockmeyer, MSC

Associates: CPT Michael G. Pappas, MSC
E-4 Robin R. Henry

Problem and Objectives

The objective is to investigate Leishmania-macrophage interaction in vitro in an effort to intervene immunologically to enhance intracellular destruction of the parasite. As animal models become available, we propose to analyze the effect of immune products or non-specific immunostimulating agents, as well as whole-cell or antigen preparations of amastigotes on the protection against leishmanial infections.

Progress:

Approaches used were: Harvest peritoneal macrophages from mice and evaluate intracellular replication of Leishmania amastigotes in macrophages in suspension culture; activate macrophages with soluble T-lymphocyte products (lymphokines) and assess effect of lymphokine activation on infection and intracellular replication of Leishmania; fractionate lymphokine supernatants and analyze lymphokines mediating intracellular killing; inoculate various inbred mouse strains with L. tropica and assess resistance or susceptibility to infection.

Treatment of mouse peritoneal macrophages with soluble products of stimulated lymphocytes (lymphokines) in vitro induces two antimicrobial activities against L. tropica: (1) pretreatment of macrophages with lymphokines 4 hr induces an increased resistance of activated macrophages to infection, and (2) treatment of macrophages with lymphokines after infection induces intracellular killing of parasite. Analysis of macrophage activation in inbred mouse strains suggested that regulation of the two antimicrobial activities may be under separate genetic control. Macrophages from A/J, C3H/HeJ, and C57Bl/10ScN mice did not respond to lymphokine pretreatment for

increased resistance to infection, but could kill intracellular L. tropica with lymphokine treatment after infection. In contrast, macrophages from BALB/cJ, C57L/J, NZW/N, and P/JN mice responded to lymphokines for resistance to infection, but were incapable of intracellular destruction of the parasite. Mice infected with L. tropica by inoculation of footpads segregated into resistant (footpad swelling < 2mm, resolution of lesion in 9-12 wks) and susceptible (footpad swelling > 4mm, necrosis of foot, visceralization of infection) strains. Susceptible strains were BALB/c, C57L/J, NZW/N, and P mice, all strains that had macrophage defects for lymphokine-induced intracellular killing of L. tropica. Different lymphokines regulate the two antimicrobial activities of activated macrophages: analysis of macrophage function of P/JN mice suggest that (1) similar numbers of lymphokine-responsive cells are present in susceptible strains, but each cell is less responsive than macrophages of resistant mice, and (2) macrophages from these mice lack killing mechanisms induced by 2 intracellular killing lymphokines.

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) development of an appropriate mouse model for visceral and cutaneous disease, 3) evaluation of nonspecific and specifically sensitized lymphocyte products for immunoprophylactic/therapeutic and diagnostic potential. 4) development of in vitro methods for assessing immunity, 5) evaluation of nonspecific immunopotentiating agents for control of leishmanial infection, and 6) evaluation of whole cell or antigen preparation of amastigotes for candidate vaccines.

Presentations:

1. Pappas, M.G., A.L. Haverly, and C.A. Nacy. 1980. Growth and lymphokine-induced killing of Leishmania tropica in murine peritoneal macrophages. Annual meeting of Am. Soc. Trop. Med. Hyg., Atlanta, GA.

2. Haverly, A.L., M.G. Pappas, R.R. Henry, and C.A. Nacy. 1981. In vitro macrophage antimicrobial activities and in vivo susceptibility to Leishmania tropica infection. Symp. on host defenses to Intracellular Pathogens, Phila, PA.

3. Nacy, C.A., M.S. Meltzer, E. Skamene, M.M. Stevenson, and E.J. Leonard. 1981. Activation of macrophages for killing of rickettsiae. Symp. on Host Defenses to Intracellular Pathogens. Phila, PA.

4. Pappas, M.G., C.N. Oster, and C.A. Nacy, 1981. Intracellular destruction of Leishmania tropica by macrophages activated *in vivo* with Mycobacterium bovis. Symp. on Host Defenses to Intracellular Pathogens. Phila, PA.

5. Nacy, C.A. 1981. Activation of macrophages to kill Leishmania tropica. Guest Lecturer, Rutgers University, New Brunswick, NJ.

6. Nacy, C.A. 1981. lymphokine-induced macrophage antimicrobial activities against Leishmania tropica Gordon Conference on Immunoparasitology. New London, NH.

7. Nacy, C.A., 1981. Activation of macrophages for intracellular destruction of Leishmania. Guest Lecturer, Catholic University, Wash. D.C.

8 Nacy, C.A. 1981. Nonspecific effector functions of macrophages. Army Veterinary Symposium, Wash, DC.

Publications:

1. Nacy, C.A., G. Radlick, and M.S. Meltzer. 1980. Activated macrophages in natural resistance to Rickettsia akari. In: Genetic Control of natural Resistance to Infection and Malignancy (a volume in the series "Perspective in Immunology"), E. Skamene, ed. Academic Press, New York, p. 555.

2. Nacy, C.A. 1980. Killing of rickettsiae by macrophages. In: Manual of Macrophage Methodology: Collection, characterization, and function, H.B. Herscowitz, H.T. Holden, J.A. Bellanti, and A. Ghaffar; eds. Marcel Dekker, Inc., New York. p 289.

3. Meltzer, M.S., C.A. Nacy and E.J. Leonard. 1981. Genetic analysis of macrophage effector functi ns: development of nonspecific tumoricidal and microbicidal activities during lymphokine activation. Proc. of Internat'l Workshop on Heterogeneity of Mononuclear Phagocytes, M. Landy, Ed. Academic Press p 384.

4. Nacy, C.A., E.J. Leonard, and M.S. Meltzer. 1981. Macrophages in resistance to rickettsial infection: Characterization of lymphokines that induce intracellular killing in macrophages. J. Immunol. 126:204.

5. Nacy, C.A. and M.G. Groves. 1981. Macrophages in resistance to rickettsial infections: early host defense mechanisms in experimental scrub typhus. Infect. Immunity 31:1239.

6. Nacy, C.A. and M.S. Meltzer. Macrophages in resistance to rickettsial infections: variation in rickettsiacidal activity of peritoneal macrophages from inbred mouse strains (In press).

7. Nacy, C.A. and C.L. Diggs. 1981. Intracellular replication of Leishmania tropica in mouse peritoneal macrophages: comparison of amastigote replication in adherent and nonadherent macrophages. Infect. Immunity 34:310.

8. Nacy, C.A., M.S. Meltzer, E.J. Leonard, and D.J. Wyler. 1981 Intracellular replication and lymphokine-induced destruction of Leishmania tropica in C3H/HeN mouse macrophages. J. Immunol. (In press).

9. 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 Against Intracellular Pathogens, Marcel Dekker (In press).

10. Haverly, A.L., M.G. Pappas, R.R. Henry, and C.A. Nacy. 1982. In vitro macrophages antimicrobial activities and in vivo susceptibility to Leishmania tropica infection. In: Host Defenses to Intracellular Pathogens, Marcel Dekker (In press).

11. Pappas, M.G., C.N. Oster, and C.A. Nacy. 1982. Intracellular destruction of Leishmania tropica by macrophages activated in vivo with Mycobacterium bovis strain BCG. In: Host Defenses to Intracellular Pathogens, Marcel Dekker (In press).

12. Nacy, C.A. and S.C. Oaks, Jr. 1980. Quantitation of the destruction of rickettsiae. In: Methods for studying mononuclear phagocytes, D.O. Adams, H.S. Koren, and P.J. Edelson, eds. Academic Press, New York (In press).

13. Nacy, C.A. and M.G. Pappas. 1980. Quantitation of the destruction of leishmania In: Methods for studying mononuclear phagocytes, D.O. Adams, H.S. Koren, and P.J. Edelson, eds. Academic Press, New York (In press).

14. Nacy, C.A., A.L. Haverly, and P.K. Russell. 1981. Microbicidal activity against Leishmania tropica by macrophages from P/JN mice. Fed. Proc. 40.

15. Pappas, M.G., T.R. Jerrells, and C.A. Nacy. 1981. Activation of C3H/HeN and C3H/HeJ mouse macrophages to kill Leishmania tropica. Fed. Proc. 40.

16. Oster, C.N. and C.N. Nacy. 1981. Kinetics of macrophage activation for microbicidal activity against Leishmania tropica. Proc. Int. Con. Chemother. Antimicro. Agents.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 2525	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWN GRADING	7. REGRADING	8a. DISTR INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. THIS UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	114
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) LIPOSOMES FOR TREATMENT OF LEISHMANIASIS

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 012600 Pharmacology

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: NA 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) 81	2.0	150
FISCAL YEAR (CURRENT) 82	2.0	111

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Exptl Therapeutics

ADDRESS: Washington, DC 20012

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

NAME: DAVIDSON, D.E., COL

TELEPHONE: (301) 427-5029

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: HENDRICKS, L.D., LTC

NAME:

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Liposomes; (U) Chemoprophylaxis; (U) Drug Development; (U) Chemistry; (U) Leishmaniasis

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

23. (U) To conduct studies in design, development and evaluation of liposomes as a delivery system of drugs for the treatment of leishmaniasis, a parasitic infection of the reticuloendothelial system which is a serious hazard of disability, disfigurement and death to military personnel operating in tropical and subtropical regions of the world, including Latin America, Asia, Africa and the Near East.

24. (U) A series of liposomal preparations will be formulated with selected constituent chemical compounds which establish the properties of the liposomes. The capacity of the preparations to transport and deliver antileishmanial drugs to infection sites and the effect on the disease will be studied in laboratory animals. Tolerance of the animals to liposomal preparations will be evaluated.

25. (U) 80 10-81 09. A series of studies of liposomal activity against visceral leishmaniasis has been conducted (four experiments in dogs and 94 in hamsters). A consistent death rate of 15% observed in infected hamsters treated with liposomal preparations was speculated to be due to a Gram-negative sepsis resulting from effects of leishmania or liposomes or both on the reticuloendothelial cells. Orally-administered tetracycline eliminated all mortality, although tetracycline encapsulated in liposomes and administered was quite toxic. Several experiments have demonstrated that liposome-encapsulated antimony compound (glucantime) eliminates parasites of both naturally-occurring and laboratory-induced antimony-resistant visceral leishmaniasis. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

* available to contractors upon originator's approval

DD FORM 1498 1 MAR 80

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 114 Liposomes for Treatment of Leishmaniasis

Investigators:

Principal: LTC Larry D. Hendricks, MSC
LTC Carl Alving, MC

PROBLEM AND OBJECTIVES:

Leishmaniasis is a parasitic infection of the reticuloendothelial system which poses a serious hazard of disability, disfigurement and death to military personnel operating in tropical and subtropical regions of the world, including Latin America, Asia, Africa and the Near East. Jungle warfare training exercises conducted in the Panama Canal Zone continue to result in cases of leishmaniasis in deployed military personnel. Currently available drug treatments are neither completely safe nor reliable in therapy or prophylaxis. Studies done under this work unit investigate the delivery of antileishmanial drugs to targeted cells by liposomes of defined composition.

RESULTS:

A series of experiments was conducted to investigate the activity of liposomal preparations against visceral leishmaniasis (L. donovani) (four experiments in dogs and 94 in hamsters). In these studies a consistent early death rate of 15% was observed in infected hamsters which had been treated with liposomes. It was considered that this might be due to a Gram-negative sepsis resulting from the effects of leishmania or liposomes or both on the reticuloendothelial cells. It was found that the early mortality was prevented by orally-administered tetracycline, although tetracycline encapsulated in liposomes was quite toxic. In several experiments, the antimony compound, glucantime, incorporated into liposomes and administered intra-cardially eliminated parasites of both drug sensitive and laboratory-induced antimony-resistant visceral leishmaniasis in hamsters. These studies suggest that antimony resistant human leishmaniasis might be successfully treated by administration of liposome-encapsulated antimonials.

FUTURE OBJECTIVES:

Investigations of liposome-encapsulated drugs will be expanded. New formulations of liposomes will be devised and evaluated as carriers of drugs to targetted host cells. A study comparing various routes of administration (subcutaneous, intraperitoneal, intracardial, intramuscular, etc.) for targetting of liposomes is underway. This information will assist in development of prospective regimens of treatment for clinical situations.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2221	81 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. GRP'S INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	C. 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	115
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

C)The Role of High Energy Substrates and Prostaglandins on Responses to Stress & Shock

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016200 Stress Physiology 008800 Life Support

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 81	1.0	50
	CURRENT 82	2.0	59

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, COL, PHILIP K.

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Surgery

ADDRESS: Washington, D.C. 20012

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

NAME: FLEMING, COL, ARTHUR W.

TELEPHONE: (202) 576-3791

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Substrates; (U) Energy; (U) Prostaglandins; (U) Stress; (U) Shock; (U) Reperfusion

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

23. (U) To establish a reproducible model of severe, progressive hemorrhagic shock in experimental animals. To assess the effects of delivering high energy substrates in this model. A long range goal is to establish a radioimmunoassay in our own laboratory for measuring prostaglandin synthesis and metabolism. Severe blood loss in combat casualties accounted for 23.9% of the deaths in patients who reached the hospital from the battlefield in a survey from Vietnam during the calendar year 1969. These studies may lead to improved methods of treating severe, progressive hemorrhagic shock.

24. (U) Hemorrhagic shock will be produced by combining the following: (1) removal of a specific percentage of the blood volume; (2) determining the optimal time:volume rate of blood removal; (3) delaying therapy for a defined period of time (representing projected scenarios); and (4) using maximal conventional therapy. Cardiovascular hemo-dynamics will be continuously monitored and survival will be determined over a specific period of time.

25. (U) 80 10 - 81 09 The use of maximal conventional therapy (returning all of the shed blood and administering a volume of Ringer's Lactate equal to three times the volume of shed blood) led to a 100 percent survival in rats subjected to a standard protocol of hemorrhagic shock. This precluded the use of animals on this protocol for treatment with high energy substrates. Using a total estimated blood volume of 7.1% of the body weight; a bleeding rate of 0.1 ml of blood per 100 gm of body weight per one minute interval for varying periods; and delaying treatment for an additional 90 minutes produced a zero to 100% mortality with maximal conventional therapy. Experimental data is accumulating to suggest a reproducible model which can be used to simulate the condition that would occur in awake battle casualties. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 80 - 30 Sept 81.

*Available to contractors upon originator's approval

DD FORM 1 MAR 86 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 86 (FOR ARMY USE) ARE OBSOLETE

Project 61110191C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 115 The Role of High Energy Substrates and Prostaglandins on Responses to Stress and Shock

Investigator:
Principal: COL Arthur W. Fleming, M.D.

Problem and Objectives:

Hemorrhagic shock contributed to the death in one out of every four combat casualties who arrived alive to a hospital in Vietnam during the calendar year 1969.[1] Excluding head injuries, it was the single most common cause of death in hospitalized patients in Vietnam. Although data is not available on those casualties killed in action, one can be assurred that excessive blood lost was a contributing factor. Alterations in prostaglandins and depletion of energy stores may or may not play a role in the propagation of shock.[2-6]

The initial objective is to develop a predictable model of hemorrhagic shock that reflects more accurately the condition in combat casualties. A second objective is to eluidate why severe hemorrhagic shock becomes progressive despite replacement of all the blood that is lost.

Progress:

A standard hemorrhagic shock model was used for some of the studies to confirm our theory that some models are sublethal if maximal conventional therapy is used. Hemorrhagic shock was also produced by combining the following: (1) removal of a specific percentage of the blood volume; (2) determining the optimal time: volume rate of blood removal; (3) delaying therapy for a defined period of time (representing projected scenarios); and (4) using maximal conventional therapy. Cardiovascular hemodynamics were continuously monitored and survial determined over a specific period of time.

Using the standard hemorrhagic shock model in a rat with treatment consisting of returning all the shed blood plus a volume of Ringer's Lactate equal to three times the volume of shed blood produced a 100% survival in those animals thus treated (over an observation period of 48 hours). This suggests the importance of using a control with maximal conventional therapy as opposed to using a volume of fluid equal only to the

vehicle for the drug(s) being evaluated. The interval of time that it takes to remove the blood from the body as well as how long therapy is delayed is critical in standardizing a reproducible model. By removing the blood at a constant rate of 0.1 ml per 100gm of body weight per minute, we have produced, preliminarily, a reasonably predictable model. The model, which needs refinement still suffers from the rather steep slope of the line at the LD50. As data accumulates, we are adjusting the model to compensate for this problem. Our present consideration is a graded hemorrhage which progressively decreases in rate over time as a response to the hypovolemia and hypotension. This approach is more consistent with the bleeding that occurs in combat casualties.

Recommendations and Future Objectives:

This project should be continued on ILIR. Initial critical manpower shortages which existed throughout FY81 have been partially reduced; essential equipment for prostaglandin assays are due to arrive within 60 days; and improved monitoring equipment has already arrived. It is anticipated that with continued funding this project will provide a useful tool for studying combined injuries (conventional and chemical injuries), and allow assessment of high energy substrates and prostaglandins on responses to stress and shock.

Project 61110191C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 115 The Role of High Energy Substrates and Prostaglandins on Responses to Stress and Shock

Literature Cited:

References:

1. Arnold, K. and Cutting, R.T.: Causes of Death in United States Military Personnel Hospitalized In Vietnam. Military Medicine, 143: 161-164, 1978.

2. Chaudry, I.H., Sayeed, M.M., and Baue, A.E.: Depletion and restoration of tissue ATP in hemorrhagic shock. Aarch Surg 108: 208, 1974.

3. Chaudry, I.H., Sayeed, M.M., and Baue, A.I.: effect of adenosine triphosphate magnesium chloride administration in shock. Surgery 75: 220-227, 1974.

4. Chaudry, I.M., Sayeed, M.M., and Baue, A.I.: alterations in high energy phosphates in hemorrhagic shock as related to tissue and organ function. Surgery 79: 66, 1976.

5. Sharma, G.P., and Eiseman, B.: Protective effect of ATP in experimental hemorrhagic shock. Surgery 59(1) 66, 1966.

6. Schloerb, P.R., Sieracki, L. and Botwin, A.J.: Winblad, J.M., McGuire, M.H.: Intravenous adenosine triphosphate (ATP) Am J Physiol 240: R52, 1981.

Publications: None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1284	81 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
80 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	119
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
002600 Biology 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: 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 PRECEDING 81	3.0	100
FISCAL YEAR CURRENT 82	3.0	100

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
Washington, DC 20012
ADDRESS:

RESPONSIBLE INDIVIDUAL
NAME: Philip K. Russell, COL, MC
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Division of Medicine
ADDRESS: Washington, DC 20012

PRINCIPAL INVESTIGATOR (Provide data if U.S. academic institution)
NAME: Daniel G. Wright, MAJ, MC
TELEPHONE: (202) 576-3358
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: MAJ Eric B. Schoomaker
NAME: Lily Tang (IPA Investigator)

21. GENERAL USE
Foreign intelligence not considered

22. KEYWORDS (Precede each with Security Classification Code) (U) Erythrocyte; (U) Acetylcholinesterase; (U) Acetylcholine receptor; (U) Structural membrane proteins; (U) Protein-membrane lipid interactions

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

23.(U) To investigate the role of the acetylcholine receptor (AChR) in red blood cell (RBC) 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 enumeration of AChR by binding of labelled agonist, for calcium flux, and for cGMP generation. Light and electron microscopy of fixed, treated cells. Assays of protein phosphorylation. Isolation of purified RBC AChE by affinity chromatography. Purification of human erythrocyte acetylcholinesterase, reconstitution of acetylcholinesterase into artificial membranes (liposomes) of diverse lipid content, measurement of acetylcholinesterase activity in erythrocyte membrane ghosts with and without acetylcholinesterase inhibitors, with and without lipophilic agents, and with variations in extracellular soluble lipid environments.

25.(U) 80 10-81 09 AChR Studies - By using non-hydrolyzable, radiolabelled cholinergic agonists and a variety of neurotransmitter antagonists, a specific, saturable muscarinic AChR has been found. Agonist stimulation of the RBC AChR has been shown to increase calcium movement into the cell and result in an increase in cGMP levels. This final effect can be abrogated by chelation of extracellular calcium. Preliminary efforts to isolate the enzyme by affinity column chromatography were carried out. In related Hemolytic Anemia Studies, we have completed a study on the mechanism of hemolysis in a patient with the heritable membrane disorder, hereditary elliptocytosis (HE), and vitamin B12 deficiency. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

*Available to contractors upon originator's approval

DD FORM 1498 (1 MAR 80) PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 JUL 68 (FOR ARMY USE) ARE OBSOLETE

3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 119 The Biochemistry and Physiology of Erythrocyte Acetylcholinesterase: Application to Chemical Defense

Investigators

MAJ Eric Schoomaker, MC; MAJ Lewis Diehl, MC (WRAMC); MAJ Daniel Wright, MC; Dr. Lily Tang (IPA Investigator)

Description

Studies of the physiology of blood cell membranes have concentrated upon the ectoenzyme, acetylcholinesterase, 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 underlie RBC shape changes and cell lysis in disorders such as hemolytic hereditary elliptocytosis. We propose to extend our preliminary studies aimed at the recognition of major changes in the conduct and character of abnormal structural proteins to those of more subtle, qualitative changes in phosphorylation and protein-protein interaction.

All laboratory studies upon receptor, enzymic and structural membrane protein are performed with whole human and rabbit RBC and isolated RBC membranes.

1. AChR Studies - This work will employ assays for enumeration of AChR binding of labelled agonist, for Ca^{++} flux, and for cGMP generation already in use in our lab. Attention shall be given to the variation in functional AChR with the age of RBC, from bone marrow erythroid progenitors to circulating mature RBC. Modulation of shape changes and deformability of cells following AChR stimulation by agonist will be studied with light and electron microscopy of fixed,

treated cells and by filtration. Finally, the effects of changes in cGMP levels upon membrane protein phosphorylation will be performed using radiolabelled phosphorylated substrates and assays of protein phosphorylation by gel autoradiography and standard liquid scintillation counting.

2. AChE Studies - The first step in this work will be the isolation of purified RBC AChE by affinity chromatography. The influence of various lipids upon enzyme activity and upon inhibition by anti-AChE agents will then be possible. An interesting in vivo model of AChE inhibition may be represented by the blood dyscrasia, paroxysmal nocturnal hemoglobinuria. This acquired disease is characterized by absence of RBC AChE activity; it has yet to be shown whether this is due to an absence of AChE protein or inhibition in situ. Purification of RBC AChE will allow us to prepare antibody against AChE for a direct visulization of the enzyme on the PNH cell. Furthermore, extraction of the PNH enzyme into liposomes (lipid microvessicles) will allow us to determine whether changing the lipid environment of the enzyme will re-activate it.

3. Hemolytic Anemia Studies - We are presently identifying patients with a variety of hemolytic and non-hemolytic membrane abnormalities in the Hematology Clinic at WRAMC. Using RBC from these patients and gel electrophoresis techniques developed for use in our lab, the quantity and quality of structural membrane proteins can be examined.

Progress

1. ACHE Studies - We have met with considerable success in identifying a AChR on human and rabbit RBC. By using non-hydrolyzable, radiolabelled cholinergic agonists and a variety of neurotransmitter antagonists, a specific, saturable muscarinic AChR has been found. Agonist stimulation of the RBC AChR has been shown to increase Ca^{++} movement into the cell and result in an increase in cGMP levels. This final effect can be abrogated by chelation of extracellular Ca^{++}.

2. AChE Studies - Work upon RBC AChE has resulted in a great deal of experience in assaying intact RBC AChE and detergent-treated, soluble AChE. Preliminary

efforts to isolate the enzyme by affinity column chromatography were unsuccessful in providing a sufficiently pure and/or potent preparation for further studies. Continued work using more specific ligands should prove more fruitful. Finally, a source of RBC from PNH patients is available and laboratory methods for isolating AChE "deficient" cells have been established.

3. Hemolytic Anemia Studies - We have completed a study on the mechanism of hemolysis in a patient with the heritable membrane disorder, hereditary elliptocytosis (HE), and vitamin B_{12} deficiency. We were able to demonstrate that cell fragmentation and subsequent hemolysis occurred during B_{12} deficiency which were reserved by B_{12} replacement. These cells had acquired a marked degree of sensitivity to heat stress--a sign of structural protein instability in HE enhanced by B_{12} depletion. Techniques used to study this patient's unusual RBC's have been more recently applied to an investigation of structural RBC proteins from a different kindred of HE patients with continuous hemolysis. As yet uncompleted, this work promises to disprove one major hypothesis for RBC destruction in this disease.

Future Plans

Investigative plans as outlined above (Description) will be pursued further as this newly established work unit is continued into its second year. In addition, studies will be undertaken to attempt a chromatographic separation of purified native RBC AChE from RBC AChE that has been irreversibly inhibited by AChE inhibitors of military relevance.

Abstracts and Presentations

1. Tang, L.C.: Erythrocyte Muscarinic Receptor - A Model for Studying Cholinergic Muscarinic Agents. Presented at the 1981 Gordon Conference on Molecular Pharmacology, June 22-26, 1981.

2. Tang, L.C.: Alteration of Dopamine-sensitive Adenylate Cyclase Activity in Mouse Caudate to be presented at the International Society of Neurochemistry Meeting, England, September 6-11, 1981.

3. Schoomaker, E.B., Butler, W.M. and Diehl, L.F.: Increased Heat Sensitivity of Red Blood Cells in Hereditary Elliptocytosis with Acquired Cobalamine (B_{12}) Deficiency. Clin. Res. 29:347A, 1981.

4. Tang, L.C. and Schoomaker, E.B.: Evidence For Muscarinic Receptor Stimulated cGMP and Ca Uptake in Human RBC Transactions of American Society for Neurochemistry 12:244, 1981.

5. Tang, L.C., Schoomaker, E.B. and Weissman, W.: Cholinergic Stimulated Ca^{++} - Uptake and cGMP Formation in Human Red Blood Cells. Clinical Research 29, 1981.

Articles Published, In Press or In Review

Schoomaker, E.B., Butler, W.M. and Diehl, L.F.: Studies of Erythrocyte (RBC) Structural Proteins in Hereditary Elliptocytosis (HE) with Cobalamins (B_{12}) Deficiency. Blood, 1981 (in review).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1296	81 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 DISB'N INSTR'N	8B SPECIFIC DATA - CONTRACTOR ACCESS	9 LEVEL OF SUM
80 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	120
b. CONTRIBUTING				
c. CONTRIBUTING				

11 TITLE (Precede with Security Classification Code) (U) Identification of Virus Polypeptides in Immune Complexes in Dengue Hemorrhagic Fever Sera

12 SCIENTIFIC AND TECHNOLOGICAL AREAS

02600 Biology 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 NA — 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 81	2.0	173
FISCAL YEAR CURRENT 82	2.0	256

19 RESPONSIBLE DOD ORGANIZATION

NAME Walter Reed Army Institute of Research
ADDRESS Washington, DC 20012

RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, PHILIP K., COL, MC
TELEPHONE (202) 576-3551

20 PERFORMING ORGANIZATION

NAME Walter Reed Army Institute of Research
Division of CD&I
ADDRESS Washington, DC 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. academic institution)
NAME BANCROFT, WILLIAM H., COL, MC
TELEPHONE (202) 576-3757
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Henchal, ERik A. CPT
NAME: Brandt, Walter E. Ph.D.

21 GENERAL USE

Foreign intelligence not 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) Dengue viruses cause substantial morbidity in US military personnel in the tropics and mortality in children of Southeast Asia from dengue hemorrhagic fever (DHF). Pathogenesis of DHF is attributed to formation of antigen - antibody complexes. Identification of the virus specified polypeptides associated with the complexes found in DHF will help explain the disease and permit more precise evaluation of the immune response to dengue vaccines.

24 (U) The approach will be to identify sera containing high levels of immune complexes followed by dissociation of the complexes and analysis of the products. Initial work will encompass standardization of the marker system for individual viral polypeptides synthesized by infected cells. The radioactive infected cells will be solublized and subjected to radioimmune precipitation by antibodies present in the sera of humans having primary and secondary dengue infections with varying degrees of severity.

25 (U) 80 10-81 09 Dengue viruses intrinsically labelled with S35 and C14, have been purified and disrupted to separate viral specified proteins by polyacrylamide gel electrophoresis. Human dengue immune sera have been tested for reactivity with dengue polypeptides by radioimmune precipitation. Acute sera collected from DHG patients do not react with polypeptides of molecular weights 43K, 36K, 32K, 12K and 11K daltons whereas convalescent sera react with all polypeptides. This evidence suggests these polypeptides may include the antigenic components of immune complexes of DHF which remove specific antibodies from the acute sera. A similar study of dengue vaccines is pending. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

DD FORM 1498 (1 MAR 86) PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 86 (FOR ARMY USE) ARE OBSOLETE

* U.S. GPO 1974-540 843/8691

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;
Dr. Walter E. Brandt, Ph.D.;
COL William H. Bancroft, MC

Associates: SP5 Matthew Seguin
SP4 Oscar E. Taylor

Purpose

Identification of all viral proteins recognized immunologically by dengue hemorrhagic fever (DHF) patients is unknown. Proper assessment of the immune response of these patients is important not only to the development of appropriate vaccines but also to the understanding of the immunopathological steps leading to dengue hemorrhagic fever. Our primary objective in this study was the identification of those viral proteins which can be immunoprecipitated by acute and convalescent sera from DHF patients. The gel electrophoresis system used for this work will serve as the standard for determining which dengue specific polypeptides are present in the immune complexes found in the sera of DHF patients.

Progress

Uninfected or dengue-infected C6/36 (Aedes albopictus) or LLC-MK_2 (monkey kidney) cells were radiolabeled continuously from 48 to 120 hours postinfection using ^{35}S-methionine and ^{14}C-amino acids. Radioimmune precipitation (RIP) antigen was prepared by detergent solubilization of radiolabeled whole cell proteins in Tris buffer, pH 8.0, containing 1% Triton X-100, 1% sodium deoxycholate and 0.1% sodium dodecyl sulfate (SDS). Ten microliter aliquots of acute and convalescent sera from Thai dengue fever patients were reacted overnight with 0.1 ml of RIP antigen ($1x10^6$ cpm) at 4C. Anti-dengue hyperimmune mouse ascitic fluid (HMAF), normal mouse ascitic fluid (NAF); normal human serum (NHS), and ascitic fluid prepared against host cell (C6/36 or LLC-MK_2) proteins (HCAF) were run as controls. Immune complexes formed were absorbed to formalin-treated Staphlococcus aureus cells and solubilized using an SDS-mercaptoethanol buffer. Samples were characterized using Laemmli

10-20% gradient polyacrylamide slab gels. Protein bands were visualized after exposure of PPO-treated (fluorography), dried gels to x-ray film. Virus-specific proteins were identified by comparison to molecular weight standards, proteins from partially purified virions, and host proteins precipitated by HCAF.

Dengue-specifc HMAF precipitated approximately ten virus-specific infected cell proteins ranging in molecular weight from 70,000 daltons to 11,000 daltons. The most predominent bands had approximate molecular weights of 58,000, 43,000, 36,000, 32,000, 17,000, 12,000, 11,000 daltons (1CP 58, 1CP 43, ICP 36, ICP 32, ICP 17, ICP 12, and ICP 11, respectively). Convalescent sera from DHF patients reacted principally with the same polypeptides; however, acute sera failed to precipitate or had much diminished reactions to ICP 43, ICP 36, ICP 32, and ICP 11. Since RIP relies primarily on the presence of uncomplexed antibody, these results suggest that certain virus-specific antibodies may be bound to circulating or fixed dengue antigens or are not present in the sera in large quantities.

Future Objectives

The development of a solid phase immune complex capturing system employing monoclonal rheumatoid factor will enable efficient binding of immune complexes in DHF sera. This will be followed by dissociation of the complexes and attempted identification of the antigen or dengue-specific polypeptide involved. We will also apply the technique of gel electrophoresis of radioimmune precipitation to sera from humans with classical dengue fever and to sera from volunteers infected with live attenuated vaccine viruses. These results will be compared with those obtained from DHF cases. The immune response to the live virus vaccines can then be evaluated in terms of antibody production to those viral specified polypeptides found in classical or severe dengue disease.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1295	81 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. DISPN INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	121
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Identification of T. rhodesiense Protective Antigens

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

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: NA — 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 81	1.0	151
CURRENT FISCAL YEAR 82	1.0	274

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
Washington, DC 20012

ADDRESS:

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL, MC
TELEPHONE: (202) 57 6-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Washington, DC 20012

ADDRESS:

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Hockmeyer, W.T., MAJ (P)
TELEPHONE: (202) 576-3544
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Esser, Klaus
NAME:

21. GENERAL USE

Foreign Intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Vaccine; (U) Trypanosomiasis; (U) Monoclonal Antibody; (U) Antigens

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish 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 prophylaxis 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 the blood form of the parasite. To identify the antigen types involved in this immunity, monoclonal antibodies are prepared as markers for specific antigens. These reagents are used for the antigen type analysis of parasites obtained from the field and will also provide the means to isolate specific antigens.

25. (U) 80 10 - 81 09 Animals have been immunized against infection by the metacyclic (insect) form of T. rhodesiense. Mice which received multiple doses of attenuated metacyclic or metacyclic-derived blood form trypanosomes remained clear of parasites after challenge with infectious metacyclics. Monoclonal antibodies have been produced which identify four distinct metacyclic antigen types. Individual tsetse flies infected with trypanosomes from one of 3 different trypanosome clones or one of 2 different human isolates had the same metacyclic antigen types. Blood form trypanosomes were also shown to express metacyclic antigens. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

* 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 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 121: Identification of Trypanosoma rhodesiense protective antigens

Investigators:

Principals: Mr. Klaus M. Esser
MAJ Wayne T. Hockmeyer, MSC

Associates: Mr. Maurice J. Schoenbechler
Mr. William L. Bowie
Sp4 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 bit 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 dies. 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 infecton 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:

Animals have been immunized against infection by the metacyclic (insect) forms of T. rhodesiense. Mice which received multiple doses of attenuated metacyclic or metacylic-derived blood form trypanosomes remained clear of parasites after challenge with infectious metacyclics. Serum from

immunized mice neutralized the metacyclic parasites, suggesting that the observed resistance to challenge was antibody mediated. Identification of the antigens relevant to the observed protection is progressing through the production and use of monoclonal antibodies as markers for discrete metacyclic antigens. Using this approach, four distinct metacylic antigen types have been identified. Each individual tsetse fly infected with the same trypanosome isolate was found to have the same metacyclic antigen types. Metacyclics from flies infected with one of three different trypanosome clones or a different trypanosome isolate also expressed the same antigen types. These findings indicate that although T. rhodesiense metacyclics are heterogeneous, this heterogeneity is restricted. Blood form trypanosomes were identified which expressed metacyclic antigens. These blood forms, in contrast to the metacyclic forms, are available in sufficient quantities for isolation of protective antigens and ultimately the genies coding for these antigens.

Recommendations·

In view of the findings 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, 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 laboratory findings. Monoclonal antibodies will continue to be the major tool for these studies. In addition, in vitro correlates of immunity should be developed to allow assessment of immunity by means other than infectious challenge. Refinement of serodiagnostic techniques is also needed to provide a clinically useful level of sensitivity and specificity.

Presentations:

1. Expression of metacyclic surface antigens on blood forms of Trypanosoma rhoedesiense demonstrated by monoclonal antibodies. K. M. Esser, M.J. Schoenbechler and J.B. Gingrich. Federation of American Societies for Experimental Biology, 65th Annual Meeting, April, 1981.

2. Composition of circulating immune complexes in experimental African trypanosomiasis. H.B. Lindsley, L.L. Janecek Societies for Experimental Biology, 65th Annual Meeting, April, 1981.

Publications:

1. Immunization with blood forms of Trypanosoma rhodesiense protects against metacyclic (fly form) challenge. K.M. Esser, M.J. Schoenbechler, and J.B. Gingrich. Submitted for publication in Nature.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA [illegible] 6750	81 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
80 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	122
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	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 81	2.5	115
	CURRENT 82	2.5	217

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL
NAME: Philip K. Russell, COL, MC
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Division of Medicine
ADDRESS: Washington, DC 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Daniel G. Wright, MAJ, MC
TELEPHONE: (202) 576-3358
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Mr. Harold Williams
NAME: LTC John A. Kark

21. GENERAL USE: Foreign intelligence not 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 antimalarial chemotherapy is of major military importance because of needs to station military personnel in regions where malaria is endemic.

24.(U) Laboratory studies include the evaluation of different B12 analogues that have different substitution 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) 80 10-81 09 The cyanide-antidotal effects of B12a were confined in studies with mice and rats. Large doses (100-400 mg/kg) given intravenously increased the LD50 of cyanide salt by up to four times. The antidotal effects of B12a were determined to be additive to those of thiosulfate and nitrite. Maximum possible antidotal effects of B12a require a molar ratio of B12a: CN of greater than 1:1. Methodologies for measuring total B12, total CN-B12 and total B12a in plasma, urine, and tissues were established. These methods have been used to study the pharmacokinetics of single I.V. doses of B12a and CN-B12 in beagles and foxhounds (doses of 5-25 mg/kg). Renal clearance of B12a has been shown to be less efficient than that of CN-B12 with a T 1/2 of clearance for B12a (about 2 hrs) that is more prolonged than that of CN-B12 (about 30 min). For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

*Available [illegible] upon author's request.

DD FORM 1498 (MAN 66) — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAN 66 (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

Mr. Harold Williams, MS (GS-13); MAJ Daniel G. Wright, MC; LTC Thomas Cosgriff, MC (Div. Exp. Therapeutics); LTC John 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} ($CN\text{-}B_{12}$) which is rapidly excreted by the kidneys if plasma levels of $CN\text{-}B_{12}$ 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 have 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 will be 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

In studies with mice and rats we have confirmed the cyanide-antidotal effects of $B_{12}a$. Large doses (100-400 mg/kg) can increase the LD5 of cyanide salt given to the animals by up to four times. The maximum possible antidotal effects of $B_{12}a$ require a molar ratio of $B_{12}a$: CN of greater than 1:1.

Methodologies for measuring total B_{12}, total CN-B_{12} and total $B_{12}a$ in plasma, urine, and tissues have been established for our use. These methods have been established for our use and have been used to study the pharmacokinetics of single I.V. doses (5-25 mg/kg) of $B_{12}a$ and CN-$B_{12}a$ in dogs. These studies have to date shown that renal clearance of $B_{12}a$ is not as efficient as CN-B_{12} with a 1/2 time of renal clearance for $B_{12}a$ (2 hrs) being more prolonged than CN-B_{12}(about 30 min).

Future Plans

1. To develop schemes of loading and maintenance doses of $B_{12}a$ concentrations at different levels within a range of 0.05-0.2 mg/ml for periods of up to 3 hours. Results of these studies will be used to plan for the second stage of this investigation in which the protective effects of different maintenance plasma levels of $B_{12}a$ against CN toxicity will be evaluated.

2. To use data obtained from these studies to design studies in which animals are challenged with escalating doses of NaCN by i.v. infusion.

3. To determine the theoretical potential of $B_{12}a$ loading in humans as a means of conferring short term (2-12 hrs.) protection against cyanide poisoning.

Abstracts, Presentations

None

Articles Published, In Press, or In Review

Kale, M.P., Grenan, M.H., Altstatt, L.B., and Wright, D.G.: Studies of hydroxocobalamin the treatment of acute cyanide poisoning, 1982 (in review).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 08 7012	81 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
80 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	123
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede 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	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 81	0.8	130
FISCAL YEAR CURRENT 82	0.8	100

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Experimental Therapeutics

ADDRESS: Washington, DC 20012

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

NAME: DAVIDSON, D.E., Jr., COL

TELEPHONE: (301) 427-5029

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: McCORMICK, G.J.

NAME

21. GENERAL USE

Foreign Intelligence Not 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) 8010-8109 Testing in detail for phototoxic characteristics which might preclude consideration for further studies leading to clinical candidacy have been conducted on two compounds of interest. WR 248332, when administered at 80 to 320 mg/kg intraperitoneally or orally, had definite but transitory evidence of phototoxicity. WR 248333 had no evidence of phototoxicity after oral administration at 40-160 mg/kg or after intraperitoneal administration at 8 mg/kg, its highest tolerated dose. Development of methodology for assessment of compounds for probable hemolytic capacity similar to that of primaquine is under way, adapting an *in vitro* radiorespirometric procedure. Studies have established the dose-related stimulatory effect of primaquine on the production of carbon dioxide by red blood cells, which is the parameter of the test. Working limits of the procedure are being determined. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

* Available to contractors upon submitter's approval

DD FORM 1498 — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 69 (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: CPT Michael Wysor
Dr. Joan E. Jackson
Dr. Gerald J. McCormick

PROBLEM AND OBJECTIVES:

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. Specific areas of current investigation are the photosensitizing characteristics of compounds such as the quinolinemethanols, and the hemolytic potential of primaquine and its analogs 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 vivax malaria.

RESULTS:

Detailed photosensitization studies have been conducted on two compounds of interest. WR 248332 when administered at 80 to 320 mg/kg intraperitoneally or orally to mice induced definite but transitory

photosensitization. WR 248333 did not photosensitize after oral administration at 40 to 160 mg/kg or after intraperitoneal administration at 8 mg/kg, its highest tolerated dose by this route of administration. In development of methodology for identification and assessment of compounds of probable hemolytic capacity similar to that of primaquine, preliminary studies have been completed in adaptation of the radiorespirometric methodology. This system measures the radioactive carbon dioxide released from erythrocytes incubated with ^{14}C-glucose. The increased release of $^{14}CO_2$ by primaquine is a reflection of drug-induced increase in hexose monophosphate shunt enzyme activity, which may lead to hemolysis of G6PD-deficient erythrocytes through accumulation of peroxides. The experimental parameters established include the pH range of suspending medium (pH 2 to 7.4), red blood cell concentration ($1x10^8$ to $2x10^{10}$ cells/ml) and incubation time periods (between 15 and 90 minutes). Standardization studies with primaquine indicate the working range to be 0.001 to 2 mg/ml.

FUTURE OBJECTIVES:

Compounds will be evaluated for photosensitization potential as required in the drug development program. Compounds will be selected on the basis of structure corresponding to chemical classes which have had members with photosensitizing characteristics. The radiorespirometric test system will be used to screen prospective antimalarial compounds for hemolytic potential using primaquine as a reference standard. Compounds or metabolites inducing a more rapid rate of CO_2 production than that observed for primaquine would be predicted to have potentially greater hemolytic activity than primaquine.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA [illegible]	81 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
81 01 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	[illegible]	124
b. CONTRIBUTING				
c. CONTRIBUTING				

11 TITLE (Include 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	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	PRECEDING	g. PROFESSIONAL MAN YRS	h. FUNDS (In thousands)
FISCAL YEAR	81	2.0	10
	CUMULATIVE		
	82	2.0	10

19 RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20 PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of CD&I

ADDRESS: Washington, DC 20012

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

NAME: Jerald C. Sadoff, MD, LTC, MC

TELEPHONE: (202) 576-3759

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS: Robert Seid, Ph.D.

NAME

NAME

21 GENERAL USE

Foreign Intelligence Not Considered

22 KEYWORDS (Provide EACH with Security Classification Code)

(U) Toxins; (U) Antibodies

23 TECHNICAL OBJECTIVE 24 APPROACH, 25 PROGRESS (Provide individual paragraphs identified by number. Provide 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 also critical 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)81 01 - 81 09 Ricin has been purified from Castor beans. The A fragment of ricin has been separated and purified from the B region and has been covalently coupled through disulfide bridges to monoclonal antibody against a model cell line B10-5 melanoma. This conjugate kills the BW-5 cell with high efficiency but retains some non-specific cytotoxicity. Gelonin has been purified from Gelonin multiflorum seeds. It has been covalently coupled through disulfide bridges to anti BW-5 monoclonal antibody. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

* Available in unrestricted form upon originator's approval

DD FORM 1498 (1 MAR 80) — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A (1 NOV 80) AND 1498-1 (1 MAR 80) FOR ARMY USE ARE OBSOLETE

U.S. GPO 1974-540-863/6601

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.
LTC Jerald C. Sadoff, MC

Problem

Techniques will be developed for producing cell directed cytotoxic agents which could be used for treatment of parasites, viral infected cells and cell related immune regulatory dysfunctions in trauma and infection. Specific aims are to use model systems to evaluate various methods of coupling monoclonal antibody to toxins or active portions of toxins. When ideal methods have been found, specific immunotoxins (coupled products) will be used in animal model systems to determine efficacy.

Progress

Methods for purification of ricin have been developed and ricin has been purified. The A fragment of ricin has been isolated and covalently coupled, using the disulfide binding reagent SPDP, to monoclonal antibody directed against melanoma cells. Some cell directed toxicity has been detected, but has not been specific. Gelonium multiflorum seeds have been obtained from India. The 27,000 Dalton protein Gelonium has been isolated from these seeds in pure form. Gelonium has been covalently coupled to monoclonal antibody using disulfide bridges. Gelonium has been found to be non-toxic in its isolated form and very stable. Cell directed cytotoxicity studies using Gelonium are currently underway.

Future Plans

Continue cell directed cytotoxicity of monoclonal antibody coupled to Gelonium. Neutralize whole ricin binding to receptor with analogues of N-acetyl-galactoseamine which covalently bind in the B (binding) region of the molecule. The neutralized whole toxin

will then be coupled to monoclonal antibody. We will also further purify ricin antibody conjugates to improve specificity. Monoclonal antibody against subclasses of T-cells and against filarial parasites will be coupled to ricin and Gelonium. Toxicity and specificity will be determined.

Bibliography

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 7011	81 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
81 07 17	D. Change	U	U		NI.	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	125
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Ecology and Biosystematics of Vectors of Rift Valley Fever Virus in Kenya

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 07	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA 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 81	2.0	9
	CURRENT 82	2.0	9

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL

TELEPHONE: (202) 576-3551

GENERAL USE

Foreign intelligence not considered

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of CD&I

ADDRESS: Washington, D.C. 20012

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

NAME: Roberts, LTC, D.R.

TELEPHONE: 202-576-3719

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Bailey, MAJ C.L.

NAME: Linthicum, CPT, K.J.

21. KEYWORDS (Precede EACH with Security Classification Code) (U) RVF; (U) Mosquitoes; (U) Taxonomy; (U) Arbovirus; (U) Ecology; (U) Vectors; (U) Kenya; (U) Epidemiology

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

23. (U) Conduct field collections of mosquitoes in Kenya for taxonomic and virus isolation studies in an effort to identify the natural vector(s) of Rift Valley Fever (RVF) virus. Describe and illustrate species groups of mosquitoes that might be involved in the natural maintenance of RVF virus. Isolate RVF virus from wild caught mosquitoes. Conduct laboratory transmission tests with species that are incriminated as vectors by virus isolation attempts. With these studies, it might be possible to obtain quick answers as to the identity of the RVF virus vector(s). Realization of these objectives may lead to prevention or control of RVF and make possible the accurate assessment of the actual threat of RVF to military troops.

24. (U) Mosquitoes will be collected in Kenya with a variety of collection methods. Mosquito eggs, larvae, pupae and adults will be collected for taxonomic rearings. The taxonomic series (from rearings) will be employed to detect morphological characters for species descriptions and for constructing identification keys. Adult mosquitoes will be collected, identified and processed for virus isolation attempts. Complete ecological data will be recorded for all collections. Specimens will be collected for colonization purposes also and, in some cases, will be collected in mass to provide laboratory populations of sufficient size for conducting laboratory transmission tests.

25. (U) 81-07 - 81-09 Purchases of supplies and equipment were made in preparation for a trip to Kenya that will be undertaken Oct - Dec 1981. Objectives for this trip are the same as outlined in paragraph 24 above. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 80-30 Sept 81.

* 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 125 Ecology and Biosystematics of Vectors of Rift Valley Fever Virus in Kenya

Investigators

Principal: Donald R. Roberts, LTC, MSC

Associate: Charles L. Bailey, MAJ, MSC; Kenneth J. Linthicum, CPT, MSC

Problem

The vector of Rift Valley Fever (RVF), a militarily important virus disease, is unknown. Vector identification will be attempted by conducting field collections of mosquitoes in Kenya for taxonomic and virus isolation studies. Included in this objective is the description and illustration of mosquito species that might be involved in the natural maintenance of RVF virus, as well as the isolation of RVF virus from wild caught mosquitoes. Laboratory transmission tests will be conducted with species that are incriminated as vectors by virus isolation attempts. Realization of this objective may lead to prevention or control of RVF and make possible the accurate assessment of the actual threat of RVF to military troops.

Progress

This is a new work unit. Supplies and equipment were purchased in preparation for a trip to Kenya that will be undertaken Oct - Dec 1981.

Recommendations for the Future

Collect mosquitoes in Kenya with a variety of collection methods. Mosquito eggs, larvae, pupae and adults will be collected for taxonomic rearings. The taxonomic series (from rearings) will be employed to detect morphological characters for species descriptions and for constructing identification keys. Adult mosquitoes will be collected, identified and processed for virus isolation attempts. Complete ecological data will be recorded for all collections. Specimens will be collected for colonization purposes also and, in some cases, will be collected in mass to provide laboratory populations of sufficient size for conducting laboratory transmission tests.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA [illegible]	81 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. DISCLOSURE AUTH'D	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H. TERM	U	U		NIL	☒ Yes ☐ No	A. WORK UNIT

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

11. TITLE (Procede with Security Classification Code)

(U) Factors Governing Access to the Nervous System

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012900 Physiology 016200 Stress physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 02	81 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE N/A — 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 80	0	0
FISCAL YEAR CURRENT 81	1.0	50

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute or Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Inst. of Research
Division of Neuropsychiatry

ADDRESS: Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)
NAME: Holaday, J.W.,
TELEPHONE: (202) 576-3028
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Tyner, C.F., LTC, MC
NAME: Kant, G.J.,

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Shock; (U) Pharmacology; (U) Stress; (U) Injury; (U) Nervous System; (U) Blood Brain Barrier

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

23. (U) To evaluate the therapeutic effects and mechanism of action of thyrotropin releasing hormone (TRH) in reversing shock and improving outcome following exposure to organophosphate poisons, including nerve agents. Neuropharmacologic factors governing access to the nervous system will be evaluated to assess the role of the blood brain barrier in altering drug, hormone, and toxin permeability. Overall objective is to explore mechanisms of action of nerve agents with emphasis on possible new therpeutic approaches to the management of soldiers exposed to chemical warfare agents.

24. (U) Experimental animals will be surgically prepared with catheters in the external jugular vein and tail artery as well as transcranial guide tubes for drug injection and blood withdrawal as well as monitoring of cardiovascular variables following systemic or central drug injections. Shock will be induced by endotoxin administration, bleeding, or cervical spinal-cord transection. Organophosphates, such as diisopropylfluorophosphate (DFP), will be injected at a 50% lethal dose, and the effects of TRH and naloxone will be determined on the cardiorespiratory depression which follows DFP administration.

25. (U) 80 10-81 09 The improvement in blood pressure, heart rate, and respiratory rate produced by TRH have been demonstrated in normal rats as well as rats subjected to experimental endotoxic and hemorrhagic shock. Moreover, these effects of TRH in endotoxic rats were shown to be dose related and to significantly improve survival. Preliminary evidence with spinally injured animals also points to the possible efficacy of TRH in that model as well. It appears that the therapeutic effects of TRH are centrally mediated, possibly involving adrenal-sympathetic outflow. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

* Applicable to contributing work unit only if approved

DD FORM 1 MAR 88 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 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 126: Factors Governing Access to the Nervous System

Investigators:

Principal: Holaday, J.W., Ph.D.

Associates: Tyner, C.F., LTC, MC; Kant, G.J., Ph.D.

Problem:

Shock due to hemorrhage, sepsis, or spinal cord injury is a common battlefield problem. In prior work, we have shown that the opiate antagonist naloxone reverses shock and improves survival in animal models of shock as outlined above. However, since the opiate antagonist naloxone also blocks the body's own pain-relieving system, the possibility exists that drugs such as naloxone could intensify traumatic pain even as they improve shock survival. In early work, we had shown that thyrotropin releasing hormone (TRH), a tripeptide involved in regulating endocrine function, may also serve as a physiologic antagonist of opiates without affecting pain responsiveness. This may provide a distinct therapeutic advantage over opiate receptor antagonists such as naloxone.

Importance:

The initial management of shock and trauma on the battlefield presently involves the rapid administration of intravenous fluids. Not only does this therapy require a skilled technician, but the problems of storage and availability of these fluids results in potentially fatal delays in treatment. More importantly, standard pharmacolgical therapies employed in treating shock (e.g. fluids, steroids, and vasoactive agents) do not reliably reverse shock states due to hemorrhage, sepsis, or spinal cord injury. Additionally, although naloxone has the advantage of reversing these forms of shock and improving survival, it has the potential adverse effect of intensifying traumatic pain. The use of TRH in these situations may improve outcome while maintaining the ability to administer analgesic drugs such as morphine.

Approaches:

Anesthetized rats were surgically prepared with cannulae to measure cardiovascular parameters and to allow for drug injections. The following day, these conscious rats were injected with E. coli lipopolysaccharide endotoxin (15 mg/kg) to produce a

shock state. A separate group of similarly prepared rats were subjected to a rapid controlled bleeding to produce hemorrhagic shock.

Results:

The initial experiments demonstrated that TRH, at a dose of 2 mg/kg, rapidly improved cardiovascular performance and respiratory function. Later experiments demonstrated that increasing doses produced even greater responses and, in addition, significantly improved survival. The rats which received saline as a inert placebo suffered a continuation in the decline of cardiovascular function as well as an earlier death. These results were complemented with studies in which it was shown that TRH, unlike naloxone, did not have any effect upon pain responsiveness by itself or following the induction of morphine analgesia. These important results require confirmation with other shock models as well as an elucidation of the site and mechanism of therapeutic effects.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA [illegible] 71 27	81 09 30	DD DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. W.A. SECURITY	7. REGRADING	8. ORGN INSTR'N	9a. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	H TEPM	U	U		NU	YES ☐ NO	A. WORK 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 (Include Title Security Classification Code)

(U) Pharmacologic modulation of effects of neural damage and stress.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012900 Physiology 016200 Stress physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 02	81 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE N/A 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 80	0	0
CURRENT FISCAL YEAR 81	1.0	25

19. RESPONSIBLE DOD ORGANIZATION

NAME Walter Reed Army Institute or Research

ADDRESS Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME Russell, Philip K., COL

TELEPHONE (202) 576-3551

20. GENERAL USE

Foreign Intelligence Not Considered

20. PERFORMING ORGANIZATION

NAME Walter Reed Army Inst. of Research

ADDRESS Division of Neuropsychiatry
Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Provide Name If U.S. Academic Institution)

NAME Holaday, J.W.,

TELEPHONE (202) 576-3028

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME Dr. Alan I. Faden

NAME

22. KEYWORDS (Provide Each with Security Classification Code)

(U) Nervous system; (U) Pharmacology; (U) Stress; (U) Injury; (U) Spinal trauma

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

23. (U) This exploratory project will assess the possible therapeutic utility of thyrotropin releasing hormone (TRH) in the prevention of functional disabilities following acute spinal-cord injury for possible application in the management of battlefield casualties. Emphasis will be placed on neurologic signs of recovery with concomitant evidence of improved perfusion of the spinal cord.

24. (U) Using established models of experimental spinal-cord injury as modified in our laboratories, anesthetized animals will be instrumented for cardiovascular monitoring. Exposure of the cervical spinal cord will be accomplished by a laminectomy in the area C_6-T_1. Separate studies will be performed with transection or blunt trauma. Acute cord transection or acute trauma to the cord will be followed by drug treatment. Physiological parameters will be monitored for two additional hours before terminating. Subsequently, these animals will be surgically repaired, and neurologic examinations will be conducted for six (6) weeks to evaluate functional recovery. The effects of TRH will be compared against the opiate antagonist naloxone as well as saline controls.

25. (U) 80 10-81 09 Neurologic deficits following acute spinal cord trauma have been shown to be reversed or blocked by the administration of the opiate antagonist, naloxone, when administered 45 min to 4 hours following injury. However, the possibility that this opiate antagonist could intensify traumatic pain led us to develop the use of TRH as a physiologic opiate antagonist. This substance has the benefit of reversing the pathophysiology of shock without intensification of pain. It is therefore critical that TRH be evaluated as a potential drug to reverse spinal cord injury since it possesses distinct therapeutic advantages over existing medications. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 80-30 Sep 81.

26. AVAILABILITY (For availability other than general release, 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 127: Pharmacologic Modulation of Effects of Neural Damage and Stress

Investigators:

Principal: Holaday, J.W., Ph.D.

Associate: Faden, A.I., M.D.

Problem:

Spinal cord injury is a common problem associated with battlefield trauma. Acute injuries of the spinal cord produce immediate, irreversible damage (usually central core necrosis) followed by gradual progressive ischemic injury to surrounding white matter tracts. This secondary ischemic damage, which is potentially reversible, is largely responsible for the motor, sensory and reflex deficits observed after spinal injury. The guiding principle in treating spinal cord trauma is to limit this progressive ischemia and thereby prevent the paralysis as well as reflex and sensory deficits that would otherwise occur.

Importance:

Drugs which are presently used, including osmotic agents and/or steroids, are employed primarily to reduce the edema subsequent to an injury, but have been of only modest benefit in the treatment of spinal trauma. Since the ability of the nervous tissue to regulate its own blood supply is lost following spinal injury, the elevation of peripheral blood pressure to increase spinal cord perfusion pressure and limit ischemia has promise as a therpeutic modality. We have shown that endogenous opiate systems are involved in the loss of blood pressure following shock or trauma and that the opiate antagonist naloxone rapidly reverses this hypotension.

Approaches:

Initial experiments demonstrated the beneficial effects of the opiate antagonist, naloxone (2 mg/kg bolus, followed by 2 mg/kg/H for 4 hours) in increasing blood pressure and enhancing recovery from spinal trauma, compared to a control treatment with physiological saline. Subsequently, the effects of delayed naloxone treatment, as well as dose responsiveness, were evaluated. Additionally, radiolabeled microspheres were used to directly measure blood flow to grey and white matter. Endorphin

concentrations in plasma and CSF were correlated with pathophysiological evidence following acute injury. Cardiovascular and neurologic consequences of delayed naloxone treatment were monitored for 6 weeks following injury.

Results:

Treatment with naloxone as long as 4 hours following spinal cord injury was shown to be as effective as early (45 min) naloxone treatment. This provides evidence for a broader therapeutic window for the time allowed to intervene pharmacologically following spinal cord trauma. Plasma levels of endogenous opiate substance, which are believed to result in the decrease in blood pressure following spinal cord injury, were significantly elevated following spinal trauma. These results confirm earlier observations and provide insights into the mechanism of action of naloxone therapy. Naloxone therapy may produce an adverse intensification of traumatic pain; future research should be directed toward development of a drug which increases spinal blood flow without altering the body's own analgesic substrates.

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	81 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'NS	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	STOC 80-7.2.2			

11. TITLE (Precede 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 81	3.0	319
FISCAL YEAR — CURRENT 82	4.0	361

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, PHILIP K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of CD&I

ADDRESS: Washington, DC 20012

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

NAME: BANCROFT, WILLIAM H. COL

TELEPHONE: (202) 576-3757

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: SCOTT, Robert McN., COL

NAME: BRANDT, WALTER E., PH.D. POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Virology; (U) Immunology; (U) Arbovirus Infections; (U) Adenovirus Respiratory Diseases; (U) Influenza; (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) To define etiology of acute infectious diseases of special hazard to military personnel, to determine and evaluate factors influencing 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 developed as needed.

25 (U) 80 10-81 09 A live attenuated dengue-2 vaccine (PR-159/S-1) was tested for reactogenicity and immunogenicity in a placebo-controlled double-blind study at Fort Bragg, NC. Results showed the 99 vaccinees experienced more symptoms and missed more duty time than the 49 placebo recipients. Geometric mean dengue-2 hemagglutination inhibition (HAI) titers at 30 days for 77 yellow fever (YF) immune and 22 YF non-immune vaccinees were 176.3 and 7.6, respectively, demonstrating again the advantage of preliminary YF vaccination. A battery of monoclonal antibodies has been produced to each of the four types of dengue. The range of reactivity clearly demonstrates an antigenic basis for differentiation of individual dengue serotypes, and dengue from other flaviviruses. Additionally, specificity of the immunofluorescent reactivity correlates closely with virus neutralization but not HAI reactivity. Monoclonal antibodies will be useful tools for distinguishing viral antigens as well as precise diagnostic reagents. A human monocyte cell line (U-937) showed differential attachment of dengue and yellow fever virus and that only cross reactive monoclonal antibodies permitted immune enhancement of dengue infections. A computer program was developed for rapid calculation and analysis of ARD rates on basic training posts. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

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 Charles H. Hoke, Jr., MC;
CPT Erik A. Henchal, MSC;
Mr. Jack McCown;
Mrs. Jeanne Burrous, M.S.;
SFC Thomas E. Simms;
SSG Kiran Jesrani;
SSG Liberato Pagaoa;
SGT Wanda Williams

Purpose

Characterization of viruses which threaten military personnel is necessary for effective disease control. Emphasis is placed on dengue viruses and respiratory viruses such as adenoviruses and influenza. Work is directed toward description of viral structural proteins, antigens, virus-cell interactions, host immune responses and means of immunoprophylaxis.

Basic research on dengue viruses is directed toward evaluation of the genetic lesions causing attenuation, the enhancement of virus replication by antibody, and their reactivity with type-specific monoclonal antibodies. Human volunteer studies are conducted to evaluate dengue vaccine safety and immunogenicity.

Research on respiratory viruses is directed toward the identification of viruses causing acute respiratory disease (ARD), evaluation of ARD incidence rates on basic training posts and the immunogenicity of influenza viral proteins.

Progress

Monoclonal dengue (DEN) antibodies were prepared to each of four prototype viruses (DEN-1, Hawaiian; DEN-2, New Guinea-C;

DEN-3, H-87; and DEN-4, H-241) in order to better ascertain the mechanism of immune enhancement and to develop an improved method for dengue virus identification. Lymphocyte hybridomas were prepared by fusing P3X63Ag8 mouse myeloma cells with spleen cells from mice immunized with dengue virus antigens. Antibody producing hybrid cells were cloned and injected into Pristane-primed mice for preparation of ascitic fluids. The resulting monoclonal antibodies included some that were DEN type-specific, some DEN group-specific and some that reacted with all flaviviruses. Curiously, a few prepared to DEN 1 or 3 cross-reacted with both viruses but not types 2 and 4, leading to the conclusion that DEN 1 and 3 form an antigenic subgroup. The type-specific DEN antibodies proved to be highly specific and sensitive in the identification of DEN viruses by indirect immunofluorescence. Some monoclonal antibodies were type-specific by hemagglutination inhibition (HAI) and one monoclone was DEN-2 specific by plaque reduction neutralization test. It has been found that the critical viral determinants for virus neutralization are distinct from those required for hemagglutination. Preliminary studies of immune enhancement of dengue virus replication demonstrated that enhancement of infection is dependent upon the presence of cross-reactive HAI antibody; type-specific antibody does not enhance.

The DEN-2 (PR-159/S-1), live, attenuated, virus vaccine was used in the sixth human volunteer study to determine the clinical and immune responses of volunteers who have or have not received yellow fever vaccine previously. The placebo-controlled, double-blind study was conducted at Fort Bragg, NC. DEN-2 vaccine was given to 99 male soldiers and placebo to 49 others. All 148 participants were followed for 21 days to determine the frequency of visits on sick call, days missed from duty and symptoms and signs of illness. Febrile participants were evaluated for viremia. The antibody responses of all participants were tested at one, two and six months following immunization. Complaints of illness were made by 54% of the vaccinees and 47% of the placebos (p=N.S.). Analysis of vaccine-related morbidity showed chills, abdominal pain, headache, fever, nightsweats and nausea to be significantly more frequent in the vaccinees. These symptoms began 9.1 $\pm$ 5.3 days after immunization and lasted 4.1 $\pm$ 2.4 days. The average duration of assignments to quarters was 1.9 days with a range of 1-4 days. Infected DEN vaccine recipients with detectable yellow fever (YF) antibody at the time of immunization were assigned to quarters more often (19.6%) than uninfected recipients (7.1%). Geometric mean titers of neutralizing antibody to DEN type 2 were higher at 60 days in infected vaccinees with yellow fever antibody (1:389) than vaccinees without YF antibody (1:58).

In FY 1981 Acute Respiratory Disease (ARD) rates were reported to be equal to or less than 2/100 men/week on most basic training posts. However on all posts the rates were higher during the cold months and at each post there was at least one sharp outbreak of ARD, usually in December, January or February which was thought to be associated with influenza. As in FY 1980, adenovirus type 4 (ADV-4) resulted in outbreaks with case rates over 1/100/week at Forts Dix and Wood. In September and October ARD rates subsided with the initiation of routine type 4 and 7 vaccination. On all posts, women had higher average ARD rates than men during the cold winter months. The female ARD rates tended to fall towards the male rates in the summer. Females do not now receive ADV types 4 and 7 vaccines which may account for this difference in rates.

Future Objectives

Monoclonal antibodies will be used to study the arrangement of type specific and group reactive antigens on the surface of dengue particles. They will also be applied to further studies of immune enhancement of dengue infections. Future studies of the dengue type 2 vaccine will evaluate the safety and immunogenicity in heterologous dengue immune recipients and explore possibilities for efficacy testing.

ARD rates on basic training posts will be monitored more efficiently in the future by utilizing an interactive automated data processing program developed during the past year.

9. Scott, R.McN
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2. Wells, R.A., Scott, R.McN., Pavanand, K., Sathitisathein, V., Cheamudon, U. and MacDermott, R.P.
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8. Butler, A.B., Scott, R.McN., Lampe, R., Schyellower, M., Lampe, R.M., Schwab, J.A. and Muelenaer, A.A.
The Immunoglobulin Response to Reimmunization with Rubella Vaccine. J. Pediatrics (In Press).

Work Unit 201 Viral Infections of Man

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1. Vanapruks, V., Scott, R.McN. and Bonyaratapan, N. Transmission of Diarrhea in a Neonatal Nursery. Thai Military Surgeons, Bangkok, Thailand, October 1980.

2. Scott, R.McN., Eckels, K.H., Bancroft, W.H., McCown, J.M., Anderson, J., Top, F.H., Jr., and Russell, P.K. Live Attenuated Dengue Type Two Vaccine in Human Volunteers Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, November 1980.

3. Millunchick, E., Burrous, J. and Hoke, C., Jr. Epidemic Viral Meningitis due to Echoviruses 7 and 11. Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA, November 1980.

4. Gentry, M.K., McCown, J.M., Harrison, S.A., Henchal, E.A., Brandt, W.E. and Dalrymple, J.M. Characterization of Monoclonal Antibodies Directed Against Dengue Viruses. American Society of Tropical Medicine and Hygiene, Atlanta, GA, November 1980.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6513	81 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
80 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
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Mechanisms of Transmission of Hepatitis Viruses

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
72 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 81	2.0	218
FISCAL YEAR CURRENT 82	3.5	225

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of CD&I

ADDRESS: Washington, DC 20012

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

NAME: BANCROFT, William H. COL

TELEPHONE (202) 576-3757

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: LEMON, Stanley M., MAJ

NAME: BINN, Leonard, N.

POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Viruses; (U) Hepatitis; (U) Antigen; (U) Immunology; (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) 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 identification and antigenic analysis of hepatitis viruses are under development. The immune response of patients infected with hepatitis viruses is studied to define sensitive parameters of infection and to define critical factors in immunity. The epidemiology of hepatitis B in military populations is defined.

25 (U) 80 10-81 09. Hepatitis A virus (HAV) was successfully cultivated in tissue culture. Replication of both PA33 and HM 175 strains of HAV was achieved in both primary African green monkey kidney cells and a continuous fetal Rhesus lung cell line certified for use in vaccine development. Both virus strains were passed four times with antigen detected in cell monolayers by direct immunofluorescence and confirmed by radioimmunoassay. These findings point the way to eventual vaccine development. The use of the New World owl monkey (Aotus trivirgatus) as a model of human hepatitis A virus infection was suggested by the demonstration that newly captured Aotus in Panama uniformly became infected with HAV. HAV was recovered from both feces and liver of monkeys. Studies are now in progress to further define the susceptibility of this species to HAV. Further studies of IgM antibody to hepatitis B virus (HBV) core antigen in chronic HBsAg carriers indicated that the presence of this antibody was highly correlated with the simultaneous presence of HBeAg, DNA-polymerase, high level HbsAg carriage and abnormal serum aminotransferases. A small scale vaccine study was initiated using HBV vaccine (Lot 751) prepared by Merck, Sharp and Dohme. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

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

Work Unit 202 Mechanisms of Transmission of Hepatitis Viruses
Investigators:

Principal: MAJ Stanley M. Lemon, MC
COL William H. Bancroft, MC

Associates: Leonard N. Binn, Ph.D.;
MAJ Maria Sjogren, MC;
Dr. Walter E. Brandt, Ph.D.;
SP4 Norman L. Gates;
Mr. Hubert Cannon
Mrs. Ruth H. Marchwicki

Problem

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 tranmsmission 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 virus diagnosis, characterization of hepatitis viruses, and the study of modes of virus transmission and evaluation of means of prevention of viral hepatitis.

Progress

Studies carried out during the past year with MAJ J. LeDuc at the Gorgas Memorial Laboratory suggest that the owl monkey, Aotus trivirgatus may be a useful animal model of hepatitis A virus (HAV) infection. Newly captured Aotus arriving at the Gorgas Memorial Laboratory (GML) in Panama were surveyed for evidence of previous HAV infection. Although only 2 of 145 newly captured monkeys were sero-positive, these monkeys rapidly acquired anti-HAV following their entry into the GML colony. In addition, HAV antigen was detected by solid-phase radioimmunoassay in fecal material collected from 11 of 13 cages housing a total of 19 monkeys during their first two months in the colony. The antigen detected was indistinguisable from HAV obtained from human sources when both were compared in cross-blocking radioimmunoassay studies. This finding represents the first recovery of HAV from non-human primates which were not experimentally infected. Viral antigen was also identified in the

liver of four monkeys dying of apparently unrelated causes. These observations indicate a high degree of susceptibility of Aotus to HAV and have led to further studies now in progress to better define the utility of this species as a model of HAV infection.

Substantial progress has been made in the propagation of HAV in vitro. Although replication of HAV in vitro has been recently described by others, (1,2) most reports have emphasized the cell-associated nature of the virus produced. HAV antigen was detected by radioimmunoassay in both cellular fractions and supernatant fluids of primary African green monkey kidney cells (AGMKC), which are certified for use in vaccine production, between 30 and 50 days after inoculation with virus recovered from Aotus at the GML. The presence of infectious virus in supernatant fluids was confirmed by the development of HAV antigen (detected by direct immunofluorescence) in AGMKC inoculated with these materials. This strain of HAV has now been passed a total of four times in AGMKC as well as in fetal Rhesus lung cells (FRhL-2) which are also certified for use in vaccine production. A second strain of HAV (HM175), acquired from NIAID, has also been successfully passed three times in AGMKC. In addition, current studies, not yet completed, indicate that other cell lines (MA104 and BSC-1) may also support HAV replication. Cytopathic effects were not observed in any infected cell culture. No antigen was detected in passage control material, nor in owl monkey kidney cells (OMK-210) or human lung carcinoma cells (A549) inoculated with virus. These studies confirm that AGMKC and other cell types support HAV replication, and suggest, by virtue of its early release into supernatant fluids, that the virus recovered from Aotus may have an enhanced ability to replicate in cell cultures.

In studies conducted during the previous year, IgM antibody to hepatitis B core antigen (IgM anti-HBc) was shown to be a useful indicator of acute hepatitis B infection (3). However, this IgM antibody persists in many chronic HBsAg carriers, raising the question of whether its presence is indicative of continued HBcAg expression and thus might correlate with other serum markers of viral replication and infectivity. Therefore, a collaborative study involving sera from 55 chronic HBsAg carriers was carried out with Dr. Jay Huffnagle of NIAMDD. The presence of IgM anti-HBc correlated significantly with serum markers of viral replication, including virus-specific DNA polymerase, HBeAg, and high titer HBsAg, as well as the presence of elevated aminotransferase enzymes. Subsequent studies suggest that in HBsAg carriers, this IgM antibody is predominantly low molecular weight or monomeric IgM (approximately 7s). Further efforts to characterize this immunological response are in progress.

Lastly, a small scale study examining the immunogenicity of the inactivated hepatitis B vaccine manufactured by Merck, Sharp and Dohme (4) was initiated. Nineteen volunteers received multiple 20 mg doses subcutaneously by jet injector. Preliminary results indicate a humoral antibody response similar to that seen with 40 mg given IM by needle (conventional route). However, thus far, 8 of 19 recipients have developed small nodular subcutaneous lesions at the sites of injections approximately two weeks after immunizations. These lesions were of minor significance and would not appear to be a major impediment to jet injection as a method of administering this vaccine.

Recommendations and future objectives.

Future research efforts should concentrate on hepatitis A virus (HAV), which is perceived to be the greatest military threat. Aotus will be tested for their susceptibility to HAV under controlled conditions. Attempts will be made to optimize the current available cell culture system for HAV and existing strains will undergo further passage in hopes of achieving high virus yields. Additional fecal material obtained from Bangkok and Alaska (5) and shown to contain HAV by radioimmunoassay will be inoculated into cell cultures in an effort to broaden the range of available HAV strains capable of in vitro replication. These isolates, in concert with an ongoing effort to develop murine hybridomas producing antibody directed against HAV, would facilitate the comparison of HAV strains isolated from different regions of the world. Virus purified from chimpanzee fecal material and possibly cell cultures will be studied by polyacrylamide gel analysis in an effort to expand existing knowledge of the polypeptide components of the virion. Such efforts will broaden the data base required for effective vaccine development.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6443	81 10 01	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SECTY	6. WORK SECURITY	7. REGRADING	8A. DISS'N INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	61102A	3M161102BS10	AB	203
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7,2:2			

11. TITLE (Provide 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 PREVIOUS 81	7.0	909
FISCAL YEAR CURRENT 82	6.0	909

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL, MC
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of CD&I
ADDRESS: Washington, DC 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Formal, Samuel B., Ph.D.
TELEPHONE: (202) 576-3344
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: J. Sadoff, A. Cross,
NAME: G. Lowell, R. Seid, W. Zollinger,
NAME: H. Schneider
POC:DA

21. GENERAL USE

Foreign intelligence not considered

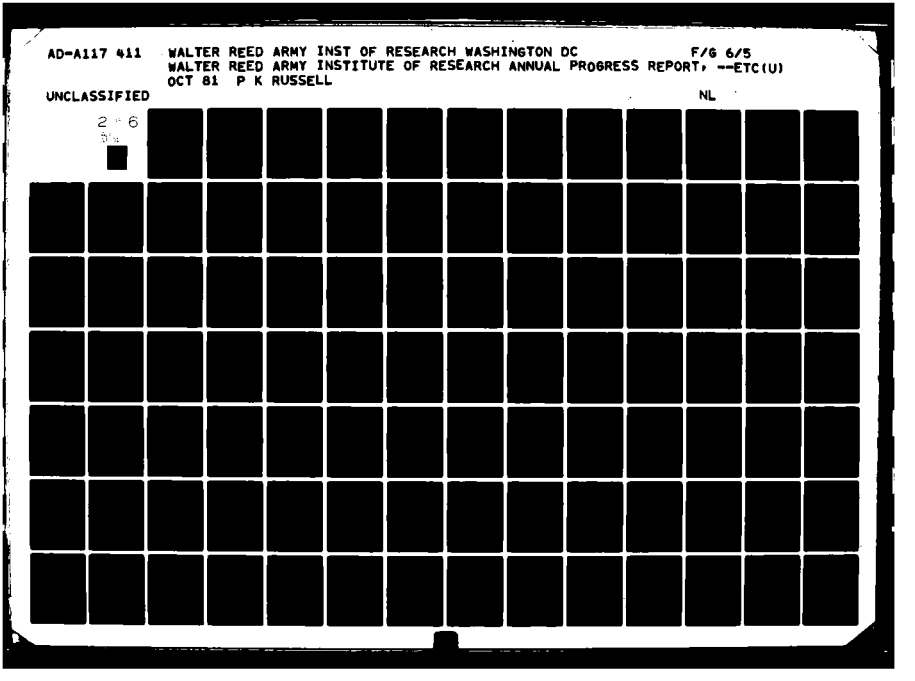
22. KEYWORDS (Provide EACH with Security Classification Code) (U) Pseudomonas aeruginosa; (U) Neisseria meningitidis; (U) Gonococcus; (U) Immunology; (U) Antibiotics; (U) Infectious Diseases; (U) Bacteria

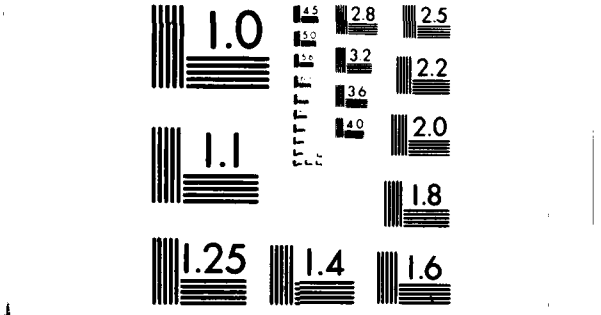
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Provide as much detail as can be confidently stated.)

23 (U) Studies on the etiology, ecology, epidemiology, [illegible] immunological and diagnostic aspects of diseases of military importance or potential problems to military forces. Current emphasis is on staphylococcal, gonococcal and pseudomonas infections.

24 (U) Basic studies on bacterial pathogenesis [illegible] genesis and result in future development [illegible]

25 (U) 80 10 - 81 09 Hybridoma [illegible] were produced and used to show [illegible] group B disease. Various [illegible] to group B polysaccharide [illegible] Pseudomonas [illegible] and shows [illegible] fied gonococcal [illegible] [illegible] [illegible]


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Project 3M161102BS10 RESEARCH ON MILITARY DISEASE INJURY AND HEALTH HAZARDS

Work Unit 203: Bacterial Diseases of Military Importance

Investigators:

Principals: Samuel B. Formal, Ph.D.
COL Edmund C. Tramont, MC
LTC Alan Cross, MC
LTC George Lowell, MC
LTC Jerald Sadoff, MC
Wendell Zollinger, Ph.D.

Associates: MAJ John Boslego, MC
Herman Schneider, Ph.D.
Robert Seid, Ph.D.

Problem

Studies are carried out on the etiology, ecology, epidemiology, pathogenesis, physiological, immunological and diagnostic aspects of diseases of bacterial origin which are present or potential problems to military forces. Current emphasis is on meningococcal, gonococcal and pseudomonas infections in military forces.

Progress

Murine hybridoma monoclonal antibodies to several key meningococcal antigens including group B polysaccharides, lipopolysaccharides, pili, and serotype proteins were produced and partially characterized. A simple, highly specific method of meningococcal serotyping which involves co-agglutination with monoclonal antibody-coated staphylococcal A cells was developed and used to serotype about 200 group B isolates from various parts of the world.

A semi-automated system for detecting and quantitating gonococci attached to either buccal epithelial cells or mammalian tissue cell lines was investigated. The Artex system that was used was unable to adequately differentiate gonococci attached to the cells from background interference. Several methods were tried to enhance the contrast between

gonococci and cells. These included a variety of conventional bacteriological and histological strains, a fluorescent method using acridine orange and a peroxidase conjugated rabbit antigonococcal antiserum to detect the organism. These methods were not capable of sufficiently enhancing the contrast between gonococci and cell to allow the Artex system to function. A system using a more sensitive detector and computer analyser of the output data is being developed. Monoclonal antibodies were produced from mice immunized either with whole GC or the gonococcal pilus vaccine. Antibodies against pili and outer membrane proteins were produced and are to be used for immunologic investigation of strain variation in N. gonorrhoeae. It is hoped that these antibodies may help to define a "common portion" shared by all GC pili that might be a future vaccine candidate. Gonococcal LPS has been detoxified using alkaline reagents and covalently coupled to gonococcal pili. The covalently coupled products inhibit bactericidal activity of human and rabbit sera for gonococci and react with anti pili antibody in solid phase immunologic reactions. These coupled products have enhanced immunogenicity for pili in mice and are potential vaccines which could induce both anti attachment and bactericidal antibody. Cyanogen bromide fragmentation of gonococcal pili revealed three fragments. Preliminary evidence indicated that a monoclonal antibody directed against the common determinant on gonococcal pili reacted with the smaller of these cyanogen bromide fragments.

A vaccine for human use was prepared using the Fisher-Devlin type 5 protype strain. A modified Phenol-Water extract with pretreatment of the culture with Formalin was the technique employed. The bottled vaccine passed rabbit and guinea pig safety tests and induced homologous protection in the mouse and rat. Chemical characterization revealed them to be high molecular weight aggregates of polysaccharides and consisting of neutral and amino sugars and lipid containing characteristic lipids of LPS. A high molecular weight polysaccharide vaccine prepared by acetic acid hydrolysis of polysaccharide obtained from the culture supernatants of a Fisher type 1 organism failed to induce protection in the burned rat against a homologous type 1 organism. Passive administration of high titered J-5 antisera prepared in rabbits also failed to protect in the burned rat model. Passive protection with high titered anti-pseudomonas

immunoglobulin protected at the 60% level in the burned rat. Pseudomonas pili protected homologously in active vaccination experiments in the burned rat model but heterologous protection was not induced. Covalently coupled conjugates of Pseudomonas aeruginosa detoxified lipopolysaccharide and pseudomonas pili induced antibody in the mouse with increased amounts of antibody being noted against the pili portion as compared to controls vaccinated with pili alone. Methods for purification of pili have been developed.

Studies on the role of capsular and LPS antigens in the pathogenesis of gram negative infections continued. We examined 498 clinical isolates of E. coli. Rough specific and K1 capsular-specific phages were found to correlate with the presence of rough LPS and K1 capsule by classical techniques. Fifty percent (97/193) of urinary E. coli were rough compared to 28% (70/248) of blood and 30% (17/57) of wound isolates. Eleven percent (21/193) of urinary and 21% (12/57) of wound E. coli were K1 positive. Forty-seven percent (33/70) of rough bacteremic E. coli were K1-positive while only 12% (122/178) of smooth E. coli were K1-positive ($p<0.0001$). This strong association between $K1^+$ and rough phenotypes was not seen with urinary E. coli. The sensitivity to killing in a phagocytic system differed with the phenotype. Among bacteremic $K1^+$ E. coli and 21% (6/28) rough K1+ E. coli were killed. In contrast, 73% (32/44) of smooth $K1^-$ and 91% (30/33) of rough $K1^-$ E. coli were killed. (81% of $K1^-$ E. coli killed versus 15% of $K1^+$ E. coli, $p<0.0001$). Thus, the combination of smooth LPS and $K1^+$ confers resistance to phagocytic kill. Capsular types other than K1 in combination with smooth LPS may confer phagocytic resistance. Only the $K1^+$ phenotype, however, confers resistance to E. coli of the rough LPS phenotype.

Thymosin fraction 5 and the synthetic [illegible]ypeptide, thymosin alpha 1, can stimulate in vitro [illegible]ion of anti-toxoid and anti-polysaccharide antibo[illegible] human peripheral blood lymphocytes by stimulating [illegible]cell helper activity. This immunopotentiating property may be useful to enhance the in vivo immunogenicity of miliarily relevant microbial antigens.

Future Plans

1. Monoclonal antibodies will be utilized to identify antigens of potential importance in the

development of group B meningococcal vaccines and gonococcal vaccines.

2. The ELISA procedure will be utilized to detect antibodies to various gonococcal antigens. Improved methods to quantitate the attachment of gonococci to epithelial cells will be sought.

3. Vaccines consisting of pseudomonas "detoxified" LPS will be prepared from additional serotypes and tested for specificity in animal models.

4. Monoclonal antibodies against E. coli antigens will be tested for protective ability.

5. Additional substances and procedures to enhance the immune respone will be investigated.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6763	81 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
80 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	61102A	3M161102BS10	AC	204
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2:2			

11. TITLE (Precede 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: 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 81	3.0	160
FISCAL YEAR CURRENT 82	2.0	206

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

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

NAME: Osterman, J.V., Ph.D.

TELEPHONE: (202) 576-2146

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Kelly, D.J., CPT, MSC, M.S.

NAME: Todd, J.M., CPT, VC, DVM POC: DA

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Rickettsiae; (U) Biochemistry; (U) Structure-Function Relationship; (U) Structure-Antigenicity Relationship

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

23. (U) Isolation and characterization of subcellular fractions of rickettsiae which have potential as experimental immunogens. Localize and identify the rickettsial surface proteins that affect virulence. Assess mice with different genetic backgrounds for optimum animal model. Evaluate in mice the immunogenic potential of rickettsial fractions. These studies will aid in development of inactivated vaccines capable of protecting troops deployed in areas endemic for rickettsial diseases.

24. (U) Isolate and evaluate the peripheral rickettsial macromolecules as experimental immunogens. Analyze the proteins using polyacrylamide gel electrophoresis coupled with enzyme-linked immunosorbent assay (ELISA). Evaluate inbred strains of mice for humoral and cell mediated immune responses to subcellular fractions of rickettsiae. Develop lymphocyte hybridomas producing monoclonal antibody to rickettsial antigens. Isolate and characterize plaque purified strains of rickettsiae.

25. (U) 80 10 - 81 09 Antigens of scrub typhus rickettsiae were separated by sodium dodecal sulfate polyacrylamide gel electrophoresis and were analyzed by ELISA. Six antigens identified in each of the 3 strains were located in the cell envelope fraction. Reactivity of these antigens with homologous or heterologous immune sera indicated that different macromolecules existed in all strains, although they exhibited similar mobilities during electrophoresis. Antigens of strain Gilliam reacted equally well with antibodies directed against Gilliam, Karp, or Kato. However, strains Karp and Kato each had two distinct antigens which did not react with heterologous antisera. R. tsutsugamushi antigens retained immunogenicity after electrophoresis, and antisera raised against them reacted with intact organisms and exhibited specificity in reactions with isolated antigens. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

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

Work Unit: 204 Rickettsiae-Host Interactions in Pathogenesis of Disease

Investigators
Principals: Joseph V. Osterman, PhD; CPT John M. Todd, VC; Christine S. Eisemann, MS
Associates: SSGT John A. Grimsley

Description

Efficacious, safe vaccines for rickettsiae are needed to preclude severe disruption of military operations when deployed troops are exposed to these organisms. One approach to vaccine development utilizes subcomponents of rickettsiae and has the potential advantage of eliciting protective immunity, while minimizing adverse side reactions due to integral endotoxic components of the rickettsiae or contaminating host cell debris. Problems associated with this approach include: (1) isolation and purification of subcellular rickettsial components responsible for eliciting immune protection; (2) localization and identification of rickettsial surface proteins that affect virulence and (3) antigenic characterization of peripheral proteins of *rickettsiae*.

Progress

Antigens of scrub typhus rickettsiae were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and were analyzed by an enzyme-linked immunosorbent assay. Six antigens were identified in each of the three prototype strains and these antigens were located in the cell envelope fraction of the organisms. Reactivity of these isolated antigens with homologous or heterologous immune sera indicated that different macromolecules existed in all strains, although they exhibited very similar mobilities during electrophoresis. Antigens of strain Gilliam reacted equally well with antibodies directed against Gilliam, Karp, or Kato rickettsiae. However, strains Karp and Kato each had two distinct antigens which did not react with heterologous antisera. Rickettsia tsutsugamushi antigens retained immunogenicity after electrophoresis, and antisera raised against them reacted with intact organisms and exhibited specificity in reactions with isolated antigens.

A latex agglutination test for antibodies to Rickettsia typhi and Rickettsia prowazekii was developed which was sensitive, group-specific, and reproducible. Erythrocyte-sensitizing

substance from these rickettsiae spontaneously adsorbed to latex, and in the presence of immune serum formed an easily observed aggregate. The test was performed in microtiter plates to conserve reagents. Sensitivity to this procedure compared favorably to the well established microimmunofluorescence assay.

Recommendations for Future

In order to characterize more carefully the peripheral proteins of scrub typhus rickettsiae, lymphocyte hybridomas will be developed which elaborate antibodies against specific cell wall antigens. In addition, it is anticipated that recombinant DNA studies will be initiated with the goal of producing rickettsial antigens in free-living bacteria. This latter study, if successful, would relieve the tedious and expensive logistic effort required for the cultivation of obligate intracellular rickettsiae.

References Cited

None

Presentations

None

Publications

1. Eisemann, C.S., and J.V. Osterman. 1981. Antigens of Scrub Typhus Rickettsiae: Separation by Polyacrylamide Gel Electrophoresis and Identification by Enzyme-Linked Immunosorbent Assay. Infect. Immun. 32: 525-533.

2. Hechemy, K.E., J.V. Osterman, C.S. Eisemann, L.B. Elliott, and S.J. Sasowski. 1981. Detection of Typhus Antibodies by Latex Agglutination. J. Clin. Microbiol. 13: 214-216.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6514	81 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
80 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	AD	205
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2:2			

11. TITLE (Procede 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)
PRECEDING FISCAL YEAR 81	6.0	451
CURRENT FISCAL YEAR 82	6.0	459

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, COL P.K.
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of CD&I
ADDRESS: Washington, DC 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Roberts, LTC D.R.
TELEPHONE: 202-576-3719
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Schneider, Dr. I.
NAME: Gingrich, MAJ J. POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Malaria; (U) Mosquitoes; (U) Trypanosomiasis; (U) Tsetse flies; (U) Scrub Typhus; (U) Trombiculid mites; (U) Immunology

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

23. (U) Develop physiological means of interrupting malaria transmission through an understanding of factors affecting parasite infectivity in vivo and in vitro. Refine model of African trypanosomiasis transmission to obtain large numbers of parasites for the study of immune mechanisms. Assess competence of closely related species as malaria vectors. Develop method of testing repellents against tsetse flies. Study mechanism that determines mite susceptibility to scrub typhus. Realization of objectives may lead to prevention or control of malaria, trypanosomiasis and scrub typhus in military troops.

24. (U) Compare different density gradients for separation of gametocytes from other forms of Plasmodium berghei and use bioassays to determine parasite infectivity after isolation and purification. Attempt infection of different vectors with cultured Plasmodium falciparum using the membrane feeder method. Acquire *anophelines* and human malaria isolates for vector competence studies. Determine locations of scrub typhus pathogens in mite vectors and search for symbionts. Identify definitive factors influencing infection rates of trypanosomes in all developmental stations of the tsetse fly.

25. (U) 80 10 - 81 09 Bloodstream forms of Trypanosoma brucei rhodesiense can be transmitted mechanically from infected to normal mice by tsetse flies. Up to 50 percent of the mice can be infected by mechanical transmission if the interval between termination of the infective feed and initiation of refeeding on a clean mouse is less than one minute. Three species of anophelines were fed via artifical membranes on cultured Plasmodium falciparum gametocytes. In 44 trials involving more than 3000 mosquitoes, 2 Anopheles stephensi were infected. Anopheles balabacensis (Perlis form) was capable of supporting growth of Plasmodium berghei only to the oocyst stage. For technical report, see Walter Reed Institute of Research Annual Report, 1 Oct 80 to 30 Sep 81.

*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 205 Vector Transmission of Militarily Important Infectious Diseases

Investigators

Principal: Donald R. Roberts, LTC, MSC

Associate: John B. Gingrich, MAJ, MSC; Michael W. Hastriter, CPT, MSC; Ronald A. Ward, Ph.D.; Imogene Schneider, Ph.D.; David E. Hayes; Lawrence M. Macken; SP5 Megan G. Dowler; SP4 Jose Ruiz; SP4 Vickie Paraschos; SP4 Sandra McMurray; PFC Pedro Quintero

Problem

Malaria, leishmaniasis and trypanosomiasis are arthropod-borne diseases of military medical importance. Consequently, any chemotherapeutic drug or vaccine for these diseases should be effective against the insect transmitted stage of parasite. Thus, the use of laboratory cyclic transmission models is an important element of any test system for a drug or vaccine development program. Current research objectives are to develop physiological means of interrupting malaria transmission through an understanding of factors affecting parasite growth, invasion and infectivity in both verte- and invertebrate hosts; determine additional infection factors in tsetse flies that would result in making the WRAIR tsetse fly colony more efficient in producing infections of Trypanosoma rhodesiense for immunological studies; determine if tsetse flies and/or stable flies can transmit T. rhodesiense by mechanical means; assess the vector competence of closely related anopheling species viz-a-viz malaria transmission and study the mechanism that determines mite susceptibility to scrub typhus. Realization of these objectives may lead to prevention or control of malaria, trypanosomiasis and scrub typhus in military troops.

Progress

Improved infection rates were obtained with tsetse flies fed on infectious mixture of red blood cells, culture medium (as a serum substitute) and blood-stream parasites. Rates in these flies were intermediate between the higher rates obtained with flies fed on a mixture of red blood cells, culture medium and culture-form (pro cyclic) parasites and the lower rates in flies fed blood-stream form parasites in defibrinated blood. The use of blood-stream forms in the artificial mixture has the advantage of greater fly survival.

Studies on mechanical transmission of trypanosomes from an infected host to an uninfected host showed that up to 51 percent of

the tsetse flies could transmit in this manner if the interruption time between feeding on the two hosts was less than a minute.

Cultures of Plasmodium falciparum, containing 1-3% gametocytes were fed to anopheline mosquitoes via an artificial membrane in an attempt to bypass the need for primate hosts in cycling this parasite. The parasite cultures were supplied by CPT Jackie Williams, Department of Immunology. According to Ifediba and Vanderberg (1981) maturity and hence infectivity of gametocytes in culture was insured or at least enhanced by reducing the erythrocyte concentration during a portion of the culture period and by the addition of hypoxanthine to the culture medium. Under these same conditions, some 4300 mosquitoes belonging to four different species (Anopheles stephensi, A. albimanus, A. dirus and A. freeborni) have been fed via the membrane technique. To date only one feed has resulted in mosquito infections and then only in A. stephensi. Especially in the more recent cultures the number of microgametocytes undergoing exflagellation upon exposure to ambient air have been impressive, e.g., up to six per 400X field. Stained preparations, however, show that the majority of the male gametes remain attached to the residual body thereby precluding fertilization of all but a small percentage of macrogametes.

Colonies of Anopheles dirus (Perlis Form) and A. freeborni were introduced into the departmental insectary. Preliminary studies indicate that the Perlis form can support the growth of the rodent malaria P. berghei only to the oocyst stage.

The four major techniques for isolating malaria sporozoites from mosquito salivary glands are (1) individual dissections, (2) decapitation followed by compression of the thoraces to extract the glands and homegization of whole mosquitoes followed by (3) density gradient centrifugation or (4) the use of DEAE-cellulose columns. The first two procedures result in far higher yields of sporozoites and in numbers of contaminating organisms in the final preparation. Combining techniques 2 and 3 with minor modifications results in high yields of greater purity than previously reported.

Scrub typhus investigations included elucidation of the distribution and ultrastructure of Rickettsia tsutsugamushi in larval and adult Leptotrombidium arenicola by TEM, elaboration of the structure and function of the genital apparatus of L. arenicola and L. fletcheri by SEM, examination of L. fletcheri eggs and spermatophores with SEM, determination of spermatophore viability during aging and development of a computer data base for literature on scrub typhus and the mite Families Trombiculidae and Leeuwenhoekiidae.

Recommendations for the future

Laboratory and field data from feeding studies coupled with data on known field infection rates in host animals, including man, should be integrated into a computer model that would predict the frequency

with which mechanical transmission might occur. Proposed studies on testing repellency of prime compounds from LAIR should be expedited. Identify those factors that are required to infect anopheline mosquitoes with cultured P. falciparum gametocytes. Initial emphasis should be on documentation of complete exflagellation of the microgametocytes. Studies on the use of liposome preparations to inhibit the exoerythrocytic stages of P. berghei malaria have been severely hampered by the erratic production of sporozoites. Future emphasis should be on selection of more susceptible mosquitoes from any of the species currently housed in the entomology insectary. Develop an ELISA field test for detecting P. falciparum sporozoites in field collected samples of mosquitoes. Such a test, if sufficiently specific, would have broad application in vector determination studies. Attempt additional refinements in isolating sporozoites from mosquito salivary glands to eliminate as completely as possible all bacterial and mosquito contaminants. Transovarial transmission of R. tsetsugamushi has never been established in any of the scrub typhus vectors that are naturally free of rickettsia. It has been proposed that certain genetic lines are susceptible to establishing transovarial transmission. To prove or disprove this theory, an attempt should be made to clear infected mites of rickettsial infection by treating with temperatures of 42°C for 24 hr periods (preliminary testing for chigger survival at this temperature has been completed) and or clearing by feeding infected larval mites on mice treated with antibiotics. Thereafter attempts would be made to reinfect progeny of the cleared line(s) by feeding on rickettsemic mice. Finally, TEM studies would be initiated on uninfected larval mites that are fed on rickettsemic animals to determine the fate of imbibed Rickettsia through all stadia.

References Cited

Ifediba, T., and Vanderberg, J. (1981) see Vanderberg, J. in "Symposium on Malaria Immunology and Vaccine Research" published by American Public Health Association, Washington, D.C. 20005. p. 19-20.

Formal Presentations

"Some phenomena associated with the development of Trypanosoma b. rhodesiense infections in the tsetse fly, Glossina morsitans" by J.B. Gingrich at 1980 meeting of the American Society of Tropical Medicine and Hygiene. Nov. 5-7, 1980.

Publications

Gingrich, J., Ward, R., Macken, L., and Esser, K. 1981. Some phenomena associated with the development of Trypanosoma brucei rhodesiense infections in the tsetse fly, Glossina morsitans. Am. J. Trop. Med. Hyg. 30:570-574.

Esser, K., Schoenbechler, M., Gingrich, J., and Diggs, C.L. 1981. Monoclonal antibody analysis of Trypanosoma rhodesiense metacyclic antigen types. Fed. Proceed. 40:1011.

Gingrich, J., Ward, R., Macken, L., and Schoenbechler, M. 1981. Trypanosoma brucei rhodesiense: factors influencing infection rates in a recent human isolate in tsetse flies, Glossina morsitans. Submitted to Journal of Med. Entomology.

Esser, K., Schoenbechler, M., and Gingrich, J. 1981. Immunization with blood forms of Trypanosoma rhodesiense protects against metacyclic (fly form) challenge. Submitted to Nature.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6436	81 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
80 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	AE	206
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7.2;2			

11. TITLE (Precede 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 81	4.0	508
FISCAL YEAR CURRENT 82	4.0	596

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, COL Philip K.

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Communicable Disease
and Immunology

ADDRESS: Washington, DC 20012

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

NAME: Baron, L.S.

TELEPHONE: (202) 576-2230

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Johnson, E.M.

NAME: Kopecko, D.J.

NAME: Wohlhieter, J.A. POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Vaccine; (U) Enteric Bacteria; (U) Antigens; (U) Virulence; (U) Salmonella; (U) Plasmids

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

23. (U) Definition in genetic and molecular terms of the properties of 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. We anticipate that it will be possible to modify genetically enteric bacteria to any desired antigenic structure and pathogenicity to 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 study of the informational micromolecules (i.e., DNA).

25. (U) 80 10 - 81 09 All Shigella sonnei strains have been found to carry a 120 Mdal plasmid that encodes for form I surface antigen. Spontaneous loss of the 120 Mdal plasmid results in loss of virulence and retransfer of this plasmid to avirulent strains makes them virulent confirming the essential nature of this plasmid in virulence. A 140 Mdal plasmid has been shown to be necessary for virulence in S. flexneri and a 42 Mdal plasmid is necessary for virulence of some Yersina strains. Further studies have demonstrated that the Shigella plasmids are needed for epithelial cell penetration, the first step in the disease process. An understanding of the genetic nature of these plasmid borne virulence properties has been valuable in the development of a bivalent vaccine strain which protects against S. sonnei and Salmonella typhi. A patent application has been prepared for this bivalent bacterial strain. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

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

Work Unit 206: Microbial Genetics and Taxonomy

Investigators:
Principal: L. S. Baron, Ph.D.
Associates: J.A. Wohlhieter, Ph.D; E.M. Johnson, Ph.D.; D.J. Kopecko, Ph.D.; C.A. Life; N.J. Snellings, M.S.; K. F. Noon, M.S.; SP5 W.C. Reid, B.S., SP5 J.N. Coulby, B.S.

Problem

Enteric bacterial infections 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 have on frequent occasions been incapacitated by enteric bacterial illnesses. These enteric organisms can produce severe stomach cramps, nausea, vomiting, bacteremia, and diarrhea. Such enteric bacterial infections generally occur soon after personnel enter an area where sanitary conditions area are deficient or disrupted. Effective prophylactic field measures do not exist for many of the severe enteric disease agents. Since native populations do develop an immunity to the enteric organisms normally present in 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 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.

Progress

We have shown that the major protective cell surface antigen, the form I antigen, of *Shigella sonnei* is plasmid-encoded in collaborative studies with the Dept. Bacterial Diseases. Until recently, no effective oral enteric vaccines had been developed. Germanier and co-workers in Switzerland, however, have recently constructed a galactose epimeraseless *Salmonella typhi* oral

vaccine strain which has proved to be safe and highly effective in recent field trials. We decided to use this already proven strain as a carrier for the form I antigen of Sh. sonnei. Using genetic manipulations, we have transferred the genes for Sh. sonnei form I antigen synthesis into this S. typhi strain. The resultant strain expressed both the S. typhi antigens and the Sh. sonnei form I surface antigen, and was protective in mouse tests against challenge with either S. typhi or Sh. sonnei cells. Thus, the hybrid strain appears to be a good bivalent vaccine against typhoid fever and shigellosis due to Sh. sonnei. It is expected that this bifunctional vaccine will serve as a model for construction of other similar multi-valent vaccines. We are employing similar technology to develop potential vaccines against Sh. flexneri and have been able to transfer the Sh. flexneri 2a antigens to the S. typhi oral vaccine strain. These studies involve the genetic manipulation of Shigella chromosomal genes encoding cell surface structures by various genetic techniques so that they can be transferred to the S. typhi oral vaccine strain. In addition to aiding in the development of vaccines against these diseases, these studies will be useful in defining each step of the pathogenic process. We have found that large plasmids in all Sh. sonnei and Sh. flexneri strains encode some property that enables these bacteria to penetrate the intestinal mucosa. Further studies are aimed at determining the plasmid-mediated products(s) that are required for virulence by cloning the virulence genes onto smaller vector plasmids for detailed examination.

Recommendations for the Future

1. Genetically modifying the S. typhi oral vaccine strain to carry the cell surface antigen determinants of Shigella flexneri serotypes 2a and 3; the new strain thus developed can be used in combination with the previously developed S. typhi form I antigen-containing strain to produce a multivalent vaccine.

2. Inserting the genes for toxoid antigens into the S. typhi oral vaccine strains to produce a vaccine protective against both typhoid fever and cholera. Similar procedures are contemplated to produce effective vaccines against other entero-toxigenic diseases.

3. Use of genetic manipulation of Shigella species to dissect the pathogenesis process, the findings of which should prove invaluable in initiating new vaccine approaches.

4. Examination of plasmids in S. typhimurium and S. typhi which we suspect may play an as yet undefined role in the virulence of these organisms.

References cited

None

Formal Presentations:

L. S. Baron - Jan 5, 1981, "Genetic, Molecular, and Biochemical Characterization of Plasmid-Mediated Atypical Utilization of Citrate by Escherichia coli". International Conference on Bacterial Plasmids, Santo Domingo, Dominican Republic.

L.S. Baron - May 3, 1981, Genetic and Molecular Studies of the Regulation of Atypical Citrate Utilization and Variable Vi Antigen Expression in Enteric Bacteria. Symposium on Genetic Engineering of Microorganism, University of Illinois, Urbana, IL.

D. J. Kopecko - Nov 6, 1980, Specialized Recombination Systems in Bacteria: Role in Gene Expression and Evolution. Department of Microbiology, Medical College of Virginia, Richmond, Virginia.

D.J. Kopecko - Jan 5, 1981, Invasive Bacterial Pathogens of the Intestine: Shigella virulence plasmids and potential vaccine approaches. International Conference on Bacterial Plasmids. Santo Domingo, Dominican Republic.

D.J. Kopecko - Mar 17-18, 1981, The Genetics of Plasmids and Their Involvement In Bacterial Pathogenicity. Invited lecturer - course on The Role of Plasmids in Bacterial Pathogenicity. Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI.

D.J. Kopecko - Apr 10, 1981, General Properties and Medical Relevance of Bacterial Plasmids. Infectious Disease Grand Rounds, Walter Reed Army Medical Center, Washington, D.C.

D.J. Kopecko - Apr 16, 1981, Genetic and Molecular Properties of Bacterial Plasmids. Invited lecturer in Course on Microbial Genetics. Department of Microbiology, Uniformed Services, University of the Health Sciences, Bethesda, MD.

D.J. Kopecko - May 8, 1981, The Involvement of Plasmids in the Virulence of Shigella. Department of Microbiology, Temple University Medical School, Philadelphia, PA.

Publications

1. Friedman, D.I., A.T. Schauer, M.R. Baumann, L.S. Baron, and S.L. Adhya (1981). Evidence that Ribosomal Protein S10 Participates in Control of Transcription Termination. Proc. Natl. Acad. Sci. USA 78:115-118.

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3. Yamamoto, N., S. Yamamoto, P. Gemski and L.S. Baron (1981). An Unusual -P22 Phage Hybrid with the c+ Region and the immI Region of P22. Abst. Ann. Meeting, Amer. Soc. Microbiol., p. 114.

4. Yamamoto, N., P. Gemski, and L.S. Baron (1981). Isolation of a Hybrid Between Salmonella Phage P22 and coli Mutation Phage Mu-1. Abst. Ann. Meeting, Amer. Soc. Microbiol., p. 115.

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11. Sansonetti, P.J., D.J. Kopecko, and S.B. Formal (1981). Shigella sonnei Plasmids: Evidence that a Large Plasmid is Necessary for Virulence. Infect. Immun. (in press).

12. Sansonetti, P.J., D.J. Kopecko, and S.B. Formal (1981). Genetic and Physical Characterization of a Large Plasmid Necessary for Virulence in Shigella flexneri. Infect. Immun. (submitted).

Patent Application

Oral Vaccine for Immunization Against Enteric Disease, L.S. Baron, S.B. Formal, and D.J. Kopecko.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6445	81 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. DOD'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	61102A	3M161102BS10	AE	207
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede 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 — PRECEDING 81	3.0	455
FISCAL YEAR — CURRENT 82	3.0	525

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of CD&I

ADDRESS: Washington, DC 20012

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

NAME: Formal, Samuel B., Ph.D.

TELEPHONE: (202) 576-3344

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: T. Hale

NAME: G. Lowell

NAME: POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede 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. Precede 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 mechansims of penetration are investigated. Attenuated living vaccines are developed.

25. (U) 80 10 - 81 09 The genetic and physical characterization of a large plasmid necessary for form I antigen synthesis and virulence in Shigella sonnei has been investigated. A large plasmid has been demonstrated to be necessary for the invasive ability of Shigella flexneri. Outer membrane proteins which are encoded by virulence-associated plasmids in Shigella flexneri have been analyzed on SDS polyacrylamide gels. Specific secretory anti-shigella LPS IgA and induce monocytes-mediated anti-shigella activity. Anti-shigella lipopolysaccharide (LPS) secretory IgA can be induced in rabbits by immunizing locally with LPS preparations containing either shigella or non-covalently complexed meningococcal outer membrane proteins. (For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.)

*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 69 (FOR ARMY USE) ARE OBSOLETE

* U.S. GPO 1974-546-043/8691

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

Work Unit 207: Pathogenesis of Enteric Diseases

Investigators:

Principal: Samuel B. Formal, Ph.D.

Associates: Thomas L. Hale, Ph.D.
LTC George Lowell, MC

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

Over 5000 doses of the Salmonella-typhi-Shigella sonnei I transconjugant strain (previously described) which is considered to be a candidate vaccine against both typhoid fever and S. sonnei dysentery have been lymphilized and tested for safety in laboratory animals.

A large plasmid (140 Mdal) in strains of S. flexneri has been shown to contribute to the virulence of these organisms. By using mini cells evidence has been obtained that these large plasmids may encode for outer membrane proteins of shigella flexneri.

The ability of specific purified secretory IgA directed against shigella X-16 lipopolysaccharide

(LPS) to induce mononuclear cell-mediated antibacterial activity against X-16 shigella has been further investigated. Monocytes, but not K or T lymphocytes are effector cells in cooperation with this anti-X-16 S-IgA. Activity is abrogated by inactivation of mononuclear cells by heat or sonication. Activity is detectable after 20 minutes, is maximal after 40 minutes and cannot be mimicked by non-immune purified S-IgA. One mechanism whereby specific anti-shigella S-IgA may protect against shigellosis may include the ability of S-IgA to induce monocyte-mediated antibacterial activity and thereby limit bacterial multiplication in secretory tissue.

Protein free LPS extracted by the Westphal method from shigellae is not antigenic when perfused in chronic rabbit Thiry-Vella loops. However LPS prepared by the Boivin procedure and which contains proderndoes produce S-IgA antibodies. Furthermore, protein free shigella LPS covalently complexed with meningococcal outer membrane protein produces IgA antibodies in the rabbit model, but is not antigenic when complexed to bovine serum albumin.

Future Studies

1. An application for an IND on the transconjuant S. typhi - S. sonnei vaccine will be made and if granted the vaccine will be tested for safety in volunteers.

2. Studies on the role of plasmid and chromosomal DNA in the virulence of shigella will continue.

3. Hybrid vaccines made from S. flexneri and the galE S. typhi 21a strain will be constructed.

4. Studies on the potentiating effect of outer membrane proteins on the antigenicity of shigella LPS will continue.

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3. Justus, P.G., J.R. Mathias, G.M. Carlson, J.L. Martin, S. Formal, and R.P. Shields. The myoelectric activity of the small intestine in response to Clostridial perfringens A enterotoxin and Clostridium difficile culture filtrate. Gastrointestinal Motility. Ed. J. Christensen. Raven Press, N.Y.

4. Mathias, J.R., G.M. Carlson, J.L. Martin, R.P. Shields and S. Formal. Shigella dysenteriae I enterotoxin: proposed role in pathogenesis of shigellosis. Am. J. Physiol. 239:G382-G386, 1980.

5. Lowell, G.H., R.P. MacDermott, P.L. Summers, A.B. Reeder, M.J. Bertovich, and S.B. Formal. Antibody-dependent cell-mediated antibacterial activity: K lymphocytes, monocytes and granulocytes are effective against shigella. J. Immunol. 125, 2778-2784. 1980

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11. P.J. Sansonetti, D. J. Kopecko, O. Washington and S.B. Formal. Evidence that a large plasmid participates in the virulence of Shigella flexneri Abst. Ann. Meeting ASM, Dallas, Texas, B48, 1981.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA OC 6435
2. DATE OF SUMMARY: 81 10 01
REPORT CONTROL SYMBOL: DD-DR&E(AR)636

3. DATE PREV SUM'Y: 80 10 01
4. KIND OF SUMMARY: B. Change
5. SUMMARY SCTY: U
6. WORK SECURITY: U
7. REGRADING:
8A. DoD'N INSTR'N: NL
8B. SPECIFIC DATA FOR CONTRACTOR ACCESS: ☒ YES ☐ NO
9. LEVEL OF SUM: A. WORK UNIT

10. NO. CODES:
PROGRAM ELEMENT: 61102A
PROJECT NUMBER: 3M161102BS10
TASK AREA NUMBER: AF
WORK UNIT NUMBER: 208
b. PRIOR:
c. CONTRACT/GRANT:
d. [illegible]: STOG 80-7.2.2

11. TITLE (Precede with Security Classification Code):
(U) Immunity in Protozoan Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
010100 Microbiology 002600 Biology

13. START DATE: 74 07
14. ESTIMATED COMPLETION DATE: CONT
15. FUNDING AGENCY: DA
16. PERFORMANCE METHOD: C. In-House

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

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING 81	2.0	375
CURRENT 82	5.0	439

19. RESPONSIBLE DOD ORGANIZATION
NAME:
ADDRESS: Walter Reed Army Institute of Research
Washington, DC 20012
RESPONSIBLE INDIVIDUAL
NAME: Russell, P.K., COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME:
ADDRESS: Walter Reed Army Institute of Rsch
Division of CD&I
Washington, DC 20012
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Hockmeyer, W.T., MAJ
TELEPHONE: (202) 576-3544
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Haynes, J.D., LTC
NAME: Williams, J.S., CPT POC: DA

21. GENERAL USE
Foreign Intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Antigens; (U) Protozoa; (U) Immunity; (U) Tropical Medicine; (U) 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) 80 10-81 09 The malaria vaccine effort is focused on obtaining monoclonal hybridoma antibodies to merozoites antigens which are involved in protective immunity. We are using an in vitro growth inhibition assay as an in vitro correlate of protective immunity and pursuing promising monoclonal antibodies from 8 fusions. Using IFA and SDS-PAGE, we are working up monoclonal antibodies with apparent specificity for merozoite surface antigens, and others which are strain-specific. Synchronization techniques which allow the harvest of naturally released merozoites continues to be improved. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

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

Work Unit 208: Immunity in Protozoan Diseases

Investigators:

Principals: LTC J. David Haynes, MC
MAJ Wayne T. Hockmeyer, MSC

Associates: LTC Stephen C. Hembree, MSC
MAJ Mills Mc Neill, MC
CPT Jeffrey A. Lyon, MSC
E5 Donald E. Raney
E3 David A. Soos
Ms. Lois A. Simonton
Ms. Theresa Jareed

Problems and Objectives

Malaria has been and continues to be a major cause of lost man hours during military operations in tropical climates. We are exploring the feasibility of developing a vaccine to protect the soldier in the field. This would have the advantage over mosquito vector control and over chemoprophylaxis of not requiring repeated application or administration in the field. We are focussing on the ability of immune antibody to interrupt the parasite cycle in the blood by preventing merozoites from reinvading red cells. We are using immune antibodies from monkeys and humans, as well as well as monoclonal hybridoma antibodies, to further define which parasite surface antigens are the best targets for antigen production by genetic engineering in bacteria.

Progress:

We have developed a method for lightly fixing merozoites released from synchronized Plasmodium falciparum cultures and attaching these intact merozoites to the bottoms of microtiter plate wells for use as the antigen in an enzyme linked immunosorbant assay (ELISA) to detect immune antibody directed against the merozoite surface. This ELISA detects antibody in high titer in the sera of monkeys that are able to block merozoite reinvasion of red cells. Several monoclonal antibodies being immunochemically analyzed also react in this ELISA. Previous monoclonal antibodies from mice have not been

able reproducibly to block merozoite reinvasion even though they have reacted with the merozoite in the ELISA, and in the immunofluorescent antibody assay. Hybridomas have now been made using immune lymphocytes from monkeys known to produce blocking antibody.

Continued work indicates that strain-specific antigen(s) play a role in protective immunity. We have begun to adapt our methods to the study of strain antigenic variation in the field. We are analysizing "pre-and post" sera obtained from troops entering areas with malaria transmission.

A collaboration with the Laboratory for Parasitic Diseases at NIH demonstrated the ability of our immune monkey serum to block the adherence of parasitized red cells to venous endothelium, thus blocking one of the ways in which the parasite is thought to avoid the host's mononuclear phagocytic system in the spleen and liver.

The importance of non-antibody aspects of protective immunity have been furthered by the discovery that the growth of parasites can be inhibited by a heat-labile complement dependent factor, and a heat-stabile "tumor necrosis factor."

Recommendations:

The prime objective is to find a monoclonal antibody that reliably blocks merozoite reinvasion of red cells, then to characterize the antigen that it reacts with, and use it as a probe to purify the corresponding RNA, make cDNA, and clone the DNA for use in producing the antigen in bacteria or yeast. A secondary objective is to characterize the surface antigens of the merozoite even if it can not now be shown which one(s) are most important. The other studies should be continued.

Presentations:

1. Williams, J.L., Haynes, J.D., and Diggs, C.L. "In vitro assays for antigenic variation in Plasmodium falciparum." at the 65th Annual Meeting of The Federation for Experimental Biology, in Atlanta, GA. April 1981. Abstract 5025.

2. Haynes, J.D, Tapchaisri, C., and Tapchaisri, P. "Plasmodium falciparum growth is inhibited by a heat-labile serum factor missing from C4-deficient serum." Ibid. April 1981. Abstract 4771.

3. Haynes, J.D. "Recent advances in the in vitro culture of parasites: Applications in biomedical resarch. Plasmodium species." Invited talk at the 30th Annual meeting of the American Society of Tropical Medicine and Hygiene. San Juan, Puerto Rico. November 1981.

4. Diggs, C.L., and Haynes, J.D. "Production and analysis of monoclonal antibodies against Plasmodium falciparum malaria parasites at WRAIR." Symposium on Malaria Immunology and Vaccine Research. Sponsored by The American Public Health Association and A.I.D., Bethesda, Maryland. January 1981.

Publications:

1. Chulay, J.D., Aikawa, M., Diggs, C. and Haynes, J.D. 1981. Inhibitory effects of immune monkey serum on synchronized Plasmodium falciparum cultures. Am. J. Trop. Med. Hyg. 30(1): 12-19.

2. Chulay, J.D., Haynes, J,D., and Diggs, C.L. 1981. Inhibition of in vitro growth of Plasmodium falciparum by immune serum from monkeys. J. Infect. Dis. 144(3): 270-278.

3. Haynes, J.D. 1981. The use of radioisotopes to study human malaria in vitro. In: Immunoparasitology: Principles and methods in malaria and schistosomiasis research. ed. G.T. Strickland. Praeger Scientific, N.Y. (In press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6440	81 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
80 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	209
b. XXXXXXXXXX				
c. XXXXXXXXXX	STOG 80-7.2:P			

11. TITLE (Provide with Security Classification Code)

(U) Parasitic Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

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	81 — 3.0	98
FISCAL YEAR — CURRENT	82 — 3.0	178

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Experimental Therapeutics

ADDRESS: Washington, DC 20012

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

NAME: DAVIDSON, David E., Jr., COL

TELEPHONE: (301) 427-5029

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign intelligence not considered.

POC: DA

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Parasite; (U) Schistosomiasis; (U) Malaria; (U) Primate; (U) Trypanosomiasis; (U) Leishmaniasis

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 physiological, biochemical, pathological and epidemiological aspects of parasitic diseases of military importance. To evaluate existing techniques and to develop new techniques for diagnosis, prevention, treatment and control.
24. (U) Culture systems and animal models of parasitic diseases will be developed and used to study the parasites of interest, the parasitic disease process, and the effectiveness of new diagnostic, preventive and therapeutic measures. Studies will emphasize but will not be restricted to malaria, leishmaniasis, trypanosomiasis, and schistosomiasis.
25. (U) 8010-8109 Further evaluation of experimental agents in the human macrophage model of leishmaniasis showed that the anti-leishmanial activity of metabolites of 8-aminoquinolines is apparently greater than that of the parent compounds and that Formycin B is very active in this in vitro system. The susceptibility of different strains of leishmania to pentavalent antimony is being investigated in this model; it may be possible to predict in vivo response to antimony treatment on the basis of in vitro susceptibilities. A long-acting antitrypanosomal cis-diammine-dichloroplatinum (II) complex was developed. It is far less toxic in use than the parent compound which requires a seven-day regimen of treatment. Preliminary evidence of the presence of nucleic acids on the surface of trypanosomes was discovered. Radiorespirometry has proven successful in rapid (three hours) identification of leishmanial species and strains. In 137 tests the technique was 99.9 percent accurate in identifications of 23 species and strains. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

Project 3M161102BS10AF RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 209 Parasitic Diseases of Military Importance

Investigators:

Principal: LTC Larry D. Hendricks, MC
MAJ Jonathan D. Berman, MC
CPT Michael S. Wysor, MSC
Dr. Joan E. Jackson
Mrs. Gloria P. Willet

PROBLEM AND OBJECTIVES:

Effective management of parasitic disease problems among military personnel is dependent upon development of improved techniques and accumulation of new information to assist in diagnosis, prevention, treatment and control. This work unit supports: (a) studies of the physiological, biochemical, pathological and epidemiological aspects of parasitic diseases of military importance; (b) development of new techniques for diagnosis, prevention, treatment and control. Parasite culture systems and animal models of the parasitic diseases of interest are developed and utilized. Emphasis is placed upon, but is not restricted to, malaria, leishmaniasis, schistosomiasis and trypanosomiasis.

PROGRESS:

Further evaluation of experimental agents in the human macrophage model of leishmaniasis showed that the anti-leishmanial activity of certain metabolites of 8-aminoquinolines is apparently greater than that of the parent compounds and that Formycin B is very active in this *in vitro* system. This model system was also employed in investigations of the susceptibility of different strains of Leishmania to pentavalent antimony. Predictions of *in vivo* response to antimony treatment may be possible on the basis of the *in vitro* susceptibilities. A long-acting cis-diammine-dichloroplatinum (II) complex was developed and tested against *Trypanosoma rhodesiense* in mice. A single dose treatment with the complex was more efficacious and far less toxic than a seven-day regimen of treatment with the parent compound. Apparently the complex releases the platinum compound slowly at a less than toxic rate and a multiple-dose regimen is not necessary.

Preliminary morphological and histochemical evidence of the presence of nucleic acids on the surface of trypanosomes was discovered. Radiorespirometry has proven successful in the rapid identification of 23 leishmania species and strains including isolates from all four types of human disease (visceral, cutaneous, mucocutaneous and diffuse cutaneous). In over 130 tests conducted to date, the technique has proven to be 99.9% accurate in identification of the species and strain of the parasitic organism under test. The test data base is being expanded; data obtained from leishmania biopsy isolates from military patients as well as from standard reference leishmania strains acquired from sources world-wide are being collected to correlate clinical disease manifestations, prognosis and drug sensitivity with the results of the radiorespirometric test.

FUTURE OBJECTIVES:

Further studies will be conducted to confirm the usefulness of the human macrophage-leishmania culture system for predicting in vivo responsiveness of patients to drug therapy. This model culture system will also be utilized to investigate the efficacy and mechanisms of action of purine analogs as antileishmanial agents, and to investigate the characteristics of liposomes and other carriers of drugs. In trypanosomiasis, studies of platinum and silver-sulfonamide compounds will continue. The nature and significance of the nucleic acids on the trypanosomal surface will be investigated. Radiorespirometry will be applied not only for identification of species and strains of leishmania but also for prediction of relative drug sensitivities. The possible application of radiorespirometric techniques to other protozoa (trypanosoma and plasmodia) will be investigated.

PUBLICATIONS:

Berman, J.D., and Dwyer, D.M. 1981. Expressions of Leishmania antigen on the surface membrane of infected human macrophages in vitro. Clin. Exp. Immunol. 44, 342-348.

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Berman, J.D. 1981. Activity of imidazoles against Leishmania tropica in human macrophage cultures. Am. J. Trop. Med. Hyg. 30, 566-569.

Berman, J.D., and Neva, F.A. 1981. Effect of temperature on multiplication of leishmania amastigotes within human monocyte-derived macrophages in vitro. Am. J. Trop. Med. Hyg. 30, 318-321.

Decker Jackson, J.E., and Fox, J.C. 1981. Rapid identification of a Leishmania sp. from the U.S.A. and preliminary drug sensitivity screening using radiorespirometry. Proceedings of the International Symposium (IAEA/FAO/WHO) on Nuclear Techniques in the Study and Control of Parasitic Diseases of Man and Animals. Vienna, Austria. 29 June - 3 July 1981. In Press.

Decker Jackson, J.E. and Tang, D.B. 1981. Rapid identification of Leishmania spp. by Radiorespirometry. II. A statistical method of data analysis to evaluate the reproducibility and sensitivity of the technique. Proceedings of the Workshop on the Biochemical Characterization of Leishmania. Washington, DC. 9-11 December 1980. In Press.

PRESENTATIONS:

Berman, J.D., Chulay, J.D., Hendricks, L.D., and Oster, C.N. Susceptibility of Leishmania from clinically sensitive and resistant lesions to pentavalent antimony in vitro.

Berman, J.D., and Lee, L.S. Sensitivity of Leishmania tropica in human macrophages to 8-aminoquinolines in vitro.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6444	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISS'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	210
b. CONTRIBUTING				
c. XXXXXXXX	STOC 80-7.2;2			

11. TITLE (Procede with Security Classification Code)

(U) Biochemical Research on Military Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

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76 07	Cont.	DA	C In-house

17. CONTRACT/GRANT

a. NUMBER / PE / PROJ / TASK / WORK UNIT
b. NUMBER
c. TYPE
d. KIND OF AWARD
e. EXPIRATION
f. AMOUNT
g. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	9.0	729
FISCAL YEAR CURRENT 82	9.0	766

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K. COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Biochemistry
ADDRESS: Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Doctor, B.P. Ph.D.
TELEPHONE: (202) 576-2594
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Wolfe, A.D. Ph.D.
NAME:

21. GENERAL USE

Foreign Intelligence Not Considered

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(U) Toxin; (U) Antigens; (U) DNA; (U) Antibody; (U) Membrane.

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 conduct studies on biochemical 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 protein 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 will be purified and characterized, using techniques of chromatography, electrophoresis, gradient centrifugation, spectroscopy and bioassays. Studies of virulence potential will be performed using cell-free enzyme assays immunochemical assays and cell culture and animal toxicity assays. The use of hybridoma technology to prepare monoclonal antibodies to components of pathogens will be employed.

25. (U) 80 10 - 81 09 Shiga toxin inhibits the aminoacyltrna binding stage of peptidyl elongation. All three biological activities of shiga toxin are associated with a 33,000 MW protein. C. difficile toxin inhibits membrane functions. Rabbit antitoxin has been produced against C. difficile toxin. New taxonomic enteric groups have been established by nucleotide sequence studies. Serological characteristics of monclonal antibodies against dengue virus antigens have been evaluated. Thiosemicarbazones cause bacteriostasis by suppression of RNA synthases. Unique purine and polyamine metabolic pathways of Leishmania were identified as potential targets of chemotherapeutic agents. See WRAIR Annual Progress Report 1 Oct 80 - 30 Sept 81.

*Available to contractors upon originator's approval

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PROJECT: 3M161102BS10 RESEARCH ON MILITARY DISEASE, IN[illegible]ND HEALTH HAZARDS

WORK UNIT: 210 Biochemical Research on Military Diseases

INVESTIGATORS:

Principle: Peter Gemski, Ph.D.
Alvarado, M., B.A., SP-4; Barreiro, [illegible]; Brown, J. E., Ph.D., CPT, MSC; Doctor, B. P., [illegible]; Emery, C.; Fanning, G., M.S.; Foret, D., SP-4; Gentry, M. K., M.S.; Griffin, D., M.S., MSC; Hansen, B., Ph.D.; Lazere, J., B.S.; Limrothert, T., B.S., SP-6; Mayo, W., SP-5; Perez-Arbelo, J., SP-4; Pritchard, D., E-3; Rothman, S., Ph.D., Sodd, M. A., M.S.; Solow, R., M.S., CPT, MSC; Wolfe, A. D., Ph.D.

In collaboration with: Brandt, W. E., Ph.D. (D.C.D. & I.), Brenner, D., (CDC, Atlanta, Ga), Collins, H., (D.C.D. & I.), Webster, C., (Div. Med.)

DESCRIPTION:

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 bacteria and parasites, and products of host responses to disease are being studied through the use of physiocochemical, biochemical, microbiological and immunological concepts and techniques. Such information provides a rational basis for immuological and chemotherapeutic protection against disease and the development of accurate diagnostic procedures.

A. Studies of Shigella and Their Toxins

1. On the Manner of Inhibition of Protein Synthesis by Shiga Toxin.

Shigella dysenteriae 1 produces a toxin known to inhibit protein synthesis in reticulocyte lysates by an inhibition of peptidyl elongation. The manner of inhibition by Shiga toxin has been further examined. The process of peptidyl elongation can be represented by three component stages: binding of aminoacyl-tRNA, the peptidyl transferase reaction and translocation. By use of cetyltrimethylammonium bromide (CTAB) to assay the concentration of peptidyl-tRNA in complete reaction mixtures, inhibition of the component reactions by inhibitors is distinquishable after addition of puromyucin. Therefore, purifield Shiga toxin, activated by pretreatment with 8 M urea, 10 mM DTT, was

added (1.1µg/ml) to actively incorporating retriculocyte lysate reaction mixtures. Incorporation of ^{3}H-leucine into TCA-precipitable material and CTAB-precipitable material was immediately inhibited. After 6 minutes, puromycin was added (40µg/ml) to the reaction mixtures. An immediate loss of ^{3}H-leucine-labeled product from the CTAB-precipitable fraction occured. In toxin-treated reaction mixtures without puromycin, no decrease of peptidyl-tRNA concentration was detected. These results are consistent with an inhibition by Shiga toxin of the aminoacyl-tRNA binding stage of peptidyl elongation.

2. Characterization of Shigella dysenteriae I (Shiga) Toxin Purified by Anti-Shiga Toxin Affinity Chromatography

Shigella dysenteriae 1 (Shiga) toxin was purified from whole-cell lysates by antitoxin affinity column chromatography, radioiodination, and Sephacryl S-200 gel fitration of ^{125}I-labeled affinity column eluates. Two chromatographic peaks were observed. The percentage of radioactivity in peak I samples immunoprecipitated with antitoxin ranged from 95 to 100%. A pool of samples from this first peak contained over 90% of the HeLa-cell-cytotoxic units applied to the column and was enterotoxic for rabbit ileal loops and lethal for rabbits. This radiolabeled material migrated as a single cytotoxic band after nondenaturing polyacrylamide gel electrophoresis, but formed three bands, of 33,000, 29,000, and 4,000 to 7,000 daltions, after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In addition, material estimated as 7,000 dalatons by Bio-Gel P-10 chromatography could be generated by treatment of S-200 peak I samples with 8 M urea. Pooled fractions from the second S-200 peak were separable into several low-molecular-weight peaks on a P-10 column. One of these P-10 peaks (7,000 daltons) was 27% immunoprecipitable with antitoxin. These data indicate that three of the known biological activities of Shiga toxin are associated with a 33,000-dalton substance which can be dissociated into 29,000- and 4,000- to 7,000-dalton components.

3. Isolation and Characterization of Minicell-Producing Mutants of Shigella spp.

Minicells are small, anucleate cells resulting from aberrant cell divisions at the polar ends of bacilli. We have isolated minicell-producing mutant strains of Shigella flexneri 2a (MC-1) and Shigella dysenteriae 1 (MC-V) after mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine. Microscopically, broth cultures of MC-I and MC-V were found to contain free minicells, normal cells, and filamentous cells with polar, attached minicells. Both strains retained their ability to prevoke keratoconjanctivitis in guinea pigs and to invade HeLa cells. Purified suspensions of minicells containing less than one whole cell per 10^{6} minicells were obtained by a combination of differential sedimentation and density gradient centfidugation (5 to 30% [wt/vol] linear sucrose gradients). Each MC-I minicell contained about 0.005 times the amount of deoxyribonucleic acid of one normal S. flexneri. The MC-V minicell had about 0.003 times the amount of deoxyribo-

nucleic acid of one whole S. dysenteriae cell. Purified MC-V minicells were treated with polymyxin B to release Shiga toxin. Shiga toxin was readily detected in MC-V minicells by means of a microtiter HeLa cell cytotoxicity assay. Our findings indicate that such a minicell-producing alteration in the cell division cycle of shigellae has not significantly affected their virulence.

B. Studies of Clostridium difficile Toxin

1. Presence of Clostridium difficile Toxin in Guinea Pigs with Penicillin- Associated Colitis

Cecal filtrates from guinea pigs treated with penicillin contained a toxin which produced cytotoxic changes in HeLa cell cultures and was lethal to guinea pigs when administered intracecally. The cytotoxicity could be neutralized by Clostridium difficile and C. sordellii antitoxins, but not by other clostridial antitoxins. Rabbit immunization with toxic cecal extracts produced antibody which neutralized the cytotoxicity of guinea pig cecal extracts, of stool extracts from humans with antibiotic-associated colitis and of culture supernatant fluids of C. difficile. Treatment with vancomycin reduced the number of deaths and increased the survival time of penicillin-treated animals. No cytotoxin was present in cecal extracts from these guinea pigs. Gram-negative bacteremia was present in half the penicillin-treated animals, the sick ones as well as the healthy ones. Treatment with vancomycin did not decrease the incidence of bacteremia. Gram-negative bacteremia and changes in fecal flora were observed in some antibiotic-treated guinea pigs; all deceased animals, however, contained this cytotoxin. C. difficile was isolated from cecal contents of sick animals and these isolatges produced the cytotoxin in vitro. The results suggest that C. difficile toxin can cause antibiotic-associated colitis in guinea pigs.

2. Inhibition of Membrane Funcitons in Intact HeLa Cells by Clostridium difficile Culture Filtrates.

The basis for cytotoxicity to intact HeLa cells by culture filtrates of Clostridium difficile has been investigated. Decrease in intracellular K^+ levels appeared to be the primary event occurring after exposure to filtrates, with essentially concurrent inhibition of both α-aminoisobutyric acid uptake and of macromolecular synthesis. Twenty-five per cent of the K^+ remained associated with the cell monolayer and amino acid uptake and macromolecular synthesis were not totally abolished. These results indicate that C. difficile culture filtrates, either by exhausting ATP supplies or by breaching the permeability barrier of the cell, inhibited membrane functions.

3. Rabbit Antitoxin Produced against Clostridium difficile Cytotoxin.

Efforts by various investigators to prepare antiserum to C. difficile cytotoxin using routine immunization procedures have met with mixed success. We have produced such antitoxin in rabbits using three distinct hyper-immunization protocols. In the first procedure, rabbits, initially primed i.v. with toxoid prepared from cytotoxic C. difficile culture filtrates, were given 9 inoculations (3 s.c., 6 i.v.) of cytotoxic culture filtrates, then 5 inoculations (1 intradermal, 4 s.c.) of cytotoxic cecal extracts from guinea pigs with penicillin-associated colitis. Neutralizing antibody was detected after 9 mos. In the second protocol, an initial intradermal injection of cytotoxic cecal extract in complete Freund's adjuvant was followed by 13 s.c. injections. Antibody was detected after 8 mos. In the third protocol, antibody was present after 8 s.c. inoculations spanning 6 mos. Less injections were needed to produce antiserum when initial inoculations had elevated cytotoxic activity. These antitoxin preprations neutralized cytotoxin in culture filtrates and in fecal extracts from humans or guinea pigs with antibiotic-associated colitis. Their neutralizing ability was about 2-8 fold less than that of C. sordellii antitoxin. Biweekly injections of immunogen were needed to maintain antitoxin levels.

C. Nucleotide Sequence Relatedness Among Enterobacteriaceae

1. Vibrio mimicus: a Newly Recognized, Cholera-like Organism.

Strains formerly considered as sucrose-negative Vibrio cholerae were shown to belong to a new species, Vibrio mimicus. In addition to its inability to ferment sucrose, negative Voges-Proskauer and corn oil reactions are of value in distinguishing V. mimicus from V. cholerae. V. mimicus strains show species-level DNA - relatedness and are 30%-50% related to V. cholerae. Most but not all strains are agglutinated by antisera to non-O1 strains of V. cholerae. Forty-one strains in our collection were isolated from 10 states, Guam, Canada, Mexico, Bangladesh, and New Zealand. Four strains were water isolates, 7 were from oyster, 1 from prawns, and 23 from humans. Of the human isolates, 4 were from ear infections, one from a wound, and the rest from stools. Three stool isolates were obtained from patients known to have eaten shellfish. Pending further study, V. mimicus should be considered as a diarrheal agent linked to consumption of shellfish and as an etiologic agent of ear infection -- probably linked to exposure to water.

2. Kluyvera, a New (Redefined) Genus in the Family Enterobacteriaceae: Identification of Kluyvera ascorbata sp. nov. and Kluyvera cryocrescens sp. nov. in Clinical Specimens.

Kluyvera is proposed as a new genus for the group of organisms formerly known as Enteric Group 8 (synonyn = API group 1). Strains of Kluyvera share the properties of most members of the family Enterobacteriaceae: they are gram-negative rods, motile with peritrichous flagella, catalase positive,

and oxidase negative; they grow on MacConkey agar, ferment D-glucose with the production of acid and gas, and are susceptible to many antibiotics. Strains are usually indole positive, methyl red positive, Voges-Proskauer negative, citrate positive, H_2S (triple sugar iron) negative, urea negative, phenylalanine deaminase negative, lysine decarboxylase positive, arginine dihydrolase negative, and ornithine decarboxylase positive. Kluyvera strains ferment many of the sugars and polyhydroxyl alcohols used in identification. By deoxyribonucleic acid-deoxyribonucleic acid hybridization, strains of Kluyvera were divided into three groups. Kluyvera ascorbata is proposed as the type species for the genus. Most strains of K. ascorbata have been isolated from clinical specimens. K. cryocreascens is proposed as the second species. It was occasionally isolated from clinical specimens, but it was isolated more commonly from the environment. Kluyvera species group 3 was heterogeneous, but was distinct from the two named species by deoxyribonucleic acid hybridization. This group was rare, so no species names will be proposed at this time. K. ascorbata can be differentiated from K. cryocrescens by its positive ascorbate test, ability to grow at 5°C in a refrigerator, and smaller zones of inhibition around carbenicillin and cephalothin disks. The test normally used for identification does not clearly differentiate these two species. Kluyvera spercies are probably infrequent opportunistic pathogens. The most common source is sputum, where they are probably not clinically significant. Five strains have been from blood cultures. More informaiton is needed about the incidence and clinical significance of the genus Kluyvera.

3. Tatumella ptyseos gen. nov., sp. nov., a Member of Enterobacteriaceae Found in Clinical Specimens

The name Tatumella ptyseos gen. nov ., sp. nov. is proposed for a group of organisms (previously called Group EF-9) isolated from clinical sources in the United States, Canada, and Puerto Rico. Sixty-eight percent of the isolates were from sputum specimens. T. ptyseos strains are Gram-negative, oxidase-negative, fermentative rods that grow on MacConkey agar. The distinctive biochemical characteristics of 44 T. ptyseos isolates were acid, no gas from D-glucose, sucrose, and, usually (71%), D-xylose (62% delayed; no acid from lactose, maltose, or D-mannitol; negative tests for indole, urea, methyl red, gelation, and L-lysine and L-ornithine decarboxylase; L-arginine dihydrolase variable; phenylalanine deaminase positive; Voges-Proskauer positive by the Coblentz method but negative by the O'Meara method; nonmotile at 36°C but 66% weakly motile (30% delayed) at 25°C; and Simons' citrate positive at 25°C (89%) but negative atg 36°C. Deoxyribonucleic acid (DNA) - DNA relatedness studies on 26 T. ptyseos strains showed them to be 80-100% related at 60°C, which indicated that they comprise a single species. The DNA relatedness to species within the Enterobacteriaceae was 7-38%. This is evidence that the group belongs in the family, is distinct from all described species, and is best placed in a new genus. The T. ptyseos isolates were susceptible to all antimicrobics

tested by broth dilution; the antimicrobics were amikacin, ampicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, tetracycline, and tobramycin. Three striking differences between T. ptyseos and other Entrobacteriaceae are its large zone of inhibition around penicillin (mean = 24 nm), its tendency to die on some laboratory media, such as blood agar, in 7 days, and its small number (usually one) of flagella. Strain H36 (=ATCC 33301 = CDC D6168, 9591-78) is the type strain of this new species. T. ptyseos is the type species for the genus Tatumella.

4. The marine bactgerium Vibrio damsella sp. nov. causes skin ulcers on the damselfish Chromis punctipinnis: association with human wound infections

A previously undescribed marine bacterium, Vibrio damsella, was isolated from skin ulcers on the temperate-water damselfish, the blacksmith (Chromis punctipinnis). Laboratory infection with V. damsella produced ulcers similar to those which have been frequently observed on the damselfish in southern California waters. V. damsella was also pathogenic for four other species of damselfish but not for members of other families of fish. V. damsella has also been isolated from water and two human wounds. During the last few years the Vibrio identification laboratory at the Enteric Section, Centers for Disease Control, has received over a dozen Vibrio strains from human wounds associated with seawater exposure or injury. These Na^+ requiring isolates were definitely excluded from belonging to the species of Vibrio usually associated with human infection -- V. cholerae, V. parahaemolyticus, V. alginolyticus, V. vulnificus, V. metschnikovii, and "Vibrio Group F." Three of the isolates from clinical specimens were almost identical phenotypically to V. damsella (Strain 2588-80). By DNA-DNA hybridization all four of these isolates were so highly related (>75% at 75°C, hydroxyapatite method) that they clearly belong to the same species. These data indicate that V. damsella may be able to infect man as well as marine fish and that further study on this point is needed.

D. Studies of Monoclonal Antibodies Produced by Hybridoma Cell Lines

1. Characterization of monoclonal antibodies directed against dengue-2 virus

Diagnosis of dengue virus infection and specific identification of isolated dengue virus serotypes have presented certain problems for the arbovirus laboratory, primarily because of extensive serological cross-reactions frequently obseved between the four dengue serotypes and other flavivirus antigens. A recently establish technology for the production of monoclonal antibodies using lymphocyte hybridoma cell cultures shows great promise for the production of type-specific

serological reagents as well as valuable research reagents to assist in the investigation of the molecular basis for the antigenic complexity of these viruses. Our primary objectives in this study were to demonstrate that monoclonal antibodies can be produced to dengue-2 virus, that type-specific determinants are recognized by certain of these antibodies, and that these immune reagents can be used in traditional serological tests.

Lymphocyte hybridoma cultures were prepared using spleen cells from BALB/c mice immunized with dengue type 2 (New Guinea C) virus fused with the P3x63Ag8 myeloma cell line. Antibody-secreting cell clones were selected by solid phage radioimmunoassay (SPIRA), and a representative collection of these cell lines were examined in some detail. Mouse ascitic fluids were prepared using these hybridomas to provide the highest-titered reagents possible.

Ten of 22 hybridoma clones examined were positive in a plaque reduction neutralization test. Of these 10, only one appeared truly type specific, in that it neutralized homologous dengue-2 virus to a titer of 1:3200 and was negative at a 1:10 dilution with the other three dengue serotypes. Seventeen antibody preparations were chosen because they inhibited dengue virus hemagglutination (HAI). Five of these antibody preparations specifically inhibited dengue-2 virus. The HAI cross-reactive clones were approximately equal in titer to each of the three serotypes tested, as well as to Japanese encephalitis virus, suggesting an antigenic component common to each of the flaviviruses examined. Complement fixation (CF) results were less precise, and certain antibody preparations were anticomplementary; however, some of those selective were CF positive.

A variety of experiments were performed using antibody from these hybridoma cultures, including immune fluorescence, immune precipitation of extracts from radiolabeled infected cells, and an examination of their ability to immunologically enhance virus replication is monocytes. These studies support the concept that monoclonal antibodies produced by lymphocyte hybridomas have enormous research potential as well as direct application as diagnostic immune.

2. Immune exhancement of dengue-2 virus replication in the U-937 human monocyte cell line by cross-reactive monoclonal antibodies

Dengue-2 virus yields from human monocytes are greatly increased when heterologous flavivirus antibodies or dilute homologous antibodies are included in both inoculum and culture medium. The increased yield is attributed to Fc receptor facilitated by complexes of virus and non-neutralizing antibody. Dengue-2 virus did not replicate in the U-937 human monocyte cell line unless dilute homologeo or heterologous antibodies were present in the culture system. We evaluated monoclonal antibodies (Mab) with differing characteristics for thier abilities to effect immune enhancement. Mab with hemagglutination-inhibition (HI) titers (1:400 to 1:12,800) and neutralization titers (1:20 to 1:150) to all

four dengue serotypes enhanced virus replication at dilutions of 1:80 to 1:2000. Type specific non-neutralizing dengue-2 Mab with HI titers of 1:80 to 1:320 did not enhance viral replication, nor did Mab with a type specific neutralization titer of 1:30,000 diluted 2- to 160-fold past the neuteralization titer. Thus, cross-reactive antibodies enhancement, and type specific antibodies directed against a neutralization determinant, or a type specific HI determinant, did not. The location of these determinants on the virion may be a major factor in effective presentation to an Fc receptor of a virion-antibody complex.

3. Evaluation of the serological characteristics of monoclonal antibodies produced against dengue virus antigens.

Lymphocyte hybridomas were prepared by fusing P3x63Ag8 myeloma cells (IgG1 secretors) with spleen cells from mice immunized with dengue-1 and dengue-2 viruses. Antibodies secreted by hybridomas were detected by solid phase radioimmunoassay (SPRIA). SPRIA positive hybridomas reactive in hemagglutination (HAI) and plaque reduction neutralization (PRN) tests were selected for cloning and further study. By immunodiffusion, most dengue-2 and dengue-1 hybridomas produced IgG1 and IgG2a. Several clones produced either IgG1 alone or IgG1, 2a and 2b; none produced IgA or IgM. Ascitic fluids prepared with four dengue-2 hybridomas were type specific by HAI and immunofluorescence (IFA) and unreactive by PRN. One dengue-2 monoclonal antibody (MAb) was type specific by PRN and IFA but did not react significantly by HAI. Ten dengue-2 MAb reacted to all four dengue serotypes by HAI (high titer) and by EAN (low titer). The distinct serological characteristics of these MAb suggests that there are separate determinants on the single glycoprotein of the varion that react by PRN and HAI.

E. Studies of Parasitic Biochemical Processes

1. The influence of a 2-acetylpyridine thiosemicarbazone on micromolecular synthesis in Escherichia coli and Plasmodium berghei.

A new class of chemotherapeutic agents, the 2-acetylpyridine thiosemicarbazones, inhibits the growth of many cellular and intracellular microganisms. The influence of the azacycloheptane derivative, (H), was studied with respect to viability and macromolecular synthesis in E. coli and P. berghei. H caused bacteriostasis, with an ED_{50} of approximately 4×10^{-6} M. Cu^{+2} at low concentration enhanced drug action, while other cations decreased potency. Analysis of the incorporation of isotopically labelled macromolecular precursors in the absence and the presence of concentrations of H between 10^{-6} M and 10^{-5} M revealed RNA synthesis to be inhibited preferentially, while linear regression analysis

of turbidimetric and isotope incorporation data showed growth inhibition to be directly related to inhibition of RNA synthesis. Preincubation of bacteria with drug indicated sensitivity of macromolecular synthesis occurred in the order RNA>DNA> protein. Similar concrentrations of H also suppressed incorporation of (^{3}H)adenosine and (^{14}C)phenylalanine into P. berghei.

2. Preferential inhibition of ribonucleic acid synthesis by a new thiosemicarbazone possessing antibacterial and antiparasitic properties

We determined the influence of the azacycloheptane derivative (H) of a 2-acetylpyridine thiosemicarbazone on growth and macromolecular synthesis in Escherichia coli AT-9. Thiosemicarbazone H caused bacteriostasis and a primary inhibition of ribonucleic acid synthesis; secondary effects included inhibition of deoxyribonucleic acid and protein synthesis. Addition of copper or other transition elements was not necessary for these inhibitions.

3. Polyamine levels in promastigotes, axenic amastigotes and tissue derived amastigotes of Leishmania mexicana mexicana.

Polyamines are cations present in all tissues and organisms but have an unclear metabolic role. Both polyamines and diamines have been implicated in the regulation of nucleic acid metabolism and tissue growth. As a consequence we have begun preliminary polyamine metabolism studies designed to identify unique leishmanial metabolic pathways as specific targets for chemotherapy. Our initial studies, begun in FY 81, helped to establish baseline levels of the polyamines spermidine, spermine and putrescine in the promastigote (life cycle form found in the insect host), axenic amastigote and the tissue derived amastigote (life cycle form found in the macrophage of the mammalian host). All quantitative and qualitative determinations were accomplished by a fluorometric high pressure liquid chromatographic method. Extremely low levels of spermine (<2 pmoles/10^{8} cells were determined for amastigotes of L.m. mexicana. This finding was in directcontrast to the reliatively high spermine values reported for amastigotes of Leishmania donovani. These results may reflect fundamental differences in polyamine metabolism between old and new world leishmania. However, the low values we reported may be due to a more thorough removal of host cell debris by isopyknic centrifugation utilizing Ludox colloidal silica. In addition, similar putrescine/spermidine and putrescine/spermine ratios from the extracts of axenic amastigotes and tissue derived amastigotes suggest that axenic amastigotes may be used for future leishmanial polyamine studies. Changes in polyamine levels were also monitored in transforming promastigotes and axenic amastigotesd. Pool levels of putrescine and spermidine in transforming promastigotes decreased significantly over a 6 hour incubation period while converse observations were made in transforming axenic amastigotes. The initial decrease in polyamine pool levels in transforming amastigotes is consistant with the known cessation of DNA synthesis during the conversion

of the promastigote stage to the amastigote form. Studies with radiolabeled polyamine procursors are currently being conducted to identify specific metabolic pathways and polyamine synthetic rates.

4. Purine metabolism is cultured promastigotes and axenic amastigotes of Leishmania mexicana mexicana.

A common characteristic among most parasitic protozoa, especially those of the family Trypanosomatidae is the absence of de novo purine synthetic capability. Purine requirements are instead met through various salvage pathways. Our studies were designed to identify and selectively inhibit unique parasite metabolic pathways of purine salvage. Initially, the uptake and metabolism of radiolabeled purine precursors (hypoxanthine, adenine, guanine, glycine and formate) were measured following a six hour incubation period. All quantitative and qualitative pool level values were determined using simultaneous radioactive-UV high pressure liquid chromatography. The following results were reported for both promastigotes and axenic amastigotes of L.m. mexicana:

1. ^{14}C-hypoxanthine (via IMP) and ^{14}C-adenine (via IMP) and^{14}C-guanine (via GMP) were readily inccorporated into adenylate and guanylate nucleotide pools.

2. A significant percentage (>60%) of the total label from these purine precursors was associated with adenylate nucleotides.

3. Amastigote nucleotide pools were consistantly larger than those of promastigotes.

4. No detectable levels of radiolabel from ^{14}C-glycine or ^{14}C-formate were observed in nucleotide pools.

Of particular significance was the observation that little detectable radiolabel from ^{14}C-glycine or ^{14}C-formate were observed in the nucleotide pools of the amastigote. Apparently amastigotes (as with promastigotes) lack de novo purine synthesis. This is particularly important from the standpoint of chemotherapy. However, other purine precursors are readily incorporated into nucleotide pools via purine salvage systems. Moreover, axenic amastigotes had consistently larger nucleotide pools which were in turn associated with a decrease in specific activity. These data suggest that amastigotes have a significantly slower rate of metabolism than promastigotyes. We also observed that in both promastigotes ans amastigotes, adenylates were the predominant class of purine nucleotides produced by salvage synthesis in L.m. mexicana.

A number of purine analogues and inhibitors (hadacidin, alanosine, bradykinin, bredinin and mycophenolic acid) were tested for activity against promastigotes and amastigotes of L.m. mexicana. Of these

substrates tested, only mycophenolic acid (an IMP dehydrogenase inhibitor) had a significant inhibitory effect on purine salvage metabolism. Specifically, mycophenolic acid blocked the synthesis of guanylates in both promastigotes and amastigotes. These observations provide the basis for an on-going study into the role of MPA and other inhibitors with similar enzymes specificities in the selective inhibition of guanylate synthesis.

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3. Farmer, J.J.,III, G.R. Fanning, G.P. Huntley-Carter, B. Holmes, F.W. Hickman, C. Richard, and D.J. Brenner. 1981. Kluyvera: A New (Redefined) Genus in the Family Enterobacteriaceae: Identification of Kluyvera ascorbata sp. nov. and Kluyvera cryocrescens sp. nov. in Clinical Specimens. J. of Clinical Microbiol. 13, 919-933.

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Abstracts and Presentations

1. Brandt, W.E., J.M. McCown, M.K. Gentry, and P.K. Russell. 1981. Immune enhancement of engue-2 virus replication in the U-937 human monocyte cell line by crossreactive monoclonal antibodies. Federation Procedings, 40: 1065.

2. Brown, J.E., and M.A. Usserg. 1981. On the Manner of Inhibition of Protein Synthesis by Shiga Toxin. Fed. Proc. 40(6):1580.

3. Brown, R.E., Stancato, F.A., and Wolfe, A.D. 1981. The influence of a 2-acetylpyridine thiosemicarbazone on macromolecular synthesis in Escherichia coli and Plasmodium berghei. Abstracts, 81st Annual Meeting, the American Society for Microbiology, Dallas, Texas, P. 3.

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6. Hansen, B.D. and Webster, H.K. 1981. Purine metabolism in cultured promastigotes and axenic amastigotes of Leishmania mexicana mexicana. Abst. Faceb. Paper No. 3104.

7. Hansen, B.D., Brown, N.D., Perez-arbelo, J., Pappas, M.G. 1981. Polyamine levels in promastigotes, axenic amastigotes and tissue derived amastigotes of Leishmania mexicana mexicana. Abst. Am. Soc. Parasitol. Paper No. 160.

8. Henchal, E.A., J.M. McCown, M.K. Gentry, J.M. Dalrymple, and W.E. Brandt. 1981. Evaluation of the serological characteristics of monoclonal antibodies produced against dengue virus antigens. Federation Proceedings, 40: 1065.

9. Rothman, S., A. Stone and H. Collins. 1981. Rabbit Antitoxin Produced against Clostridium difficile Cytotoxin. Abstracts of Ann. Meet. ASM 1981 p.20.

10. Webster, H.K., Hensen, B.D., Berman, J.D. and Hendricks, L.D. 1981. Purine metabolism in Leishmania: Effect of mycophenolic acid on the synthesis of guanosine nucleotides. Abst. Am. Soc. Trop. Med. Hyg.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6744	81 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
80 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	211
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Biochemistry of Parasitic Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 010100 Microbiology

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 81	3.0	201
FISCAL YEAR CURRENT 82	3.0	321

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Biochemistry

ADDRESS:
Washington, D.C. 20012

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

NAME: Alving, Carl R., COL, MC

TELEPHONE: (202) 576-3248

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Owens, Roberta L. Ph.D.

NAME POC: DA

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Drug Carriers; (U) Antibody; (U) Antigens; (U) Toxins; (U) Parasites

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

23. (U) The work unit is concerned with all aspects of drugs against parasitic diseases of military relevance that involve interactions with membranes. The objectives are: to improve and revise novel methods for delivery of liposome-encapsulated durgs in leishmaniasis, malaria, and other diseases; to elucidate stages in the life cycle of malaria that are not currently understood and that might be amenable to drug therapy; to develop improved methods utilizing lipid A from endotoxin for formulating vaccines against leishmaniasis, malaria, viruses, or other diseases; and to study receptors for parasites and exotoxins. It is anticipated that enhance efficacy of treatment of several diseases having military importance will be achieved. These include: malaria, leishmaniasis, and endotoxemia.

24. (U) The approach will be to formulate liposomes from a variety of lipids and entrap drugs, adjuvants or other substances within the liposomes. The liposomes will then be applied for use in animals that have appropriate infections, such as leishmaniasis or malaria. For synthesizing vaccines, antigens will be included within the liposomes. For work on parasitic or toxin receptors, the materials will be extracted from cell membranes, reconstituted in vitro and tested with appropriate analytical techniques.

25. (U) 80 10 - 81 09 The conditions for utilizing liposomes as drug carriers was achieved with more than 1000-fold enhanced efficacy. Liposomes cured antimony-resistant leishmaniasis, and the liposomes were effective in cutaneous leishmaniasis infections. Liposome-encapsulated primaquine, was more than 46,000-fold more effective than primaquine alone in treating malaria. One patent was issued, one approved, and one submitted. For technical report, see WRAIR Annual Progress Report of 1 Oct 80 to 30 Sep 81.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 88

PROJECT: 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

WORK UNIT: 211 Biochemistry of Parasitic Drugs

INVESTIGATORS:

Principal: Carl R. Alving, M.D., COL, MC

Associates: Roberta L. Richards (Owens), Ph.D., DAC; Benoy Banerj, Ph.D., NRC Associate; Earl C. Richardson, DAC; SP-4 Elizabeth Graves; SFC Reginald MacDaniel.

DESCRIPTION:

The main objectives of this work were to study: immunological and biochemical properties of membranes of infectious organisms; and use of synthetic membranes (liposomes) as drug carriers in treatment of leishmaniasis and malaria, and as vehicles for vaccines. Further objectives included detailed study on the chemistry and immunology of lipid A from endotoxin, and immunologic aspects of natural antibodies against membrane glycolipids.

1. Therapeutic potential of liposomes as carriers in leishmaniasis, malaria and vaccines.
2. Liposomes as vehicles for vaccines.
3. Anti-liposome antibodies induced by lipid A. Influence of ceramide, glycosphingolipids, and phosphocholine on complement damage.
4. Interaction C-reactive protein with liposomes. Membrane requirements for binding.
5. Immune reactivities of antibodies against glycolipids.

1. Therapeutic Potential Of Liposomes As Carriers In Leishmaniasis, Malaria, and Vaccines

We, and others, have demonstrated that when standard antimonial drugs, such as meglumine antimoniate (Glucantime) or sodium stibogluconate (Pentostam) are employed, the liposome-encapsulated drugs are hundreds of times more effective than unencapsulated drugs in treating experimental visceral leishmaniasis of rodents. Actually, the quantitative assessment of the relative effectiveness of the drugs can be readily inflated, or deflated, depending on numerous variables in the rodent model. One such variable is the length of time that elapses between infection and initiation of treatment.

Chemotherapy of malaria typically is keyed to a single stage of the life cycle. For example, chloroquine affects only erythrocytic forms, whereas primaquine affects only hepatic schizonts. Our initial approach was to utilize liposome-encapsulated primaquine to treat sporozoiteinduced malaria. We decided to try "intrahepatic targeting" of liposomes. Our reasoning was as follows. The hepatic schizont stage is known to reside in hepatocytes. The liposome-encapsulated drug might have been taken up by Kupffer cells (and other macrophages) and degraded or diluted in body fluids at a distance from hepatocytes. We felt that if the liposomes contained a glycoconjugate, such as a glycolipid, then they might have an increased affinity for hepatocytes

because of the carbohydrate-binding lectin on hepatocyte plasma membranse earlier described by Ashwell and colleagues. Previous reports indeed have shown increased hepatic uptake of liposomes containing glycolipids.

We found, to our amazement, that control liposomes that contained glycolipid in the lipid bilayer, but which lacked primaquine, had strong antimalarial properties. Although not shown, even glycolipids alone, without liposomes, were equally effective as antimalarial agents. After obtaining the above results, the question arose whether liposomal glycolipids would enhance the therapeutic efficacy of liposome-encapsulated primaquine? The answer is that they cause an enormous enhancement. Liposomal glycolipids still cause 50% suppression (SD_{50}) when diluted by 1:46,000. The same liposomes, containing primaquine, caused 50% suppression at a dilution of 1:104,000. Thus, it can be said that primaquine increased the efficacy of liposomal glycolipids by approximately two-fold. Based on the lack of increased efficacy of liposomal primaquine in the absence of glycolipids, apparently it can also be said that liposomal glycolipids increased the efficacy of primaquine by approximately more than 46,000 fold!

Despite these interesting observations, it has not yet been determined whether glycolipids alone, or liposome-encapsulated primaquine targeted by liposomal glycolipids, are equivalent to, superior to, or inferior to standard clinical methods for oral administration of primaquine in treating hepatic states of malaria.

Although primaquine acts exclusively on hepatic schizonts, and causes a radical cure of established infection, the mechanism, and indeed, the exact site of action, of primaquine is still unknown. Liposomal glycolipids also apparently cure 24 hr sporozoite-induced infections in mice. It is evident that liposomes might be useful in helping to elucidate not only the mechanism of action of primaquine, or other drugs, but also may help to clarify certain biological aspects of the malaria infection itself. If the glycolipids only block entry of the parasite into the hepatocyte, then it is unlikely that they would have any clinical application.

To illustrate the efficacies of liposomes as adjuvants for protein antigens, a model immunogenic particle was constructed that utilized cholera toxin (CT) as an antigen. Liposomes containing the receptor for CT, ganglioside G_{M1} were coated with CT in such a concentration that all of the CT was bound to the outer surface of the liposomes, and all of the G_{M1} molecules had CT attached to them. These vesicles were then injected i.v. or s.c. into rabbits, and serum antibody levels against CT were measured by a solid-phase radioimmunoassay. When lipid A was included as an adjuvant in the liposomal lipid bilayer, a very strong anti-CT response was obtained, and the response was approximately equivalent to that found when the same amount of CT was emulsified with complete Freund's adjuvant. When lipid A was omitted, the anti-CT

response of the liposome-bound CT was greater (by 18-fold) than CT alone, but it was not strikingly impressive when compared with complete Freund's adjuvant as a standard.

The results show that the immune response against a liposomal antigen can be approximatley the same as that induced by complete Freund's, provided that the liposomes contain lipid A.

2. Liposomes As Vehicles For Vaccines.

Lipid A from Shigella flexneri LPS, or acylated derivatives of muramyl dipeptide (MDP), were incorported into the liid bilayer of liposomes to enhance the adjuvanticity of the liposomes in rabbits. Liposomes containing lipid A induced antibodies against lipid A, phosphocholine, phosphatidylcholine, and sphingomyelin. Lipid A was resolved into eight fractions, some of which did, and others of which did not, induce antibodies against liposomes. Anti-liposome antibodies also were induced (in the absence of liposomes) by complete Freund's adjuvant, and by acid-treated bacterial cells coated with lipid A, but were not induced either by lipid A or liposomes alone, or by liposomes containing acylated MDP. We tested the liposomes for the ability to enhance the immunogenicity of a protein antigen. The antigen consisted of liposomes having acylated MDP and ganglioside G_{M1} (the receptor for cholera toxin, or CT) in the lipid membrane, and CT bound to the outer surface of the liposomes. The liposomal antigen (having surface-bound CT) produced a much greater anti-CT titer than that obtained by injection of CT alone. We conclude that liposomes containing only phosphatidylcholine, cholesterol, and dicetyl phosphate are poorly immunogenic, but that antibodies against them can be induced by inclusion of lipid A. Liposomes that are appropriately formulated can strongly enhance the immunogenicity of a liposome-bound protein antigen.

3. Anti-liposome Antibodies Induced By Lipid A. Influence of Ceramide, Glycosphingolipids, and Phosphocholine on Complement Damage.

Anti-liposome (anti-phosphatidylcholine) antibodies were produced in rabbits either by injection of phosphatidylcholine liposomes containing lipid A or, in the absence of phosphatidylcholine, by injection of acid-treated bacterial cells coated with lipid A. Complement-(C) dependent membrane damage mediated by anti-liposome antibodies was markedly enhanced by inclusion of glycosyl ceramide, or ceramide alone, in the liposomes. Adsorption studies demonstrated that, with the antiserum tested, liposomes containing ceramide absorbed fewer antibodies than did liposomes lacking ceramide, and therefore enhancement by ceramide was due to increased C activation, rather than due to increased binding of anti-liposome antibodies. None of the enhancing effect of glycosyl ceramide was due to presence of anti-glycosyl ceramide antibodies in the antiserum. Bona fide anti-galactosyl ceramide antibodies did not react significantly with liposomes lacking glycolipid, or with liposomes containing ceramide,

glucosyl ceramide, or lipid A. Incubation of liposomes containing membrane glycolipid with soluble phosphocholine caused either inhibition or stimulation of anti-phosphatidylcholine antibody activity, depending on the antiserum used, liposome composition, and phosphocholine concentration. Phosphocholine could be bound to liposomes under certain conditions, and resultant agglutination of liposomes was observed. Increased immune damage in the presence of phosphocholine, when it occurred, was due to binding of phosphocholine to the liposomes, resulting in increased antigen concentration at the membrane surface.

We conclude that: a) even in the absence of liposomes, or in the absence of injected phosphatidylcholine or sphingomyelin, lipid A can induce anti-liposome (mainly anti-phosphatidylcholine) antibodies; b) a glycosphingolipid, or even ceramide alone, can enhance C damage mediated by antibodies against certain lipid antigens in lipid membranes; and c) phosphocholine can bind nonspecifically to liposomes and cause further binding of anti-liposome (anti-phosphocholine) antibodies.

4. Interaction of C-Reactive Protein (CRP) With Liposomes. III. Membrane Requirements for Binding.

We have previously presented a model for CRP-membrane interactions using liposomes composed of dimyristoyl phosphatidylcholine (DMPC), cholesterol (CHOL), stearylamine (SA), and galactosyl ceramide. We have shown that the interaction of CRP with these liposomes in the presence of human serum results in consumption of hemolytic C and membrane lysis. In the present paper we have directly examined the binding reaction between CRP and liposomes using radiolabeled CRP. We have found that this binding is more characteristic of CRP interactions with polycations than CRP interactions with phosphocholine-(PC) containing molecules. CRP binding to liposomes was dependent on the presence of SA in the membrane and could occur with dimyristoyl phosphatidylethanolamine in place of DMPC. The binding was not inhibited by ethylenediaminetetraacetate (EDTA) but could be inhibited by $CaCl_2$, whereas CRP binding to PC-Sepharose was inhibited by EDTA and required $CaCl_2$. We have further examined the effects of changes in membrane composition on CRP binding to liposomes. In liposomes with a limiting density of SA, we found increased CRP binding with changes in composition that would increase membrane fluidity. In most cases, the amount of CRP binding correlated with the amount of C activation observed previously. However, increasing the amount of CHOL in the membrane was found to increase C activation while decreasing CRP binding. These findings indicate that CRP binding to membranes and subsequent C activation can occur through cationic molecules as well as phospholipids.

5. Immune Reactivities of Antibodies Against Glycolipids. Natural Antibodies.

The major purpose of the present study was to describe, and to quantify, the widespread occurence of natural complement-fixing

autoantibodies against numerous simple glycolipids. We show that every individual rabbit and human serum tested had complement-fixing autoantibodies against glycolipids that are widely distributed in circulating blood cells and other tissues.

Patents

Chemotherapy of Leishmaniasis. E.A. Steck and C.R. Alving, Canadian No. 1093465 (issued 13 January 1981).

Two other U.S. patents pending.

Publications: Full Papers

1. Mold, C.M., Rodgers, C.P., Richards, R.L., Alving, C.R. and Gewurz, H. Interaction of C-reactive protein with liposomes. III. Membrane requirements for binding. J. Immunol. 126:856-860 (1981).

2. Banerji, B. and Alving, C.R. Anti-liposome antibodies induced by lipid A. I. Influences of ceramide, glycosphingolipids, and phosphocholine on complement damage. J. Immunol. 126:1080-1084 (1981).

3. Alving, C.R. Therapeutic potential of liposomes as carriers in leishmaniasis, malaria, and vaccines. in "Targeting of Drugs", NATO ASI, series A, edited by G. Gregoriadis, Plenum, NY (in press).

4. Alving, C.R. and Richards, R.L. Immunologic aspects of liposomes, in "The Liposomes", edited by M. Ostro, Marcel Dekker, Inc., NY (in press).

5. Fredman, R.L., Roerdink, F., Iglewski, B.H. and Alving, C.R. Suppression of cytotoxicity of diphtheria toxin by monoclonal antibodies against phosphatidylinositol phosphate. Biophys. J. 37(1) 000-000 (1982) (in press).

Abstracts and Presentations

1. Richardson, E.C., Banerji, B., Seid, R. and Alving, C.R. Mitogenic and Limulus activities of lipid A and lipid A fractions in liposomes. Fed. Proc. 40(3) 1131 (1981).

2. Roerdink, F., Berson, B.J., Richards, R.L., Swartz, Jr., G.M., Lyons, J.A. and Alving, C.R. Specificity of a hybridoma monoclonal antibody against liposomes containing phosphatidylinositol monophosphate. Fed. Proc. 40(3) 996 (1981).

3. Alving, C.R., Moss, J., Richards, R.L. and Alving, L.I. liposomes as vehicles for vaccines. Increased antigenicity and lack of toxicity of a toxin bound to liposomes. Clin. Res. 29(2) 531A (1981).

4. Wright, D.G., Meierovics, A.I., Richards, R.L. and Alving, C.R. Studies of cytoplasmic granules in human neutrophils (PMN): Differences in the membrane phospholipid content of azurophil and specific granules. Fed. Proc. 40(3) 375 (1981).

5. Invited Seminar speaker, Univeristy of Illinois School of Medicine, Chicago, Ill., 26 Jan 1981.

6. Invited Seminar speaker, Rush Medical College, Chicago, Ill., 27 Jan 1981.

7. Invited Seminar speaker, Amer. Red Cross Blood Research Laboratory, Bethesda, MD, 28 Jan 1981.

8. Faculty member, NATO Advanced Study Institute on Targeting of Drugs, held at Cape Sounion Beach, Greece, 24 June-5 July 1981.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 64/1	81 10 01	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
80 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	CS	212
b. CONTRIBUTING				
c. [illegible]	STOG 80-7.2.4			

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 Impulse Noise

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
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)
FISCAL YEAR PRECEDING 81	3.0	241
FISCAL YEAR CURRENT 82	3.0	290

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, Philip K., COL, MC
TELEPHONE: 202 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
ADDRESS: Div of Med, Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Provide last name, first name, middle initial)
NAME: PHILLIPS, Yancy Y., MAJ, MC
TELEPHONE: 202 576-3014
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: LORENZ, Patrick E. CPT, MSC
NAME: VERMA, Pritam, CPT, MSC

21. GENERAL USE

Foreign Intelligency Considered

22. KEYWORDS (Precede each with Security Classification Code)

(U) Blast (verpressure; (U) Pulmonary Physiology; (U) Pulmonary Biochemistry; (U) Pulmonary Receptors; (U) Impulse Noise

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 physiologic effects of blast overpressure generated by firing Army weapon systems and to determine the limits of human safety for exposure to impulse noise. There is military relevance in this research.

24. (U) Approach includes use of biochemical assays and monitoring of physiologic tests before and after blast and impact injury. Water jet impact will be used to simulate blast injury in the laboratory. Blood from blast and impact injured animals will be analyzed for various enzymes, hormonal markers, elastin and surfactant related products and protein changes as detected by 2 dimensional gel electrophoresis. Pulmonary tissue will be examined histologically and assayed for hormone receptor concentrations.

25.(U)80 [illegible]81 09 Although the search for a biochemical marker of blast injury has shown (U) bradykinin and prostaglandins to be unreliable, our program has broadened to include a search for elastin and surfactant related moieties in blood and urine and to use two-dimensional gel electrophoresis as a screening tool for examination of blood, bronchoalveolar lavage, and thoracic lymph. The technique of chronic thoracic lymph duct cannulation in sheep is being developed. High affinity thyroid hormone nuclear receptors have been identified in rat lung. After characterization of their properties, they will be studied in the context of blast overpressure effects. Techniques were developed for determination of air and saline filled pressure-volume curves of excised rat lungs. Blood and urine samples from sedentary and exercise controls and animals injured by one 34 psi blast (IP 1*) and by 50 15 psi blasts were collected over a one week period after injury. 10Oct80-30Sep81.

*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: 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 212: Physiology of Systemic Effects of Blast Overpressure

Investigators:

Principal: Yancy Y. Phillips, MAJ MC
Associate: Patrick E. Lorenz, CPT MSC; Pritam S. Verma, CPT MSC; Robert C. Smallridge, LTC MC; Nancy E. Whorton, GS-11; Carolyn Umstott, GS-6

The WRAIR blast overpressure (BOP) project was established to define the physiologic effects and evaluate the potential for non-auditory injury after exposure to blast wave impulses generated by the firing of Army weapons systems.[1-9] A major area of investigation has been the search for potential biochemical markers of blast injury.

Plasma and serum samples were obtained from severely blast-injured animals exposed to the shock tube at the Lovelace Foundation. There were no consistent changes in bradykinin levels correlating with severity of the blast. While there was possibly a positive correlation between pre- and post-blast levels of prostaglandins in an individual animal, there was no difference between population pre- and post-blast levels. Any changes in these mediators of inflammation are likely to be nonspecific but they will be investigated in conjunction with other assays. Serum lactate dehydrogenase and creatine phosphokinase isoenzymes have, so far, proven to be unreliable markers of blast injury.

Current studies are directed at the development of specific radioimmunoassays for measurement of elastin and surfactant associated moieties in biologic samples. High titer anti-desmosine antibody is now being harvested from hyperimmunized rabbits. Two-dimensional gel electrophoresis is being developed for use as a screening tool to examine blood, urine, bronchoalveolar lavage, and thoracic lymph for identification of possible protein/peptide markers of injury. Thyroid hormone receptors have been identified in pulmonary tissue. After further characterization, they will be studied in the context of BOP effects[2,3,9] since thyroid hormone affects phospholipid metabolism and ultrastructure in the lung.

Present plans call for development of an antibody to surfactant associated apoprotein. Intratracheal elastase will be used to create acute lung injury in sheep to allow study of

elastin degradation product metabolism and characterization of injury by in vivo pulmonary function tests. Tissue and serum from blast and impact injured animals will be tested for angiotensin converting enzyme levels and with the methods described above.

LITERATURE CITED;

References:

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2. Chiffelle, T.L., Pathology of direct air-blast injury. Technical Progress Report (Contract No. DA-49-146-X2-055), Lovelace Foundation for Medical Education and Research, Albuquerque, NM, April, 1966.

3. Jonsson, A., Experimental investigations on the mechanisms of lung injury in blast and impact exposure. Linkoping University Medical Dissertations No. 80, Stockholm, Sweden, 1979.

4. Viano, D.C., Evaluation of biomechanical response and potential injury from thoracic impact. Aviat Space Environ Med, 49:125, 1978.

5. White, C.S., R.K. Jones, E.G. Damon, E.R. Fletcher, and D.R. Richmond. The biodynamics of air blast. Progress Report on contract No. DASA 01-70-C-0075, submitted to the Defense Nuclear Agency, Washington, D.C., Lovelace Foundation, Albuquerque, NM, 1 July 1971.

FORMAL PRESENTATIONS:

1. Jurney, T.P., L. Wartofsky, P.A. Routledge, D.G. Shand, and R.C. Smallridge. Propranolol infusions decrease serum thyroxine (T_4) as well as triiodothyronine (T_3) in rats. Presented at the Fifty-sixth Meeting of the American Thyroid Association, San Diego, CA, November, 1980.

PUBLICATIONS:

1. Sander, G.E., P.S. Verma, P.E. Lorenz, and T.D. Giles. Clonidine interactions with canine lung angiotensin I converting enzyme in vitro. Clin Res, 28:880A, 1980.

2. Sander, G.E., P.E. Lorenz and P.S. Verma. Inhibition of the partially purified canine lung angiotensin I converting enzyme by opioid peptides. Biochem Pharmacol, 21:3115, 1980.

3. Jurney, T.H., L. Wartofsky, P.A. Routledge, D.G. Shand, and R.C. Smallridge. Propranolol infusions decrease serum thyroxine (T_4) as well as triiodothyronine (T_3) in rats. Endocrinology 107 (Suppl): T-17, 1980.

4. Burman, K.D., R.C. Smallridge, L. Jones, E.A. Walker-Ramos, J.T. O'Brian, F.D. Wright and L. Wartofsky. Glucagon kinetics in fasting: A metabolic event associated with physiologic alterations in serum T_3. J Clin Endocrinol Metab 51:1158, 1980.

5. Smallridge, R.C. Thyroid hormone effects on the heart, In "The Heart and Heart-like Organs", Vol 2 (Geoffrey Bourne, ed). Academic Press, pp. 93-160, 1980.

6. Smallridge, R.C., K.D. Burman, K.E. Ward, R.C. Dimond, F.D. Wright, K.R. Latham and L. Wartofsky. 3',5'-diiodothyronine to 3'-monoiodothyronine conversion in the fed and fasted rat: Enzyme characteristics and evidence for two distinct 5'-deiodinases. Endocrinology 108:2336, 1981.

7. Smallridge, R.C., K.D. Burman, C.E. Smith, K.R. Latham, F.D. Wright and L. Wartofsky. Metabolic clearance and production rates of 3'5'-diiodothyronine in hyperthyroidism and hypothyroidism in man: Comparison of infusions using radiolabeled versus unlabeled iodothyronine. J Clin Endocrinol Metab 52:722, 1981.

8. Smallridge, R.C., H.L. Wray, and M. Schaaf. Hypocalcemia with osteoblastic metastases in a patient with prostate carcinoma: A cause of secondary hyperparathyroidism. Am J Med 71:184, 1981.

9. Burman, K.D., Y.D. Lukes, K.R. Latham, A.R. Glass, R.C. Smallridge, and L. Wartofsky. The effect of dexamethasone, diet control and hyperglycemia on murine hepatic T_3 receptors. Life Sciences 28:1701, 1981.

10. Smallridge, R.C., L. Wartofsky, and K.D. Burman. Thyroid carcinoma and Hodgkin's disease. Ann Intern Med 94:412, 1981.

11. Smallridge, R.C. and N.E. Whorton. 3'-monoiodothyronine degradation: Similarity to thyroxine (T_4) 5'-deiodination in fed and fasted rat liver. Endocrinology 109 (Suppl): T-42, 1981.

12. Smallridge, R.C., I. Mehlman, C.L. Pamplin III, N.E. Whorton, R.C. Dimond, T. Doyle, and L. Wartofsky. An assessment of pituitary and thyroid function in the male cynomolgus monkey. Lab Anim Sci (in press).

13. Smallridge, R.C., L. Wartofsky, and K.D. Burman. The effect of experimental hyper- and hypothyroidism on 5'-monodeiodination of reverse T_3 and 3',5'-diiodothyronine by rat liver and kidney. (Submitted for publication).

14. Smallridge, R.C., A.R. Glass, L. Wartofsky, K.E. Ward, K.R. Latham, and K.D. Burman. Investigations into the etiology of elevated serum T_3 levels in protein-malnourished rats. (Submitted for publication).

15. Holland, J.C., R.A. Vigersky, R.C. Smallridge, A.N. Martins, and M. Schaaf. Cushing's disease: Repetitive recurrence after two selective pituitary tumor removals. (Submitted for publication).

16. Smallridge, R.C. and A.N. Martins. Transsphenoidal surgery for prolactin secreting tumors: A study of 28 cases and review of the literature. (Submitted for publication).

17. Burman, K.D., K.R. Latham, E.W. Ferguson, R.C. Smallridge, Y-Y Djuh, and L. Wartofsky. Relationship of thyroid hormone receptor and acetylase activity in circulating human mononuclear cells: Studies in critically ill subjects and during exercise. (Submitted for publication).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6451	81 10 01	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	9a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	213
b. CONTRIBUTING				
XXXXXXXXXXXX	STOG 80-7.2:4			

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	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 81	5.0	447
FISCAL YEAR CURRENT 82	7.0	580

19. RESPONSIBLE DOD ORGANIZATION
NAME:* Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME:* Walter Reed Army Inst. of Research
Division of Neuropsychiatry
ADDRESS:* Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)
NAME:* Elsmore, T.F., Ph.D.
TELEPHONE: (202) 576-3037
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Hursh, S.R., MAJ
NAME: Wylie, R.M., Ph.D. POC: DA

21. GENERAL USE
Foreign Intelligence Not Considered

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 (Provide 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) 80 10 - 81 09 Major findings: Economic concepts of elasticity of demand and substitution appear to be useful in accounting for the behavior of laboratory animals in a variety of experimental settings. Monkeys required to learn a sequence of responses to obtain food show little variation in errors in acquisition of a new sequence as a function of time of day, but show large circadian rhythms in the time of acquisition. Loud noise may disrupt the normal circadian patterns of behavior. Spinal origins of sensory neurons innervating the esophagus, stomach, and duodenum were defined. Animals deprived of sensory input from a limb show deficits in adapting to altered requirements for limb movement, but are able to learn appropriate limb movements with practice. Accuracy deficits in animal short-term memory experiments may be due to attentional rather than memory processes. For technical report, see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*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 213: Biological Modulation of Military Performance

Investigators:

Principal: Elsmore, Timothy F., Ph.D.

Associate: Hursh, MAJ S.R.; Raslear, CPT T.G., Kaufman, CPT L.K.; 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 situations outside of the laboratory. Techniques and methods are drawn from a broad spectrum of neuroscience disciplines including psychology, neurophysiology, neuroanatomy, and neuropharmacology.

Progress:

It has been known for some time that human performance varies with time of day. Circadian variability in animal performance has been investigated in a series of experiments that are nearing conclusion. These experiments have demonstrated that the amplitude of circadian changes in behavior depends upon several variables including the economic context within which the behavior occurs, the effortfulness of the behavior, the amount of reinforcement the behavior produces, and the position of the behavior in the sequence of behaviors leading to reinforcement.

The overall economic context in which behavior occurs has been largely ignored in laboratory studies of animal behavior. In several experiments the economic concepts of demand elasticity and substitution were found to be useful in accounting for behavior of animals working to obtain food. When the price of food is varied within a situation such that the lower priced alternative can be substituted for a higher priced alternative, preference will be for the low price. When price of food is varied between situations such that no substitution can occur, more behavior is expended for higher priced alternatives, and demand is said to be inelastic.

Knowledge of how stimuli are perceived and how such perceptions are modified by physiological or environmental stressors is crucial in constructing animal models of military performance. The bisection method was used in an experiment in which rats were trained to respond differently to long and short duration stimuli. During tests, stimuli of intermediate duration were presented and the animal required to report whether the test stimuli resembled the long or the short duration training stimulus. The "bisection" point, therefore was the value of the test stimulus which was judged equally often to be long and short. This bisection point was found to be dependent upon the spacing of the set of test stimuli.

Continuing work on recovery of limb function in monkeys lacking sensory input from the limb following dorsal rhizotomy suggests that both the physical properties of the muscles controlling limb movement and cues from other sensory modalities are important in the recovery process. Equipment has been built, and initial training begun to investigate manual tracking (i.e. pointing) in limbs lacking sensory input.

Using the horseradish peroxidase technique, sensory neurons originating in the esophagus, stomach, and duodenum were traced to the spinal cord. Each of these regions of the gut has a characteristic pattern of spinal innervation with considerable overlap between the regions.

Future Objectives:

Applicability and utility of economic concepts in the explanation of animal behavior will be further evaluated. The variables mediating accuracy on long-delay trials in delayed discrimination performances will be investigated. Effects of stress on temproal bisection are being investigated. Research on recovery of function following dorsal rhizotomy will continue.

Presentations

Raslear, T.G. Context effects in the bisection of a temporal interval by rats. Eastern Psychological Association, 1981.

Wylie, R.M. Computer simulation of the performance of a weight-lifting task by normal and deafferented monkeys. Neuroscience Society, 1980. Neuroscience Abstracts 6:465, 1980.

Publications

Hursh, S.R. and Natelson, B.H. Electrical brain stimulation and food reinforcement dissociated by demand elasticity. Physiology & Behavior, 1981, 26, 509-515.

Kaufman, L.W. and Collier, G. The economics of seed handling. The American Naturalist, 1981, 118, 46-60.

Raslear, T.G. Differential responding without differential reinforcement: Intensity difference, continuum position, and reinforcement density effects. Journal of the Experimental Analysis of Behavior, 1981, 35, 79-91.

Raslear, T.G. On the use of bisection procedures in animal psychophysics. Psychometrika, in press.

Wylie, R.M. and Tyner, C.F. Weight-lifting by normal and deafferented monkeys: evidence for compensatory changes in ongoing movements. Brain Research, 1981, 219, 172-177.

Manuscript Submitted

Raslear, T.G. Stimulus intensity dynamism: a reconsideration. Journal of the Experimental Analysis of Behavior.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6755	81 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. DOD'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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
b. CONTRIBUTING				
XXXXXXXXX	STOG 80-7.2:A			

11. TITLE (Precede with Security Classification Code)

(U) Millimeter Wave Biophysics and Biohazards

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
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 81	1.0	398
FISCAL YEAR — CURRENT 82	2.0	299

19. RESPONSIBLE DOD ORGANIZATION

Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL, MC
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Dept of Microwave Research
ADDRESS: Div of Neuropsychiatry
Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: L.E. Larsen
TELEPHONE: 202-576-3615
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: J.H. Jacobi
NAME:

21. GENERAL USE

Foreign Intelligence Not Considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Biophysics; (U) Millimeter Wave; (U) Bioeffects; (U) Permittivity

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with 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. There is military relevance in this research.

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) 80 10 - 81 09 A millimeter wave anechoic chamber was designed, tested and installed. It exhibits more than 100 dB attenuation at frequencies between 18 and 96 GHz. Free space VSWR tests were performed with reflectivity less than -40dB. Development of the six-port network analyzer has resulted in a patent disclosure for reflection coefficient measurement of improved accuracy. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

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

Work Unit 214: Millimeter Wave Biophysics and Biohazards

Investigators.
Principal: LTC Lawrence E. Larsen, M.D.
Associate: John H. Jacobi, M.S.

Objectives

Millimeter wave area spectral region of great technological interest to the Army. This is a result of the fact that tactical deployment of millimeter wave radars and imaging systems is projected to maintain weapon system operability in battlefield environments designed to render electro-optical components ineffective.

This is a new technology area not only for weapon systems but also for biomedical study. Since these systems are projected to have vast tactical deployment in this decade and little reliable biomedical information exists in this spectral region, we have begun a millimeter wave research program. This program consists of two sections: instrumentation development and exposure system development. Once these stages are completed, biomedical experimentation will begin (projected for FY 82-83).

Progress

Instrumentation development consists of a 40-60 GHz digital synthesizer and six-port network analyzer which will be completed in late FY 82. These systems will be applied to studies of dielectric relaxation for cell suspension and various cell free systems such as enzymes and biopolymers.

The exposure systems consist of a 1 kilowatt continuous wave transmitter at 35 GHz, a 35 kilowatt peak (pulse) transmitter and a 1 meter elliptical antenna. These systems are presently under development and have delivery dates from late FY 82 to FY 84. These will be used for ocular studies with emphasis on cornea for heating and corneal endothalium for thermoacoustic expansion. Lens and retina will also be examined by ultrastructural methods.

Future Plans

Spectral scanning will be extended to the 60-90 GHz range. Pulse transmitter capability will extend to 94 GHz. High level effects will also be explored with special emphasis on thermoacoustic expansion.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA 0C 6449	2. DATE OF SUMMARY: 81 10 01		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUM'RY: 80 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISP'N INSTR'N: NI	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	61102A	3M161102BS10		CD	215		
b. CONTRIBUTING							
XXXXXXXX NO	STOG 80-7.2.4						

11. TITLE (Precede with Security Classification Code): (U) Mechanism of Response to Stress

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 012900 Physiology 002300 Biochemistry 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	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 81	3.0	143
	CURRENT 82	1.0	298

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute or Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Inst. of Research
Division of Neuropsychiatry
ADDRESS: Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)
NAME: Meyerhoff, J.L., M.D.
TELEPHONE: (202) 576-3559
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Kant, G.J., Ph.D.
NAME: Belenky, G.L., M.D., LTC

21. GENERAL USE: Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Stress; (U) Cyclic Nucleotides; (U) Neurotransmiters; (U) Neurochemistry; (U) Microwave Inactivation; (U) Lateralization of Cerebral Function

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

23. (U) To examine neurochemical mechanisms regulating neuroendocrine responses involved in adaptation to stress, providing database for interpretation of military field studies and recommendations for prevention and/or treatment of breakdown in soldiers. To examine neurochemical mechanisms mediating lateralization of function, spatial abilities and recovery from cerebral injury.

24. (U) Analysis of role of neurotransmitter pathways in regulation of hormonal response to stress. Effect of stimulation or lesion of specific pathway (i.e., noradrenergic dopaminergic, cholinergic, or serotonergic). Effect of stress or centrally-acting hormones on cyclic nucleotides and neurotransmitters in specific brain regions. In-vivo determination permitted by use of microwave enzyme inactivation system. Role of dopamine in lateralization of cerebral function.

25. (U) 80 10 - 81 09 Plasmacorticosterone and pituitary cyclic AMP levels were elevated by a range of stressors, listed in order of increasing response: cold exposure, forced running, forced immobilization and inescapable footshock. Footshock increased pituitary cyclic AMP over 10 fold and plasma prolactin over 60 fold with no increases in cyclic AMP. No differences were seen between male and female responses for several of the stressors. We have further demonstrated that psychological stress alone is sufficient to produce this response by exposing rats to stimuli previously paired with inescapable footshock. Pituitary cyclic AMP appears to be a transducer by which neural systems regulate the hormonal response to stress. In continuing our studies of neurochemical responses to cholinergic drugs, we have found that muscarinic agonists cause increases of cyclic GMP in the septal region of the brain. For technical report, see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

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

Work Unit 215: Mechanism of Response to Stress

Investigators:

Principal: Meyerhoff, J.L., M.D.

Associate: Kant, G.J, Ph.D., Belenky, G.L., M.D., LTC, MC, Bates, V.E., M.D., MAJ, MC, Mougey, E.H., M.S., Collins, D.R., B.S., Pennington, L.L, B.S.

Objectives:

Evaluation of neurochemical mechanism of response to stress, brain injury and other factors which produce psychiatric incapacitation or brain syndromes pertinent to military medicine. Included are CNS regulation of pituitary function in acute and chronic exposure to stressors, effects of stress on neurotransmitter turnover and receptor function, as well as studies of neurochemical system interactions (e.g. cholinergic-dopaminergic interactions). These studies are intended to provide a database for interpretation of psychoendocrine studies of stress, and for understanding the mechanisms by which traumatic factors decrement CNS function.

Progress:

Plasma corticosterone, prolactin, and pituitary cyclic AMP levels were elevated by a range of stressors, listed in order of increasing magnitude of response elicited: cold exposure, forced running, forced immobilization and inescapable footshock. Footshock increased pituitary cyclic AMP over 10 fold and plasma prolactin over 60 fold. No increases in cyclic AMP were seen in brain. Comparison of male and female responses were made for several of the stressors and no differences were observed. Having shown that physical stressors increase pituitary cyclic AMP, we have further demonstrated that psychological stress alone is sufficient to produce this response. This was accomplished by exposing rats to stimuli previously paired with inescapable footshock. Thus, pituitary cyclic AMP appears to be a transducer by which neural systems regulate the hormonal response to stress. We have continued our studies of neurochemical responses to cholinergic drugs. We have found that stimulation of muscarinic receptors produces elevations of cyclic GMP in the septal region of the brain. This effect was not produced by nicotinic stimulation. By contrast, both nicotinic and muscarinic drugs produce increases in cyclic AMP in the interpeduncular

region. The increases cited are not due to stress or locomotor activity, and are blocked by appropriate pharmacological antagonists.

Future Objectives:

We will attempt to determine which neurotransmitters and hypothalamic releasing factors are mediating the pituitary cyclic AMP response to stress and to explore pharmacologic or endocrinologic means of blocking this response. We will explore the relationship between the pituitary cyclic AMP increases and the release of various pituitary stress hormones as well as the feedback effects of hormones on brain, and how they may affect behavior. Also we will study the effect of chronic stress on neurotransmitter receptors in brain. We will continue studies of neuroendocrine responses in animals that have either won or lost in aggressive interactions. We will continue studies on cholinergic interactions with neurochemical systems in specific brain regions both in-vivo and in-vitro.

Presentations

1. Society for Neuroscience, Cincinnati, Ohio, November 1980. Bates, V.E., Meyerhoff, J.L., Kant, G.J., and Lenox, R.H. "In vivo cyclic AMP levels in rat brain regions following kindling."

2. Society for Neuroscience, Cincinnati, Ohio, November 1980. Collins, D.R., Meyerhoff, J.L., Kant, G.J., Pennington, L.L., and Lenox, R.H. "Regional brain cyclic nucleotide and hormonal response in genetically hypertensive rats (SHR) to aminergic and cholinergic stimulation."

3. Society for Neuroscience, Cincinnati, Ohio, November 1980. Kant, G.J., Bates, V.E., Lenox, R.H., and Meyerhoff, J.L. "Isoproterenol-induced cyclic AMP increases in vivo in pineal and other rat brain regions."

4. Society for Neuroscience, Cincinnati, Ohio, November 1980. Meyerhoff, J.L., Kant, G.J., Lenox, R.H., Pennington, L.L., and Collins, D.R. "Effects of muscarinic and nicotinic agonists and antagonists on brain regional cyclic nucleotides."

5. Society for Neuroscience, Cincinnati, Ohio, November 1980. Sessions, G.R., Kant, G.J., Lenox, R.H., and Meyerhoff, J.L. "Cyclic nucleotide levels in the pituitary, hypothalamus, pineal and cerebellum of female rats during the estrus cycle."

6. Symposium on Drug Effects on Rapidly Metabolized Compounds in the CNS. Tokyo, Japan, July 1981. Meyerhoff, J.L., Kant, G.J. and Lenox, R.H. "Effects of Cholinergic Agonists, Locomotor Activity and Stress on Brain and Pituitary Cyclic Nucleotides in the Rat."

Publications

1. Kant, G.J., Bunnell, B.N., Lenox, R.H., Pennington, L.L., Collins, D.R., Mougey, E.H., and Meyerhoff, J.L. Stressors elevate pituitary cyclic AMP in the rat. Neuroscience Abstracts 7:333 (1981).

2. Bunnell, B.N., Kant, G.J., Lenox, R.H., Pennington, L.L., Collins, D.R., Mougey, E.H., and Meyerhoff, J.L. Pituitary cyclic AMP is increased by psychological stress. Neuroscience Abstracts 7:918 (1981).

3. Lenox, R.H., Kant, G.J., Meyerhoff, J.L., and Annau, A. Brain cyclic nucleotide response following central cholinergic activation in rats exposed to chronic lead. Neuroscience Abstracts 7:918 (1981).

4. Sessions, G.R. and Meyerhoff, J.L. Dissociation of pituitary-adrenal activation and behavioral stress response following taste aversion conditioning. Neuroscience Abstracts 7:870 (1981).

5. Kant, G.J., Meyerhoff, J.L., and Corcoran, M.E. Release of norepinephrine and dopamine from brain regions of amygdaloid-kindled rats. Experimental Neurology 70:701-705 (1980).

6. Kant, G.J., Bates, V.E., Lenox, R.H., and Meyerhoff, J.L. Increases in cyclic AMP levels in rat brain regions in vivo following isoproterenol. Biochemical Pharmacology (in press).

7. Kant, G.J., Sessions, G.R., Lenox, R.H., and Meyerhoff, J.L. The effects of hormonal and circadian cycles, stress, and activity on levels of cyclic amp and cyclic GMP in pituitary, hypothalamus, pineal and cerebellum of female rats. Life Sci. (in press).

8. Meyerhoff, J.L., Kant, G.J., Sessions, G.R., Mougey, E.H., Pennington, L.L., and Lenox, R.H. Brain and pituitary cyclic nucleotide response to stress, in: Perspectives on Behavioral Medicine, Vol. II, (Redford B. Williams, Jr., ed), Academic Press, New York, 1981 (in press).

9. Lenox, R.H., Kant, G.J., and Meyerhoff, J.L. Rapid enzyme inactivation, in: Handbook of Neurochemistry, Vol. II, (ed. A. Lajtha), Plenum (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6473	81 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. DISB'N INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	XX YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CD	216
XXXXXXXXXX				
XXXXXXXXXX	STOG 80-7.2;4			

11. TITLE (Precede with Security Classification Code)

(U) Military Stress: Non-Invasive Monitoring of Health and Performance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016200 Stress Physiology 013400 Psychology

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 81	4.0	22[illegible]
FISCAL YEAR CURRENT 82	4.0	356

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K. COL, MD

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Sivision of Neuropsychiatry

ADDRESS: Washington, DC 20012

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

NAME: Hegge, F.W. Ph.D.

TELEPHONE: (301) 427-5521

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Genser, LTC S.G.

NAME: Sing, H.C. POC: DA

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Electrophysiology; (U) Psychophysiology; (U) Psychophysics; (U) Stress; (U) Performance; (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) Objective is the development of non-invasive human psychophysiological monitoring technology in support of field studies of stress in military environments.

24. (U) Approach is to exploit advances in signal acquisition and processing technologies to enlarge the scope of psychophysiological measurements that can be made under field conditions. Techniques are validated in the laboratory prior to deployment in controlled field trials.

25. (U) 80 10 - 81 09 This work unit provides the technology base for Work Unit 043, Military Stress: Circadian Ultradian Factors (Accession Number DA OC 6457).

The Canadian developed ingestible encapsulated temperature sensitive oscillator/transmitter combined with a belt mounted receiver has been used to monitor core body temperature in a number of studies of human circadian rhythmics. A readout system for this temperature data (recorded on a Medilog tape recorder) using an Apple Microcomputer has been developed and work is progressing towards increasing the reliability of reception of the tranmitted signal.

The microcomputer administered Performance Assessment Battery (PAB) is being refined using studies of whether requiring a set number of correct responses per task versus using a set time period per task leads to increased sensitivity to circadian performance rhythms. Complex demodulation programs are being developed to explore the two oscillator theory of human performance rhythms using rest-activity and core body temperature data as well as ECG. Questions of stress related phasic coupling among these oscillators as well as synchrony with performance rhythms are being explored using these tools. A computer based war game permitting the ongoing monitoring of complex cognitive performance is being developed (using contract as well as in-house resources) both to assess team and individual performance under a variety of common military stresses.

* Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND WILL BE USED FOR ARMY USE. DA FORMS 1498A, 1 NOV 65 ARE OBSOLETE

Project 3M161102BS10 RESEARCH ON DISEASE, INJURY AND HEALTH HAZARDS

Work Unit 216: Military Stress: Non-invasive Monitoring of Health and Performance

Investigators:

Principal: Frederick W. Hegge, Ph.D.

Associates: LTC Sander G. Genser, MC; MAJ R. Curtis Graeber, MSC; CPT Bruce N. Cuthbert, MSC; Stanley Hall, M.S.; Helen Sing, M.S.; Alison L. Lee; Jacob Karen; John Jackson

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 645/), 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

Working under an Memorandum of Understanding (MOU) with the Canadian Defense Civil Institute of Environmental Medicine, the development of a continuously recording, ingestible, body core temperature telemetry system is nearing completion. The temperature transmitter, which takes the form of a readily swallowed pill, tumbles as it moves through the gastro-intestinal system. This tumbling action produces an constantly changing orientation between the antennas of the pill, inside the body, and the receiver, which is worn at the waist. Since the signals being transmitted are extremely weak, and all components of the system must be kept as small as possible, the tumbling led to a loss of signal which interfered with the goal of continuous monitoring.

This problem has been largely overcome during the reporting period through changes in the receiver design that have resulted in the reception of stronger signals and a less cumbersome apparatus that is worn at the waist. In addition to hardware improvements, a microprocessor signal analysis system has been implemented which greatly increases our ability to discriminate real signal from noise and to acquire sufficient signal to permit the necessary analysis of continuous core temperature changes.

The microprocessor controlled Performance Assessment Battery (PAB) continues to undergo refinements designed to improve the motivational structure, to increase sensitivity to time-of-day effects, and to ensure that elements of the battery are related to significant military performance requirements. Initial development of a more elaborate PAB that is configured as a computerized war game has been completed under contract with the Midwest Research Institute. The game structure was selected because its command, control and communication elements are functional analogs of similar military tasks and because it maintains participant motivational levels during sustained testing around the clock.

Future Objectives

Evidence is accumulating rapidly that the human circadian rhythm system consists of two independent, but coupled, complex biological oscillators. Under normal conditions, these oscillators appear to operate in the closely synchronized manner that has been so well documented in the scientific literature. However, it now appears that when the human organism is subjected to any of a wide variety of stressors, the association between these two oscillator systems breaks down and a state of desynchrony results. There is strong evidence that psychobiological well-being is to a considerable degree dependent upon the integrity of rhythm synchronization. The jet lag that afflicts the transoceanic travellers, certain aspects of psychiatric bipolar depression, and the fatigue associated with shift-work provide a few examples of the consequences of rhythm desynchronization.

The core temperature monitoring system, the two Performance Assessment Batteries and a data analysis technique developed in this laboratory will be used to explore, develop, and test the implications of the two oscillator model for previously intractable problems related to military stress and performance deterioration. At the same time, the core temperature monitoring technology will be disseminated to the U.S. Army Human Engineering Laboratory to support work on the monitoring of the temperature change in personnel wearing protective garments. The Performance Assessment Battery will continue to be used in the testing of Army aviation personnel under a cooperative program with the U.S. Army Aeromedical Research Laboratory.

Presentations and Publications

1. Cuthbert, B.N., Ackles, K.N., & Graeber, R.C. Microcomputer-based analysis of rhythmic temperature pill data. The XV International Conference of the International Society for Chronobiology. Minneapolis, Minn., Sept 1981.

2. Cuthbert, B.N., Sing, H.C., & Hall, S.W. Jr. A microcomputer-based test battery for assessment of human performance rhythms. The XV International Conference of the International Society for Chronobiology. Minneapolis, Minn., Sept 1981.

3. Sing, H.C., Hegge, F. W. Complex demodulation -- technique and applications. The XV International Conference of the International Society for Chronobiology. Minneapolis, Minn., Sept 1981.

4. Sing, H.C., Redmond, D.P., & Hegge, F.W. Multiple complex demodulation: A method for rhythmic analysis of physiological and biological data. The Fourth Annual Symposium on Computer Applications in Medical Care. Washington, D.C., Nov, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6448	81 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	9b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	AG	217
b. XXXXXXXXX				
c. XXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Basic Pharmacological Studies

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 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 81	3.5	196
	CURRENT 82	3.5	289

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, COL P.

TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of Experimental Therapeutics

ADDRESS: Washington, DC 20012

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

NAME: HEIFFER, Dr. M.H.

TELEPHONE: 301-427-5393

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: KORTE, CPT D.W. Jr.

NAME: LOWENSOHN, Dr. H. POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Pharmacology; (U) Medicinals; (U) Drugs; (U) Toxicity; (U) Quantitation Methodology

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

23. (U) Studies are designed to investigate the basic pharmacology of militarily significant drugs. The research effort includes characterization of the drug effects on different organ systems, the interaction of the drugs and determination of their mechanism of action. In addition studies are directed to developing new animal models or improving existing animal models for defining drug actions.

24. (U) Drugs of military importance are tested in animal models designed specifically to elucidate the mechanism of their pharmacological effects and to delineate the physiological responses to drug administration.

25. (U) 80 10 - 81 09 The cardiovascular activity of the potent new antimalarial, WR 225,448, and primaquine were compared in anesthetized dogs. A cumulative, bolus, intravenous dosing regimen of primaquine produced significant changes in the electrocardiogram which progressed to ventricular fibrillation and death while an equivalent regimen of WR 225,448 produced no significant effects. Infusion of equivalent doses of the two compounds over a 30 minute period confirmed that primaquine possessed more potent cardiovascular actions than WR 225,448. Development of a bile collection system in the dog continued. A new catheter system was developed which provided improved bile flow rates over an eight hour collection period. A thin layer chromatographic method for the analysis of chloroquine and its primary metabolite, desethylchloroquine, was developed and utilized to analyze the pharmacokinetic profile of chloroquine. Chloroquine was shown to have an elimination half-life of 12.6 days as described by a two compartment open model. For technical report see WRAIR Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

Work Unit 217 Basic Pharmacological Studies

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
Associate: CPT D. Korte, Jr., Dr. H. Lowensohn, LTC B. Schuster, SP5 C. Basamania, MAJ J. von Bredow, Dr. L. Fleckenstein

1. Description.

Basic research studies undertaken by the department during the past year were a comparison of the cardiovascular effects of WR 225,448 with those of primaquine, the continuing development of a bile collection system in the dog, and a pharmacokinetic evaluation of chloroquine. Studies on development of antidotes to chemical agents which previously had been reported in this unit have been incorporated in Work Unit 163, Preclinical and Clinical Assessment of Antidotes.

2. Progress.

The cardiovascular effects of the candidate antimalarial drug, WR 225,448, which is a potent analog of primaquine, were investigated in comparison with primaquine using a pentobarbital anesthetized dog as the experimental model. Primaquine when administered as a bolus injection, decreased mean arterial pressure and increased the PR interval of the electrocardiogram prior to causing ventricular fibrillation and death at a cumulative dose of 14.4 mg(base)/kg. An equivalent dosing regimen of WR 225,448 produced no significant effects. Infusion of primaquine, 10 mg(base)/kg, over a 30 minute period produced significant decreases in the heart and respiratory rate while increasing amplitude of T wave and the duration of the PR and QTc intervals of the electrocardiogram. The only change observed following infusion of an equivalent regimen of WR 225,448 was a prolongation of the PR interval. Both primaquine and WR 225,448 attenuated the increase in pulse pressure following carotid occlusion and reduced the increased heart rate response to bolus injections of isoproterenol. Based on these studies it was concluded that WR 225,448 produced less severe changes of cardiovascular indices than primaquine in the pentobarbital anesthetized dog.

Development continued on a canine model for collection of bile. Early attempts were hampered by a faulty catheter design

which has been corrected. The new catheterization system provided for long-term implantation with no overt tissue damage at the site of catheter placement in the neck of the gall bladder. Preliminary studies with the new catheter indicated that improved bile flow rates could be obtained during the eight hour collection period. A problem encountered with this system is the diminished rate of bile flow observed during the eighth hour of collection.

A thin-layer chromatographic method was developed for quantitating blood levels of chloroquine and its major metabolite, desethylchloroquine. This method was sensitive at blood levels ranging from 80 to 600 ng/ml which were required to investigate the pharmacokinetic profile of chloroquine. The pharmacokinetic profile of chloroquine in the beagle dog was assessed and shown to be consistent with a two compartment open model with the following parameters: mean elimination half-life, 12.6 days; clearance, 2.9 L/kg/day; volume of central compartment, 10.4 ml/kg; VD_{SS}, 53.8 L/kg and VD_{β}, 52.4 ml/kg.

3. Future Objectives.

Development of the canine bile collection model will continue. Immediate goals are to develop a hydraulic valve system for the bile duct and to prevent the reduction of bile flow during the eight hour observation period. The objectives of these studies are to develop an acute model for the study of the enterohepatic circulation and metabolism of candidate drugs and to develop an unanesthetized and unrestrained canine model for long term sampling of bile for determining the pharmacokinetic profile of candidate drugs.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6449	81 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
80 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
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:Z			

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 81	4.0	430
FISCAL YEAR — CURRENT 82	2.0	351

19. RESPONSIBLE DOD ORGANIZATION

NAME

ADDRESS: Walter Reed Army Institute of Research
Washington, DC 20012

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

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of CD&I

ADDRESS: Washington, DC 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Hockmeyer, W.T., MAJ
TELEPHONE: (202) 576-3544
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Oster, C.N. POC: DA
NAME

21. GENERAL USE

Foreign Intelligence not 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 (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) The objective of this work unit is to elucidate the mechanisms operative in the natural and artificial induction of immunity to a variety of parasitic infections of military importance. This includes the study of infections in model systems and the development of methodologies for the study of the immune reaction in humans for research as well as diagnostic evaluations.

24 (U) The approaches used for these studies involve the measurement of various parameters of disease and of the immune response to disease in both in vivo and in vitro experiments. A variety of different diseases are also studied.

25 (U) 80 10 - 81 09 Twelve strains of inbred mice were infected with Leishmania donovani. Various hematologic, immunologic and other parameters were monitored throughout the infection to develop a murine model for Leishmaniasis which correlates with the human disease. Resistant and susceptible strains are now available for studies on mechanism of immunity and the genetics of resistance. In clinical studies lymphocyte-transformation was performed on patients with recurrent and/or drug resistant Leishmanial infections in an attempt to document previously observed decreases in lymphocyte response to antigen during active Leishmanial disease and correlate lymphocyte responsiveness with clinical cure. To date lymphocytes from 6 patients have been tested for reactivity to a variety of Leishmanial antigens over several courses of drug treatment. Correlation of clinical data with in vitro lymphocyte reactivity will be done when a significantly meaningful sample number has been obtained. Studies are underway to analyze monocyte/macrophage function in patients infected with cutaneous leishmaniasis. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

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

Work unit 218: Immunological Mechanisms in Microbial Infections

Investigators:
Principals: MAJ Wayne T. Hockmeyer, MSC
LTC Charles N. Oster, MC

Associates: John F. Barbaro, Ph.D.
Donald T.O. Wong, Ph.D.
Mr. Joseph S.Williams
Mr. Rufus W. Gore
Mr. Andre J. Toussaint
Ms. Anne L. Haverly
E5 Perry J. Sayles

Problem and Objectives:

The study of leishmaniasis is hampered by the lack of a suitable animal model for visceral leishmaniasis that correlates with the pathogenesis of the human disease. The objective of this study is to find an animal model that can be used to elucidate immune mechanisms operative in visceral leishmaniasis. This includes the development of methodologies for the study of the immune reaction by measurement of various parameters of leishmaniasis by both *in vitro* and *in vivo* experiments.

Progress:

Leishmania donovani infections were studied in 12 strains of inbred mice. Early resistance (innate resistance) is controlled by a single autosomal Leishmanial (Lsh) gene. Acquired immunity or resistance developing later in the infection is controlled by a gene or genes with or close to chromosome 17. The phenotypic expression of these genetically controlled traits is not known. Of those parameters studied during the infection, resistance correlates most closely with maintenance of cell mediated immune response, particularly lymphocyte blastogenesis and delayed type hypersensitivity. Resistant mice possess reduced parasite burdens and limited hepatosplenomegaly. Clinical studies of lymphocyte transformation are continuing to be performed on patients with recurrent and/or drug resistant leishmanial infections to document previously observed decreases in lymphocyte response to leishmanial antigens and mitogens during active disease.

Recommendations:

Validation of the mouse model system developed to study visceral leishmaniaisis by examining pathogenesis and immunologic response of at least one more Leishmania donovani isolate. We feel it is important to make certain the work to be done on mechanisms of immunity and vaccine studies is done with a typical representative strain. These isolates will be also verified by kDNA analysis. Initiate basic immunologic studies of lymphocyte transformation are continuing to be performed on patients to further document observed decreases and to correlate lymphocyte responsiveness with clinical cure. Studies are underway to analyze monocyte/macrophage function in patients infected with cutaneous leishmaniasis).

Presentations:

1) Bergman, J.D., J.D. Chulay, L.D. Hendricks, and C.N. Oster, 1981. Susceptibility of Leishmania from clinically sensitive and resistant lesions to pentavalent antimony in vitro. The 21st Interscience Conference on Antimicrobial Agents and Chemotherapy. November 1981

Publications:

1) Hockmeyer, W.T., P.A. Kager, P.H. Rees, and L.D. Hendricks. 1981. The culture of Leishmania donovani in Schneider's insect medium; its value in the diagnosis and management of patients with visceral leishmaniasis. Trans. Royal Soc. Trop. Med. Hyg. 75: In press

2) Kager, P.A., P.H. Rees, B.T. Wellde, W.T. Hockmeyer, and W.H. Lyerly. 1981. Allopurinol in the treatment of visceral leishmaniasis. Trans. Royal Soc. Trop. Med Hyg. 75: 556.

3) Peterson, E.A., F.A. Neva, C.N. Oster, and H.B. Diaz. 1981. Diffuse cutaneous leishmaniasis in the Dominican Republic. Adherent supressor cells inhibit lymphocyte proliferative response to leishmanial antigens. New Eng. J. Med. In press.

4) Berman, J.D., J.D. Chulay, L.D. Hendricks, and C.N. Oster. 1981. Susceptibility of clinically sensitive and resistant Leishmania to pentavalent antimony in vitro. Am. J. Trop. Med. Hyg. In press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6480	81 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. DISS'N INSTR'N	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	EB	219
b. CONTRIBUTING				
c. [redacted]	STOG80-7.2:1			

11. TITLE (Precede with Security Classification Code)

(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: 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 81	11.0	531
FISCAL YEAR CURRENT 82	11.0	716

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, P. K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Biochemistry

ADDRESS: Washington, D.C. 20012

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

NAME: Doctor, B.P., Ph.D.

TELEPHONE (202) 576-3001

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Sleeman, H. K., Ph.D.

NAME Brown, N. D.

21. GENERAL USE

Foreign Intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Organophosphates; (U) Nerve Agent Antidotes; (U) Acetylcholinesterase; (U) Receptors

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

23. (U) The objectives of this work unit are: (1) to provide the military with a safe and effective prophylactic/therapeutic formulation against chemical agents; (2) to investigate the effects of organophosphates on acetylcholinesterase activity, cellular biochemical processes and organ receptor sites; (3) to determine the pharmacokinetics, distribution, transport and metabolism of both chemical and antidotal agents; (4) to investigate metabolites and degradation products of chemical and antidotal agents including their identification, synthesis and quantitation; and (5) to develop the necessary methodology for conducting the studies.

24. (U) Classical biochemical, pharmacological and physiological procedures will be used to assess the effectiveness of quinuclidines and oximes as nerve agent antidotes. The masking and protection of sites on acetylcholinesterase by scavenger peptides to preclude organophosphate binding will be explored. Conformational changes in acetylcholinesterase upon forming complexes (aging) with organophosphates will be studied with a view to understanding and preventing these molecular alterations. The effects of organophosphates and nerve agent antidotes on biochemical mechanisms of inhibition and reactivation will be systematically investigated to include effects on respiration.

25.. (U) 80 10 - 81 09 A zinc-citrate complex was identified as the toxic product arising from prolonged storage of autoinjectors containing nerve agent antidote formulations. High performance liquid chromatography methodology was developed for the analyses of the nerve agent antidotes, aprophen and N-methyl pyridinium-2-aldoxime (2-PAM-Cl). Absorption, distribution, metabolism and excretion of ^{14}C- prophen were studies in rats. Stability and degradative fate of 2-PAM-Cl under various conditions of pH and temperature were determined. Studies underway are concerned with the determination and identification of metabolites of aprophen and 2-PAM-Cl in experimental animals. For Technical Report see WRAIR Annual Progress Report 1 Oct 80 to 30 Sep 81.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 72

PROJECT: 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

WORK UNIT: 219 Biochemical Aspects of Medical Defense Chemical Agents

INVESTIGATORS:

Principal: Bhupendra P. Doctor, Ph.D.

Associate: Nesbitt D. Brown, M.S.,; PVT Lynn Decker; SSG Theodore A. Gegoux, B.S.; Judith M. Gemski, B.S.; R. Richard Gray, M.S.; Leo Kazyak, B.S.; SFC Evelyn Moore; SP4 Gregory A. Schoo; H. Kenneth Sleeman, Ph.D.; M. Patricia Strickler, Ph.D.

DESCRIPTION:

The objective of this work unit is to provide the military with a safe, effective prophylactic and therapeutic product against nerve agent poisoning by evaluating potential nerve agent andidotes, insuring the quality control and stability of nerve agent antidote formulations, and the isolation and identification of possible toxic material resulting from degradation products or contamination. New and necessary methodologies for performing the research are also developed.

1. Toxic Substance Formation in Autoinjectors Containing Citrate Buffer.

2. Degradative Fate of N-Methyl Pyridinium-2-Aldoxime Chloride in Aqueous Solutions at Various pH and Temperatures.

3. Determination of Aprophen in Biological Samples.

4. Purification of Commercially Prepared Trypsin.

5. Collaborative Research Studies on Polyamines and Chloroquine.

A. Toxic Substance Formation in Autoinjectors Containing Citrate Buffer

The extraction or leaching of materials from plastics and synthetic rubbers into the solution in which they are in contact presents a variety of problems. These problems may result from the extracted product (plasticizers, hardening agents, degradation products) or from complexes formed between these products and the constituents of the solution. These substances may be innocuous, interfere with chemical analyses, or exhibit toxin properties. In our investigation on a toxin substance formed in the solution from aged (10 years) autoinjectors, it was found that the toxin production was due in part to a constituent of the butyl rubber components. The toxicity of the solutions was assayed by mouse lethality, HeLa cell cytotoxicity, and aconitase inhibition. Isolation and purification of the toxic substance was accomplished by high performance liquid chromatography, and column chromatography on DEAE-Sephacel and Sephadex G-10 and P-6. The purified toxic substance was analyzed by IR, C_{13}NMR, and mass spectrometry and was found to be a polycarboxylic acid that contained 3 distinct carbonyl groups. Analysis by atomic absorption showed the presence of Zn and traces

of Mn. Citrate analyses were positive. Extraction of butyl W rubber stoppers at 80°C for 8 weeks with the constituents of the autoinjectors showed that the toxic substance was found only when citrate was present. When other butyl rubbers were treated with citrate, results varied directly with the zinc content of the rubber. The toxic product formed appears to be a complex of citrate and zinc, and is most toxic when the ratio of citrate to zinc is 2:1.

B. Degradative Fate of N-Methyl Pyridinium-2-Aldoxime Chloride in Aqueous Solutions at Various pH and Temperatures

N-methyl pyridinium-2-aldoxime (2-PAM-Cl) is a therapeutic drug employed for reactivating organophosphorus-inhibited acetylcholinesterase poisoned by various chemical agents. The stability of this oxime in formulation, under prolonged storage conditions in different climatic areas is therefore of importance since the potency of this therapeutic is directly dependent on the amount of 2-PAM-Cl present. In order to thoroughly study the stability of this compound and its breakdown products, a reverse phase high performance liquid chromatography (HPLC) gradient method along with an isocratic paired-ion method were developed. Experimental samples of 2-PAM-Cl were prepared, in water, dilute HCl or dilute NaOH, and analyzed by HPLC. The degradative fate of 2-PAM-Cl followed closely the decomposition scheme postulated by Ellin.

Solutions of 2-PAM-Cl at pH >7 rapidly degrade to 1-methyl-2-pyridone and 2-carboxy-N-methyl pyridinium chloride. A small amount of 2-carbamoyl-1-methyl pyridinium chloride also forms, but is rapidly converted to the pyridone and the carboxyl compound. 2-Cyano-N-methyl pyridinium chloride, the intermediate breakdown product of 2-PAM-Cl, is extremely labile and is completely undetectable in basic solution. In contrast, 2-PAM-Cl in aqueous solutions at pH>3<7 is quite stable. More than 90% of a 2-PAM-Cl remains intact after heating at 95°C for two hours. The degradative products formed under these conditions are 1-methyl-2-pyridone, 2-carbamoyl-1-methyl pyridinium chloride and 2-Cyano-N-methyl pyridinium chloride. The cyano compound is also relatively stable under these conditions, but when heated, slowly forms the pyridone and the carbamoyl compound. Elevated temperatures had a negligible affect on the stability of 2-PAM-Cl in acidic solutions (pH <3). During a 2 hour period at 95°C, 2-formyl-N-methyl pyridinium chloride was the only degradative product formed. Mass spectral analyses of all isolated peaks separated in this system were used to confirm peak identity of each degradative product.

C. Determination of Aprophen in Biological Samples

While it is possible to accurately and reproducibly quantitate low concentrations of aprophen in neat solutions by reverse phase HPLC, the limited solubility of aprophen in these chromatographic systems necessitated the application of normal phase HPLC to the determination of aprophen in biological fluids and tissues. The chromatographic conditions were chosen by comparing the resolution of standards of aprophen and

benactyzine on a partisil-5 silica column while varying the mobile phase composition. A solvent composition of 30% methanol/70% acetonitrile provided the best compromise for resolution and reproducibility.

Aprophen was extracted from serum and tissues prior to injection into the column. An extraction step was necessary to concentrate the aprophen for greater sensitivity and to remove biomolecules that could possibly interfere in the assay technique. The recovery of aprophen from spiked serum samples ranged from 75-77% while the extraction efficiency from spiked tissues was 89% to 95%. The chromatographic method employed clearly demonstrated the usefulness of normal phase chromatography for the analysis of compounds with limited stability in the aqueous/organic mobile phases used in reverse phase HPLC.

D. Purification of Commercially Prepared Trypsin

A reverse phase high performance liquid chromatographic method was developed for the purification of milligram amounts of commercially prepared bovine trypsin. Increased specific enzymatic activity was observed in the purified material. The removal of impurities in the commercial trypsin that interfere with peptide mapping is particularly critical when large amounts of trypsin are needed for complete digestion or when tryptic maps are monitored at high sensitivities in the HPLC. The purification procedure was also able to resolve the α and β forms of trypsin, thus demonstrating the potential of reverse phase chromatography for enzyme purification.

E. Collaborative Research Studies on Polyamines and Chloroquine

1. In collaboration with the Department of Hematology, Division of Medicine, WRAIR, experiments were conducted to investigate the role of polyamine metabolism to cell growth. The recent development of an *in vitro* erythrocyte culture system coupled with the use of an ultrasensitive (femtomolar) high performance liquid chromatographic method permitted investigations into the relationship of polyamines and cell growth in normal and malaria-infected (*Plasmodium falciparum*) red blood cells.

A series of ornithine decarboxylase inhibitors and various vitamin B_6 antagonists severely reduced the concentration of polyamines in parasitized red blood cells. Biochemical consequences of this reduction were shutdowns in the biosyntheses by the parasite of DNA, RNA and protein. Morphological changes were also noted. The parasites of infected red blood cells not exposed to inhibitors or antagonists increased in number and progressed to other stages of development whereas the parasites within exposed red cells decreased in population and did not progress beyond the early trophozoite stage. No appreciable differences were detected in the polyamine levels of exposed or unexposed non-parasitized erythrocytes.

2. In collaboration with the Division of Experimental Therapeutics, WRAIR, and the U.S. Army Medical Research Unit, Kenya, the metabolic

disposition of chloroquine was determined. Although extensive research has been conducted on this antimalarial, the metabolic fate of chloroquine in mammals remains unknown.

A recently developed ion-pair reverse phase high performance liquid chromatographic method was employed to determine the metabolic degradation products of chloroquine produced over various time periods. After the administration of therapeutic doses, the pharmacokinetics of chloroquine were investigated in both man and monkey. Upon analysis of plasma extracts obtained from the two experimental groups, subtle biotransformations of the parent drug were apparent. Desethylchloroquine and bidesethylchloroquine were the principal end-products of degradation. Trace amounts of an unknown metabolite were also found. Since certain metabolites of chloroquine (for example, hydroxychloroquine found in urine) have been found to be therapeutically effective, peak fractions of each separation are being collected and the antimalarial efficacy of each compound will be tested.

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3. Whaun, J.M., and N.D. Brown. 1981. Effects of two ornithine decarboxylase (ODC) inhibitors on polyamine synthesis in Plasmodium falciparum. Gordon Research Conference on Polyamines.

4. Strickler, M.P., N.D. Brown, and B.P. Doctor. 1981. Degradative fate of N-methyl pyridinium-2-aldoxime in aqueous solutions at various pH and temperatures. U.S. Army Medical Research and Development Command Chemical Program Meeting.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6464	81 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
80 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	220
b. XXXXXXXX				
c. XXXXXXXX	STOG 80-7.2:5			

11. TITLE (Precede with 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 81	9.0	633
FISCAL YEAR CURRENT 82	9.0	669

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, COL Philip K.

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Medicine

ADDRESS: Washington, D.C. 20012

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

NAME: BUTKUS, COL DONALD E.

TELEPHONE: (202) 576-2300

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: DUARTE, LTC MC C.

NAME: JOHNSON, LTC MC J.P.

NAME: WEISMANN, MAJ MC W. POC: DA

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Renal Failure; (U) Renal Hemodynamics; (U) Heat Stress; (U) Shock; (U) Fluid and Solute Homeostasis; (U) Dialysis; (U) Kidney Function

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

23. (U) To investigate mechanisms 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 bases for prevention and treatment of renal failure.

24. (U) Clearance methods, micropuncture, membrane transport, tissue culture, radioimmunoassay, enzyme kenetics, isotope dilution, chromatography, and dialysis.

25. (U) 80 10-81 09 the role of vasoactive anines in the maintenance of renal ischemia and induction of renal failure was investigated in a hemorrhagic shock model of renal ischemia. These studies revealed significant net production of nor-epinephrine by the kidney both during and following hemorrhage suggesting that endogenous renal nor-epinephrine may contribute to maintenance of renal ischemia. Studies on the renal effects of thiopental anesthesia indicated that they too were mediated by endogenous catecholamine release. Gentamicin nephrotoxicity was shown to occur in two phases, the first characterized by functional changes and accompanied by increased renal prostaglandin and the second characterized by renal failure and decreased prostaglandin production. Urinary epithelial cells grown in tissue culture have now been characterized as to their hormonal and metabolic responses and will be used to study toxic and metabolic factors accompanying renal failure. Studies in uremic patients revealed abnormalities of purine metabolism suggesting a potential mechanism for the increased susceptability of these patients to infection.

For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65

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

Work Unit 220: Pathogenesis of Renal Disease of Military Importance

Investigators

Principal: COL Donald E. Butkus, MC
Associates: LTC Cristobal G. Duarte, MC;
Mr. John A. Gagnon;
LTC John P. Johnson, MC;
Mrs. Natalie L. Lawson;
Mr. James S. McNeil;
MAJ William Weismann, MC

Problem and Objectives

The subject undergoing investigation in this study is the high incidence and persistently high mortality rate associated with acute renal failure in the combat casualty. The incidence of acute renal failure declined from frequencies as high as one in four seriously wounded in WWII [1,2] to one in 200 and one in 600 in the Korean [3,4] and Vietnam Conflicts [5-7], respectively. The reason for this decline is related primarily to more rapid evacuation of the sick and wounded to definitive treatment centers and to initiation of earlier and more efficacious resuscitative measures during the latter two conflicts [6-8]. Engagement in more conventional warfare is likely to reverse this trend by hampering evacuation efforts. While the mortality associated with acute renal failure declined from 90% to 68% between WWII and Korea, largely due to availability of hemodialysis during the latter conflict, a further decrease was not seen in Vietnam despite greater technical experience with the latter procedure [3-8]. A similarly persistent high mortality rate has been noted in the civilian community [9]. Therefore the major objectives of this study are to delineate those factors of importance in initiating and maintaining acute renal failure so that (1) prophylactic measures may be defined which may be initiated early in the treatment of combat casualties to (a) prevent development of acute renal failure and (b) reverse early renal ischemic changes before cell death and necrosis occur; (2) means of hastening recovery of renal function may be employed; and (3) measures may be taken to reduce mortality from infections and complications.

Progress

Numerous studies since the 1940's have demonstrated that decreased renal blood flow secondary to increased renal vascular resistance occurs early in the initiation of human as well as experimental acute renal failure [10,11]. During the 1970's it was hypothesized that the increased renal vascular resistance was primarily the result of increased systemic or renal production of angiotensin II from renin and this peptide was incriminated as etiologic in acute renal failure [10]. Numerous lines of evidence suggest that this is not the case and that renal vascular resistance and renal blood flow in the stressed state are modulated by the balance of effects of a number of vasoactive factors. These include vasoconstrictors: renin-angiotensin, catecholamines, vasopressin, renal sympathetic tone; and vasodilators: prostaglandins, kinins [10,11]. It is therefore likely that the increased renal vascular resistance resulting in acute renal failure in various states of stress results from an imbalance of one or more of these factors. For this reason studies in the past year have continued to concentrate on elucidating the inter-relations of these effectors, on their production during renal ischemia and renal failure, on their mechanisms of action on the cellular level and on potential methods of inhibiting local constrictor effects.

Studies in an Ischemic Model of Renal Failure

Hemorrhagic hypotension of significant degree to produce renal failure generally results in acute death of the animal from extra renal causes whereas lesser degrees of hypotension fail to induce acute renal failure [11]. Studies have therefore been conducted to develop a model combining both significant hemorrhage and aortic constriction in order to produce both renal underperfusion and release of systemic amines without producing mortality from non-renal causes [12,14]. Studies were performed with 30 cc/kg hemorrhage and aortic constriction for three hours. This model resulted in definite acute renal failure in 30% of animals and lesser lesions in the remainder. Moreover, there was no immediate mortality from non-renal causes and the course of renal failure was followed for seven days. Both glomerular filtration rate and renal plasma flow remained severely depressed with plasma urea nitrogen and creatinine rising to 83-255 mg% and 3.8-20 mg% respectively. During the initial insult, renal perfusion pressure was maintained at an average of 54 mmHg and all animals were oliguric throughout the three hours. This suggests that either systemic or local release of vasoconstrictors contributed to the suppression of glomerular filtration and renal

contributed to the suppression of glomerular filtration and renal plasma flow during this period.

In this regard, plasma renin activity increased from 1.5 to 10 ng/ml and plasma norepinephrine and epinephrine increased from 78 to 440 pg/ml and 28 to 1844 pg/ml, respectively. Plasma PGE, however, increased only minimally (from 53 to 92 pg/ml). In addition net renal venous addition of renin increased from 0.7 to 81 ng/ml and renal venous norepinephrine and epinephrine increased from 176 to 3079 pg/ml and -4 to 13,178 pg/ml, respectively. Net renal venous addition of PGE increased from -2 to 241 pg/ml. These observations reveal significant increases in renal generation of vasoconstrictors during ischemia which may contribute to the reduced filtration rate [12,14].

Seven days after ischemia plasma renin activity remained elevated above control levels as did renal venous norepinephrine whereas the other parameters returned to normal. Plasma kinin and vasopressin levels in this study are yet to be measured. At this point, it is unclear what the relationship of the various vasoconstrictors is to the development of and maintenance of renal failure in this model. Although this model produced classical acute renal failure in only 30% of animals, there was no mortality in the remaining animals. We therefore feel confident that the percentage of animals developing renal failure can be increased significantly by extending the period of ischemia without inducing unacceptable degrees of mortality.

Studies in Gentamicin-Induced Acute Renal Failure

Studies in the gentamicin-induced acute renal failure model in the dog have demonstrated biphasic effects of this agent on renal function [13]. In the first phase, which begins within 24 hours of gentamicin administration, urinary prostaglandin excretion increases dramatically. This is accompanied by a progressive decrease in urinary concentrating ability commensurate with the known antagonistic effect of prostaglandins on vasopressin responsiveness. Mild pre-renal azotemia then ensues, accompanied by increasing plasma renin activity. On day 15, urinary prostaglandin excretion declines dramatically and this decline coupled with a progressive rise in plasma renin is accompanied by progressive acute renal failure. This suggests that the elevated renal prostaglandins may serve to maintain glomerular filtration and that their decline allows the overriding effect of renin and possibly other vasoconstrictors to produce renal ischemia and renal failure. Studies with prostaglandin inhibitors are currently in progress to test this hypothesis.

Cis-Platinum Nephrotoxicity

Limited studies have been conducted to attempt to define a mechanism to prevent the acute nephrotoxicity associated with cis-platinum administration. Over 200 patients are scheduled to undergo treatment with this agent at WRAMC alone. It had been previously demonstrated that disulfuram in very large doses can protect against cis-platinum nephrotoxicity in the dog. The toxicity of this agent in man, however, is great enough to preclude its use. We have shown that dithiotheitol has a much greater protective effect in the rat than disulfuram. The findings are similar to those reported from this laboratory with other heavy metals in the past. Studies are currently in progress to test the efficacy of the penicillamine derivatives dimercaptosuccinic acid and dimercaptopropane sulfonic acid which have much better therapeutic indices [unpublished observations]. These agents are currently in use in Europe as chelating agents for sodium arsenite and have high affinity for platinum.

Effects of Potassium Depletion on Renal Function

Potassium depletion is known to produce a state of nephrogenic diabetes insipidus and to predispose to the development of renal failure in several laboratory models. Studies in potassium depleted rats revealed significant magnesium wasting concommitant with elevation of serum magnesium [3, abstracts and publications]. Administration of DOCA to similarly K-depleted animals revealed a restoration of plasma magnesium toward normal without changes in external magnesium balance. Measurement of plasma volume indicated that these changes could be accounted for partly on the basis of contraction and re-expansion of plasma volume in the two groups respectively. Changes in plasma volume in the two models of potassium depletion may play a significant role in the susceptibility to acute renal failure. Studies are currently underway to test this hypothesis in the gentamicin-induced model of acute renal failure.

Altered Immunity in Acute Renal Failure

Infection is a leading cause of death in patients with acute renal failure and these patients have increased susceptibility to infection. Studies were undertaken to evaluate the mechanism of this altered susceptibility to infection. Studies in uremic patients demonstrated significant alterations in purine nucleoside metabolism in red blood cells and leukocytes centered around the metabolism of adenosine [12, abstracts and publications]. As altered adenosine metabolism is the primary defect in a number of

hereditary immune deficiency diseases, it is likely that this abnormality contributes to the altered immunity seen in acute renal failure. Studies are being conducted to define this abnormality precisely in patients and to assess its development in the experimental model.

Studies on Urinary Epithelial Cells in Tissue Culture

A line of urinary epithelial cells grown in tissue culture for the past three years has been characterized for its hormonal responsiveness and transport characteristics [1-4, publications]. These cells respond to aldosterone, dexamethasone, insulin and kinins but not to vasopressin. Active transport can be demonstrated as well as formation of urinary vasoactive amines such as kinins and prostaglandins. In addition characterization of several metabolic pathways including krebs cycle intermediates and pentose shunt activity have been accomplished. Studies in these cells have demonstrated aldosterone mediated kallekrein stimulation and have documented that activation of citrate synthase, the entry enzyme to the krebs cycle, is not necessary for the development of a physiologic response to aldosterone. The latter observation is important because activation of this enzyme has been used in the past as a marker of aldosterone response in many tissues. Studies will now be undertaken to test the response of this cell line to various noxious stimuli, as anoxia and nephrotoxins, to determine the mechanisms of renal epithelial cell injury and regeneration.

Future Plans and Recommendations

Plans are in progress to expand studies in the ischemic model of acute renal failure by increasing the period of ischemia in order to produce a consistently higher incidence of renal failure. Following this, studies will be implemented to assess the relative importance of the various vasoconstrictors in the genesis and maintenance of renal failure and attempts will be made to interrupt the ischemic cycle. Studies will continue to assess the mechanisms of increased susceptibility to infection in acute renal failure and to further define the described alterations in purine metabolism. In addition, studies will be initiated on isolated epithelial cells in culture to determine the cellular mechanisms of renal injury and regeneration. These studies would be greatly aided by more sophisticated histologic support which might require extramural contracts in the future.

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ARTICLES SUBMITTED

1. Johnson, J.P., McCauley, C. and Copley, J.B. The quality of life of hemodialysis and transplant patients - a more comprehensive methodology and some herestic data. Submitted.

2. Gagnon, J., Moore, J., Verma, P., Sander, G. and Butkus, D. Plasma kinin levels in acute renovascular hypertension in dogs: relation of Kallekrein-Kinin, renin-angiotensin and prostaglandin systems. Submitted.

3. Gagnon, J., Felipe, I. and Butkus, D. Influence of thiopental anesthesia on renal sodium and water excretion in the dog. Submitted.

4. Butkus, D.E. and Schwartz, J.H. Reversal of dithiothreitol induced vasopressin inhibition by oxidizing agents and guanlyl inidodiphosphate. Submitted.

5. Butkus, D.E. and Jones, F.T. Reversal of copper induced vasopressin inhibition by reducing agents. Submitted.

ABSTRACTS AND PRESENTATIONS

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2. McNeil, J.S., Batista, S.L., Jackson, B.D., Nelson, L.D. and Butkus, D.E. Plasma renin activity and urinary prostaglandin excretion in gentamicin induced acute renal failure. Fed. Proc. 40:A3414, 1981.

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4. Duarte, C.G. Effect of DOCA administration on the urinary excretion of electrolytes in sodium depleted rats. Clin. Res. 29:460B. 1981. abst.

5. Old, C.W., Duarte, C.G., Lehrner, A.R., Henry, A.K. and Sinnott, R.C. A prospective evaluation of mannitol in the prevention of radio contrast acute renal failure. Clin. Res. 29:472A, 1981. abst.

6. Butkus, D.E. and Jones, F.T. Redox reversal of copper-induced inhibition of vasopressin responsiveness in B. marinus. Clin. Res. 29:475A, 1981.

7. Butkus, D.E. and Schwartz, J.H. Effect of Dithiothreitol on vasopressin-sensitive adenylate cyclase in B. marinus. Kid Int. 19:236A, 1981.

8. Johnson, J.P. and Green, S.W. Aldosterone increases Na^+ transport in cultured cells without a change in citrate synthase activity. Proc. 13th Ann. Mtng. Amer. Soc. Neph. 138A.

9. Old, C.W., Durate, C.G. and Siedlecki, M. Effects of DOCA in K-depleted rats. Proc. 13th Ann. Mtng. Amer. Soc. Neph. 144A, 1980.

10. Butkus, D.E. Histophysiology of the Nephron. Presented by invitation at the course entitled "Pathology of Medical Renal Diseases", April 1981. Co-sponsored by the Armed Forces Institute of Pathology, the American Registry of Pathology and the National Kidney Foundation.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 06 6753	81 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
80 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	EA	221
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7.2.1			

11. TITLE (Precede with Security Classification Code)

(U) Neural Mechanisms of Chemical Defense-Related Compounds

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 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

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 81	1.0	122
CURRENT FISCAL YEAR 82	2.0	376

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Inst. of Research
Division of Neuropsychiatry

ADDRESS: Washington, D.C. 20012

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

NAME: Campbell, C.B.G., LTC

TELEPHONE: (202) 576-3067

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Tyner, C.F., LTC(P)

NAME: Meyerhoff, J.L., M.D.

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Chemical Defense; (U) Chemical Interactions; (U) Sensory-Motor Processing

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

23. (U) Investigators are directed at understanding the effects on nervous system function of chemical defense-related compounds, extrinsic and intrinsic. There is military relevance in this research.

24. (U) Animal experiments are based on anatomic methods for locating critical sites of agent/antidote action; on pharmacologic and biochemical methods for elucidating interactions between agents/antidotes and the body's chemistry; and on physiologic methods for studying the effects of agents/antidotes on neural signal processing.

25. (U) 80 10 - 81 09 A pattern of brain damage has been demonstrated following soman exposure in rats affecting limbic and motor systems, but sparing the afferent systems and cerebellum. Carbamate cholinesterase inhibitors were found to elevate plasma betaendorphin, which can produce blood pressure and respiratory depression, two characteristics of nerve agent toxicity. The efficacy of endorphin antagonists as therapeutic agents is being investigated. An organophosphate (DFP) has been shown to enlarge peripheral receptive fields and lower thresholds for motor-sensory cortical neurons. Atropine reverses these changes. Other studies are also in progress. For technical report, see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available in 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

☆ U.S. GPO 1974-540-843/8691

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

Work Unit 221: Neural Mechanisms of Chemical Defense-Related Compounds

Investigators: C.B.G. Campbell, M.D., Ph.D., LTC, MC
C.F. Tyner, M.D., LTC(P), MC
J.L. Meyerhoff, M.D.
J.M. Petras, Ph.D.
G.J. Kant, Ph.D.

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.

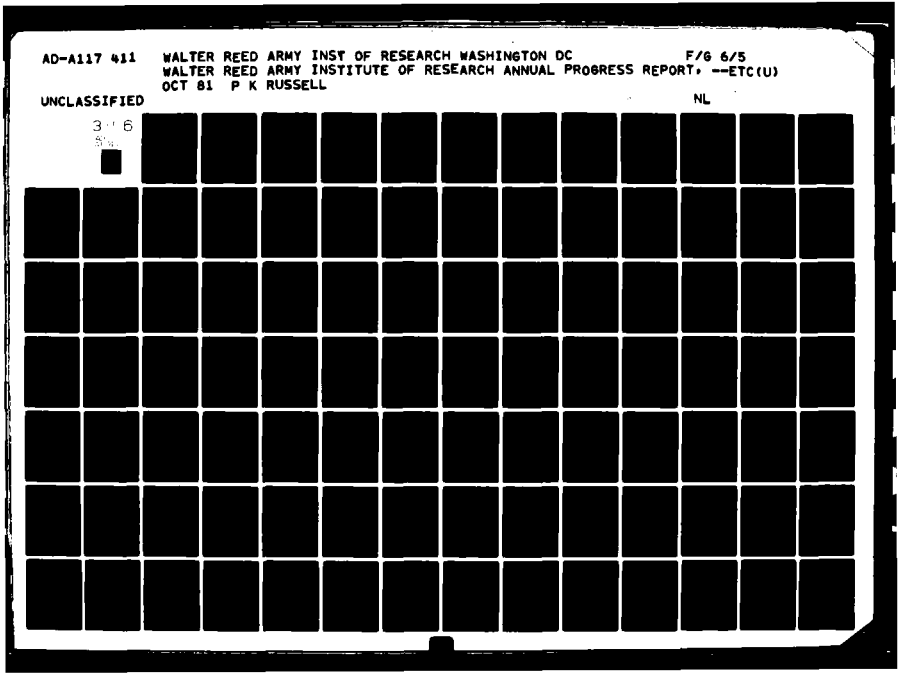
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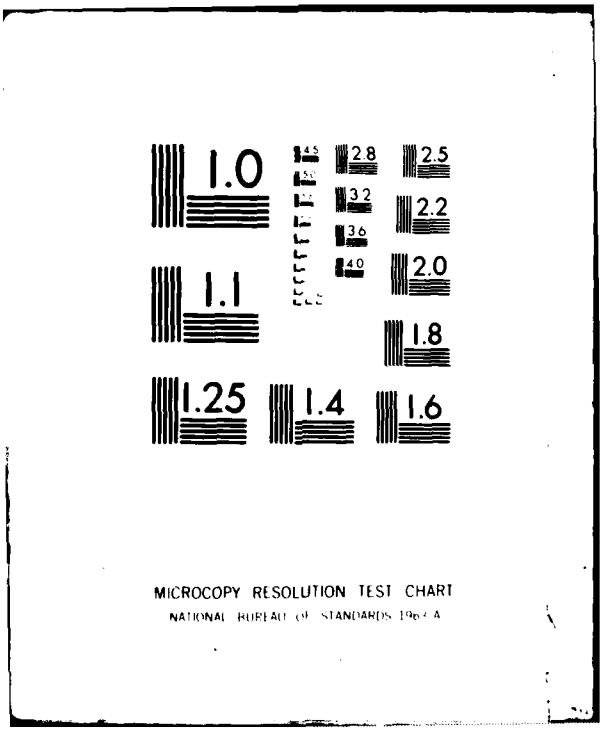
During the past year the brains of rats exposed to single doses of the nerve agent Soman ranging from 79.4 ug/kg to 114.8 ug/kg (LD50 approximately 110 ug/kg), and which survived this exposure, were examined with sensitive experimental neurohistological methods. These methods, the Nauta-Gygax and Fink-Heimer techniques, deposit silver preferentially in the axoplasm of degenerating nerve cells. These methods are more sensitive to early degenerative changes and fine axon degeneration than are routine neurological stains. Extensive degeneration of nerve cells was seen in the cerebral cortex, elements of the limbic system, and the efferent systems of the brain and spinal cord. The major afferent systems, cranial nerves and cerebellum were unaffected. This finding raises the possibility of direct or secondary neurotoxic effects of a permanent nature which could affect survivors of nerve agent exposure.

Preliminary work has shown that an organophosphate, DFP (Diisopropylfluorophosphate), enlarges peripheral receptive fields and lowers the threshold of response of motor-sensory cortical neurons in cats. Atropine reverses these changes. Ethyl alcohol, for example, has an opposite effect to that of DFP.

Many of the physiologic alterations seen with organophosphate poisoning bear a strong resemblence to those seen with exposure to beta-endorphins. Work by our neurochemistry and neuroendocrinology group has shown that several chemical defense related compounds produce elevated plasma beta-endorphin levels. These same investigators have demonstrated elevations in the levels of the cyclic AMP and GMP after central cholinergic stimulation. This suggests that these cyclic nucleotides play a role in cholinergic transmission similar to that seen with other neurotransmitters.

Recommendations for Future Work: We plan to determine whether or not the anatomical changes produced by Soman in rats are primary or secondary effects, and whether or not otner species, including primates, are equally susceptible. Further, do other nerve agents produce similar neurological damage. Beta-endorphin antagonists such as naloxone will be tested for possible therapeutic effects. Soman will be tested to determine if it also raises beta-endorphin levels which can be antagonized by naloxone or TRH. Physiologic studies on cortical neurons affected by agents or antidotes will be continued. The effects of various chemical defense-related compounds on elements of the central respiratory system using acetylcholine and other neurotransmitters will be studied. The central respiratory system is a prime target of organophosphate nerve agents and the cessation of respiration is usually the cause of death after exposure. Preliminary data suggest a significantly greater number of striatal muscarinic receptors in the rat at 1000 hours vs 2200 hours. If confirmed, this would be consistent with the finding in a previous study that cholinergic agonists and anticholinesterases would be more effective (lethal) at 1000 than at 2200 hours. This study will be pursued.


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PRESENTATIONS

Petras, J.M. Brain damage following non-lethal soman (GD) poisoning. Symposium on Prophylaxis and Treatment of Organophosphate Poisoning, San Diego, CA, 5-6 March 1981.

Campbell, C.B.G. Brain damage following non-lethal soman (GD) poisoning (a paper by J.M. Petras). USAMRDC Chemical Scientific Program Review, Fort Detrick, MD, 8-9 June 1981.

Tyner, C.F. Brain damage following non-lethal soman (GD) poisoning (a paper by J.M. Petras). Preventive Medicine Symposium hosted by the Israel Institute for Biological Research.

Campbell, C.B.G. Review of chemical defense-related research in the Division of Neuropsychiatry presented for the Research Area Managers at the Walter Reed Army Institute of Research.

Meyerhoff, J.L., Kant, G.J., Lenox, R.H., Pennington, L.L., and Collins, D.R. Effects of muscarinic and nicotinic agonists and antagonists on brain regional cyclic nucleotides. Society for Neuroscience Meeting, Cincinnatti, OH, November 1980.

Meyerhoff, J.L., Kant, G.J., and Lenox, R.H. Effects of cholinergic agonists, locomotor activity and stress on brain and pituitary cyclic nucleotides in the rat. Symposium on Drug Effects on Rapidly Metabolized Compounds in the CNS, Tokyo, Japan, July 1981.

PUBLICATIONS

Lenox, R.H., Kant, G.J., and Meyerhoff, J.L. Regional sensitivity of cyclic AMP and cyclic GMP in rat brain to central cholinergic stimulation. Life Sciences 26:2201-2209, 1980.

Mougey, E.H. and Meyerhoff, J.L. Effect of cholinomimetics and cholinesterase inhibitors. Neuroscience Abstracts 7:153, 1981.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6446	81 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. DISTR'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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
b. ********				
c. ********	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)
(U) Histopathologic Manifestations 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	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 81	5.0	40
	CURRENT 82	5.0	324

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL, MC
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Division of Pathology
ADDRESS: Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. academic institution)
NAME: Takeuchi, Akio, M.D.
TELEPHONE: 202-576-2024
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Hase, T.
NAME: Tseng, J. POC: DA

21. GENERAL USE
Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Immune responses; (U) Intestine; (U) Immunoglobulin A; (U) Rickettsia

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 histopathologic manifestations of injuries and diseases which have current or potential problems in military personnel. The current effort is directed toward studies of enteric diseases and immunologic responses to enteric and other infections. These studies provide a basis for a comprehension of pathogenesis, therapy, and determination of prognosis in infectious diseases of military personnel.
24(U) Various morphologic techniques including histology, histo- and cytochemistry, autoradiography, immunofluorescent microscopy, transmission and scanning electron microscopy are employed. Various immunologic techniques have also been utilized.
25(U) 80 10-81 09 Initial electron microscopic studies of the morphogenesis of Rickettsia tsutsugamushi in mammalian cells have been completed and more definitive studies are underway. The origins, genesis, and traffic dynamics of IgA - producing cells in the intestine were studied. The relative role of spleen and Peyer's patches in providing cells for intestinal immunity was determined. Studies on the long-term culture of intestinal explants were completed. The role of macrophages and T cells in IgA plasma cell regulation in irradiated mice was investigated. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*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 72 (FOR ARMY USE) ARE OBSOLETE

PROJECT: 3M161102BS10 RESEARCH ON MILITARY DISEASES, INJURY AND HEALTH HAZARDS

WORK UNIT: 222 Histopathologic Manifestation of Military Diseases and Injuries

INVESTIGATORS:

Principal: Akio Takeuchi, M. D.
Associate: Han Y. Cho, Ph.D., Tatsuo Hase, M. D., SFC Garnett Henley, M. S., Jeenan Tseng, Ph.D.

Description:

To define histopathologic manifestations of injuries experimentally produced and diseases which present current or potential problems in military personnel. The current effort is directed toward studies of diseases of the digestive tract and immune response due to infeciton. These studies provide a basis for a comprehension of pathogenesis, scientific treatment, and determination of prognosis in diseases and injuries in military personnel. A multi-disciplinary approach including conventional histology, histo- and cytochemistry, autoradiography, radiotracer methods, various immunological techniques, immunofluorescent microscopy, transmission and scanning electron microscopy is employed.

Problem and Progress:

This work unit consists of studies of histologic and immunologic manifestations of acute diarrheal diseases of infectious origin and collaborative studies of experimental rickettsial infections with other research divisions of the WRAIR.

I. Studies on IgA-Producing Cell Dynamics in the Gut.

The work done in the past year was mainly focused on the population dynamics of IgA plasma cells in the gut lamina propria, and on exploring a radiation problem which arose during the study. The population study was stimulated by the reported finding that humoral immunity in the gut is mainly manifested by IgA which is produced by the plasma cells in the gut lamina propria. Although it is known that Peyer's patches (pp) are the major sources supplying the precursors for the IgA plasma cells, the migration route, differentiation steps, and population processes are still unknown. To study these problems, we developed a lymphocyte transfer system between CB-20 and BALB/c mice. This system solves the technical problems of histoincompatibility and of differentiating donor and recipient cell because CB-20 and BALB/c mice are genetically identical except the allotype genes controlling the production of immunoglobulins which can be used as a marker to differentiate donor and recipient cells. When CB-20 lymphocytes were transferred into BALB/c recipients, pp lymphocytes were the best among all the lymphyoctes tested. The donor (CB-20) IgA plasma cells started to appear at day 6, increased exponentially, and repopulated essentially the entire gut lamina propria at day 12-14 after cell transfer. By sequential transfer and triggering maturation of IgA plasma cells in culture, the CB-20 IgA precursors, when transferred into BALB/c, were found in the spleen of the recipients during the first 5 days. In the spleen, the patch IgA precursors probably divided and differentiated to a stage(s) that was able to migrate to the dut lamina propria. After arriving in the gut lamina propria, the IgA precursors divided and differentiated further into plasma cells. Although the spleen is the intermediate tissue in the migration route, it is not completely obligatory because only partial

reduction of the repopulation was seen in the gut of splenectomized recipients. The stay of the patch IgA precursors in the spleen was not due to trapping, but rather to the characteristics of the precursors. This is supported by the results that patch IgA precursors did not migrate to the gut lamina propria of the splenectomized recipients. All these results provide information concerning the first interaction of IgA precursors with antigens in pp and the expansion and differentiation of IgA plasma cells in the gut lamina propria. The report has been written, accepted by Journal of Immunology and will appear in November issue (1981) of the Journal.

During the study on the population of IgA plasma cells in the gut lamina propria, we have found that gamma-irradiation of recipient mice can induce large numbers of IgA plama cells in the spleen. Further studies reveal that IgM and IgG plasma cells also appear in the same time and tissues as the IgA plasma cells. The radiation induced plasma cells appear virtually in all the lymphoid tissues except the thymus which is an organ containing only T cells. Thus B-cell containing lymphoid tissues supplied the precursors. The maximal time of the appearance is day 6 after irradiation and maximal radiation dose is approximately 500 r/mouse. The appearance of the plasma cells in the spleen can be enhanced by passive transfer of lymphoid cells, and the enhancement occurs only in the spleen. This is probably due to the lodging of donor cells in the spleen, which regulates the appearance. Histocompatible lymphoid cells are better than nonhistocompatible cells, including those from an xenogeneic source, in the enhancement. T-cells are responsible for the enhancement. When T-cells were replaced by macrophages, a suppression of the appearence occurred. Thus T-cells and macrophages regulate the expression/appearance of the radiation-induced plasma cells. These results provided

base-line information on the humoral immunity of the irradiated animals used of in immunological studies and in irradiated patients undergoing clinical treatments. The report has been accepted by Journal of Immunology and will be published in the November issue of the Journal (1981).

II. Morphogenesis of Rickettsia tsutsugamushi in Cultured Cells.

In electron microscopic studies of the infection of cultured L cells and mouse peritoneal mononuclear cells with Rickettsia tsutsugamushi, we have observed a unique finding regarding the life cycle of this organism. In this life cycle, rickettsia enter the host cells as small structures which we prefer to call protoplasmic bodies. The protoplasmic bodies dissolve and release their content into the host cell cytoplasm with concomitant formation of an amorphous, granular matrix in the cytoplasm. Subsequently, multiple rickettsiae appear within the matrix. The above observation indicates that in the obligatory intracellular phase of R. tsutsugamushi, the organisms undertake a virus-type reproduction within the host cell cytoplasm and rickettsia are assembled within the matrix thus formed.

In collaboration with the Department of Rickettsial Diseases, confirmation and definition of this unique reproductive cycle of R. tsutsugamushi is in progress.

III. Culture of Intestinal Explants.

Development of long term organ culture techniques for fetal mouse intestine were developed. Using these techniques, viable cultures were maintained for up to 20 days, whereas other investigators had been able to maintain intestinal explants up to a few days. Sequential study of cultured explants by light, electron, and phase

microscopy revealed that structural differentiation of intestine occurred in culture. Starting at 12 hours after explantation, explants developed rhythmic contractions which continued for most of the culture period. Fibroblastic proliferation, with formation of a monolayer, preceded epithelial proliferation. By day 3 epithelial cells had assumed a columnar shape and on day 4 rudimentary villi were formed, followed by evagination to form crypts. Goblet cells first appeared on day 4 and argentaffine cells on day 7. Survival of the explants was dependent on the type of maintenance chamber; explants in Rose chambers began to degenerate on day 10, while those in Falcon dishes or flasks survived for as long as 20 days.

Synthesis of protein and glycoprotein peaked on day 5, but was evident from day 1 through day 14. Fetal explants were observed to absorb a marker macromolecule, horseradish peroxidase, as early as 4 hours after explantation. Amounts of the enzymes lactase and ornithine carbamoyl transferase secreted by cultured expalnts reached peaks on day 7 of culture. Cyclic AMP levels remained relatively constant throughout the culture period.

The development of this system has provided an *in vitro* system which will permit long term study of the structural and functional effects of various infections and toxic agents in the intestine. Additionally, *in vitro* studies of the interaction of various substances and factors such as stage of villus genesis, enyzme activity and macromolecular synthesis will be possible using this system.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6537	81 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
80 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	223
b. CONTRIBUTING				
XXXXXXXXX	STOG 80-7.2:P			

11. TITLE (Precede 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	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 81	8.0	31
	CURRENT 82	8.0	397

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL, MC

TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Pathology

ADDRESS: Washington, D.C. 20012

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

NAME: Bunte, Ralph M., LTC, VC

TELEPHONE: 202-576-2183

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Keenan, Charlotte M., CPT, VC

NAME: POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Animal model; (U) Trypanosomiasis; (U) Leishmaniasis; (U) Pathogenesis; (U) Morphologic pathology;

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 and define the pathology and pathogenesis of experimental trypanosomiasis and leishmaniasis and the effects of other infectious, toxic, and environmental biohazards 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 utilize conventional gross and histopathology, clinical pathology, histochemistry, immunohistochemistry, and electron microscopy techniques.

25(U) 80 10-81 09 Hematologic and pathologic changes occuring in German Shepherd dogs experimentally infected with Leishmania have been determined, establishing this system as an ideal model of experimental visceral leishmaniasis. Histologic changes occuring in dogs and rodents being treated with antiprotazoan drugs were identified and assembled into formal reports. Studies to determine the efficacy of a cutaneous antigen in the clinical diagnosis of salmonellosis were completed in collaboration with the Division of Veterinary Medicine. The pathogenesis of gentamicin nephrotoxicity is being resolved. A study to determine the functional and morphologic conelates of Hepatitis A infection in Aotus monkeys has been initiated. Several studies resolving response of the tracheal epithelium to injury and cellular dynamics of the repair process were completed. For technical report see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

*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 DISEASES, INJURY AND HEALTH HAZARDS

Work Unit 223 Pathologic Manifestations of Zoonotic Diseases of Military Importance

Investigators:

Principal: Ralph M. Bunte, LTC, VC

Associate: Anthony J. Johnson, LTC, VC; Kevin P. Keenan, MAJ, VC; Charlotte M. Keenan, CPT, VC; James E. Sanders, CPT, VC; Richard E. Long, CPT, VC; Charles B. Clifford, CPT, VC

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 had 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 macroscopic pathology, histopathology, clinical pathology, ultrastructural pathology, histochemistry, and immunohistochemistry.

Progress:

During the reporting period research activities have included: (1) The histologic changes in rats and dogs treated with candidate antimalarial compounds, WR-171,669, WR-172,435 and WR-180,409; (2) The amelioration of nephrotoxicity of candidate antitrypanosomal compound WR-163,577 in calves; (3) The assessment of local tolerance to parenteral administration of various salts of a candidate antitrypanosomal compound (WR-163,577) in calves; (4) The histopathology of rats and dogs treated with antiprotozoan compound WR-228,548; (5) The use of the German Shepherd dog as an experimental model for evaluation of human isolates of visceral leishmaniasis; (6) The pathologic and clinicopathologic evaluation of the owl monkey (Aotus trivirgatus) as a model of hepatitis A; (7) The pathogenicity of a coronavirus isolated from the peritoneal fluid of a cat with effusive peritonitis; (8) The pathology of intrapalpebral and intracutaneous injection sites of a protein extract of Salmonella typhimurium in guinea pigs to detect Salmonella carriers; (9) The pathologic changes in rabbit esophagi exposed to different concentrations of bile salts, trypsin, and hydrogen ions to develop an animal model of reflux esophagitis and define the pathogenetic mechanisms; (10) The assessment of toxicity of a tissue adhesive in rats; (11) Respiratory epithelial injury and regeneration in hamsters;

(12) The interaction of vasoconstrictor and vasodilator hormones released systemically and intrarenally in response to the combined insult of hemorrhagic hypotension and suprarenal aortic constriction in dogs; (13) The pathology of rabbits used on feeders for tsetse flies (Glossina spp); and (14) Clinical pathology laboratory and histopathology laboratory support and collaborative studies.

I. Histologic Changes in Rats and Dogs Associated with Candidate Antimalarial Compunds.

Interdivisinal study with the Division of Experimental Therapeutics, WRAIR.

Two studies were performed: lymphoid and hematopoietic tissues including spleen, thymus, lymph nodes and bone marrow from 41 rats receiving compound WR-172,435 orally once daily for 28 days were examined microscopically. It was found that dosages of 90 and 270mg/kg/day produced increased numbers of vacuolated macrophages in these tissues. The threshold for this effect was between 30 and 90 mg/kg/day. Lymphoid and hematopoietic tissues including spleen, thymus, lymph nodes, tonsils, and bone marrow from 6 Beagle dogs receiving compound WR-172,435 orally once daily for 28 days were examined microscopically. It was found that dosages of 45 and 135 mg/kg/day produced increased numbers of vacuolated macrophages in these tissues. The threshold for this effect was between 15 and 45 mg/kg/day.

Four similiar studies conducted previously with antimalarial compounds WR-171,669 and WR-180,409 produced similiar results.

These three drugs, each with a different chemical classification, have demonstrated antimalarial activity which makes them promising chemotherapeutic agents against this disease. The consistent production of increased numbers of vacuolated (foamy) macrophages in the lymphoreticular system in the rats and dogs was most dramatic in animals treated with the highest dosages of these drugs, indicating a definite dose-related effect. These observations suggest that the antiprotozoan activity of these drugs may be augmented through modulation of macrophage metabolism. Further studies to determine whether antiprotozoan activity and macrophage vacuolation and hyperplasia are related, as well as investigation of the cytochemical basis of those phenomena might provide insight to understanding biological mechanisms of host defense against protozoan infections.

II. Amelioration of Nephrotoxicity of Candidate Antitrypanosomal Compound (cis-Diaminedicholoroplatinum II) by Saline Hydration and Disulfarim Treatment.

Interdepartmental study with the Department of Parasitology, WRAIR.

Cis-Diaminedichloroplatinum II (DDP) is a cancer chemotherapeutic agent which also has antitrypanosomal activity. It use is limited, however, because of dose-related nephrotoxicity. Mannitol diuresis or sulphahydryl compounds such as dithiocarbamate had been known to diminish the nephrotoxicity. Subsequent studies in this Institute then demonstrated that when DDP was given per os to mice along with disulfarim per os and physiologic saline subcutaneously there was a decrease in the nephrotoxicity as determined by microscopic examination of the kidneys. The curative action of DDP against Trypanosoma rhodesiense was not changed. This study was designed to measure more definitively the toxicity in mice of DDP and the ameliorative effect of disulfarim and physiologic saline. The LD_{50}, serum chemistries, and microscopic examination of the kidneys were the parameters used for measurement. Results are preliminary, but it appears that saline hydration or disulfarim treatment decreases the nephrotoxicity and raises the LD_{50} of DDP in mice. This effect is maximum when saline hydration and disulfarim treatment are combined.

III. Assessment of Local Tolerance to Parenteral Administration of Various Salts of a Candidate Antitrypanosomal Compound (WR-163,577) in Calves.

Interdepartmental study with the Department of Parasitology, WRAIR.

WR-163,577 is a bisquinaldine with antitrypanosomal 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 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. There appeared to be minimal absorption of the salts and the quantity observed correlated with the dosage injected. 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 which contained Staphylococcus aureus.

Microscopic evaluation of tissues is underway to complete this pilot study. Additional studies are needed to assess at least the dihydrochloride and acetate salts over longer periods of time.

IV. Histopathology of Rats and Dogs treated with Antiprotozoan Compound WR-228,548.

Interdivisional study with the Division of Experimental Therapeutics, WRAIR.

Eighty rats and 36 dogs were given various dosages of compound WR-228,548 orally for 28 days. Twenty-one tissues from the rats and 24 tissues from the dogs were selected and examined microscopically. Single or multiple drug-associated lesions were graded for severity in each tissue. No threshold for effect could be determined but the severity of lesions increased with dosage.

V. The Use of the German Shepherd Dog as an Experimental Model for Visceral Leishmaniasis.

Interdepartmental 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 is 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 there were six 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 that 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, tonsil, bone marrow, intestine, and lung.

The clinicopathologic findings are consistent with those observed in human visceral leishmaniasis. Two manuscripts are in preparation for publication.

VI. Pathologic and Clinicopathologic Evaluation of the Owl Monkey (Actus trivirgatus) as a Model of Hepatitis A.

Interdepartmental 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 experiment is to confirm the susceptibility of Aotus monkeys to HAV and to record the clinical, viral, pathological, and serological response to experimental infection.

VII. Pathogenicity Studies of a Coronavirus Isolated from the Peritoneal Fluid of a Cat with Effusive Peritonitis.

Interdepartmental study with the Department of Clinical Investigation and Research, WRAIR.

Feline infectious peritonitis (FIP) is a viral disease of cats with protean signs and lesions. Classically there is an insidious onset with persistent nonresponsive fever, progressive debilitation, and eventual death. Lesions are granulomatous inflammation in the abdomen with or without fluid accumulation. Other manifestations include chronic respiratory disease, pleuritis, pericarditis, ophthalmitis, encephalitis, hepatitis, reproductive failure and increased kitten mortality. Inapparent infections are common also. The disease is caused by a coronavirus, but the pathogenesis of the infection is not understood.

Studies of FIP have been severely limited because of failure to isolate and passage the virus in-vitro. There are now several preliminary reports of isolation and cultivation of the putative causative agent, but there is also information that there may be two feline coronaviruses, and that they may interact synergistically to produce the severe, fatal form of the disease. This study was designed by the Department of Clinical Investigation and Research to assess the pathogenicity of a coronavirus isolated and passaged in-vitro from the peritoneal fluid of a WRAIR at with FIP with effusive peritonitis. In view of recent information that cats develop a more rapid and severe fatal infection if they have FIP (coronavirus) antibody then if they are seronegative, the study included cats that were seropositive and seronegative.

The results are very encouraging. Of five seropositive cats inoculated with the isolate, two developed severe granulomatous peritonitis with abundant fibrinopurulent abdominal fluid, and the remaining three developed moderate-to-marked granulomatous peritonitis with little or no abdominal fluid. Two seronegative cats inoculated

with the isolate developed no gross or microscopic lesions characteristic of FIP. These results suggest that the severe fatal form of FIP in cats may be an immune-mediated disease.

VIII. Pathology of Intrapalpebral and Intracutaneous Injection Sites of a Protein Extract of Salmonella typhimurium in Guinea Pigs to Detect Salmonella Carriers.

Interdepartmental study with the Department of Clinical Investigation and Research, WRAIR.

For several years the WRAIR guinea pig colony was infected with Salmonella typhimurium group B var. Copenhagen. An attempt was first made to eradicate the infection by periodically culturing cage droppings and culling all animals over culture-positive cage pans. The method proved ineffective and was stopped because S. typhimurium is not shed consistently in the feces and carrier animals can spread the infection before they are detected. A second attempt to eradicate the disease was to treat all animals with neomycin. This second method was ineffective and stopped because the feces were only temporarily cleared by the antibiotic; after cessation of neomycin therapy the animals began shedding Salmonella again. This is because the organisms are sequestered in the liver, spleen, lymph nodes and intestines of chronically infected animals and neomycin clears only the intestines. This study was designed by the Department of Clinical Investigation and Research to assess the efficacy of injecting a small quantity of protein extract from S. typhimurium into the eye lid and flank skin to produce a delayed hypersensitivity skin reaction in infected animals.

Thirty-nine infected and control animals were used. All received complete necropsies and all organs and injection sites were examined grossly and microscopically. Injection sites were graded according to the amount of necrosis, the inflammatory cell types present, the amount of edema, and the extensiveness of the changes. Only a few animals developed lesions characteristic of delayed hypersensitivity; most were more typical of an Arthus reaction. Also there were no apparent differences between control and infected animals in terms of type and severity of lesions at injection sites. These results indicated that cutaneous hypersensitivity testing will not provide an effective means of detecting Salmonella carrier guinea pigs.

IX. Pathologic Changes in Rabbit Esophagi Exposed to Different Concentrations of Bile Salts, Trypsin, and Hydrogen Ions.

Interdepartmental study with the Department of Surgical Gastroenterology, WRAIR.

Bile, gastric acid, and probably trypsin play a major role in the development of reflux esophagitis, a significant problem in man. The pathogenetic mechanism(s) of injury is poorly understood.

Two studies were designed by the Department of Surgical Gastroenterology to define the pathogenetic mechanism(s). In one, the changes in transmucosal electrical potential and tissue resistance along with hydrogen ion back diffusion were measured in rabbit esophagi exposed to different concentrations of taurodeoxycholic acid, trypsin, and hydrogen ions. In the second, identical parameters were measured in rabbit esophagi exposed to different concentrations of taurochenodeoxycholic acid, tauroursodeoxycholic acid, and hydrogen ions. In both studies the perfused esophageal segments were removed and examined grossly and microscopically in a blinded fashion to determine histomorphologic changes and any differences there might be in lesion development or evolution between the different substances at varied concentrations. A quantitative scoring system for evaluating tissue changes was developed and used. Correlation of changes in electrical potential, tissue resistance, and hydrogen ion back diffusion with pathological changes for each substance are incomplete. Preliminary results suggest a positive correlation between increasing concentrations of trypsin with the severity of tissue injury.

X. Assessment of Toxicity of Tissue Adhesive in Rats.

Interdivisional study with the Division of Surgery, WRAIR.

Tissue adhesives are being evaluated to find improved methods of handling massive trauma to organs, such as those seen in war wounds. Such wounds with excessive bleeding are difficult or impossible to control with suture hemostasis. The Division of Surgery and Pathology have conducted studies with Bulgarian surgical glue, a cyanoacrylate, to assess its efficacy in controlling bleeding and bile leakage from surgically created trauma to the livers of rats. Gross and microscopic examination of such traumatized livers treated with glue have revealed good hemostasis and a tissue response essentially no different from that seen in rats with hepatic surgical trauma controlled with suture hemostasis.

One report in the literature indicates that cyanoacrylates may induce sarcomas when injected subcutaneously in rats. A preliminary study was therefore designed by the Division of Surgery to assess this possible carcinogenicity. Approximately 100 rats have been injected subcutaneously with the compound and examined grossly and often microscopically up to 9 months post injection. No evidence of neoplasia has been observed. A more comprehensive protocol is in the planning stages.

XI. Respiratory Epithelial Injury and Regeneration in Hamsters

Collaborative Studies with Dr. Elizabeth M. McDowell, University of Maryland at Baltimore (Protocol No.: P-05-79).

Most environmental and infectious diseases of the large conducting airways involve changes in the mucous cell populations. These changes may involve metaplastic and/or hyperplastic responses by the mucous cells. The cellular origin(s), differentiation and renewal of the mucous cells in the tracheobronchial epithelium are poorly understood at present.

Four experiments were conducted in this study to determine the roles of basal, mucous, ciliated and undifferentiated "indifferent cells" in regeneration of hamster tracheal epithelium.

In the first experiment on epithelial regeneration all stages of the process were quantified in hamster tracheal epithelium (middle third) following production of a focal denuding wound by mechanical injury. Every epithelial cell around the entire mid-tracheal circumference was counted and categorized according to cell type. The epithelium was divided into wound and non-wound sites.

In the second experiment regeneration of hamster tracheal epithelium (middle third) following multifocal mechanical injury was studied with the combined use of 4-hour colchicine metaphase blockade and 6-hour single pulse ^{3}HTdR labeling.

In the third experiment the histogenesis of epidermoid metaplasia and the restoration of mucociliary epithelium was studied and compared in large and small wounds in the same hamster, in the middle third of the trachea. Large and small wounds were made mechanically in the ventral and dorsal tracheal semi-circles, respectively. Single pulse and continuous infusions of ^{3}HTdR was used in conjunction with 6-hour colchicine blockade of metaphase mitoses.

In the fourth experiment the detailed population dynamics of the regeneration of hamster tracheal epithelium was studied based on continuous ^{3}HTdR infusion via intraperitoneal Alzet® osmotic minipumps and mitotic blockade. Cells were scored as ^{3}HTdR labelled and unlabelled interphase and mitotic cells in each morphologic category. The counts were processed by simple linear algorithms to estimate mitotic cell production, and the number of cells lost and gained in each morphologic category at intervals after wounding.

Except for some electron microscopic evaluations, these four experiments are completed.

The data indicate that secretory cells play the major role in the regenerative response and that this cell type is much more important than basal cells during the entire regenerative process including restoration of ciliated cells. This conclusion is contrary to current dogma. Four manuscripts are in preparation on these experiments and another experiment is in progress.

XII. The Interaction of Vasoconstrictor and Vasodilator Hormones Released Systemically and Intrarenally in Response to the Combined Insult of Hemorrhagic Hypotension and Suprarenal Aortic Constriction in Dogs.

Interdepartmental study with the Department of Nephrology, WRAIR.

Post-traumatic acute renal failure has been recognized as a major complication of combat casualties since WWII. Studies of this problem have been hampered by the lack of an experimental model strictly comparable to humans. This study was designed by the Department of Nephrology to determine the relationships between the vasoconstrictors angiotensin II, norepinephrine, and AVP, and the vasodilators PGE and bradykinin, when renal perfusion pressure is decreased by hypotensive hemorrhage and aortic constriction. Also, the study is designed to determine if the severity of renal dysfunction, as measured by changes in renal blood flow, glomerular infiltration rate, and extraction of PAH, can be correlated to an imbalance between renal vasocontrictors and renal vasodilators. Pathologic studies of kidneys from 18 dogs are being performed. Gross and microscopic examinations, including the use of thin sections imbedded in plastic, are being conducted to screen for pre-existent morbid changes, to determine the morphologic changes resulting from the experimental manipulations, and to permit correlations of changes in morphologic and physiologic parameters.

XIII. Pathology of Rabbits Used as Feeders for Tsetse Flies (Glossina spp).

Interdepartmental study with the Department of Entomology, WRAIR.

Many New Zealand White and Flemish Giant rabbits used as feeders to maintain the WRAIR tsetse fly (Glossina spp) colony have rapidly developed a syndrome characterized by progressive weight loss, unthriftiness, diarrhea, and elevated serum BUN and creatinine. In pathologic studies on approximately 25 rabbits, most have had extensive deposits of amyloid in many organs, most

notably in the kidneys, spleen, stomach, intestines and pancreas. Many of the animals have also had bacteremias with associated lesions such as lung and testicular abscesses, otitis externa and media, septic renal thrombi and infarcts, and septic vegetative endocarditis. The flies feeding on these diseased rabbits have experienced a marked decrease in reproductivity, thus jeopardizing the supply available for research studies. Also, the decreased life-span of the rabbits has increased the number of replacement rabbits needed and the financial costs. Studies are planned to reveal the pathogenesis of the lesions and, thereby, hopefully preclude their development in the future.

XIV. Clinical Pathology Laboratory and Histopathology Laboratory Support and Collaborative Studies.

The clinical pathology laboratory handled approximately 11,000 requests for hematology and 39,000 determinations for serum biochemistry during the reporting period. The histopathology laboratory processed approximately 10,000 paraffin blocks and 15,000 microslides during the reporting period. These two 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.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OG 6751	2. DATE OF SUMMARY: 81 10 01		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 80 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8a. GROUP HIST'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	AG	224
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2:2			

11. TITLE (Precede with 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	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 81	2.0	25
FISCAL YEAR CURRENT 82	2.0	95

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL, MC
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Division of Pathology
ADDRESS: Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN in U.S. Academic positions)
NAME: MOE, James B. LTC, VC
TELEPHONE: 202-576-2677
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Clifford, Charles B., CPT, VC
NAME: POC: DA

21. GENERAL USE
Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Functional correlation; (U) Exposure factors (U) Pathogenesis (U) Blast overpressure; (U) Vascular permeability; (U) Vascular ultrastructure

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 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 blasts 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. Compare the fragility of the pulmonary vascular bed with that of blood vessels in other organs and tissues throughout the body. Determine the effects of blast injury on other parameters e.g., susceptibility to infectious diseases.

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) 80 10-81 09 Basic pathologic studies of changes in sheep exposed repeatedly to blast overpressure generated in the working area around field artillery weapons were completed and a summary report forwarded. Experiments were initiated to determine the cellular and subcellular bases of blast-induced injury in the conducting airways. Collaborative efforts initiated with other blast overpressure investigators included studying the effects of repeated laporatomy in sheep and developing a suitable model of experimental emphysema. Studies were initiated to utilize small laboratory animals in future blast overpressure experiments. For technical report see Walter Reed Army Institute of Research Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, 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.
Associate: CPT Charles B. Clifford, DVM

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.

Problem and 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.

Results

The pathologic non auditory effects in sheep repeatedly (50 shot) exposured to blast overpressure generated by a field artillery weapon were determined. The range of overpressure exposures included 3.5 pounds per square inch (psi), approximately the maximum exposure received by an artilleryman operating a weapon firing extended range ammunition. Other exposures were 7.5 psi and 15.0 psi. The only changes induced in sheep exposed repeatedly to 3.5 psi were small foci of petechial hemorrhage and minimal focal loss of epithelium in the larynx and trachea (Table I). The hemorrhagic foci were limited, both in extent and number, and were judged to be minor lesions and probably would have resolved quickly. There was only a slightly greater incidence of hemorrhage in the respiratory tracts of sheep exposed repeatedly to 7.5 psi, and the hemorrhage was only slightly more severe on a subjective basis. In the tracheal of one sheep exposed to 7.5 psi, there were small foci in which the surface epithelium was stripped. In sheep exposed repeatedly to 15 psi, airway hemorrhages and epithelial stripping occurred more frequently and were noticeably more severe than in the groups exposed to lower overpressures. Additionally, in the 15 psi groups 12 of 15 sheep had hematomas in the rumen, whereas only 2 of 30 in the 7.5 psi group had ruminal hematomas. There was no microscopic evidence of blast-induced parenchymal lung damage in any sheep exposed repeated to blast overpressures in the range of pressures from 3.5 to 15 psi.

These results suggest that mammalian tissues are relatively resistant to blast-induced damage at 3.5 psi. There was only a slight increase in susceptibility to blast-induced injury at 7.5 psi, while at 15 psi airways and gastrointestinal tracts contained significant evidence of damage. The lung parenchyma was apparently resistant to injury over the entire range of overpressure exposures studied. The only life-threatening lesions were contained in the gastrointestinal tracts of sheep exposed repeatedly to 15 psi of overpressure.

TABLE I

	Exposure Group			
	Controls 0 psi	3.5 psi	7.5 psi	15 psi
Number of Sheep	29	15	30	15
Larynx				
No. with Hemorrhage	2[a]	4[a]	6[a]	14
Trachea				
No. with Hemorrhage	1[a]	2[a]	9[a]	14
No. with Epithelial stripping	0	1	2	7
Gastrointestinal				
No. with Hemorrhage in Rumen	0	0	2	12

a. Hemorrhage in airways in these groups consisted mainly of small, pinpoint foci of blood in the epithelial lining.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6767	81 10 01	DD DR&E/AR(6)5

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'S	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:5			

11. TITLE (Precede with Security Classification Code)

(U) Pathophysiology of Blast Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Stress Physiology 017100 Weapons Effects

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMING 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 (Th. Thousands)
FISCAL YEAR	PREVIOUS 81	1.0	152
	CURRENT 82	1.0	216

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, COL, Phillip K.

TELEPHONE: (202) 576-3551

21. GENERAL USE

Foreign Intelligence not considered

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

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

NAME: GRAEBER, MAJ, Geoffrey M.

TELEPHONE: (202) 576-3791

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

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 creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) in the peripheral serum subsequent to blast injury, more must be known concerning the enzymes distribution in the various parts of the G.I. tract. If a difference in enzyme distribution could be detected, then earlier stages of injury may be able to 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. Important gains are anticipated when the next set of serum samples is analyzed in the next few months.

25 (U) 80-10 - 81-09 Preparatory work with sera obtained from sheep exposed to low level blast injuries showed two important results. a. CPK and its isoenzymes may be altered in sheep secondary to stress and/or exercise. b. Changes in LDH system secondary to the same stimuli are very little. Continuing work in the field of identifying serum isoenzyme markers which can be used as markers of intestinal injury has shown that: a. All three isoenzymes of CPK are located throughout the G.I. tract from the cervical esophagus to the rectum. b. The concentrations of CPK are highest in the seromuscular layer. c. Severe ischemic injury to the colon causes elevations of CPK-MB and CPK-BB in the peripheral serum. Plans are being made currently to study the changes in these peripheral serum isoenzymes secondary to higher level blast exposure. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct. 80 - 30 Sept. 81

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DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NOV 75

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

Work Unit 225 Pathophysiology of Blast Injury

Investigator:
Prinicipal: MAJ (P) Geoffrey M. Graeber, M.C.

Background and Objectives:

Blast overpressure injury has particular predilictions for injuring lung and portions of the gastrointestinal tract. A variety of lesions of varying severity may be seen in the G.I. tract depending on the amplitude and duration of the injuring blast. Organs particularly affected are the stomach and colon. Whether or not these injuries may be detected by monitoring enzymes or other proteins in the peripheral serum is not known. If such were the case, the military physician monitoring these injuries might find particular help in following these enzymes in the peripheral serum of soldiers being treated for blast injuries. If these enzymes were very sensitive, then an early return to duty might be anticipated in soldiers with minimal injury. More severe levels of injury might cause early serum elevations and prompt life-saving surgical intervention.

Publications from our laboratory have demonstrated that severe injury to the small and large bowel can cause changes in peripheral serum creatine phosphokinase and its isoenzymes.[1-3] Similar but less dramatic changes have also been noted in serum lactic dehydrogenase and its isoenzymes.[3] Continuing work along these lines conducted this year has pursed three courses: Delineation of possible new serum markers of gastrointestinal injury, analysis of sheep serum to determine whether minimal injury (exposure) can cause alterations in these serum isoenzyme systems, and identification of the distribution of creatine phosphokinase in the G.I. tract.

Progress:

Delineation of possible new markers of gastrointestinal injury in the peripheral serum focused on the distribution of alkaline phosphatase in the G.I. tract and comparison of the various isoenzymes found in

the different organs with that found normally in serum. Initial results suggest that this isoenzyme system will be most helpful in delineating liver and pancreatic injury. It may have some usefulness in assessing bowel injury, but this is not clear at the present time.

Studies have been conducted in conjunction with the Department of Clinical Physiology, Division of Medicine on sera obtained from sheep that have been subjected to low level blast injuries. So far, this work has shown that minimal exposure to blast injuries does not cause substantial changes in any of the serum isoenzyme systems under study, but that severe exercise may cause elevations of total enzyme content in the peripheral serum. Storage of serum samples appears to have detrimental effects on the isoenzyme content in that certain isoenzymes deteriorate even when stored at -70° Celsius. This finding has important implications for future studies with blast exposed animals.

In a recently conducted experiment, the distribution of creatine phosphokinase within the G.I. tract was determined. This study showed that the vast majority of this enzyme in the G.I. tract is located in the seromuscular layer. Very little of it is located in the mucosal layer. These findings suggest that only severe injury (i.e. transmural injury) is capable of causing enzyme release to the peripheral serum.[4]

Work conducted so far has revealed that the study of peripheral serum enzyme systems have the potential and the distribution to be able to detect and diagnose severe gastrointestinal tract injury. The utility of assessing the extent and severity of blast injury to the gastrointestinal tract has yet to be delineated. Work in this area will be conducted in the next fiscal year.

Recommendations for the Future:

Plans are being made to study the changes in peripheral serum enzyme systems secondary to severe stress and exercise and to exposure to blast overpressures in the range which would be expected to cause moderately severe gastrointestinal tract injury. Three groups of sheep will be studied. One group will have only two hours of severe exercise. They will serve as controls for the two groups of blast exposed animals. One group of experimental animals will be subjected to blast at the LD 15% level; the second group will be exposed to an LD 30% blast. Serum isoenzymes will be taken on all the sheep before the experiment for two days to determine baseline levels. Samples will be collected and assayed three times a day for the four

days after injury or exercise. All the sheep will then be killed and autopsies will be conducted on all of the animals so that an accurate correlation may be conducted between the injuries created and the eneyzme changes seen in the sera of each animal.

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

Work Unit 225 Pathophysiology of Blast Injury

Literature Cited:

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Publications:

See Citations 3 and 4 above.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6768	81 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
80 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. XXXXXXXX	STOG80-7.2:5			

11. TITLE (Precede with Security Classification Code)

(U) Pathophysiologic Studies of Blast Injury to the Gastrointestinal Tract

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016200 Stress Physiology 008800 Life Support

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 81	2.0	116
FISCAL YEAR CURRENT 82	2.0	233

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, PHILIP K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Surgery

ADDRESS: Washington, DC 20012

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

NAME: HARMON, JOHN W., LTC

TELEPHONE: (202) 576-3391

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: LILLEMOE, KEITH D., CPT

NAME:

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Blast injury; (U) Gastrointestinal hemorrhage; (U) Gastrointestinal perforation; (U) Combat Casualty Management

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. 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 Explosive (FAE) Mine Neutralization System.

24(U) Observations of the gastrointestinal results of blast injuries over a range of intensities, 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, respiratory rate and white blood cell count.

25 (U) 80 10 - 81 09 During the prior year the state of knowledge regarding blast injury to the gastrointestinal tract was reviewed and a protocol was prepared to initiate studies using the approach outlined above. We visited the Aberdeen Proving Ground and the Lovelace Facilities for studying blast injuries. We plan to utilize both facilities. Currently we will start a pilot project to develop our methodology for sequential laparotomies in sheep in WRAIR. For technical report see Walter Reed Army Institute of Research Annual Progress report 1 Oct 80 - 30 Sep 81.

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

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-investigator: Keith D. Lillemoe, 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.[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.

Our experimental approach will be to study the gastrointestinal effects of various blast regimens on sheep. Open air explosions and blast tube will be used, with blasts over a range of intensities, durations, and frequencies.

Progress:

During this fiscal year, our first with the mission to study the effects of blast overpressure injury or combat casualties, we have devoted our efforts to developing a data base and an experimental approach. We have visited the facilities for studying blast overpressure at Aberdeen Proving Grounds in Maryland, as well as those of the Lovelace Foundation, in Albuquerque, New Mexico. We have identified facilities at both locations which will be helpful in our future studies.

We have developed a collaborative relationship with the blast overpressure group at WRAIR, which is determining the safe threshold levels of blast exposure for our troops..

They study lower levels of blast overpressure than we will be investigating, but we share an interest in the gastrointestinal effects of blast overpressure injury, and expect to collaborate on both in-house and contract projects.

Recommendations for the future:

A pilot protocol entitled "Evaluation of the feasability and effects of repeated laparotomies on sheep" has been approved by the WRAIR LAIRB. This will allow us to begin a project to assess the natural history of the gastrointestinal lesions which we have previously observed.

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-Investigator: Keith D. Lillemoe, CPT, MC

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6761	81 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	9. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. MO CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CD	228
b. CONTRIBUTING				
c. [illegible]	STOG 80-7..			

11. TITLE (Precede with Security Classification Code) (U) Regulatory Mechanisms and Pathophysiology of Hematopoiesis Application to Military Hematology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
008800 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. 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 81	3.5	197
FISCAL YEAR — CURRENT 82	5.0	137

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL
NAME: Philip K. Russell, COL, MC
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Division of Medicine
Washington, DC 20012

PRINCIPAL INVESTIGATOR (Provide DoAN if U.S. Academic position)
NAME: Daniel G. Wright, MAJ, MC
TELEPHONE: (202) 576-3358
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: William H. Crosby, COL, MC
NAME: August J. Salvado, LTC, MC

21. GENERAL USE
Foreign intelligence not considered.

22. KEYWORDS (Precede 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 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) under military personnel may be exposed to 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) 80 10-81 09 A naturally occurring regulator of erythroid stem cell proliferation and differentiation, Burst Promoting Activity (BPA), has been isolated from mixed spleen cell cultures and its effects upon hematopoietic stem cell growth have been characterized in mice. A reproducible, micro assay for erythropoietin (Ep), an important regulator of red blood cell production, has been developed using cryopreserved rabbit bone marrow cells. A technique for purifying Ep from small amounts of plasma and urine has been developed using HPLC. An oral iron tolerance test has been developed that very sensitively detects subtle iron deficiency states. Techniques for creating cross-species bone marrow chimeras in animals have been developed for studies of marrow growth and structure. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

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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 228 Regulatory Mechanisms and Pathophysiology of Hematopoiesis Application to Military Hematology

Investigators

LTC August Salvado, MC; COL William Crosby, MC; MAJ Pedro Mora-Urdaz, MC; Dr. Michael Sorrell (Fellow, GWU); LTC John Foxley (Visiting Fellow, British Army Medical Corps); MAJ Ramona Chapman, MC: MAJ Daniel Wright, MC; Mr. Harold Williams, GS-13; Ms. Mary Cutting, MS (Fellow, GWU)

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 hematopoiesis precursor cells from human, mouse, and rabbit marrow, using leukemic cell lines, and using allogenic transplantation of bone marrow tissue into mice.

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 Burst Promoting Activity (BPA) and Erythropoietin (Ep) - mediators that regulate the production of red blood cells:

BPA has been found in medium conditioned by pokeweed mitogen stimulated spleen cells. This medium when added to short term suspension cultures of murine

marrow will promote the survival of significant numbers of both pluripotent and committed erythroid stem cells for up to five days. It has also been observed that the morphology of pluripotent spleen colonies in irradiated hosts is altered by the spleen cells conditioned medium. Specifically, a greater number of erythroid to myeloid or megakaryocyte colonies is observed after five day incubation of marrow in the presence of conditioned medium. BPA therefore appears to affect both survival and differentiation of pluripotent cells. In regards to purification, the factor has been partially purified by means of $(NH_4)_2SO_4$ precipitation and a Concanavolin A affinity column. In the first step it is found in the 60% supernatant and in the second step it is bound to the immobilized lectin and eluted with 2 methyl mannose. Further purification is presently underway.

A critical requirement for development of purification techniques effective for small quantities of Erythropoietin (Ep) was the development of a sensitive, reliable and convenient way to analyze multiple samples for Ep activity. A bioassay was developed which was sensitive to 1 milliunit of Ep. The assay utilized rabbit marrow grown in microwells as target cells and was thus capable of analyzing multiple samples. Moreover, the target cells were functional after cryopreservation in 10% DMSO, 90% fetal calf serum. This allows for the convenience and reproducibility of a large pool of cells which could be used for many Ep assays. With this capability, we looked at the possibility of purifying Ep frm sheep plasma or human urine by high performance liquid chromatography (HPLC). We have found that reverse phase HPLC using a C18 column with a mobile phase of 0.1% TFA and a 25-65% gradient of CH_3 CN yielded excellent results. Greater than 50% recovery of activity loaded onto the column is routinely obtained. When fractions are analyzed by discontinuous polyacrylamide slab gel electrophoresis, the peak fractions have 3-4 lightly stained bands using a silver stain technique sensitive to nanogram quantities of protein. The same fractions, however, show no increment above baseline for A^{280} or A^{215} on the chromatograms. We are presently gathering several HPLC runs from a concentrated sample of human urine to pool active fractions and re-chromatograph the material. In

this way we hope to increase the purity and have enough material to quantitate more reliably for specific activity.

2) Studies of Iron Absorption:

An oral iron tolerance test (ITT) has been developed and refined to demonstrate that individuals with normal blood hemoglobin but partially depleted iron stores (blood donors) react to oral doses of iron as small as 10 mg by a significant elevation of plasma iron concentration. Radiotracer experiments indicate that 5 mg of iron is absorbed from both 10 mg and 20 mg carrier doses. Iron replete subjects do not show increases in plasma iron. These studies indicate that it may be possible to study the absorption of "dietary amounts" of iron in normal humans without using radioactive tracers. This oral ITT also is expected to be a useful, sensitive, and clinically accessible technique for detecting very subtle iron deficiency states.

Iron distribution in the crypt region of gut mucosa of mice has been studied histologically. Iron, as demonstrated by the prussian blue staining procedure, is located, in part, in macrophages. These iron-laden macrophages have never been observed to be in direct contact with epithelial cells. Their numbers vary depending upon the animal's iron status. Also present and demonstrable only at the electron microscopic level are deposits of iron at the basal surface of crypt epithelial cells. A portion of this iron is found within and on the surface of fibroblastic cells that partially envelop the intestinal crypts. Iron is also found within the basal lamina and in the basal intercellular spaces between adjacent cryptic epithelial cells. Intraperitoneal injection of iron dextran results in increased amounts of iron within the crypt region within 24 hours. Especially noticeable are increases in amounts of iron in the intercellular spaces at the base of epithelial cells.

These studies are to be extended to study iron distribution in intestinal crypts and villi:

a) At various times after IP injection of iron dextran,

b) At various times after placing mice on an iron free diet,

c) Of normal, iron deficient, and iron overloaded mice,

d) Of neomatal mice whose intestinal epithelium responds as if the animal were iron deficient.

3) Studies of bone marrow structure and development with marrow transplantation:

We have attempted to establish a rat-mouse chimera by providing rat bone marrow to a mouse irradiated with 900r whole body irradiation. The rat marrow has been introduced into the mouse by two different methods: 1) implantation of a core of intact marrow tissue under the skin of the mouse's abdomen, and 2) intraperitoneal injection of a suspension of 10^7 viable marrow cells. We have measured success of the chimera by survival of the mouse and by a positive assay for leukocyte alkaline phosphatase (LAP), an enzyme present in rat granulocytes but not in those of mice. As yet, no mice have survived indefinitely with the evidence of chimerism (i.e. positive LAP). Based on the concept that there may be a critical number of cells necessary to repopulate marrow after whole body irradiation, the procedure has been modified by increasing the size of tissue implanted and increasing the number of marrow cells injected.

Future Plans

1) Studies of the regulation of erythropoiesis (red blood cell production):

Basic studies of erythropoiesis regulators will continue. Studies with BPA will continue to focus upon its purification and its effects upon pluripotential stem cells. We plan to pursue BPA purification using HPLC techniques. We also plan to study the influence of BPA upon stem cell survival after exposure to ionizing radiation. Purification of Ep will continue with hopes of establishing preparative techniques. Effects of purified Ep on stem cell growth and survival can then be assessed.

2) Studies of iron absorption:

There will be continued evaluation of the oral ITT in normal human volunteers with and without prior blood donation. The accuracy of this technique will be assessed by comparisons with radiotracer studies of iron absorption. The technique will then be applied to the clinical evaluation of iron deficiency states in selected patient populations.

Ultrastructural studies of iron deposition in the intestinal mucosa under differing conditions of iron repletion or deficiency will be carried out.

3) Studies of bone marrow structure and development

Attempts to establish bone marrow chimeras will be contiued in rodents with the ultimate goal of being able to study human bone marrow structure and function once established in an animal host.

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21. Sorrell J.M. and L. Weiss: A light and electron microscopic study of the region of cartilage resorption in the embryonic chick femur. Anat. Rec., 198:513, 1980.

22. Sorrell J.M. and L. Weiss: Development of the embryonic chick phagocytic system. Intraembryonic erythrophagocytosis induced by phenylhydrazine (in press), J. Morphol., 1981.

23. Sorrell J.M. and L. Weiss: The cellular organization of fibroblastic cells and macrophages at regions of uncalcified cartilage resorption in the embryonic chick femur as revealed by alkaline and acid phosphatase histochemistry. Anat. Rec. (in review), 1981.

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27. Chapman R.M.: Effect of cytotoxic therapy on sexuality and gonadal function. (Article submitted by invitation), Seminars of Oncology, (in press), 1981.

28. Chapman R.M., Sutcliffe S.B.: Protection of ovarian function by oral contraceptives in women receiving chemotherapy for Hodgkin's disease. Blood, (in press), 1981.

29. Sutcliffe S.B., Chapman R.M.: Pregnancy in lymphomas and leukemias, in Pregnancy and Cancer (by invitation), (in press), 1981.

30. O'Neil-Cutting M.A., Bomford A. and Munro H.N.: Effect of excess dietary zinc on tissue storage of iron in the rat. J. Nutr., 1981 (in press).

31. Johnson D.J., Williams H.L., Slater S., Haut M.J. and Altstatt L.B.: The in vitro effect of selected enviromental toxicants on two heme synthesis enzymes. J. Environmental Pathology and Toxicol., 1981 (in press).

32. Williams H.L. and Johnson D.J.: Hematopoietic toxicity of selected aromatic compounds. Proceedings of 7th Annual Meeting of National Organization for the Professional Advance of Black Chemists and Chemical Engineers, 1981.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6762	81 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
80 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	61102A	3M161102BS10	EB	229
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.211			

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 81	3.0	259
	CURRENT 82	3.0	118

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
Washington, DC 20012
ADDRESS:

RESPONSIBLE INDIVIDUAL
NAME: Philip K. Russell, COL, MC
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Medicine
ADDRESS: Washington, DC 20012

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)
NAME: Daniel G. Wright, MAJ, MC
TELEPHONE: (202) 576-3358
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Drs. Lucas, Mora, Schoomaker, Alving
NAME: Salvado, Crosby, and Kark

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Coagulation; (U) Hematopoiesis; (U) Blood; (U) Marrow Failure; (U) Erythrocytes; (U) Leukocytes; (U) Sickle Cell Trait

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 modalities to restore hemostasis, to augment host defense systems against infection; to evaluate the pathophysiology of sickle hemoglobin (Hgb S) in military personnel with sickle trait. 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 environmental, 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) 80 10-81 09 Assays for measurement of changes in the kallikrein-kinin system in humans and animals have been developed and have been used to predict the best animal models for study of this plasma protein system in shock syndromes. Studies of the effects of coumadin on coagulation proteins and of L-Asparaginase on fibrinogen synthesis and turnover were undertaken. A clinical study of the therapeutic potential and toxicities of intravenous immunoglobulin preparations has been begun. Interactions of human neutrophils with monocytes in acute inflammation via secreted, soluble mediators have been done. Characterization and quantification of acetylcholine receptors on blood and exudate neutrophils, and on neutrophil precursor cells have been accomplished. Studies of the leukemia cell line, HL60, have shown that production of guanosine nucleotides may be important in the regulation of myeloid cell maturation. Vitamin B6 compounds, pyridoxal and pyridoxal-phosphate, have been shown to modify Hgb S and to have potent antisickling effects. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

*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 69 (FOR ARMY USE) ARE OBSOLETE

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

Work Unit 229 Military Hematology

Investigators

MAJ Daniel G. Wright, MC; MAJ Barbara Alving, MC; Dr. Diane Lucas, GS-12; LTC John Kark, MC; Mr. Charles Barr, GS-12; MAJ Phil Baldwin, MC (WRAMC); MAJ Pedro Mora-Urdaz, MC; COL William Crosby, MC; LTC August Salvado, MC; MAJ Eric Schoomaker, MC

Description

Three distinct research tasks have been carried out under this work unit during FY81. (Three additional research tasks that were part of this work unit during FY80 have been continued under individual work units and are described elsewhere.)

1. Studies of coagulation and plasma proteins: Blood coagulation factors, blood platelets, and plasma proteins (e.g. the kinin-kallikrein, fibrinolytic 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 intravenous immunoglobulins that have military relevance.

2. Studies of blood phagocytes: Phagocytic blood leukocytes are critical to host defenses 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 and utilization of these phagocytes in peripheral tissues.

3. Studies of sickle cell disease and other hemoglobinopathies:

Although congenital diseases resulting from abnormal hemoglobin or disordered hemoglobin production are very rare among military personnel (e.g. sickle cell anemia, thalassemia major), military personnel may carry the trait for these diseases which can predispose these individuals to health risks if they are exposed to unusual or physically stressful circumstances (e.g. high altitude or low oxygen environments) in the course of military activities. Studies have been begun that concentrate upon the pathophysiology of disease associated with "sickle" hemoglobin (Hbg S) and other abnormal hemoglobin variants, and upon the effects of unusual environmental conditions on individuals with sickle trait (normally healthy carriers of an Hbg S gene).

Progress

1) Studies of coagulation and plasma proteins

Assays to measure changes in the kallikrein-kinin system that occur in humans or animals exposed to vasoactive agents or to the stress of acute trauma have been developed. These assays have been used to predict the most appropriate animal models for studies of the kallikrein-kinin system in shock that are relevant to human pathophysiology.

Studies of the effects of commonly used drugs on coagulation have also been undertaken. Identification of a coumadin-resistant patient at WRAMC stimulated the development of an HPLC assay for measurement of coumadin plasma levels. This assay has been used to correlate drug levels with prothrombin time measurements in a patient population undergoing chronic coumadin treatment. The observation that the drug L-Asparaginase (used in treatment of leukemia) causes hypofibrinogenemia prompted the development of an animal model using rabbits for studying the effects of this drug on fibrinogen synthesis and catabolism.

A protocol for study of the hazards and efficacy of intravenous immunoglobulins in a population of hospitalized, immunosuppressed, neutropenic patients

has been approved and initiated. This double-blind clinical trial represents a first step in ultimately evaluating the use of intravenous immunoglobulin preparations to treat serious, militarily relevant infections.

2) Studies of blood phagocytes

Various studies of human blood neutrophils and monocytes have been carried out relevant to their function in inflammatory responses. Purified secondary granule protein has been shown to stimulate monocyte motility in a variety of assay systems. This neutrophil product has chemokinetic rather than chemotactic effects upon monocytes. It is secreted from human neutrophils under experimental conditions that promote exocytosis of neutrophil secondary granules. In the development of normal inflammatory responses, this neutrophil secretory product may promote the participation of monocytes in the response and may influence monocyte functional transformations.

Cholinergic stimuli have been shown to modulate various functions of human blood phagocytes important in their various host defense activities. Studies of human neutrophils have identified a high affinity muscarinic receptor for acetylcholine. Numbers of these receptors on the surface of peripheral blood neutrophils are equivalent to or exceed those reported for chemotactic factor receptors. Receptor numbers are decreased in exudate neutrophils compared to cells purified from peripheral blood. Receptor numbers are most numerous on immature neutrophils recovered and purified from bone marrow aspirates, suggesting that cholinergic stimulation in the marrow (a highly ennervated organ) may have a role in the maturation or mobilization of neutrophils in the marrow.

Techniques have been developed to purify human blood monocytes and selected marrow myeloid precursor cells using counter-flow elutriation.

Studies of the human promyelocytic cell line, HL-60, have been carried out to analyze purine metabolism by these cells as they are maintained in continuous tissue culture and as they undergo induced, terminal maturation to cells resembling peripheral blood phagocytes. These studies have demonstated that the

production of guanisine nucleotides through salvage purine synthesis may have important regulatory influences upon the terminal maturation of immature myeloid cells.

3) Studies of sickle cell disease and other hemoglobinopathies

Expert consultation has been provided to the Dept. of Defense and to the Office of The Surgeon General, U.S. Army, concerning sickle trait in military personnel. Laboratory studies have been directed towards defining the effects of Vitamin B_6 compounds upon sickle hemoglobin and upon intact red blood cells from patients with sickle cell anemia. It has been shown that the natural aldehyde forms of B_6, pyridoxal and pyridoxal phosphate, are potent anti-sickling agents. These non-toxic compounds may have a role in preventing red blood cell sickling in soldiers with sickle trait who by assignment necessity may be exposed to hypoxia or heat stress during performance of duties.

Future Plans

1) Studies of coagulation and plasma proteins

a) Studies of the kallikrein-kinin and coagulation systems in patients with hereditary deficiency of C1-inhibitor to determine if bradykinin has a role in the onset of symptoms in the unusual disease that may in some ways be analogous to shock syndromes associated with acute infections.

b) The fibrinolytic activity found in urine of patients with idiopathic hematuria will be studied to determine if measurement of urinary urokinase may be used as a marker for this disorder or for certain physical stress syndromes associated with hematuria.

c) Studies will also be carried out with sera obtained from patients with selected diseases in which anti-coagulant antibodies against phospholipids are present. It is anticipated that lipisomes of differing compositions may be designed as a consequence of these studies that may be used in sensitive tests of certain disease states.

2) Studies of blood phagocytes

a) The neutrophil secondary granule protein shown to influence monocyte mobility and maturation will be further characterized and purified. This protein will be evaluated as a natural adjuvant that promotes or modulates antigen recognition and processing by macrophages.

b) The effects of cholinergic stimulation on the proliferation and maturations of myeloid precursor cells will be evaluated.

c) Continued studies of the induced maturation of HL-60 cells and the regulatory role of purine and pyrimidine synthesis upon this process.

d) Studies of the effects of extracellular lipid environments as influenced by diet or starvation upon normal phagocyte host defense functions will be begun.

3) Studies of sickle cell disease and other hemoglobinopathies

a) Our laboratories will participate in interagency studies of sickle trait in military personnel and will continue to serve as expert consultants to the SGO concerning this subject.

b) Studies of the effects of B_6 compounds upon Hgb S and upon red cell sickling phenomena will be continued.

c) Studies of the pathophysiology of sickling in sickle trait under conditions of hypoxia and low atmsopheric pressure will be begun.

Abstracts and Presentations at National and International Meetings

1) Coagulation and Plasma Proteins

1. Alving, B.M., Imanari, T., Mason, B.L., Tankersley, D.L. and Pisano, J.J.: Determination of plasma prekallikrein by direct activation with Hageman factor fragment. Clin. Res. 29:328A, 1981.

2. Tankersley, D.L., Alving, B.M., Mason, B.L. and Finlayson, J.S.: Properties of an altered plasma kallikrein purified from Cohn fraction IV-I. Clin. Res. 29:350A, 1981.

3. Alving, B.M., Tankersley, D.L., Mason, B.L. and Finlayson, J.S.: Altered properties of human kallikrein purified from Cohn fraction IV-I. Thrombos. Haemostasis 46(1):230, 1981.

4. Tankersley, D.L., Alving, B.M. and Finlayson, J.S.: Activation of Factor XII by dextran sulfate: a convenient assay for Factor XII. Thrombos. Haemostasis 46(1):231, 1981.

2) Blood Phagocytes

1. Wright, D.G., Meierovics, A.I., Richards, R.L., and Alving, C.R.: Studies of cytoplasmic granules in human neutrophils: Differences in the membrane phospholipid content of azurophil and specific granules. Fed. Proc. 40:375, 1981.

2. Wright, D.G. and Meierovics, A.I.: Assessing the numbers of neutrophils supplied to tissues: Studies with neutrophils recovered from mouthwash specimens. Clin. Res. 29:353A, 1981.

3. Lucas, D.L., Webster, H.K., and Wright, D.G.: Purine metabolism in myeloid precursor cells during maturation: Studies with the HL-60 cell line. Blood (in press), 1981.

4. Wright, D.G.: Leukocyte Transfusions, presented at the annual meeting at the National Blood Club, San Francisco, Calif., April 26, 1981.

3) Sickle Cell Disease and other Hemoglobinopathies

1. Hannah, J.S., Kark, J.A., Goodman, A., Agamanolis, D.P., Hines, J.D., and Harris, J.W.: Altered central nervous system lipids in experimental vitamin B_{12} deficiency. Presented to Midwest AFCR, Chicago, November 1980. Clin. Res. 28:728A, October 1980.

2. Kark, J.A., Tarassoff, P.G., Hicks, C.U., and Bongiovanni, R.: Contrasting mechanisms of the antisickling activity of pyridoxal and pyridoxal phosphate. Clin. Res. 29:337A, 1981.

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4. Kark, J.A., and Hicks, C.U.: Enchanced permeability of membrane anion channels in sickle erythrocytes. Blood (in press), 1981.

Articles Published, In Press, or In Review

1) Coagulation and Plasma Proteins

1. Alving, B.M., Tankersley, D.L., Mason, B.L., Rossi, F., Aronson, D.L., and Finlayson, J.S.: Vasoactive enzymes in immunoglobulin preparations. In Immunoglobulins: Characteristics and uses of intravenous preparations. Alving, B.M. and Finlayson, J.S., editors. (DHEW Publications No. (FDA)-80-9005). Washington, DC, US Government Printing Office, 1980.

2. Tankersley, D.L., Alving, B.M., Yi, M., Blou, M.G., Mason, B.L. and Finlayson, J.S.: Predictive tests for fragmentation of immune globulins. In Immunoglobulins: Characteristics and uses of intravenous preparations. Alving, B.M. and Finlayson, J.S., editors. (DHEW Publication No. (FDA)-80-9005). Washington, DC, US Government Printing Office, 1980.

3. Alving, B.M., Tankersley, D.L., Mason, B.L., Condie, R.M. and Finlayson, J.S.: Biologic activities of enzymic contaminants in immunoglobulin preparations. Proceedings of a conference on Immunohemotherapy, Interlaken, August 24-26, 1981 (in press),
Academic Press.

4. Marks, E., Alving, B., and Pisano, J.J.: Plasma prekallikrein and the hypotensive response of the Brown Norway rat to kinin-generating agents. Experientia (in review).

5. Tankersley, D.L., Alving, B.M., and Finlayson, J.S. Preparation of B-XIIa (Hageman Factor Fragment) from Human Plasma. Thrombosis Research (in review).

6. Spencer, C.D., Crane, F.M., Kumar, J.R., and Alving, B.M.: Treatment of postpartum hemolytic uremic syndrome with plasma exchange. Ann. Int. Med. (in review).

2) Blood Phagocytes

1. Wright, D.G.: The activation and deactivation of neutrophils. In: The Biochemistry and Physiology of Acute Infections, Powanda and Canonico, eds., North-Holland, Amsterdam, 1981.

2. Wright, D.G.: The neutrophil as a secretory organ of host defense. In: Advances in Host Defense Mechanisms, Raven Press, N.Y., 1981.

3. Wright, D.G., Karsh, J., Fauci, A.S., Klippel, J.H., Decker, J.L., O'Donnell, J.F. and Deisseroth, A.B.: Lymphocyte depletion and immuosuppression with repeated leukapheresis by continuous flow centrifugation. Blood 58:451-458, 1981.

4. Wright, D.G., Fells, G.A., Gadek, J.I., Kelman, J.A., Gallin, J.I. and Crystal, R.G.: Extracellular release by human neutrophils of a latent collagenase: A differentiation of collagenases stored in the neutrophil primary and secondary granules, 1981 (in review).

5. Wright, D.G., Robichaud, K.J., Pizzo, P.A. and Deisseroth, A.B.: Lethal pulmonary reactions associated with the combined use of Amphotericin B and leukocyte transfusions. N. Eng. J. Med. 304:1185-1189, 1981.

6. DeShazo, R.D., Hase, T., Diem, J.E. and Wright, D.G.: Evidence for histamine mediated inhibition of monocyte chemotaxis in atopic dermatitis. 1981 (in review).

7. Harlan, J.M., Killen, P.D., Harker, L.A., Striker, G.E. and Wright, D.G.: Neutrophil mediated endothelial injury *in vitro*: Mechanisms of cell detachment. J. Clin. Invest., 1982 (in press).

8. Lucas, D.L., Dragsten, P., Robinson, D.M. and Bowles, C.A.: Increased lateral diffusion of a lipid probe in the plasma membrane of elicited macrophages. J. Reticuloendothelial Soc. 30:107, 1981.

3. Sickle Cell Disease and Hemoglobinpathies

1. Kark, J.A., Haut, M.J., Hicks, C.U., McQuilkin, C.T., Reynolds, R.D.: A rapid flurometric assay for erythrocyte pyridoxal kinase. Biochemical Medicine (in press), 1981.

2. 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 (in press), 1981.

3. Kark, J.A., Haut, M.J., Schechter, G.P., McQuilkin, C.T., Duffy, T.P., and Vigersky, R.A.: The biochemical response to vitamin B_6 in sideroblastic anemia (RSA). I. Pyridoxal 5'-phosphate. Blood Cells (in press), 1981.

4. Kark, J.A., Haut, M.J., Schechter, G.P., Hicks, C.U., 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), 1981.

5. Kark, J.A., Tarassoff, P.G., and Bongiovanni, R. In vitro inhibition of sickling by pyridoxal 5'-phosphate (in review), 1981.

PROJECT 3M263750A808

DRUG AND VACCINE DEVELOPMENT

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6481	81 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
80 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	3M263750A808	AA	001
b. [redacted]				
c. [redacted]	CARDS 1411A			

11. TITLE (Procede with Security Classification Code)

(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: NA 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 81	3.0	192
	CURRENT 82	3.0	178

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, COL P.
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of Experimental Therapeutics
ADDRESS: Washington, DC 20012

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

ASSOCIATE INVESTIGATORS
NAME: COSGRIFF, LTC T.
NAME: BOUDREAU, MAJ E.

21. GENERAL USE

Foreign intelligence not considered

POC: DA

22. KEYWORDS (Procede 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. Procede 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 individual 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 subject 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) 80 10 - 81 09 Studies were performed to determine the lowest dosage regimen of WR 171,669 that produced a 100% cure rate in twelve volunteers experimentally infected with Plasmodium falciparum Smith strain. A split dose of 1500 mg administered as 1000 mg at 0 hours and 500 mg at 6 hours produced a cure in all 5 treated subjects. Three patients continued to receive WR 638 for cystinosis in a study performed in conjunction with the National Institutes of Health. A pharmacokinetic comparison of a capsule and tablet formulation of WR 180,409 revealed no significant differences in pharmacokinetic parameters between the two formulations. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE.

Project 3M263750A808 DRUG AND VACCINE DEVELOPMENT

Work Unit 001 Phase II Antimalarial Drug Trials

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
Associate: LTC C. Pamplin, LTC T. Cosgriff, COL C. Canfield, MAJ E. Boudreau, COL R. Dimond, LTC B. Schuster, Dr. L. Fleckenstein, 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.

WR 171,669 was tested for efficacy versus blood-induced Plasmodium falciparum (Smith) infections. Twelve volunteers were studied in order to define the lowest dosage regimen that would produce a 100% cure rate. The oral dose levels were:

WR 171,669 Rx Levels	Number of Subjects
1000 mg single dose	3
1500 mg single dose	4
1500 mg split dose	5

The split dose regimen consisted of a 1000 mg dose at 0 hours followed by a 500 mg dose at 6 hours. Recrudescence was observed in volunteers from both single dose treatment groups. All five subjects receiving the split dose were treated successfully with no recrudescence after 28 days. The average parasite clearance time for the split dosage regimen was 56.2 hrs and the average fever clearance time was 64.6 hrs.

WR 638 is being studied as a treatment regimen for cystinosis in conjunction with the Institute of Child Health and Development at the National Institutes of Health. Currently three

patients receive WR 638 on a daily basis. The study was initiated with one patient in January 1980 and he currently is receiving a daily dose of 70 mg/kg. The second patient has received treatment since September 1980 and has reached a daily dose of 100 mg/kg. The third patient was started on WR 638 in February 1981, and is currently receiving 150 mg/kg/day. Only the first patient shows signs of poor compliance, however this is probably not attributable to drug intolerance as he had poor compliance with other therapeutic regimens.

The pharmacokinetic profile of two formulations of WR 180,409 was assessed in twelve volunteers. A dose of 750 mg was administered either as a capsule or tablet in a cross-over study. The results were tabulated from the eight subjects completing the entire study. Absorption half-lives were 0.088 ± 0.037 days for tablets and 0.087 ± 0.037 days for capsules. The elimination half-lives were 7.01 ± 1.85 days and 6.13 ± 1.52 days for tablets and capsules, respectively. Bioavailability parameters for the tablets and capsules were, respectively: area under the curve, 3.13 ± 0.82 µg days/ml and 2.89 ± 0.57 µg days/ml; measured peak concentrations 0.367 ± 0.90 µg/ml and 0.359 ± 0.079 µg/ml; and measured peak times, 0.48 ± 0.18 days and 0.43 ± 0.27 days.

3. Future Goals.

Efficacy studies on WR 171,669 will be completed. Studies will be initiated to establish the efficacy of WR 180,409. The three patients with cystinosis currently receiving WR 638 will be continued on WR 638 during the next year.

4. Publications.

1. Fleckenstein, L., Pamplin, C., von Bredow, J., Heiffer, M. and Canfield, C.: Bioavailability and pharmacokinetics of a new antimalarial drug, WR 180,409. Drug Intel. Clin. Pharmacol. 15:[illegible], 1981.

2. Kitihara, M., Cheng, D. and Cosgriff, T.: Adult Wiskott-Aldrich syndrome. Amer. J. Med. Sci., Aug-Sep 1981.

3. Cosgriff, T.M. and McCloskey, D.W.: Hodgkin disease as the terminal malignancy in Richter syndrome. Med. Ped. Oncol. 9:265-272, 1981.

4. Cosgriff, T.M. and Martin, B.A.: Low functional and high antigenic anti-thrombin III level in a patient with the lupus anticoagulant and recurrent thrombosis. Arth. Rheuma. 24(1):94-96, 1981.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2527	81 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
80 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	63750A	3M263750A808	AC	002
b. CONTRIBUTING				
c. XXXXXXXXX CARDS 1411A				

11. TITLE (Precede with Security Classification Code)

(U) Evaluation of New Antiparasitic Drugs and Vaccines in the Tropics

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 81	6.0	850
FISCAL YEAR CURRENT 82	6.0	1,101

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, P.K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: US Army Medical Component, AFRIMS

ADDRESS: Bangkok, Thailand

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

NAME: BENENSON, M.W., LTC

TELEPHONE: (02) 281-7776

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS POC:DA

NAME: DIXON,K.E.,LTC;GILBREATH,M.J.,CPT;

NAME: HARRISON,B.A.,LTC;WHITMIRE,R.E.,LTC

21. GENERAL USE

Foreign Intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Malaria; (U) Mefloquine; (U) Fansidar; (U) Monkey; (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 objective of this task is to establish the efficacy of new drugs for both prophylaxis and treatment of tropical infectious diseases of military importance. Particular emphasis is placed on malaria, a disease of worldwide endemicity and resistance to conventional drugs, which continues to cause high attack rates (up to 50%) in unprotected troops. The effect of conventional and experimental antimalarials in treatment, prophylaxis and transmission of drug resistant falciparum malaria will be determined.

24. (U) Army investigational antimalarial drugs are compared with standard drugs and new combinations of standard drugs in the treatment and prophylaxis of drug resistant falciparum malaria in hospitalized human volunteers. Advanced development and field testing of new techniques supporting this task will be accomplished. Candidate antimalarial drugs will be evaluated using simian malaria, as a model for human malaria.

25. (U) 80 10-81 09 Field studies have documented the continued failure of Fansidar in the treatment of falciparum malaria in areas of Thailand. New treatment regimens with a Quinine-Tetracycline combination are being investigated in hospitalized volunteers with naturally occurring malarial infections. These have shown that the combination is very effective and presently the minimum length of treatment necessary for acceptable cure rates is being determined. Mefloquine continued to show excellent results in therapy, but of concern was the documentation of at least two cases of mefloquine resistant malaria in study subjects. Studies on the cynomologus monkeys for a model for the malaria drug screening program were disappointing. The establishment of a functioning cynomologus and rhesus breeding colony was begun with excellent initial success. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITION OF THIS FORM ARE OBSOLETE

PROJECT 3M263750A808 DRUG AND VACCINE DEVELOPMENT
Work Unit 002: Evaluation of New Antiparasitic Drugs and Vaccines in the Tropics
PRINCIPAL INVESTIGATORS: LTC M.W. Benenson,MC; LTC K.E. Dixon, MC: LTC R.E. Whitmire,VC; LTC B.A. Harrison,MSC; LTC D.S. Burke,MC; LTC P.E. Echeverria,MC; LTC G.S.Ward,VC; MAJ R.G.Andre,MSC; CPT M.J. Gilbreath,MSC; CPT M.A. Ussery,MSC; CPT T.A. Klein,MSC; CPT R.R. Graham,VC; MG P. Phintuyothin,MC,RTA (Ret); A.Nisalak, MD
ASSISTANT INVESTIGATORS: SFC A.L. McFarland; SSG P.K. Gandhi; SSG F.Wilson.

1. Treatment of Falciparum Malaria in Thailand with Fansidar

PROBLEM: Falciparum malaria is becoming increasingly resistant to Fansidar (sulfadoxine-pyrimethamine) in Thailand. In studies last year at the AFRIMS study sites in Chantaburi and Phrabuddhabat, Fansidar had cure rates of 9 percent (3/33) for the two tablet dose and 19 percent (6/31) for the three tablet dose. Most of the resistant cases were classified RII. The study was continued at the Phrabuddhabat site to further evaluate the three tablet regimen.

PROGRESS: Eight patients were treated with Fansidar between 25 September 1980 and 21 April 1981, when this regimen was stopped. Two patients had an RI response and six had an RII response. None were cured. However, in all eight cases there was a significant reduction of parasitemia and alleviation of symptoms. Mean fever clearance time was 71 hours.

RECOMMENDATIONS: No further research is planned with Fansidar. Further research is planned with alternate single-dose therapies for falciparum malaria. Evaluation of mefloquine will continue and a new project to evaluate WR171669, a phenanthrene methanol, will be initiated in January 1982.

2. Treatment of Falciparum Malaria in Thailand with a Combination of Quinine and Tetracycline

PROBLEM: In Thailand, P. falciparum has long been resistant to the 4-aminoquinolines, is becoming increasingly resistant to quinine, and within the last two years has become resistant to Fansidar. Various combinations of quinine and tetracycline were tested in the early 1970's and were effective. It was decided to re-examine the effectiveness of this combination against the current, more resistant strains of P. falciparum.

PROGRESS: Between July 1980 and March 1981, soldiers of the 1st Division, 1st Army Area, Royal Thai Army who presented with malaria at the medical treatment center in Sa Kaeo were treated with either quinine alone (650 mg. tid x 10 days) or quinine at the same dose for seven days plus tetracycline started concurrently at a dose of 250 mg. qid for 10 days. Twenty-seven patients treated with quinine alone completed the 28 day follow-up period, with a cure rate of 63 percent (17/27). The ten treatment failures all showed an RI pattern of resistance. Fifty-six patients treated with the quinine tetracycline combination completed the 28 day follow-up period with a cure rate of 96 percent (54/56). The two treatment failures were both classified as RI. A further 26 patients were treated with a seven day course of quinine-Tetracycline and all were cured.

RECOMMENDATIONS: A project is currently underway at Sa Kaeo to further reduce the course of treatment with quinine and tetracycline. A second study will be started in January 1982, to investigate the pharmacokinetics of quinine and tetracycline when the combination is used in acutely ill malaria patients.

3. Treatment of Falciparum Malaria in Thailand with Mefloquine

PROBLEM: With the advent of widespread resistance to Fansidar in Thailand, it becomes increasingly important to find alternative drugs which are effective in a single dose and thus suitable for outpatient treatment programs. Mefloquine has proven itself effective in a single dose in over 95 percent of patients treated. There have been no fully documented cases of mefloquine resistance when the patient has received 1500 mg of the drug. The Department of Medicine at AFRIMS continues to monitor the effectiveness of mefloquine in the rapidly changing drug resistance patterns of Thailand.

PROGRESS: Twenty of twenty-two patients treated with 1500 mg of mefloquine between September 1980 and September 1981 were available for the complete 28 day follow-up period. All but one were cured. Mean initial parasitemia was 21,220, parasite clearance time was 81 ± 68 hrs ($\bar{x}$ ± 2 S.D.) and fever clearance time was 30 ± 38 hrs. ($\bar{x}$ ± 2 S.D.). The single treatment failure had an initial parasitemia of 4,089, but took 147 hours to clear his parasitemia and then relapsed on day 20. A macro *in vitro* test showed no evidence of resistance and the patient responded promptly to a second dose of mefloquine. The patient left the area and was not available for further follow-up. His serum will be tested for mefloquine levels.

RECOMMENDATIONS: The effectiveness of mefloquine will continue to be monitored and will be compared to that of a new antimalarial drug, a phenanthrene methanol designated WR171669.

4. Treatment of Falciparum Malaria in Thailand with Quinine and a Combination of Quinine and Primaquine

PROBLEM: Although quinine is still a useful drug in the treatment of falciparum malaria, especially when the parasite becomes resistant to the newer antimalarials, resistance to quinine itself seems to be increasing. Last year at the AFRIMS study site at Sa Kaeo in eastern Thailand, only 17/27 (63%) of cases treated with 650 mg of quinine tid for 10 days were cured, the rest showing an RI pattern of resistance. Further studies were done this year at Phrabuddhabat in central Thailand.

PROGRESS: During the period September 1980-June 1981, 18 patients were treated with 650 mg quinine sulfate tid for seven days and 17 were treated with the same regimen to which primaquine, 15 mg per day for five days was added. Of the 18 patients treated with quinine alone, 15 were available for the full 28 day follow-up period. Nine of these (60%) were cured, six had an RI resistance and one may have been reinfected. Of the 17 patients treated with quinine and primaquine, 16 were available for the 28 day follow-up period. Only three were cured (19%) while ten were resistant (RI) and three may have been reinfected. In July 1981, the course of quinine was increased to ten days. The cure rate with quinine alone improved to 75% (6/8) with one RI failure and one possible reinfection, while all six patients treated with the quinine-primaquine regimen were cured. For all patients treated with quinine alone, the initial parasitemia was 20,621 ± 54,952 (all figures represent mean ± 2 S.D.), the parasite clearance time (PCT) was 90 ± 50 hours and the fever clearance time (FCT) was 35 ± 35 hours. For all patients treated with quinine plus primaquine, the initial parasitemia was 19,188 ± 32,462, the PCT was 91 ± 68 and the FCT was 39 ± 44. Gametocytemia cleared more quickly when primaquine was given. With quinine alone, 13/26 (50%) of patients had gametocytes on day three and 17/26 (65%) on day five. With the addition of primaquine, gametocytes were found in only 10/23 (43%) of patients on day three and 2/23 (9%) on day five.

RECOMMENDATIONS: Quinine still remains a useful drug in the chemotherapy of malaria, but resistance to it is increasing. Much remains to be learned about the pharmacokinetics of quinine and its interaction with other drugs in patients with malaria. Two new projects will be initiated in January 1982 to study the pharmacokinetics of quinine and a combination of quinine and tetracycline in patients with falciparum malaria.

5. In Vitro Response of Plasmodium falciparum to Chemical Constituents Isolated from Thai Medicinal Plants

PROBLEM: In 1950, clinical trials (1) on the treatment of P. vivax and P. falciparum infected patients with preparations of various Thai medicinal plants confirmed the schizonticidal effect of a number of these plants. To date there have been no further reports published concerning the antimalarial activity of the components or the chemical characterization of their active substances. In collaboration with local Thai researchers we are attempting to identify and chemically isolate components of Thai Medicinal Plants exhibiting an inhibitory effect on in vitro growth of Plasmodium falciparum. In addition, compounds which show activity will be used in various cellular assays (mitogenesis, mixed lymphocyte cultures) to investigate the effect of these substance on the immune response of human lymphocytes.

PROGRESS: An evaluation of *in vitro* effects of the plant extracts on the freshly collected *P. falciparum* is in progress. Further chemical isolation of the different constituent will be performed on the plant extract exhibiting the activity that inhibits parasite growth.

RECOMMENDATIONS: An investigation was carried out to examine the effect of chemical extracts of five different Thai medicinal plants on the *in vitro* development of *P. falciparum* isolated from naturally infected patients. Different concentrations of the crude water and alcoholic extracts were added to the culture medium. A control with ethanol at the concentration introduced with the plant preparations was included in each experiment. An evaluation of the effect on the intraerythrocytic development of asexual parasites was made by microscopic examination of stained blood smears after 24 and 48 hours incubation. The schzonticidal effects of test substances were compared with that of chloroquine and quinine. The result showed that ethanol at the concentration introduced in the culture exerted no effect on normal development of the parasites. Schizonticidal effect of the plants was demonstrable by the alcoholic extracts except *Morinda corcia*, this effect was detected only with high concentration of water extract (170.12 ug/ml). The schizonticidal effect of alcoholic root extract of *Erycoma longifolia* was as potent as the alcoholic stem bark extract of *Cinchona succirhubra* (1.68 ug/ml). The alcoholic extracts of *Oroxylum indicum* and *Tinospora rumphii* exerted the same effect at a higher concentration than that of *Cinchona succirhubra*, (565.56 and 360.25 ug/ml respectively). Further chemical analysis was carried out on *Eurycoma longifolia*. Five different fractions of various quantities were obtained from a combined solvent extraction and chromatographic technique. Among these five fractions, only one was obtained in sufficient yield for study. An intensive chemical study was made on this fraction and a purified lactone was identified and isolated. The study of *in vitro* effect of Eurycomalactone and other isolated fractions from this plant on the development of asexual *P. falciparum* is in progress.

REFERENCES:

1. Ketusinh, O. Report on Experimental Anti-Malarial Therapy of Thai Medicinal Plants. Proceedings of the Siriraj 60th Anniversary Meeting, 271-281, April 1950.

6. Antimalarial Drug Testing

PROBLEM: To test candidate drugs for antimalarial activity using a primate model.

PROGRESS: During FY 81 radical curative antimalarial screening tests were completed on eight compounds. Screening tests were initiated on eight new compounds. Fifty-one rhesus and two cynomolgus monkeys were utilized. Indian rhesus monkeys became unavailable and testing was discontinued during the spring and summer. During late FY 80 and early FY 81 an attempt to produce a consistant and adequate infection for a new antimalarial drug testing model in Macaca fasicularis (Cynomolgus monkeys) was not successful. Eight young cynomolgus monkeys were used. Intravenous sporozoite injections into both intact and splenectomized animals were made. Infections, when established, resulted in extremely low parasite counts and inconsistant relapses. This model was determined to be unsatisfactory for drug efficacy testing.

RECOMMENDATIONS: Continued testing should be done. Recently twenty-nine young male rhesus monkeys were purchased from Davis Primate Center and shipped to AFRIMS. The Plasmodium cynomolgi infection has been reestablished and antimalarial compound testing will continue at a decreased level in FY 82. More primates should be purchased and shipped to AFRIMS to begin to decrease the backlog of candidate drugs waiting to be tested.

7. Primate Breeding Colony

PROBLEM: The Indian moratorium on exporting rhesus monkeys three years ago has led to a lack of research animals and has adversely affected the malaria drug screening program. To provide animals a primate breeding program was initiated at AFRIMS this year.

PROGRESS: A primate breeding colony for rhesus and cynomolgus monkeys has been started. Ten gang-cage breeding units with a total of 93 female and ten male rhesus monkeys have been established. Two gang cage breeding units with 19 female and two male cynomolgus monkeys have been established. Recent receipt of 38 cynomolgus monkeys will provide breeders for three more gang cages after quarantine has been completed. Seventeen young cynomolgus have been weaned and are individually caged ready for experimentation. Fifteen infant and young cynomolgus monkeys are presently in the gang cages. Twenty-six infant and young rhesus monkeys are in the gang cages. In addition four orphan rhesus infants are being bottle fed by caretakers. Bite wounds among the rhesus monkeys are the primary medical problem. Many emergency suturing, treatment, and follow-up treatment procedures occur weekly. If equipment and supplies can be obtained, transections and pulp capping of all rhesus canine teeth will be done in FY 82.

RECOMMENDATIONS: Continued expansion of the program including new caging facilities and importation of female potential breeders from the States.

8. Prophylactic Doxycycline for Travelers' Diarrhea in Thailand

PROBLEM: This study was designed to evaluate the efficacy of daily doxycycline in preventing travelers' diarrhea among Americans during their first five weeks in Thailand.

PROGRESS: A randomized double blind study to determine the efficacy of doxycycline (100 mg daily) in preventing travelers' diarrhea was performed among 63 American Peace Corps volunteers during their first five weeks in Thailand. Eight (24%) of 33 volunteers taking placebo and three (10%) of 30 taking doxycycline developed travelers' diarrhea during their first three weeks in country ($p = 0.12$). During the period of prophylaxis enterotoxigenic Escherichia coli were isolated from only one volunteer with travelers' diarrhea (placebo group). Aeromonas hydrophila, bacteria of uncertain enteropathogenicity, were isolated from four (50%) of eight volunteers with travelers' diarrhea in the treatment group ($p = 0.21$). In this study in which the attack rates were low and in which 77 percent of enterotoxigenic E. coli, and 11 percent of A. hydrophila were resistant to doxycycline, this antibiotic was only partially effective in preventing travelers' diarrhea.

RECOMMENDATIONS: No further trials of antibiotics to prevent travelers' diarrhea are planned. This population would, however, be excellent for other treatment or prophylactic regimens although none are planned at this time.

9. Evaluation of the Efficacy of Selected Antiviral Drugs in Japanese encephalitis Virus Infections

PROBLEM: Japanese encephalitis virus (JEV) is endemic in many parts of Southeast Asia with case fatality rates between ten and 90 percent. In addition, large epidemics have been reported in Taiwan, Thailand, Korea and India since WWII. JEV infection is therefore a serious threat to local populations and military forces deployed anywhere in this region. Recent progress in rapid diagnosis of the subpopulation of JEV patients at highest risk makes the use of an effective antiviral drug more attractive. We are therefore screening likely candidate drugs for their in vitro and in vivo efficacy.

PROGRESS: Isoprinosine, an antiviral drug with reported immunopotentiating properties, has been tested in vitro for its ability to inhibit JEV replication in LLC-Mk2 cells and for its ability to stimulate specific antiviral antibody production by human peripheral blood lymphocyte (PBL) in culture. As anticipated there was no significant inhibition of JEV replication in LLC-Mk2

cell monolayers which were preheated for 24 hour before infection and continuously during infection with a range of drug concentration from 0.1 - 300 ug/ml. Isoprinosine (100 ug/ml) failed to cause any stimulation of flavivirus - specific IgG or IgM synthesis by PBL in culture as measured by IgM or IgG antibody capture radioimmunoassays.

The antiviral drug ribavirin, which has been previously shown to inhibit the replication of a related flavivirus, dengue type 2, has been tested against JEV. Concentrations of 30 and 100 ug ribavirin/ml evidenced significant inhibition of JEV replication in LLC-Mk2 monolayers when present continuously beginning 24 hour before infection. Maximal virus titers were reduced from 10^7 to less than 4×10^4 PFU/ml in the presence of a high therapeutic dose (100 ug/ml).

RECOMMENDATIONS:

1. In vivo ribavirin treatment should be initiated in experimentally infected rhesus monkeys.

2. Isoprinosine should also be evaluated in monkeys since immunopotentiation may involve cell mediated immunity or an earlier step in the regulation of B cell function than can be measured in the in vitro PBL antibody production assays.

10. Production of Flavivirus Temperature Sensitive Mutants

PROBLEM: The existance of a battery of temperature sensitive (ts) mutants of flaviviruses and the demonstration of complementation would allow investigation of the biochemical functions of nonstructural virus specified proteins, the identification and characterization of the lesion in candidate vaccine viruses, and the relationship of protein function to virus virulence.

PROGRESS: Heat resistant strains of Japanese encephalitis virus (JEV) and dengue type 2 virus (DEN-2) have been selected by plaque purification of survivors of treatment of 60°C.

The JEV HR strain has been treated with hydroxylamine to induce mutation and 40 ts mutants have been cloned for characterization.

RECOMMENDATIONS:

1. Other mutagens should be utilized to isolate DEN-2 ts mutants and more JEV mutants since each mutagen tends to select certain mutants more often than others.

2. The isolated mutants should be tested for reversion frequency and leakiness.

3. Stable mutants should be characterized as to mutant function and complementation with other mutants.

Presentations:

1. Dixon, K.E. Recent Chemotherapeutic Malaria Studies at AFRIMS. Annual Malaria Conference in Haad Yai, Thailand, November 1980.

Publications:

1. Tingpalapong, M., Watson, W.T., Whitmire, R.E., Chapple, F.E., Marshal. J.T. Unilateral, Congenital Cleft Palate in a White-Handed Gibbon. Accepted for publication in the J. Med. Primatology.

2. Tingpalapong, Mr., Watson, W.T., Whitmire, R.E., Chapple, F.E., Marshal, J.T. Reactions of Acptive Gibbons to Natural Habitat and Wild Conspecifics. Nat. His. Bull. Siam Society, 29:31-40, 1981.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6448	81 10 01	DD-DR&E(AR)636

3 DATE PREV SUMMARY	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
80 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	3M263750A808	AB	003
b. CONTRIBUTING				
c. XXXXXXXXX	CARDS 1413A			

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)
PRECEDING FISCAL YEAR 81	4.0	371
CURRENT FISCAL YEAR 82		633

19 RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL, MC

TELEPHONE: 202-576-3551

21 GENERAL USE

Foreign intelligence not considered

20 PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of CD&I

ADDRESS: Washington, D.C. 20012

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

NAME: Berman, S., Ph.D.

TELEPHONE: 301-427-5208

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Altieri, P.L.

NAME: Dubois, D. POC: DA

22 KEYWORDS (Precede EACH with Security Classification Code) (U) Biological products; (U) Dengue virus vaccine; (U) Pseudomonas vaccine; (U) Typhoid-Shigella hybrid vaccine; (U) E. coli soluble pilus protein vaccine; (U) Bioassays; (U) Freeze-drying

23 TECHNICAL OBJECTIVE, 24 APPROACH, 25 PROGRESS (Furnish individual paragraphs identified by number. Precede text of each 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) 80 10 - 81 09 Investigations on the development of new and improved biologics for military use have continued. 1. A purified polysaccharide Pseudomonas (strain T-5) vaccine has been prepared for human studies and vaccines from 2 additional strains are currently in progress. 2. A freeze-dried, living oral vaccine, suitable for human studies, has been produced from cultures of a Salmonella typhosa-Shigella sonnei hybrid. 3. Studies were initiated on developing a mouse potency assay for the Typhoid-Shigella hybrid vaccine. 4. Production of an oral vaccine derived from the pili of Escherichia coli is currently in progress. 5. Continuing studies on developing a mouse potency assay for A and C meningococcal polysaccharide vaccines indicated that a degree of protection in mice can be obtained by the elimination of mucin from the challenge culture. 6. The C6/36 clone of Aedes albopictus mosquito cells was tested and found to be suitable as a virus vaccine substrate. 7. A mosquito cell plaquing system was developed and used to isolate Den-2 and Den-3 clones which are currently being evaluated for potential candidates for vaccine production. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*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

* U.S. GPO 1974-540 843/6691

Project 3M263750A808 DRUG AND VACCINE DEVELOPMENT

Work Unit 003: Advanced Vaccine Development

Investigators:

Principal: Dr. Sanford L. Berman, Ph.D.

Associates: Mrs. Patricia Altieri
Dr. Doria Dubois, Ph.D.
Mr. Calvin Powell

Problem

The objectives of this work unit is to continue investigations on the development of manufacturing methods and production of new and/or improved vaccines for military use. Methods developed for producing purified polysaccharide vaccines from cultures of the T-5 strain of Pseudomonas aeruginosa were applied to cultures of 2 additional strains of the organism. Studies were completed on methods for producing a stable, freeze-dried vaccine from agar grown harvests of a Salmonella typhosa-Shigella sonnei hybrid and investigations initiated on developing a mouse potency assay for this product. The production of an oral vaccine derived from the pili of Escherichia coli was initiated. Studies were also continued on the development of a mouse potency assay for the A and C meningococcal vaccines. Application of mosquito cells to vaccine production and testing was continued.

Progress

In this year a purified polysaccharide Pseudomonas aeruginosa (strain T-5) vaccine has been prepared and made available for human studies. In addition, purified polysaccharides from cultures of the 1244 and 134 VA strains of the Pseudomonas organism have been prepared and are currently being evaluated for suitability as potential vaccines. A freeze-dried, living oral vaccine, has been produced from cultures of a Salmonella-typhosa-Shigella sonnei hybrid and is available for human trials. Initial studies on the development of a mouse potency assay for this product show that mice respond to both components of this hybrid and that the mice are protected against a homologous challenge but not against a cross challenge. Nine production runs were required to accumulate 2.4 grams of soluble pilus protein derived from agar grown cultures of Escherichia coli and these materials are currently being evaluated for suitability

as an oral vaccine for human use. Continuing studies on developing a mouse potency assay for A and C meningococcal polysaccharide vaccines have indicated a degree of protection in mice can be demonstrated by immunizing the mice by the subcutaneous route and by eliminating the mucin from the challenge culture. The C6/36 clone of Aedes albopictus mosquito cells was tested and found to be suitable as a virus vaccine substrate. A mosquito cell plaquing system was developed and used to isolate Den-2 and Den-3 clones which are currently being evaluated as potential candidates for vaccine production.

Future Objectives

Work will continue on evaluating the Pseudomonas polysaccharides and E. coli soluble pilus proteins as to their suitability as potential vaccines. If the studies so indicate, the materials will be processed to final vaccines suitable for human use. Studies will continue on developing stable, freeze-dried Dengue vaccines from harvests of Dengue strains grown in the mosquito cell line now available as a vaccine substrate. Future investigations on developing mouse potency assays for both the meningococcal polysaccharide and the Typhoid-Shigella hybrid vaccines will include studies on dose response and sensitivities of the assays. Renewed efforts will be made to produce an improved meningococcal group B protein-polysaccharide vaccine over the group B vaccine tested previously in human trials. Work will be initiated on developing the techniques for producing and isolating pili from agar grown cultures of Neisseria gonorrhoeae.

Publications

1. Williams, J.E., Altieri, P.L., Berman, S., Lowenthal, J.P., and Cavanaugh, D.C. Potency of killed plague vaccines prepared from avirulent Yersinia pestis. Bulletin of the World Health Organization 58(5): 753-756, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 7009	81 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 INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 06 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	63750A	3M263750A808	AB	004
b. CONTRIBUTING				
c. XXXXXXXXX	CARDS 1413A			

11. TITLE (Precede 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)
FISCAL YEAR PRECEDING 81	3.0	100
FISCAL YEAR CURRENT 82	4.0	80

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of CD&I

ADDRESS: Washington, DC 20012

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

NAME: Tramont, Edmund C., COL, MD, MC

TELEPHONE: (202) 576-3601

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS NAME: D. McChesney, CPT, MSC, J. Boslego, MAJ, MD, MC, M. Piziak, CPT, MC

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Neisseria; (U) Gonorrhoeae; (U) Gonococcal Vaccine; (U) Antigen; (U) Immunity

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each 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 pilus, cell wall protein appendages known as pili which function to attach the gonococcus to epithelialmucosal 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) 80 10 - 81 09 A lyophilized PGH 3-2 gonococcal pilus vaccine was administered locally to human females and the antibody response quantitated. A formalin sterilized PGH 3-2 pilus vaccine was tested for safety and immunogenicity. Local genital antibody produced in response to the parenteral Pgh 3-2 gonococcal pilus vaccine are directed against both homologous and heterologous pili. Monoclonal antibodies to whole gonococcus and to the gonococcal pilus vaccine were produced in mice. Techniques used in the solid phase are being adapted to a radioactive IEA. (For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 80 - 30 Sep 81.)

*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 66 AND 1498 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE © U.S. GPO: 1974-540-843/8691

Project 3M263750A808 DRUG AND VACCINE DEVELOPMENT

Work Unit 004: Gonococcal Vaccine Development

Investigators:

Principals: COL Edmund C. Tramont, MC
Samuel B. Formal, Ph.D.

Associates: LTC Jerald Sadoff, MC
MAJ John Boslego, MC
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. The objectives of the WRAIR program are to determine the antigens of the gonococcus with eliciting an immune response, to study the mechanisms of immunity to the gonococcus and to develop vaccines to protect against clinical disease.

Progress

Twenty human volunteers were injected intramuscularly with varying doses (2000 µg, 1000 µg, 500 µg, 250 µg) of a formalin sterilized PGH 3-2 gonococcal pilus vaccine (Lot 004). The serum antibody response was quantitated using the SPRIA and was shown not to be dose related. The adverse reactions (sore arm, malaise, joint pain) that were observed were subjectively more severe than observed with the filter sterilized lot, (Lot 001). However, these reactions were dose (volume) related. The formalin sterilized vaccine was determined to be both safe and immunogenic.

The antibody response to a local administered gonococcal pilus vaccine was investigated. The lyophilized vaccine was administered to women either in a cream or a special tampon impregnated with pili. Initial studies showed that there was no detectable level of gonococcal pilus antibodies in the vaginal secretions.

Future Studies

1. Test efficacy of the gonococcal pilus vaccine.
2. Correlate antibody level (serum & local) with vaccine efficacy.
3. Correlate pilus antigenic variability with vaccine efficacy.
4. Determine other cell wall antigens involved with attachment.

Bibliography

1. McChesney, D., C. Woodbury, J. Boslego, J. Ciak, E. Tramont and C. Brinton. Cross-reactivity of Human Genital Antibodies from Volunteers Vaccinated with Gonococcal Pilus Vaccine PGH 3-2. Abstract E65. ASM, Dallas, Texas, 1981.

2. Tramont, E., D. McChesney, J. Boslego, J. Ciak, J. Sadoff and C. Brinton. Induction of Local Genital 11s Antibody by a Parenteral Gonococcap Vaccine. Abstract, Am Fed. Clinical Res., San Francisco, CA, 1981.

3. Tramont, E., J. Sadoff, J. Boslego, J. Ciak, D. McChesney, E. Takefuji. Gonococcal Pilus Vaccine: Studies of Antigenicity and Inhibition of Attachment. JCI, October 1981.

4. Tramont, E., J. Ciak, D. McChesney, J. Boslego and C. Brinton. Inhibition of Attachment of N. gorrhoeae by Antipilus Antibodies Induced by Pilus Vaccines. Abstract, Sexually Transmitted Disease Meeting, Antwerp, Belgium. 1980.

5. Brinton, C. Jr., S. Wood, A. Brown, A. Labik, J. Bryan, S. Lee, S. Polen, E. Tramont, J. Sadoff, W. Zollinger. The Development of a Neisserial Pilus Vaccine for Gonorrhea and Meningococcal Meningitis. Reviews Inf Dis International Symposium on Bacterial Vaccines, submitted for publication, 1980.

6. Harrison, S., E. Tramont, W. Herald, D. Norenburg, R. Morrison. Treponemal Levels Amoxicillin Achieved in CSF by Oral Treatment of Syphilis. ICAAC, Chicago, November 1981.

7. Harrison, S., W. Herald, R. Morrison, E. Tramont, D. Norenburg. Amoxicillin in the Treatment of Rabbit Syphilomas. Submitted for publication.

PROJECT 3M162770A870

RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA UG 6764	81 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
80 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	3M162770A870	AC	071
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code):
(U) Biosystematics of Arthropods of Military Medical Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
002600 Biology 005900 Environmental Biology

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 81	2.0	137
FISCAL YEAR CURRENT 82	3.0	178

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012
RESPONSIBLE INDIVIDUAL NAME: Russell, COL P.K.
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Div of CD&I
ADDRESS: Washington, D.C. 20012
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Roberts, LTC D.R.
TELEPHONE: 202-576-3719
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Faran, CPT M.E.
NAME: Harbach, CPT R.E.

21. GENERAL USE
Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Biosystematics; (U) Disease Vectors; (U) Arthropods; (U) Mosquitoes; (U) Epidemiology; (U) Malaria; (U) Arboviruses

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

23. (U) Conduct biosystematic research of medically important arthropod groups in support of epidemiological studies and disease control strategies of importance to the military. Disease vector groups currently under systematic investigation are (1) Anopheles of Neotropical Region (malaria), (2) Anopheles (Cellia) of Oriental Region (malaria) (3) Pipiens Complex of Middle East and Afrotropical Region (arboviruses and filariasis), and (4) Aedes (stegomyia) of Afrotropical Region (arboviruses). Build a computer data base on 1,000,000 specimens of mosquitoes to provide information on ecology, distribution and medical importance of vector species.

24. (U) Comparative morphological study of medically important mosquito groups in regions of military interest, electrophoretic and cross-breeding studies of vector populations, and correlation of these data with other pertinent information to result in (1) description and illustration of species, (2) development of effective identification keys, (3) provision of data on ecology, medical importance and distribution. Computerization of data on 1,000,000 specimens of mosquitoes to facilitate the conduct of biosystematic research.

25. (U) 80 10 - 81 09 Published two large monographic revisions (Scutellaris Group of Aedes (Stegomyia) and Myzomyia Series of Anopheles (Cellia) in Thailand) and an identification handbook (Amazonian species of Anopheles (Nyssorhynchus)). A new species was described and keys to vector species of Aedes (stegomyia) were prepared and published. Continued research on malaria vectors of the Balabacensis Complex. Obtained, individually reared and prepared, 1,300 specimens of Cx. pipiens for study. Incorporated 3,640 collection forms into the computer data base. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 80 - 30 Sep 81.

*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 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

Work Unit 071 Biosystematics of Arthropods of Military Medical Importance

Investigators:
Principal: Donald R. Roberts, LTC, MSC
Associate: Michael E. Faran, CPT, MSC; Ralph E. Harbach, CPT, MSC; Kenneth J. Linthicum, CPT, MSC; E.L. Peyton, Y.-M. Huang, Ph.D.; PFC Richard Soltero; James E. Pecor

Problem

Epidemiological studies and disease control strategies concerning arthropod-borne diseases 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, for providing the above support, are (1) to describe and illustrate all the species in these groups, (2) to reconcile any systematic problems, (3) to develop effective keys for all stages of the life cycle of these species, (4) to provide basic biological and ecological data useful in the control of vector species, (5) to provide data concerning the medical importance of each species, and (6) to train personnel in field studies and systematic research. Current studies have concentrated on (1) malaria vector-groups of the Oriental region {Leucosphyrus Group of *Anopheles* (*Cellia*)}, and of the Neotropical region (*Nyssorhynchus* of *Anopheles*), and (2) arbovirus and filariasis vectors of the Middle East and Afrotropical region, *Aedes* (*Stegomyia*) and the Pipiens Complex of *Culex*. In conjunction with the study of the mosquito-vector groups, the detailed systematic and ecological data available for approximately 1,000,000 specimens are being used to develop a computer based master file. This study is directed at providing easily accessible, coordinated ecological and vector data to the military, public health organizations, and other scientific and environmental agencies concerning vector species of mosquitoes.

Progress

Research continued, in collaboration with AFRIMS Bangkok, Thailand, toward the final revision of the malaria vectors of the Leucosphyrus group of *Anopheles* (*Cellia*), with primary emphasis on resolving problems within the Balabacensis Complex. A new morphologically distinct form was discovered from Thailand. The taxonomic status of this form is pending more detailed character analysis of the various life stages and comparison of these with those of other members of this complex. The revision of the Argyritarsis Section of *Anopheles* (*Nyssorhynchus*) has been completed and is currently being edited for

publication. This monograph will be approximately 200 pages in length and will include descriptions, discussions of bionomics and medical importance, keys, and illustrations for the 8 species included in the Section. It is based on the study of approximately 9,000 specimens: 1,500 males, 3,800 females, 3,000 larvae and 700 pupae, including 526 individual rearings (268 larval, 226 pupal and 32 incomplete) and 15 progeny rearings. Associated with this latter work and other previous research, an 81 page handbook on the Amazonian species of Anopheles (Nyssorhynchus) was published. Regarding the Pipiens Complex of Culex (Culex), extensive crossbreeding experiments have demonstrated genetic compatability among five strains of "pipiens" from Egypt, U. S. and Brazil. Detailed morphological studies on pipiens centered on the immatures and adults, particularly of populations from the Middle East. A technique was developed to freeze-dry adult specimens for taxonomic study. In conjunction with the morphological study of this complex, an electrophoresis apparatus was designed and developed to study specific isozyme electromorphs among various populations of Culex pipiens; electrophoretic study is currently underway. For the subgenus Stegomyia of Aedes, 3 new species in the Simpsoni Subgroup, 2 species in the Pseudonigeria Subgroup and one species in the Poweri Subgroup were discovered and described. Also, current work on the South African Stegomyia specimens collected during the previous year should resolve taxonomic problems related to the following taxon pairs: simpsoni-bromeliae, demeilloni-heischi and aegypti aegypti-aegypti formosus. An illustrated key was prepared to the African vector species of Stegomyia to be published in late 1981. A paper describing a new species, Aedes ledgeri, was published. In addition to the above research, two large monographic revisions were published during the year. These treat the Scutellaris Group of Aedes (Stegomyia) of Tonga, and Myzomyia Series of Anopheles (Cellia) in Thailand.

During the year 3,640 collection records were added to the computer data base: 1,939 pertain to Anopheles (Nyssorhynchus), 1,110 to Panama, 540 to Colombia and 61 to Ecuador. Six separate files have been established for countries of Middle and South America. Narrative concerning the medical importance, bionomics and distribution, with important references, cross-referenced by a "species code" number on the collection form, have been entered onto floppy discs for 25 species.

The project has acquired, through transfer from the project Mosquitoes of Middle America, University of California to the Smithsonian, 500,000 Neotropical mosquito specimens, 11,400 collection forms, 5000 topographic maps, reprint files and an extensive collection of taxonomic literature.

Future Plans

Research will continue on the Leucosphyrus group of Anopheles (Cellia), the Argyritarsis Section of Anopheles (Nyssorhynchus), the Afrotropical Aedes (Stegomyia) and the Pipiens Complex of Culex (Culex). The revision of the Argyritarsis Section and a paper describing the Mosquito Information Management Project will be submitted for publication. Depending on projected priorities, work will begin on an Afrotropical arbovirus and/or malaria vector group of mosquitoes. It is recommended that additional field collection trips take place this fiscal year to the Afrotropical Region and Middle East.

Formal Presentations

Faran, M.E. 1981a. Organized and presented introduction to symposium entitled "Determination and Recognition of a Disease Vector." Presented at annual meeting of the American Mosquito Control Association, March 15-18, San Antonio, TX.

Faran, M.E., C.B. Klarman and C.L. Bailey. 1980. Application of a computerized information management system (SELGEM) to medically important insects. Presented at annual meeting of the American Society of Tropical Medicine and Hygiene, November 5-7, Atlanta, GA.

Harbach, R.E. 1981. Pipiens complex: A preliminary morphological study. Presented at annual meeting of the American Mosquito Control Association, March 15-18, San Antonio, TX.

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Linthicum, K.J. and M.E. Faran. 1981. Hypothesized phylogenetic relationships within the subgenus Nyssorhynchus of Anopheles. Presented at the annual meeting of the American Mosquito Control Association, March 15-18, San Antonio.

Publications

Bailey, C.L., M.E. Faran, T.P. Gargan, II and D.E. Hayes. Winter survival of blood-fed and nonblood-fed Culex pipiens. Submitted to American Journal of Tropical Medicine and Hygiene.

Faran, M.E. 1981. Synonymy of Anopheles (Nyssorhynchus) noroestensis with An. (Nys.) evansi, with a description of the male genitalia of the lectotype of An. (Nys.) evansi (Diptera: Culicidae). Mosq. Syst. 13:86-91.

Faran, M.E. and K.J. Linthicum. 1981. A handbook of the Amazonian species of Anopheles (Nyssorhynchus) (Diptera: Culicidae). Mosq. Syst. 1(3):1-81.

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Harrison, B.A. 1980. Medical Entomology studies - XIII. The Myzomyia series of Anopheles (Cellia) in Thailand, with emphasis on intraspecific variations (Diptera: Culicidae). Contr. Am. Entomol. Inst. 17(4):1-195.

Huang, Y.-M. 1981. A redescription of Aedes (Stegomyia) calceatus Edwards and description of a new Afrotropical species, Aedes (Stegomyia) ledgeri (Diptera: Culicidae). Mosq. Syst. 13:92-113.

Huang, Y.-M. and R.A. Ward. A pictorial key for the identification of mosquitoes associated with yellow fever in Africa. {In press} Mosq. Syst. 13.

Mattingly, P.F. 1981. Medical entomology studies - XIV. The subgenera Rachionotomyia, Tricholeptomyia and Tripteroides in the Oriental Region (Diptera: Culicidae). Contr. Am. Entomol. Inst. 17: 1-147.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6489	81 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
80 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	3M162770A870	AA	072
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.232			

11. TITLE (Precede 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
72 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 81	3.0	51
	CURRENT 82	3.0	68

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Division of Preventive Medicine
Washington, DC 20012

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

NAME: Miller, Richard N., COL, MC

TELEPHONE: (202) 576-3553

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Park, Jung Han, MAJ, MC

NAME: Prier, Ronald E., MAJ, MC POC: DA

21. GENERAL USE

Foreign intelligence not considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Epidemiology; (U) Infectious Disease; (U) Risk Assessment; (U) Data Bases

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish 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 infectious diseases in military populations.

24. (U) Contemporary epidemiologic methods are applied to causes of disability in military populations. Multi-disciplinary collaborative approaches are utilized and new methods developed as required.

25. (U) 80 10-81 09. Completed are: cost/benefit analysis of use of hepatitis B vaccine in selected military populations; hepatitis antigen/antibody prevalence in Special Forces troops; etiology of viral hepatitis in U.S. military personnel; assessment of ARD at Fort Leonard Wood. Analyses of the following studies are in progress: assessment of risk of coccidioidomycosis at Fort Irwin; the importance of splenectomy in troop deployment; long term follow-up of soldiers with hepatitis B; development of better diagnostic methods for leishmaniasis; assessment of risk of infectious diseases to the Rapid Deployment Force; evaluation of reduced dosage of Human Diploid Cell Rabies Vaccine given by jet injector. For technical Report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

*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 68 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE

Project 3M162770A870 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; MAJ Mary K. McKenna, ANC; MAJ Ronald E. Prier, MC; CPT (P) Wayne M. Lednar, MC; SFC George L. Rockenbaugh, Jr.; L. Charlene Evans

Objective: To assess the actual or potential impact of selected infectious 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 usually represent cooperative efforts with other divisions of WRAIR.

Progress:

1. Coccidioidomycosis Risk at Fort Irwin, California: The 197th Infantry Brigade (Sep) at Fort Benning underwent pre-exercise skin testing in July 1981. Ten days of active surveillance of the brigade during training at Fort Irwin failed to yield significant numbers of overt infections. Post-exercise testing will be done in October 1981.

2. Hepatitis Follow-up Study: A follow-up study of 284 hepatitis B and 94 Non-A, Non-B hepatitis infections identified during a study in 1978-79 is underway. Chronic effects of hepatitis to include chronic active hepatitis, cirrhosis, and the chronic hepatitis B carrier state will be determined for those active duty members who have remained on active duty (approximately one-third).

3. Development of better diagnostic methods for Leishmaniasis: The original research protocol entitled "Improved Skin Testing Antigen for the Diagnosis and Prognosis of Leishmaniasis" has been revised. The Division of Communicable

Disease and Immunology and the Division of Experimental Therapeutics have initiated guinea pig innoculations with L. enrietti and will be performing challenge studies with a strain of L. braziliensis panamensis. With successful completion of testing in the animal model, limited skin testing in a human population is projected in the near future, followed by field testing during an actual deployment of troops to an endemic area with leishmaniasis.

4. Assessment of risk to the RDF: In an effort to define the role of the Problem Definition and Assessment Team and elucidate infectious disease threats for U.S. troops in projected areas of RDF deployment, the medical surveillance of RDF units involved in OCONUS training is desirable. Protocols for the medical surveillance of Special Forces units deploying OCONUS and for units involved in Operation Bright Star '82 have been initiated by the Department of Epidemiology.

5. Evaluation of Human Diploid Cell Vaccine (HDCV) in Reduced Dosage by Jet Gun Injector: Approximately 120 Special Forces troops who, by virtue of their mission, require HDCV immunization will participate in a volunteer study of 0.1 ml dose of the vaccine given with the jet gun injector intradermally. Preliminary studies by the Center for Disease Control indicate that this approach will probably produce protective levels of antibody in more than 95% of immunized persons. If successful, the test method will allow mass immunization of troops at one-tenth the present cost.

Recommendations: An increase in resources, both budgetary and personnel, should be devoted to this vital area of research. Epidemiologic studies should address the assessment of risk of Korean hemorrhagic fever; Rift Valley fever; Congo Crimean fever; sandfly fever; dengue fever; schistosomiasis; rickettsial diseases; diarrheal diseases worldwide; toxoplasmosis; histoplasmosis; and leishmaniasis in Panama (as recommended by WRAIR RDF Workshop). Penicillinase producing N. gonorrhea (PPNG) is emerging as a significant military problem worldwide, and epidemiologic studies to better define this disease are indicated.

Formal Presentations:

1. "Coccidioidomycosis Surveillance of U.S. Army Personnel Training at Fort Irwin, California." 26th Annual Meeting of the Coccidioidomycosis Study Group, San Francisco, California, 28 March 1981. COL Richard N. Miller, M.D.

2. "Etiology of Viral Hepatitis Occurring in American Military Populations." 1981 International Symposium on Viral Hepatitis, New York, NY, 30 November 1981. COL Stanley M. Lemon, M.D., CPT (P) Wayne M. Lednar, M.D., COL Richard N. Miller, M.D.

3. "Outbreak of Norwalk-like Agent Gastroenteritis Among Residents and Staff of a Nursing Home in Frederick, Maryland." 14th Annual Meeting of the Society for Epidemiologic Research, Snowbird, Vermont, 18 June 1981. CPT (P) Wayne M. Lednar, M.D., Joseph V. Horman, M.D., MAJ Jung Han Park, M.D.

4. "Medical Threat Briefing." The Infectious Disease Threats to the Rapid Deployment Force: Preventive Strategies Workshop, WRAIR, Washington, D.C., 14 July 1981. MAJ Ronald E. Prier, M.D.

Bibliography:

1. McKenna, M.K., Hales, P.W. "Application of the Nursing Process to Improve the Quality of Nursing Service Provided to a Military Community in Germany." In Press - to be published in Military Medicine.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	3. REPORT CONTROL SYMBOL
DA OG 6760	81 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
80 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	3M162770A870	AB	073
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7.2.2			

11. TITLE (Precede with Security Classification Code)

(U) Threat Assessment of Diseases of Military Importance in the Tropics

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

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 81	11.0	500
FISCAL YEAR CURRENT 82	11.0	680

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, P.K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: US Army Medical Component, AFRIMS

ADDRESS: Bangkok, Thailand

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

NAME: BENENSON, M.W., LTC

TELEPHONE: (02) 281-7776

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: BURKE, D.S.,LTC;HARRISON,B.A. LTC;ECHEVERRIA,P.D.,LTC;GILBREATH,M.J.

NAME: CPT POC:DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Dengue; (U) Hepatitis; (U) Gonorrhea; (U) Diarrhea; (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 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) 80 10- 81 09 A number of suspected malarial vectors have been successfully colonized and species complex definition has progressed significantly. At least four species are recognized in the Dirus complex and initial studies of malaria suspectibility have been done. A study of human malarial susceptibility in 10 different species is presently underway. A DNA hybridization probe was developed in the laboratory and was shown to be as reliable as the suckling mouse assay and the adrenal cell system to identify LT and ST toxin of E. coli. Diarrheal etiology studies are continuing in both the village and hospital setting. The antibody capture RIA was successfully adapted for JEV IgM in CSF. The epidemiology of hepatitis B in Thai monks was studied to determine the role of close personal contact for transmission in Bangkok. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

* Available to contractors upon originator's approval

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

PROJECT 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS
Work Unit 073: Threat Assessment of Diseases of Military Importance in the Tropics

INVESTIGATORS: LTC M.W. Benenson,MC; LTC D.S. Burke,MC; LTC B.A.Harrison,MSC; LTC P.E. Echeverria,MC; LTC R.E.Whitmire, VC; CPT M.J. Gilbreath,MSC; CPT M.A. Ussery, MSC; CPT R.R. Graham, VC; CPT T.A. Klein, MSC

1. Hemorrhagic Fever in Bangkok - 1980: Epidemiologic Studies

PROBLEM: Previous studies of dengue hemorrhagic fever in Bangkok have shown that severe illness is usually associated with a secondary antibody response to infections. However, studies from other countries have not shown an association of severe disease and sequential infections, and the validity of the reported association in Thai population has therefore been called into question. We undertook a carefully controlled study of hemorrhagic fever in Bangkok to determine if sequential infections can be implicated in severe disease.

PROGRESS: During the calendar year 1980, 169 dengue virus strains were isolated and typed from cases of DHF and 39 strains from cases of undifferentiated pyrexia at Bangkok Children's Hospital. On the basis of the hemagglutination inhibition (HAI) antibody response, cases were classified as showing a primary or a secondary flavivirus seroresponse. Seroresponse patterns varied markedly according to the infecting virus type. In DHF patients over the age of 12 months, D2 infected children showed a secondary seroresponse pattern in 95% (102/107) of all isolate proven cases, while D1 infected children showed a secondary seroresponse pattern in 50% (9/18) of isolate proven cases. Similarly, in cases of undifferentiated pyrexia, 95% (20/21) of D2 isolate proven cases had a secondary seroresponse pattern while only 33% (5/15) of D1 isolate proven cases did so.

All 17 dengue infected, isolate proven children under age one year showed primary seroresponse pattern irrespective of the infecting virus type.

These results suggest that sequential infections are important in the pathogenesis of illness due to DEN-2 but not DEN-1.

RECOMMENDATIONS:

1. Virologic manitoring of the dengue virus serotypes causing human infections in Bangkok should be continued into the future to determine temporal patterns of appearance and disappearance of the serotypes.

2. Systematic monitoring of dengue virus activity should be extended to the entire Southeast Asian and western pacific regions in order to detect geographic patterns of spread of dengue virus serotypes.

3. The role of immune antibody in the interaction of the Bangkok 1980 DEN-1 and DEN-2 strains with macrophage cell lines should be studied in vitro.

2. A Prospective Study of Dengue Hemorrhagic Fever in a Defined Bangkok School Population

PROBLEM: A considerable body of evidence has been accumulated showing that severe dengue virus infections occur more regularly in flavivirus immune subjects than would be predicated on the basis of the known antibody prevalence in Bangkok children. However, doubts persist as to whether the DHF cases may come from select subpopulations in which the antibody prevalence is higher, or that a "secondary" antibody response pattern might be seen in first flavivirus infections under unusual circumstances. Unimpeachable proof that severe dengue infections occur predominantly in flavivirus immune subjects must therefore be obtained by prospective studies.

PROGRESS: Paired venous blood samples were obtained from 1745 healthy school children of the Phibunprachasan School in Bangkapi Amphur in June 1980 and again in January 1981. All specimens were tested for heamgglutination inhibiting antibodies to DEN 1, 2, 3, and 4 and JEV. In June 1980, 736 students lacked detectable antibodies (all < 1:10); 39 of these children had developed new antibodies by January 1981 (infection rate 39/736 = 6.3%). Of the 1009 children seropositive in June 1980, 60 (5.9%) showed a four fold or greater rise to three or more antigens by January 1981.

Twenty-five paired sera showing a primary seroconversion were tested for dengue neutralizing antibodies and the infecting serotype identified by a monospecific seroresponse pattern. There were 11 DEN-1, 8 DEN-2, 0 DEN-3, 2 DEN-4, 2 "no rise," 1 secondary, and 1 uninterpretable seroresponse patterns. Clinical illness was monitored in all children. Students who were absent from school for two days or more with Febrile illnesses were interviewed and a blood sample obtained when they returned to school. Dengue infected children were identified as those with IgM anti-dengue antibodies detected by radio-immunoassay or an HAI titer $\geq$ 1:2560 in blood samples obtained at the time of return to school

Of 49 children absent from school for febrile illness serologically shown not to be dengue, none were hospitalized with a clinical diagnosis of DHF. Similarly, none of four children ill with primary dengue were hospitalized. However, seven of nine children absent from school with secondary dengue were hospitalized with a clinical diagnosis of DHF. Thus, antibody positive children were at greater risk for hospitalization than antibody negative children (7/1009 vs 0/736, Fisher's exact test p = .021, one tail). Also serologically identified secondary infections were more likely to result in hospitalization than primary infections (7/60 vs 0/39, Fishers' exact test p = .026, one tail).

RECOMMENDATIONS:

1. Vaccination strategies should be formulated to take into account the likelihood that partially immune subjects are at risk of severe disease.

2. Further studies should be conducted to define the mechanism whereby partial immunity enhances susceptibility to dengue virus infection.

3. An Annual Cycle of Acute Hepatitis in Thailand

PROBLEM: In a recent year long AFRIMS study of acute hepatitis in a Bangkok hospital, a pronounced seasonality was observed with cases peaking in the rainy season and declining during the cool and dry seasons. In recent clinical studies of acute hepatitis in Bangkok we have learned that most acute hepatitis in children less than 15 years of age is due to hepatitis A, while most hepatitis in young adults is due to type B. As essentially all rural as well as all urban Thai adults are probably immune to hepatitis A but not to hepatitis B, it is reasonable to suppose that the etiologic pattern in parts of Thailand outside Bangkok is similar to that found in Bangkok, i.e. A in children and B in adults. If this is so, then an analysis of age specific attack rates could provide a clue to possible seasonal changes in hepatitis A and B, and perhaps non A-non B.

PROGRESS: Acute hepatitis in Thailand is a notifiable disease. Accordingly, standardized forms are available to health facilities which include blanks for diagnosis, patient age, sex, and residence. These forms are regularly sent by mail to the Department of Epidemiology for tabulation.

Partial tabulation of this data is published annually by the Ministry of Public Health as a booklet entitled "Epidemiological Surveillance Report, Thailand." However, an analysis of the data according to the age, sex, and geographical local of the cases is not routinely compiled and reported; this analysis is presented here. Seasonality of hepatitis was found in provinces from all four major geographical regions (north, central, south, and north-east) but the amplitude was small (peak: nadir = 2:1) except in the north-east where seasonality was pronounced in all four provinces analyzed (peak: nadir = 5-10:1). Hepatitis seasonality was clearest among children and young adults. Thus, hepatitis A is probably a seasonal disease in northeast Thailand. The finding of seasonal hepatitis among adults in the northeast suggests that the anti-HAV prevalence rate is lower in this region than in others, or else there is another endemic seasonal hepatitis virus (Non-A Non-B) in this region.

RECOMMENDATIONS: The transmission of hepatitis A, B., and non A non B viruses in northeast Thailand should be carefully investigated with integrated clinical and epidemiologic studies.

4. Detection of Enterotoxigenic Escherichia coli by Colony DNA Hybridization in Thailand

PROBLEM: To determine the applicability and limitations of examining specimens with a DNA hybridization technique in which genes encoding for enterotoxin are detected.

PROGRESS: To determine the applicability and limitations of examining specimens with a DNA hybridization technique in which genes encoding for enterotoxins were detected, 24 heat-labile and heat-stable (LT+ST+), 17 heat-labile (LT+ST-), and 22 heat-stable (LT-ST+) enterotoxigenic E. coli isolated in Thailand were examined. All enterotoxigenic E. coli identified with the Y-1 adrenal and suckling mouse assays were homologous and thus identifiable with radiolabelled fragments of DNA encoding for heat-labile (LT) or heat-stable toxins of procine (ST_P) or human origin (ST_H). LT-ST+ strains from rural Thailand were homologous with only ST_H and not ST_P while strains isolated in Bangkok were homologous with either ST_H, ST_P, or both. All LT-ST+ E.coli isolated from children in Thailand were homologous with ST_H. In contrast to Bangladesh where 70 percent of LT-ST+ E. coli were homologous with ST_P only 27 percent of strains in Thailand were detected with this probe. This technique detected DNA homologous with the three probes in bacterial growth of all stools from patients with diarrhea from whom enterotoxigenic E. coli inoculated water containing other species of bacteria. The DNA hybridization assay is a useful technique to characterize LT-ST+ E. coli and identify environmental sources of these enteric pathogens.

RECOMMENDATIONS: This technology is currently being used to screen environmental sources for enterotoxigenic Escherichia coli in an on going longitudinal study in Soongnern.

5. Serotypes of Enterotoxigenic Escherichia coli in Thailand and the Philippines

PROBLEM: This study was performed to determine the serotypes of enterotoxigenic Escherichia coli in Thailand and the Philippines and compare the serotypes of these pathogens in Southeast Asia with other locations.

PROGRESS: The serotypes of 386 enterotoxigenic Escherichia coli isolated from 82 individuals with and without diarrhea in

Thailand and the Philippines were determined. The 136 strains producing both heat labile and heat-stable toxin belonged to 12 different O serogroups however 83 percent (113/136) were of one of four serogroups (06, 08, 025, and 078) and 76 percent (104/136) belonged to one of seven O:K:H serotypes. The 196 strains producing only heat-labile toxin belonged to 35 different serotypes and only 14 percent (28/196) belonged to serogroups most common among heat-labile and heat-stable producing strains. Three O serogroups (020, 027, and 078) accounted for 94 percent (51/54) of strains producing only heat-stable toxin, however only seven percent (14/196) of heat-labile only and four percent (2/54) of heat-stable only toxigenic E. coli belonged to the seven serotypes most commonly found among strains which produced heat-labile and heat-stable toxin. Serotypes previously found to be common among enterotoxigenic E. coli (06:H16, 08:H9, 025:H42, and 078:H12) were found to be as common among enterotoxigenic E. coli isolated from children with or without diarrhea (23/150 vs 5/54) ($p > 0.3$). Among Peace Crops volunteers, however, enterotoxigenic E. coli of these serotypes were more common among enterotoxigenic E. coli isolated from volunteers with and without diarrhea (38/78 vs 7/30) ($p < 0.025$). Forty-six percent (37/80) of enterotoxigenic E. coli of serotypes (06:H16, 08:H9, 025:H42, and 078:H12) were resistant to two or more antibiotics in comparison to 68 percent (208/306) of enterotoxigenic E. coli of other serotypes ($p < 0.001$). In Thailand and the Philippines E. coli which produced heat-labile and heat-stable toxin, but not either toxin alone, were restricted in their O:K:H serotypes and these serotypes were less resistant to antibiotics than other serotypes.

RECOMMENDATIONS: This study is complete. Enterotoxigenic Escherichia coli isolated in future studies will be serotyped to verify if these conclusions are correct.

6. Enteropathogenicity of Aeromonas hydrophila and Plesiomonas shigelloides: Prevalence Among Individuals With and Without Diarrhea in Thailand

PROBLEM: To evaluate the enteropathogenicity of Aeromonas hydrophila and Plesiomonas shigelloides in Thailand.

PROGRESS: To evaluate the enteropathogenicity of Aeromonas hydrophila and Plesiomonas shigelloides, the rate of isolation of these organisms was compared among individuals with and without diarrhea in Thailand. In two groups of American travelers A. hydrophila, but not P. shigelloides was associated with episodes of travelers' diarrhea more often than when individuals did not have diarrhea ($p < 0.025$). Among three populations of Thais A. hydrophila and P. shigelloides were isolated with similar frequencies from individuals with and without

diarrhea. The biochemical characteristics, production of cytotoxin, and ability to distend suckling mouse intestine was similar among A. hydrophila isolated from individuals with and without diarrhea. However, cytotoxic A. hydrophila distended rabbit and suckling mouse intestine and produced destructive lesions in intestinal mucosa of both species of animal. P. shigelloides produced neither cytotoxin nor distended intestine. Oral administration of 10^9 cytotoxic A. hydrophila or P. shigelloides failed to cause diarrhea in rhesus monkeys. Volunteer studies or intestinal biopsies of patients with diarrhea may be required to establish whether A. hydrophila is a gastrointestinal pathogen in humans.

RECOMMENDATIONS: Dr. H. DuPont of the University of Texas plans to feed several of the A. hydrophila isolates from Thailand to human volunteers to further evaluate the enteropathogenicity of this organism.

Thirty patients in Thailand will be investigated by quantitatively culturing their large and small bowel and visualizing these areas by endocopy and biopsing abnormal lesions.

7. Antibiotic Sensitivities of Neisseria gonorrhoeae in the Far East: Comparison of Plasmid Species in Isolates from Six Countries

PROBLEM: To determine the sensitivities of 36 Neisseria gonorrhoeae isolated in the Philippines, Thailand, Indonesia, Malaysia, Singapore, and Hong Kong in 1979 and 1980 and to compare the plasmids encoding for antibiotic resistance in these isolates.

PROGRESS: In vitro susceptibility testing of 36 Neisseria gonorrhoeae isolated in the Philippines, Thailand, Indonesia, Malaysia, Singapore, and Kong Kong in 1979 and 1980 by the agar dilution method demonstrated that 27 penicillinase producing isolates(PPNG) and nine non-penicillinase producing isolates (NPPNG) were susceptible to chloramphenicol, thiamphenicol, spectinomycin, sulfamethxazole-trimethoprim, cefoperazone, moxalactam, cefotoxime, and ceftriaxone. The latter two compounds were tenfold more active (on a MIC basis) than cefoperazone and moxalactam. In comparison 50 percent of the strains required tetracycline MICs of >2 μg/ml, and 75 percent required streptomycin MICs of >128 μg/ml. The MICs of erythromycin to inhibit 90 percent of the isolates were 7.3 μg/ml and 6.0 g/ml for the PPNGs and NPPNGs respectively. Eighty-two percent of PPNGs and 100 percent of NPPNGs examined required kanamycin MICs of >32 μg/ml. None of the MICs of the nine NPPNG strains for ampicillin and penicillin was greater than 1 μg/ml.

β-lactamase production was associated with a 4.4 megadalton plasmid in all 27 PPNGs examined; 93 percent of PPNG and 22 percent of NPPNG contained a 24 megadalton plasmid. Single isolates from Malaysia, Indonesia, the Philippines, and Singapore contain 12.5 and/or 7.5 megadalton plasmids which could not be associated with resistance to any specific antibiotics. Two NPPNG from Thailand contained a 3.2 megadalton plasmid which was similar in size to the plasmids encoding for β-lactamase in strains traceable to West Africa.

RECOMMENDATIONS: This study is complete. A further study to search for spectinomycin and kanamycin resistant isolates is currently underway.

8. Sensitivity of the C-Test for Diagnosis of Gonorrhoeae from Specimens Sent from Thailand

PROBLEM: To determine the clinical applicability of the C-test, a method of detecting gonococcal DNA in Thailand.

PROGRESS: A transformation test for the diagnosis of gonorrhoeae, specifically the C-test, was used to detect gonococcal DNA in 37 male urethral and 159 cervical specimens sent from Bangkok, Thailand to Philadelphia, Pennsylvania. The results from C-testing the specimens mailed to Philadelphia were compared with those from immediate culturing in Thailand. In the urethral specimens there was 100 percent concordance between the C-test and the cultures. In the cervical specimens the C-test identified 31 of 41 (76%) that were cultured positive. An additional two were cultured negative but C-test positive. The sensitivity of the C-test on the cervical specimens sent from Thailand was 76 percent compared to a sensitivity of 94 percent previously reported in the United States. It is suggested that the mixed flora in the cervical swabs, which could flourish in the heat and humidity during the collection, storage, and transportation of the specimens can adversely affect the results of the C-test.

RECOMMENDATIONS: More cervical specimens will be collected with modified swabs to try to improve the sensitivity of this test.

Either the temperature sensitive mutant will be sent from philadelphia or another will be made at AFRIMS to establish this assay in Thailand.

9. Epidemiological Studies on Leptospirosis in Northeast Thailand

PROBLEM: To identify the serotypes of leptospirosis present in man, domestic and wild animals and to develop diagnostic techniques to study human populations.

PROGRESS: The epidemiological survey involving various domestic and trapped wild animal species in Northeast Thailand has been completed. Definitive identification and confirmation of serotypes identified at AFRIMS was accomplished at the Leptospirosis Reference Laboratory in Brisbane, Australia.

Microagglutination screening tests for Leptospiral antibodies were completed on the following:

Human Sera	215	specimens
Water buffalo and cattle sera	1,510	"
Dogs	214	"
Horses	108	"
Rodents	188	"
Pigs	50	"

An addition culturing of 267 human blood and urine specimens as well as 251 trapped rodent kidneys for leptospiral organisms was completed in FY 1981. Specimens from suspect human cases at the Children's Hospital are being cultured and a microagglutination test completed to further characterize human incidence and serotypes present in Thailand.

RECOMMENDATIONS: During FY 82 a solid phase antibody capture enzyme linked immunoabsorbant assay for rapid diagnostic screening of fever of unidentified origin cases in humans will be developed using cultures from the two human serotypes obtained in this study. Continued studies of FUO will then be carried out.

10. Mosquito Survey and Taxonomic Studies

PROBLEM: To elucidate the mosquito fauna of Thailand and Southeast Asia, with primary emphasis on the identification of diagnostic characters for the separation of vector species and groups containing vector species of human pathogens.

PROGRESS: Major efforts continued during this period to assist in the monographic revisionary study of the Leucosphyrus Complex of Anopheles (Cellia). Studies comparing the affects of rearing progeny immatures of colonized An. dirus and An. balabacensis Perlis forms at different water temperatures, on pupal branching and adult wing spot characters, have identified potential wing differences in these two species.

A morphological analysis of the Subpictus Group of the Pyretophorous Series, Anopheles (Cellia), in Thailand was completed during this period (1). Keys were prepared and a manuscript is in preparation. The status of Anopheles nivipes as a separate species from Anopheles philippinensis was

definitely established during this period by cytogenetic and cross mating studies. The question of Anopheles maculatus as a single species or a complex of several species, received considerable attention during this period.

Excellent progress was made on the taxonomic revision of the Kochi Group of Aedes (Finlaya) during this period. Description, illustrations, and keys have been made for most of the species. Two new species have been identified. A monographic study on the Myzomyia Series of Anopheles (Cellia) in Thailand was published (2). A paper (3) elevating Anopheles takasagoensis Morishita, to full species status was also published. A guide to the genera of mosquitoes of Thailand, with illustrated keys, biological notes and preservation and mounting techniques has been submitted for clearance.

RECOMMENDATIONS: Morphological studies will continue on the Leucosphyrus Complex, the Maculatus Complex, and the philippinensis-nivipes group. Work on the Kochi Group of Aedes (Finlaya) will be terminated following acceptance of the manuscript for publication. The surveillance of vector species densities and distributions in Thailand will continue in order to delineate important vector-parasite interactions.

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1. Kittayarak, P. Intra-Interspecific Morphological variations in the Subpictus Group of Anopheles in Thailand. Master of Science Thesis, Faculty of Science, Mahidol Univ., Bangkok, Thailand. 142 p., 1981.

2. Harrison, B.A. Medical Entomology Studies-XIII. The Myzomyia Series of Anopheles (Cellia) in Thailand, with Emphasis on Intrainterspecific Variation (Diptera: Culicidae). Contrib. Am. Entomol. Inst. (Ann Arbor). 17(4):1-195, 1980.

3. Peyton, E.L. and Harrison B.A. Anopheles (Cellia) takasagoensis Morhishita 1946, an additional species in the Balabacensis Complex of Southeast Asia (Diptera: Culicidae). Mosq. Syst. 12:335-347, 1980.

11. Entomological Evaluations of Human Malaria Transmission in a Village-Rice Field Scenario on the Korat Plateau of Thailand

PROBLEM: The overall objectives of this study were: (a) to obtain epidemiological and vector information regarding human malaria transmission; (b) to confirm the presence of known primary, secondary and/or suspected Anopheles vectors of human

malaria parasites; (c) to identify the vector(s) of malaria parasites by dissection; (d) to determine the human malaria prevalence and ratio of endemic: migrant infections; (e) to confirm the presence or absence of An. philippinensis and/or nivipes, and determine their role in the transmission of human malaria parasites; (f) determine the parity rates, nocturnal biting cycle and host propensity of the vector(s), particularly that for philippinensis and/or nivipes; and (g) determine the natural larval habitats of potential vector(s), particularly philippinensis and/or nivipes.

PROGRESS: This project was terminated early in the FY due to insufficient funds and personnel. Before termination, however, several aspects of the study were completed. A blood smear survey (1) was conducted for malaria parasites in 519 people in the study village. This survey revealed one boy positive for P. vivax. Mosquito collections prior to and during the project revealed that Anopheles nivipes was very common in the area, while An. philippinensis was not collected. A colony of An. nivipes was established from specimens collected in the study area. The methods and techniques used for colonizing this species are described elsewhere (2). Anopheles nivipes was an avid human biter in human bait collection in the village and in the laboratory, but is very reluctant to feed on hamsters.

RECOMMENDATIONS: This project has been terminated due to lack of funds and personnel. Anticipated projects with a higher priority most likely will preclude the reinitiation of this project should resources again become available.

REFERENCES:

1. Klein, T., Harrison, B.A., Malikul, S. Entomological Evaluation of Human Malaria Transmission in a Village-Rice Field Scenario on the Korat Plateau of Thailand. AFRIMS Annual Progesss Report, Oct 79-Sep 80.

2. Klein, T.A., Harrison, B.A., Inlao, I., and Boonyakanist, P. Colonization of Thailand Strains of Anopheles philippinensis Ludlow and Anopheles nivipes (Theobald) (Diptera:Culicidae). (Manuscript in preparation).

12. Comparative Susceptibility of Known and Suspected Species/Strains of Anopheles to Plasmodium Parasites

PROBLEM: The objectives of this study are: (a) to determine and compare the susceptibility of colonized primary and suspected secondary vectors of malaria to human and simian Plasmodium

parasites; (b) to observe the development of malaria parasites in anopheline species with varying degrees of susceptibility to malaria parasites; and (c) to observe the feeding behavior of colonized primary and suspected secondary vectors of malaria under laboratory conditions.

PROGRESS: During this reporting period and the previous one (1), studies testing the susceptibility of seven mosquito species/strains to the simian parasite, Plasmodium cynomolgi were concluded. In summing up these studies, An. balabacensis (Perlis form), An. takasagoensis, An. maculatus (IMR-Kuala Lumpur strain), and the An. maculatus (Thailand-Nakhon Nayok strain) demonstrated a high degree of susceptibility to P. cynomolgi (B strain) when compared to An. dirus, while An. maculatus (Huai Kuum strain), and An. philippinensis (Rayong strain) demonstrated a low susceptibility to this parasite. The differences detected here demonstrate the importance of intraspecific strain variation in the susceptibility to malaria parasites.

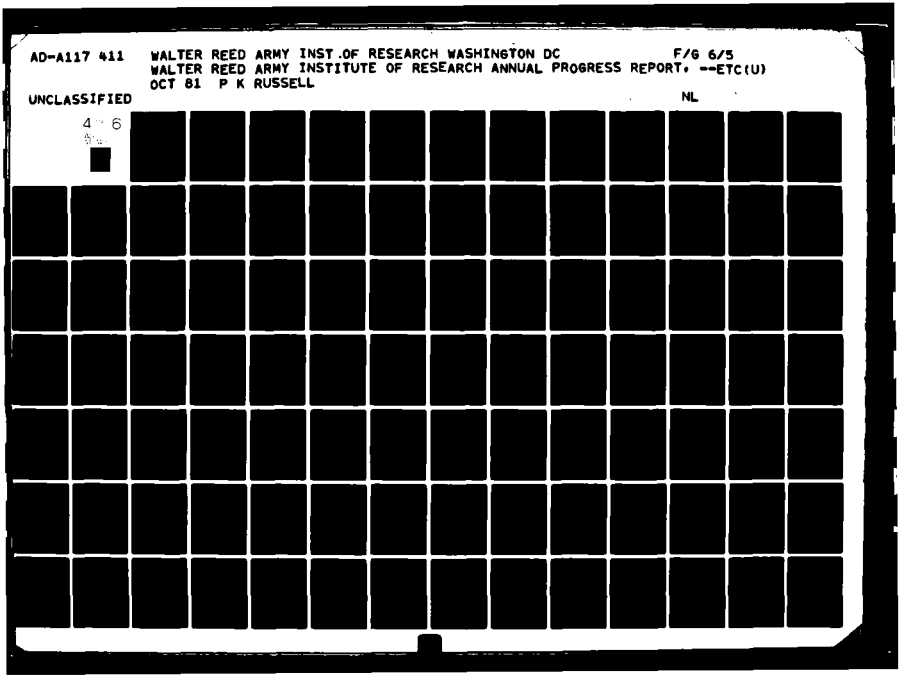
Beside testing the susceptibilities of these species/strains to P. cynomolgi, these efforts were designed to develop and evaluate the methods and techniques that are necessary for conducting human malaria susceptibility studies. The P. cynomolgi studies were terminated in preparation for the beginning of P. falciparum and P. vivax studies. A manuscript (2) regarding the P. cynomolgi susceptibility studies is in preparation.

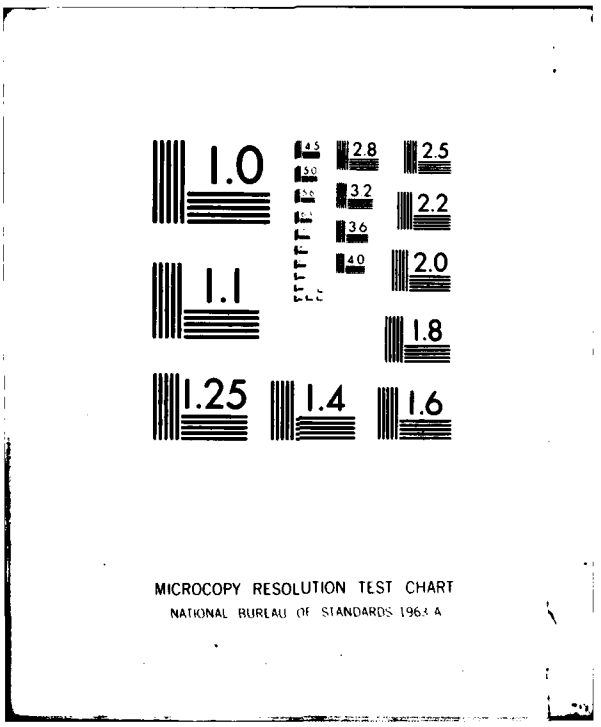
Preliminary aspects of the human malaria susceptibility studies were initiated early this FY with the submission of a protocol for clearance by respective health officials and Human Use Committees. This protocol was approved by the Thailand Ministry of Health and the Human Use Committee, Office of the Surgeon General, U.S. Army, in May 1981. Subsequently, arrangements for a field study site have proceeded rapidly. The human malaria susceptibility studies were actually initiated in Tha Muang, Kanchanaburi, on 5 October 1981.

RECOMMENDATIONS It is anticipated that this study as presently designed will have to continue for at least two rainy seasons (over a 2-year period).

REFERENCES:

1. Harrison, B.A., Klein, T.A. Comparative Susceptibility of Known and Suspected Species/Strains of Anopheles to Malaria Parasites. AFRIMS Annual Progesss Report, [illegible]


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2. Klein, T.A., Harrison, B.A., Vongpradist, S., and Inlao, I. Comparative Susceptibility of Known or Suspected Vector Species/ Strains of Anopheles to Plasmodium cynomolgi (B strain). (Manuscript in preparation).

13. Detrimental Effects of Plasmodium Infections on the Survival Rate of Anopheles dirus

PROBLEM: The overall objectives applying to this report are: (a) to determine if the longevity of mosquitoes infected with Plasmodium spp. is significantly different from that of mosquitoes that are not infected; (b) to determine if the longevity among mosquitoes with different "infection rates" of Plasmodium spp. is significantly different; and (c) to determine if the longevity of mosquitoes infected with different Plasmodium spp. is significantly different among groups.

PROGRESS: The P. cynomolgi-Anopheles dirus studies were terminated shortly after the beginning of FY 81. Nearly all aspects of the results of those studies were discussed in last year's annual report (1). Since then two manuscripts have been prepared and cleared for publication. The 1st (2) describes differences found in the mortality rates of infected versus non-infected groups of An. dirus. Non-infected mosquitoes exhibit only one peak period of mortality, while the infected mosquitoes exhibit two peak periods of mortality. The second manuscript (3) correlates the differences found in the survival rates of infected and non-infected groups with the infection density. These data show that there is an indirect relationship between survival rates and infection density, i.e. the most heavily infected mosquitoes have the lowest survival rate, while lightly infected mosquitoes have a survival rate comparable to that of non-infected mosquitoes. Both papers stress the impact of these findings on WHO malaria epidemiological models, if the same results are obtained in human malaria studies. Both papers have been submitted to journals for publication.

RECOMMENDATIONS: Human malaria aspects of this project were delayed and only began as of 5 October 1981. Results of these studies will be presented in next year's report. These studies on the human malaria aspects are continuing.

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1. Klein, T.A., and Harrison, B.A. Detrimental Effects of Plasmodium cynomolgi Infections on the Survival Rate of Anopheles dirus (Diptera: Culicidae). AFRIMS Annual Progess Report, Oct 79-Sep 80.

2. Klein, T.A., Harrison, B.A., Andre, R.G., Whitmire, R.E, and Inlao, I. Detrimental Effects of Plasmodium cynomolgi Infections on the Longenity of Anopheles dirus (Diptera: Culicidae). (Manuscript submitted for publication).

3. Klein, T.A., Harrison, B.A., Grove, J.S., Vongpradist, S. Correlation of Survival Rates of Anopheles dirus (Diptera: Culicidae) with Different Infection Densities of Plamodium cynomolgi. (Manuscript submitted for publication).

Project Number: 3M162770A870
Work Unit Number: 073
Title: Threat Assessment of Diseases of Military Importance in the Tropics

Presentations:

1. Burke, D.S., Jatanasen, S., Watts, D.M., Tang, D.B. Correlation Between Cool Season Environmental Temperatures and Dengue hemorrhagic Fever (DHF) Case Rates in Bangkok, Thailand. Twenty-ninth Annual Meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, USA, November 1980.

2. Watts, D.M., Burke, D.S., Harrison, B.A. Effects of Temperature on the Extrinsic Incubation Period of Dengue Virus in Aedes aegypti. Twenty-ninth Annual Meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, USA, November 1980.

3. Burke, D.S., Jatanasen, S., Watts, D.M., Tang, D.B. Correlation Between Cool Season Environmental Temperatures and Dengue Hemorrhagic Fever (DHF) Case Rates in Bangkok, Thailand. II. Effects of Temperature on the Extrinsic Incubation Period of Dengue Virus in Aedes aegypti. Tenth International Congress on Tropical Medicine and Malaria, Manila, Philippines, November 1980.

4. Burke, D.S., Chutichedej, P., Boongrapu, P., Pal, S.N. Etiology of Acute Hepatitis in Thailand. Tenth International Congress on Tropical Medicine and Malaria, Manila, Philippines, November 1980.

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2. Burke, D.S., Jatanasen, S., Watts, D.M., Tang, D.B. Correlation Between Cool Season Environmental Temperatures and Dengue Hemorrhagic Fever (DHF) Case Rates in Bangkok, Thailand (abstract). Proceedings of the Tenth International Congress on Tropical Medicine and Malaria, Manila, Philippines, November 1980.

3. Burke, D.S. Chutichedej, P., Boongrapu, P., Pal, S.N. Etiology of Acute Hepatitis in Thailand (abstract). Proceedings of the Tenth International Congress on Tropical Medicine and Malaria, Manila, Philippines, November 1980.

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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 6538	81 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
80 10 01	D. Change	U	U		NL	XYes ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AA	151
b. CONTRIBUTING				
XXXXXXXXX	STOG 80-7.2.2			

11. TITLE (Precede with Security Classification Code)
(U) Characteristics of Attenuated Dengue Viruses

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 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)
PREVIOUS FISCAL YEAR 81	2.0	217
CURRENT FISCAL YEAR 82	2.0	246

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL, MC
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Div of CD&I
ADDRESS: Washington, DC 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Eckels, Kenneth H., Ph.D.
TELEPHONE: 301-427-5208
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Harrison, Venton R.
NAME: Summers, Peter L. POC: DA

21. GENERAL USE
Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Attenuation; (U) Human volunteer; (U) Dengue; (U) Vaccine; (U) Immunity; (U) Cell culture

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 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 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 virus.

25. (U) 80 10 - 81 09 1. Lot 1 of dengue-2 vaccine continued to be tested in groups of human volunteers. A placebo-controlled, randomized study involving 149 volunteers was conducted at Ft. Bragg. As observed in previous studies, yellow fever immune individuals seroconverted to a higher percentage than non-immune volunteers when given the S-1 vaccine. Clinical symptoms of dengue fever were similar in both groups following vaccination. (This was a collaborative study done in conjunction with the Dept. of Virus Diseases). 2. The C6/36 clone of Aedes albopictus mosquito cells was further evaluated as a vaccine substrate. Tests for adventitious agents as well as tumorigenicity and karyologic analysis indicate that the C6/36 line is acceptable for use in vaccine preparation. Passage of dengue-2 and dengue-3 viruses in C6/36 cells causes a selection for a small plaque phenotype concomitant with suckling mouse attenuation. Clones are being prepared for further evaluation in rhesus monkeys. 3. Lot 1 of dengue-4 vaccine, has been prepared at WRAIR following development of an acceptable seed virus by Dr. S.B. Halstead of the University of Hawaii. Neurovirulence tests in rhesus monkeys and other safety tests are being completed. Submission of an IND to the Bureau of Biologics and plans for phase I human trials are being formulated. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 151: Characteristics of Attenuated Dengue Viruses

Investigators:

Principal: Venton R. Harrison

Associates: Kenneth H. Eckels, Ph.D.
Peter L. Summers

Problem and Objectives

The project involves the development, production, and assay of live-attenuated vaccines against various strains of dengue viruses. Isolates of dengue types 1 and 3 viruses 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 lots and vaccine lots.

Progress

1. Lot 1 of dengue-2 vaccine was continued to be tested in groups of human volunteers. A placebo-controlled, randomized study involving 149 volunteers was conducted at Ft. Bragg starting in March, 1981. The study was designed to test groups of yellow fever immune and non-immune recipients for their clinical and serologic responses to dengue-2 vaccination. A control group receiving a virus-free placebo was also included. Preliminary results indicate a higher seroconversion rate in yellow fever immune volunteers which has been observed in previous smaller groups receiving the vaccine. Although this was the case, approximately 70% of non-immune volunteers stimulated the production of neutralizing antibodies. The rate of clinical symptomatology was similar in the two groups while a greater number of yellow-fever immune vaccinees removed themselves from duty assignments. This may indicate an increased severity of dengue-related disease symptoms. (This was a collaborative study done in conjunction with the Department of Virus Diseases). 2. A clone (C6/36) of Aedes albopictus mosquito cells was evaluated as a possible vaccine substrate. Dengue-2 and dengue-3 viruses reach titers of 10-100 fold higher in the C6/36 cells compared to mammalian cells. It was also found that continuous passage of several

dengue viruses resulted in a selection for small plaque virus which appears to be temperature sensitive. Several clones of dengue-2 and dengue-3 viruses derived from small plaque-enriched seeds appear to be genetically stable. The mouse neurovirulence of a dengue-2 clone is reduced compared to virus taken from an earlier passage. These clones are currently being tested for attenuation and immunogenicity in rhesus monkeys. Tests to detect adventitious microbial agents were negative when the C6/36 cells were inoculated in various cell cultures and animals. Additionally, the cell line is not tumorigenic and is karyologically normal. An RNA dependent RNA polymerase was found to be associated with cell membranes and preliminary evidence indicates that it is a poly-G-specific polymerase. A contracting laboratory, the Salk Institute at Swiftwater, PA, is in the process of establishing a large volume cell bank that will be used for vaccine development in the future. Testing of this bank is underway. 3. The first lot of dengue-4 vaccine was prepared at WRAIR following delivery of an acceptable seed virus by Dr. S.B. Halstead of the University of Hawaii. Both the vaccine and production seeds have passed all safety tests; neurovirulence tests in rhesus monkeys are being completed. Submission of an IND to the Bureau of Biologics and plans for phase I human trials are being formulated.

Future Objectives

In the coming fiscal year, a master seed for a new dengue-2 vaccine (clone S-1/3C) will be prepared and tested. Production and vaccine lots will be prepared following the completion of testing and "future need" analysis. A dengue-3 clone derived from C6/36 cells will be chosen for further seed and vaccine preparation. Acceptable levels of attenuation and immunogenicity will determine the feasibility of using currently available clones or if other clones will have to be isolated. Dengue-4 vaccine trials will be arranged in a small group of volunteers located at Ft. Detrick. The phase I trials for dengue-4 will follow the dengue-2 vaccine human trial protocols.

Formal Presentations

Early interactions of attenuated dengue viruses with mammalian and mosquito cells. K.H. Eckels, P.L. Summers, and P.K. Russell (Presented to the American Society of Tropical Medicine and Hygiene Annual Meeting, Atlanta, GA, Nov, 1980).

Publications

Bancroft, W.H., F.H. Top, K.H. Eckels, J.H. Anderson, Jr., J.M. McCown, and P.K. Russell. 1981. Dengue-2 vaccine: Virological, immunological, and clinical responses of six yellow fever-immune recipients. Infect. Immun. 31: 698-703.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6766	81 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
80 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	AB	152
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7,2:2			

11. TITLE (Precede 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. 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 81	1.0	213
	CURRENT 82	1.0	185

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of CD&I

ADDRESS: Washington, DC 20012

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

NAME: Formal, Samuel B., Ph.D.

TELEPHONE: (202) 576-3344

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: B. Brandt

NAME:

21. GENERAL USE

Foreign intelligence not considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Vaccines; (U) Human Volunteers; (U) Meningococci; (U) Pseudomonas; (U) Shigella

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS. (Furnish 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 group B, Y and 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.

24. (U) Experimental vaccines, consisting of living attenuated bacteria, killed bacteria, or 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 experimental products are tested in soldier volunteers for safety and antigenicity.

25. (U) 80 10 - 81 09 An experimental tetravalent (A,C,Y,W135) meningococcal polysaccharide vaccine was administered at two different doses, (45 or 90 micrograms of total polysaccharide to 300 basic training recruits at Ft. Dix. Greater than 90per cent of the recruit volunteers with no pre-existing antibody showed a four-fold or greater increase to each of the polysaccharide components independent of dose. No adverse side reactions were observed. Clinical evaluation of a novel meningococcal group B vaccine consisting of a noncovalent complex of group B capsular polysaccharide and serotype-specific outer membrane proteins showed it to be safe and immunogenic. (For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.)

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

t 3M162770A871 PREVENTION OF MILITARY
E HAZARDS

nit 152: Role of Polysaccharide Antigens in
ty

igators:
ncipals: Wendell D. Zollinger, Ph.D.
Samuel B. Formal, Ph.D.
Brenda Brandt

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nfectious diseases continue to be a threat to
ry operations. Effective vaccines are a means to
l infections, and several have reached the stage
elopment which requires preliminary testing in
beings for safety and antigenicity. Current
is is on the testing of meningococcal group B,
W-135 vaccines and on a gonococcus pilus vaccine.
inary safety and antigenicity studies in human
of experimental vaccines are necessary before
ry studies of experimental vaccines can be under-
Experimental vaccines, consisting of living
ated bacteria, killed bacteria, or purified pro-
extracted from bacteria, are prepared in pilot
y the Dept. of Biologic Products, WRAIR. These
sted for safety and antigenicity in the labora-
Following review by the SGO and the Bureau of
ics, FDA and with the consent and cooperation
ld Commanders, these experimental products are
in soldier volunteers for safety and antigeni-

ss

vaccine composed of group A, C, Y and W135
ar polysaccharides from Neisseria meningitidis
epared and tested in volunteers. The 288
eers were randomly divided into three groups;
l received 45 µg of polysaccharide containing
A, 15 µg C, 7.5 µg Y, and 7.5 µg W135; group 2
ed 90 µg of polysaccharide or twice the 45 µg
group 3 received 100 µg of the standard A & C
ococcal vaccine (50 µg A and 40 µg C) given to
coming basic trainees. Serum samples were
ed from each volunteer at the time of vaccination
weeks later. Reactogenicity of the vaccine was

evaluated 18-24 hours after immunization. None of the volunteers had a complaint severe enough to go to sick call. Independent of vaccine dose, the homologous serum bactericidal test demonstrated that greater than 90% of the volunteers responded with a four fold or greater rise in antibody to the group A, C and W135 components of the tetra-valent vaccine, and greater than 80% demonstrated a four fold or greater rise to the group Y. Volunteers with a pre existing bactericidal antibody titer to group Y of less than or equal to 1:16 all show a four fold or better change in titer to the group Y component. When the geometric mean antibody titer (Farr and Bactericidal) of the A and C components of the tetravalent vaccine were compared to the titer of the standard A and C meningococcal vaccine (Connaught Lot 2689LF) by one way analysis of variance, a significant difference was found among the three vaccines. This difference may not be of any great significance to the volunteer since all three preparations produced greater than 90% conversions of volunteers to A and C. In addition, the geometric mean antibody response induced by two different lots of standard A & C vaccines (Connaught A & C, Lot 2358KE and Lot 2689LF), when analyzed by the t test, were also found to be significantly different (Farr data), indicating a possible lot to lot variation.

A meningococcal group B vaccine consisting of a noncovalent (hydrophobic) complex of group B capsular polysaccharide and serotype 2 outer membrane protein has been tested in volunteers. Serologic analysis of the serum samples indicate that the dose-response curve for antibody response to the group B polysaccharide is flat over the range from 30 g to 120 g. The antibody response to the outer membrane proteins was weakly dose dependent over this range with the 120 g dose inducing a stronger response than the 30 g or 60 g doses. Reactogenicity also showed only a weak dose response effect. Local reactions, which typically consisted of erythema (2 to 3 cm diameter) and sometimes induration at the vaccination site, were slightly greater in frequency and intensity at the 120 g dose than at the 30 and 60 g doses.

A vaccine composition study was conducted to determine the optimal ratio of polysaccharide to outer membrane protein in the vaccine. Five lots of vaccine

were compared. These consisted of purified group B capsular polysaccharide alone, the outer membrane protein alone, and three lots prepared by complexing the polysaccharide and protein together in the relative proportions of 3:1, 1:1 and 1:3. The strongest antibody response to the B polysaccharide was obtained with a polysaccharide to protein ratio of 1:3, and the strongest antibody response to the proteins was obtained with the 1:1 ratio. The polysaccharide alone was non-immunogenic and the protein alone was only slightly immunogenic. The results clearly demonstrated that non-covalent complexing of the polysaccharide and protein enhanced the immunogenicity of both. In conjunction with the increased immunogenicity, however, a somewhat increased level of reactogenicity was also observed. A statistically significant correlation was found between the occurrence of a number of febrile reactions and the administration of the group B vaccine within 12 hours after the recruits had received the meningococcal A+C vaccine.

Future Plans

A tetravalent meningococcal vaccine consisting of group A, L, Y and W135 capsules polysaccharides prepared by a commercial company will be tested for reactogenicity and antigenicity in volunteers.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6442	81 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
80 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	AC	153
b. CONTRIBUTING				
c. [redacted]	STOG 80-7,2,2			

11. TITLE (Provide with Security Classification Code)

(U) Rickettsial Diseases of Military Personnel

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 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 81	4.0	348
	CURRENT 82	4.0	407

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

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

NAME: Osterman, J.V., Ph.D.

TELEPHONE: (202) 576-2146

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Bernier, D.R., LTC, MC, M.D., Ph.D.

NAME: Jerrells, T.R., Ph.D. POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Rickettsial Infections; (U) Laboratory Diagnosis; (U) Vaccines; (U) Epidemiology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish 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; 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. Determine the genetic basis of resistance and sensitivity of the mouse model to scrub typhus infection.

25. (U) 80 10 - 81 09 A mouse model system was used to analyze the in vivo response of lymphocytes to rickettsial antigens. Sensitive and resistant strains of mice expressed a delayed-type hypersensitivity (DTH) early in the course of infection with R. tsutsugamushi. The sensitive animals exhibited a marked decline in reactivity just prior to death. Reactivity of resistant mice remained high through day 9 and declined slowly through day 15. Rechallenge of resistant mice elicited a rapid increase in reactivity, suggesting a secondary memory response. Transfer of DTH was accomplished using immune thymus-derived splenic lymphocytes. Abrogation of the ability of immune spleen cells to transfer DTH following treatment with anti-Thy alloantiserum and complement indicated these responses to scrub typhus rickettsiae were mediated by T-lymphocytes. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 80 - 30 Sep 81.

*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: 1974-546-043/6691

PROJECT 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit: 153 Rickettsial Diseases of Military Personnel

Investigators
Principals: Joseph V. Osterman, PhD; LTC Ralph D. Bernier, MC; CPT(P) Daryl J. Kelly, MSC; Thomas R. Jerrells, PhD
Associates: CPT Jeffrey A. Havenner, MSC; SP4 Miriam R. Pedersen; PFC Bradley R. Oelmann

Description

Investigations are designed to develop experimental rickettsial immunogens, define the pathogenesis of rickettsial infections in laboratory animals, and determine the sequence of events leading to immunity following vaccination or infection. These studies are directed toward development of safe, efficacious rickettsial vaccines which will protect deployed troops, and development of accurate, sensitive immunoassays to evaluate the extent of immunity induced by vaccination.

Progress

Research efforts have continued toward the development of an efficacious vaccine against scrub typhus (1,2). Rickettsiae propagated in embryonated eggs and inactivated by gamma radiation elicited long-lasting protection in mice against a number of antigenically diverse challenge strains of Rickettsia tsutsugamushi. Current efforts are focused on propagating rickettsiae in tissue culture cells suitable for human vaccine use. Preliminary experiments have indicated that chicken embryo fibroblasts are a suitable substrate for growth of rickettsiae, and that substantial purification of these rickettsiae is possible by Renograffin density gradient centrifugation. Studies are in progress to determine the culture conditions optimal for maximum rickettsial yield.

Pathogenesis studies have been conducted in a mouse model system. The inflammatory response of susceptible mice to intraperitoneal infection with Rickettsia tsutsugamushi was evident approximately 5 days postinfection, and the magnitude of the cellular influx increased until death of the animal. The inflammation consisted of an early polymorphonuclear leukocyte response, followed by a mononuclear cell influx which persisted until death of the animal. Resistant mice evidenced similar kinetics of cell influx, but the inflammatory response was

significantly reduced in magnitude, and was predominatly mononuclear in nature, with little influx of polymorphonuclear leukocytes into the peritoneal cavity. Resistant mice could be rendered susceptible to infection by induction of a nonspecific inflammation prior to infection. Conversely, treatment of susceptible animals with an anti-inflammatory agent prolonged survival after infection.

Both sensitive and resistant strains of mice expressed a delayed-type hypersensitivity early in the course of infection (5-7 days) with Rickettsia tsutsugamushi. The sensitive animals exhibited a marked decline in reactivity just prior to death. In contrast, reactivity of resistant mice remained high through day 9 and declined slowly through day 15 after infection. Rechallenge of resistant mice elicited a rapid increase in reactivity, suggesting a secondary memory response. Transfer of delayed-type hypersensitivity reactivity was accomplished using immune thymus-derived splenic lymphocytes isolated on nylon wool columns. Abrogation of the ability of immune spleen cells to transfer delayed-typed hypersensitivity reactivity following treatment with anti-Thy 1.2 alloantiserum and complement further supported the view that these responses to scrub typhus rickettsiae were mediated by thymus derived lymphocytes.

Recommendations for Future

Studies will continue toward preparation of a gamma-irradiated scrub typhus vaccine from rickettsiae propagated in tissue culture cells suitable for human vaccine use. Immunogens produced will be evaluated for efficacy in the mouse model system. Subsequently, it is anticipated that sufficient quantities of immunogen will be produced to permit extensive evaluation in a subhuman primate model.

The cellular nature of inherent resistance in mice will be explored further using an irradiation chimera model, nude mice, and by selective elimination of effector cells with an appropriate antiserum and complement. In vitro and in vivo correlates of cell mediated immunity will be examined in mice following vaccination with gamma-irradiated immunogens, and will be compared with the protective immune response determined by direct challenge.

References Cited

1. Eisenberg, G.H.G., Jr., and J.V. Osterman. 1978. Gamma-irradiated scrub typhus immunogens: Development and duration of immunity. Infect. Immun. 22:80-86.

2. Eisenberg, G.H.G., Jr., and J.V. Osterman. 1979. Gamma-irridated scrub typhus immunogens: Board-spectrum immunity with combinations of rickettsial strains. Infect. Immun. 26:131-136.

Presentations

1. Jerrells, T.R., and J.V. Osterman. Parameters of cellular immunity in acute and chronic Rickettsia tsutsugamushi infections of inbred mice. 4th Biennial Basic Science Symposium, Host Defenses to Intracellular Pathogens, Philadelphia, Pennsylvania, June 10-12, 1981.

Publications

1. Jerrells, T.R., and J.V. Osterman. 1981. Host Defenses in Experimental Scrub Typhus: Inflammatory Responses of Congenic C3H Mice Differing at the Ric Gene. Infect. Immun. 31:1014-1022.

2. Osterman, J.V., and G. Rapmund. 1981. Rickettsial Infections. Medicine International 1:129-134.

Manuscripts Submitted

1. Jerrells, T.R., and J.V. Osterman. 1981. Host Defenses in Experimental Scrub Typhus: Delayed-Type Hypersensitivity Responses of Inbred Mice. Infect. Immun. (submitted).

2. Kelly, D.J., J.V. Osterman, and E.H. Stephenson. 1981. Rocky Mountain Spotted Fever in the Southeastern United States: Survey for Canine Antibodies to Spotted Fever Rickettsiae. Am. J. Vet. Research (submitted).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6531	81 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
80 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	AH	154
b. CONTRIBUTING				
c. ~~XXXXXXX~~	STOG 80-7.2:2			

11. TITLE (Procede 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 81	3.0	292
FISCAL YEAR CURRENT 82	1.0	306

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL, MC

TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of CD&I

ADDRESS: Washington, DC 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. academic participant)

NAME: Williams, James E., MAJ, MSC

TELEPHONE: 301-427-5176 or 5110

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Harrison, Daniel N., Ph.D.

NAME: POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Yersinia pestis; (U) Plague; (U) Vaccines; (U) Immunization; (U) Serological tests; (U) Genetics

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

23. (U) Determine the factors influencing outbreaks of plague and the most appropriate methods to prevent the infection of troops engaged in field operation.

24. (U) Specimens and sera from humans and animals are tested for the presence of Y. pestis and antibody to Y. pestis. Strains of Y. pestis are characterized for determinants of virulence and antibiotic susceptibilities.

25. (U) 80 10 - 81 09 A prototype ELISA field kit with presensitized microtiter plates and lyophilized reagents was developed for surveillance of rodent plague and serodiagnostic confirmation of human plague. The ELISA, using F1 antigen for detection of antibody and an inhibition control to confirm reaction specificity, was successfully tested in South Africa on sera of clinically suspect plague cases from Ovamboland, Namibia. ELISA proved more sensitive than the standard indirect hemagglutination (IHA) test and increased serodiagnostic confirmations of plague from 6 percent to 42 percent. Killed plague vaccines prepared from avirulent Y. pestis were found by mouse potency tests to be as effective as Plague Vaccine, USP, for preventing plague. A staphylococcal radioimmune precipitation (St-RIP) technique being considered for serological potency assays of plague vaccines correlated with IHA in tests of sera from vaccinated humans, but St-RIP demonstrated several advantages over IHA, including greater sensitivity. Studies of plague genetics showed that certain attenuated live vaccine strains of Y. pestis that lack the pigmentation virulence factor become pigment-positive and virulent when induced with bacteriophage. A computer program in Fortran to store and sort data for an epidemiological forecasting system was completed and was employed to analyze data from historical outbreaks of plague in North America. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

* Available to contributors upon supplement's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE (1) FORMS 1498-1 NOV 68

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 154: Prevention and Treatment of Plague

Investigators:

Principal: COL Dan C. Cavanaugh, MSC

Associates: COL David M. Robinson, VC
MAJ James E. Williams, MSC
CPT Michael W. Hastriter, MSC
Dr. Daniel N. Harrison, Ph.D.
Michael K. Fortier
SSG Carol A. Braden

Problem

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 to typical plague, there exist potentials for plague from variant bacilli that are resistant to the more efficacious drugs used in therapy and from nonencapsulated strains that are not readily detected by current diagnostic tests. Objectives focused on three requirements for the protection of soldiers in combat environments: i) forecasting systems that rapidly and accurately indicate where and when plague surveillance or control activities should be instituted, ii) rapid, sensitive and field-adaptable procedures to confirm plague infection, characterize the pathogen and evaluate drug susceptibilities, and iii) sufficient supplies of safe, efficacious plague vaccine during emergencies.

Progress

A computer program in Fortran was completed to store and analyze data for an epidemiological forecasting system. Data from historical outbreaks of plague in America were entered and processed to demonstrate how the disease was apparently confined to California by high mountain barriers for about 20 years after which several years of mild weather permitted the disease to travel over mountains and become established in 11 other western states. Climatic conditions favorable for plague outbreaks in various localities can be ascertained with the system. A prototype ELISA field kit was developed for rapid serodiagnostic confirmation of human plague and for surveillance of rodent plague. The kit includes presensitized microtiter plates and lyophilized reagents. The ELISA, using Fl

antigen for detection of antibody and an inhibition control to confirm reaction specificity, was successfully tested in South Africa on sera of clinically suspect plague cases from Ovamboland, Namibia. ELISA proved more sensitive than the standard indirect hemagglutination (IHA) test and increased serological confirmations of plague from 6 percent to 42 percent, depending upon the sensitivity of the IHA test used in the comparison. A portable microphotometer was perfected for use with ELISA in the field. The microphotometer should be useful for a variety of other chemical tests. Investigations continued on killed plague vaccines prepared from avirulent strains of Y. pestis in order to reduce requirements for high-containment facilities during emergency vaccine production. Experimental vaccines were as effective as Plague Vaccine, USP, in preventing plague in laboratory mice and rats. To further reduce high-containment facilities, a staphylococcal radioimmune precipitation (St-RIP) technique was considered for serological potency assays of plague vaccines. In tests of sera from vaccinated humans, the St-RIP demonstrated several advantages over IHA, including greater sensitivity. In studies of live plague vaccines, certain attenuated vaccine strains of Y. pestis that lack the pigmentation virulence factor were made pigment-positive and virulent following exposure to bacteriophages.

Recommendations

The computerized forecasting system operational for plague in the United States can be applied globally; data entry and analysis should emphasize regions presently of greatest interest to the Department of Defense. Development and field-testing of improved diagnostic capabilities for plague should be directed towards rapid detection of specific Y. pestis antigens using ELISA or other technologies for confirmation of clinically suspect morbidity or mortality and rapid tests for drug susceptibilities to determine effective therapy. Investigations of an alternative killed plague vaccine prepared from avirulent Y. pestis should ascertain if seroimmunity from vaccination is comparable to that derived from Plague Vaccine, USP.

Publications

1. Cavanaugh, D.C. and Williams, J.E. 1980. Plague: Some ecological interrelationships. IN: Traub, R. and Starcke, H. (ed.) Fleas - Proceedings of the International Conference on Fleas, Ashton Wold, Peterborough, UK, 21-25 June 1977. Balkema, Rotterdam, pp. 245-256.

2. Williams, J.E., Hudson, B.W., Turner, R.W., Sulianti Saroso, J., and Cavanaugh, D.C. 1980. Plague in Central Java, Indonesia. Bulletin of the World Health Organization 58(3): 459-468.

3. Williams, J.E., Altieri, P.L., Berman, S., Lowenthal, J.P., and Cavanaugh, D.C. 1980. Potency of killed plague vaccines prepared from avirulent Yersinia pestis. Bulletin of the World Health Organization 58(5): 753-756.

4. Hastriter, M.W. and Cavanaugh, D.C. 1981. An apparatus for colonizing fleas (Siphonaptera) and collecting pupal cocoons. Journal of Medical Entomology 18: 251-252.

5. Schaffer, F.L., Soergel, M.E., and Williams, J.E. 1981. Antibody response to plague vaccination in humans as assayed by staphylococcal radioimmune precipitation (St-RIP) test. Journal of Biological Standardization 9: 265-276.

Submitted for Publication

1. Cavanaugh, D.C., Cadigan, F.M., Williams, J.E., and Marshall, J.D. Plague. IN: Infectious Diseases, History of Internal Medicine in Vietnam and Southeast Asia, Surgeon General Office, Department of Army (In press).

2. Williams, J.E. and Robinson, D.M. Requirement to confirm the specificity of ELISA reactions. Transactions of the Royal Society of Tropical Medicine and Hygiene.

3. Williams, J.E., Arntzen, L., Robinson, D.M., Cavanaugh, D.C., and Isaäcson, M. Comparison of passive hemagglutination and enzyme-linked immunoassay for serodiagnosis of plague. Bulletin of the World Health Organization.

Patents

Patent No. 4,299,493 was awarded for a portable microphotometer designed to function in the field on battery or on line current.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 08 6536	81 10 01	DD DR&E/AR(A)36

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
80 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	AF	155
b. XXXXXXXXX				
c. XXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Determination of Pharmacological Effects of Antimalarial Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 07	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 81	3.0	213
	CURRENT 82	3.0	435

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, COL P.

TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of Experimental Therapeutics

ADDRESS: Washington, DC 20012

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

NAME: HEIFFER, Dr. M.H.

TELEPHONE: 301-427-5393

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: CHUNG, Dr. H.

NAME: FLECKENSTEIN, Dr. L. POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Pharmacodynamics; (U) Pharmacokinetics; (U) Toxicology; (U) Antimalarial Drugs; (U) Preclinical Pharmacology; (U) Quantitation Methodology

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

23. (U) Research objectives are the development of in vivo and in vitro models to study the pharmacodynamics and the metabolism and pharmacokinetics of antiparasitic compounds being developed for use in man. These studies are designed to provide the developmental rationale for the introduction of candidate drugs to man as well as satisfying all regulatory requirements for granting an IND for clinical trial of the candidate drugs. These compounds are developed to permit maximum utilization of military personnel in areas where parasitic diseases are endemic.

24. (U) Studies are performed in non-infected, healthy animals to determine the manner in which the candidate drug is metabolized by the animal in addition to determining how the drug produces its effect. These studies are necessary to predict human tolerance to the candidate drug (Phase I). Pharmacokinetic analysis of the drug actions using analytical techniques specifically developed for each candidate drug provides a rational basis for dosing during human studies.

25. (U) 80 10 - 81 09 Technical management continued for 13 extramural pharmacology contracts. Administrative direction and support was continued or initiated for 8 IND's in an Active status and two potential IND compounds. WR 238,605 and WR 242,511 were shown to be 3 to 6 times more potent than primaquine in their ability to produce methemoglobinemia in vivo. The cardiorespiratory profiles of WR 6026 and WR 228,258 were determined. Intravenous infusion of either compound decreased heart rate and prolonged PR intervals. Evidence that WR 6026 may induce the release of histamine was also observed. The sensitivity of a thin layer chromatographic assay for mefloquine currently being developed was shown to be limited by degradation of the mefloquine during an evaporative step in the analytical procedure. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available in conjunction with originator's approval

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

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 155 Determination of pharmacological effects of antimalarial drugs

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
Associate: Dr. R. Rozman, MAJ J. von Bredow, Dr. H. Chung, Dr. L. Fleckenstein, CPT D. Korte, Jr., CPT J. Anders, LTC C. Pamplin, Dr. H. Lowensohn, SFC J. Baker, J.H. Digiovanni, SP5 J. Ferri, SP5 P. Porter, SP4 Lena Dokes, PFC S. Ivory

1. Description.

Studies undertaken by the department in support of the Army Drug Development Program have continued in two major areas. The first area is concerned with how the body affects the action of the candidate drug and includes studies to determine the pharmacokinetic and metabolic profiles of the drugs. The second major area is concerned with how the candidate drug affects the body or the pharmacodynamics of the drug. Sensitive drug assay methods and in vitro techniques necessary for the study of the drugs continue to be developed. In addition, the department continues to direct extramural contract work in support of active IND's and those compounds being groomed for IND status.

2. Progress.

A thin layer chromatographic technique for analysis of mefloquine in plasma, sensitive over a range of 100 to 1200 ng/ml, has been developed. Studies indicate greater than 90% recovery of mefloquine following extraction although there is a recovery rate of less than 70% for the entire analytical procedure. Reanalysis, using radiolabeled mefloquine, revealed a second peak suggesting partial decomposition of mefloquine during the procedure. Studies demonstrated that the mefloquine decomposed during the evaporative step used to concentrate the drug. This degradation was consistent in both prepared standards and clinical patient sets. Although this degradation limits the sensitivity, it does not appear to affect the accuracy or precision of the techniques.

The in vivo methemoglobin-producing potential of two primaquine analogs, WR 238,605 and WR 242,511, was studied in dogs using primaquine as a reference control. Previous in vitro work had suggested that WR 238,605 was equipotent with

primaquine while WR 242,511 had very little potential for methemoglobin production. However, the in vivo study results indicated that WR 238,605 was two to three times more potent and WR 242,511 was six to seven times more potent than primaquine in producing methemoglobinemia.

The cardiopulmonary profile in pentobarbital anesthetized beagles of two new antiparasitic drugs was assessed. WR 6026, a potent 8-aminoquinoline, produced a dose-related decrease in mean arterial pressure following bolus intravenous injections. This hypotension was attenuated by administering the WR 6026 as a slow infusion. This infusion of 17.8 mg/kg of WR 6026 over a 45 minute period also decreased heart rate and decreased the PR, QTc and QRS intervals of the lead II electrocardiogram. A most interesting phenomenon observed in these dogs during the various WR 6026 dosing regimens was the presence of urticaria and angioneurotic edema which was suggestive of a possible drug-induced release of histamine. Bolus injections of the amodiaquine analog, WR 228,258, produced dose-related decreases in mean arterial pressure, increases in respiratory rate and a biphasic heart rate response. Smaller doses of WR 228,258 reflexively increased heart rate while larger doses decreased heart rate. Infusion of 17.8 mg/kg WR 228,258 produced a decrease in heart rate and QTc interval and an increase in the PR interval. WR 228,258 pretreatment enhanced the vasopressor phase of the cardiovascular response to serotonin injections.

The department provided technical management for 13 extramural contracts in pharmacology. The department also provided administrative direction and support of intramural and extramural work on 8 drugs classified in an active IND status, as well as for two drugs being groomed for IND status.

3. Future Work.

Newly developed 8-aminoquinolines to include potential metabolites will be screened for methemoglobin-producing potential utilizing both the in vitro and in vivo assays. The metabolism of radiolabeled candidate drugs will be studied. The histamine-releasing potential of WR 6026 will be investigated. Additional studies are planned to increase the sensitivity of the mefloquine TLC analytical procedure.

ılications.

ıng, J., Jimmerson, V.R., Sanders, J.E., Bounds, D.W.,
, R.S., and Thorne, J.: The disposition of DL-3-di-n-
ımino-l-[2,6-bis(4-trifluoromethyl)-4-pyridyl]-propanol
:sulfonate (WR 172,435·CH_3SO_3H) in mice. Drug Metab.
9:65-66, 1981.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6495	81 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	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	AG	156
b. CONTRIBUTING				
c. XXXXXXX	STOG 80-7.2.2			

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
012100 Organic Chemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 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 81	5.0	343
	CURRENT 82	5.0	466

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS:
Washington, DC 20012
RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, P., COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
Div of Experimental Therapeutics
ADDRESS: Washington, DC 20012
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Pick, Robert O., MAJ MSC
TELEPHONE: (301) 427-5422
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Canfield, C.J., COL MC
NAME: POC: DA

21. GENERAL USE
Foreign intelligence not considered.

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Malaria; (U) Leishmaniasis; (U) Trypanosomiasis; (U) Schistosomiasis; (U) Antiparasitic Drugs; (U) Chemical Synthesis; (U) Antimalarials

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 manage, integrate, and provide technical direction for both a contract and in-house program to obtain potentially active antiparasitic agents for military use through rational organic syntheses.

24. (U) Necessary research areas are defined, proposed research evaluated, ongoing research guided, evaluated, and integrated with the other program elements. Technical advice is obtained through an Ad Hoc Study Group on Medicinal Chemistry. Information is exchanged by contractors through technical meetings.

25 (U) 80 10--81 09: Synthesis in the acridinedione-acridinedioneimine series continues. One resolved pair has been submitted for testing. In aotus monkey testing with human strains, one compound has shown good efficacy against both multi-resistant and sensitive strains. Quassinoids which show in-vitro activity are now in hand in sufficient quantity for multiple in-vivo testing. Work in the area of tissue schizonticides has decreased, but feedback from newly restarted rhesus testing will soon be available for guidance in this area. Antimalarial activity is still present in new members of the thiosemicarbazone series, as well as activity against resistant strains of N. gonorrhoeae. Efforts in the areas of schistosomiasis, leishmaniasis, and trypanosomiasis remain low, however, an oxime derivative exhibiting the best oral antitrypanosomal activity observed in the program to date has been produced. Data processing conversion to a new in-house computer has begun. Sample number verification of the old chemistry data base is complete, and structure-screen file verification has begun. Approximately 465 samples were submitted from the synthesis program. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 80 - 30 Sep 81.

* Available to contractors upon originator's approval

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 156 Synthesis of Antiparasitic Drugs

Investigators:

Principal: MAJ Robert O. Pick, Ph.D.
Associates: COL Craig J. Canfield, MC; William Y. Ellis, B.S.; Bing T. Poon, Ph.D.; Daniel L. Klayman, Ph.D.; CPT John P. Scovill, Ph.D.; Edgar A. Steck, Ph.D., Hikmat A. Musallam, B.S.

Efforts in this work unit were in four main areas described below.

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 7, leishmaniasis 1, trypanosomiasis 2. Two preparations laboratories and a radiolabel synthesis contract also supported the program. A portion of these last three contracts also supported the Chemical Defense Program. The decreased antiparasitic contractual effort is in line with the lack of activity noted in the last annual report.

The 8-aminoquinolines continue to show good blood as well as tissue schizonticidal activity. Efforts continue in two principal areas: (a) investigate chain length variation and branching in the 5-OR series; and (b) investigate the effect of substitution in the 2 and 3 position instead of, and in addition to, a CH_3 group in position 4. It is thus hoped to circumvent toxicity problems.

Two naphthalene analogs of the 8-aminoquinolines have been received and are awaiting tissue schizonticidal testing. Efforts will continue in this area. Also, synthetic efforts continue in the areas of amodiaquine analogs, pyrimethamine types, and acridinoneimines.

In leishmaniasis, a pyridine bis amidine type and its oxime precursor have provided the most orally active compound screened to date. Testing and synthetic work continue.

Efforts in the nucleoside contracts as antitrypanosomiasis agents have encountered problems in producing sufficient quantities for *in vivo* testing. *In vitro* testing and limited *in vivo* results have shown limited activity.

2. Data Processing

Efforts at converting the Chemical Information Retrieval System (CIRS) to a new in-house computer have begun utilizing the Walter Reed Army Institute of Research VAX11/780. Programs for the inventory system have been converted and work has begun on the chemistry system. The biology system will be largely re-written with remote interactive data entry included. Verification of the chemistry file continues. This should greatly aid the data base conversion effort to the new system.

3. Acquisition of Compounds

The following table summarizes the various classes of compounds received for screening/testing during FY 80.

	Originals	Duplicates	Total
Purchased	30	13	43
Gifts	125	51	176
Synthesized	330	65	395
Discreet	2049	122	2171
Prep Labs	30	42	72
	2564	293	2857

4. Organic Synthesis Section

About 90 compounds were synthesized for biological testing during the past year, virtually all of which are new to the chemical literature.

The construction of a series of 2-acetylquinoline and 1- and 3-acetylisoquinoline thiosemicarbazones is close to completion. Concomitantly, a parallel series has been made in which the azomethine bond is reduced. The latter technique has provided derivatives with lessened toxicity. It appears that the quinolines and isoquinolines have a level of antimalarial activity comparable to the pyridines.

In addition, a modest group of 2-acetylpyrazine thiosemicarbazones has been synthesized and is being evaluated. Initial indications are that the pyrazines are less active than the related pyridines. A new series of pyridine 1-oxide derivatives is now being prepared.

Some unusual reactions in the synthesis of thiosemicarbazones have been encountered. For example, the reaction of 2-acetylpyridine with 4-methyl-4-phenyl thiosemicarbazide in the presence of acetic acid gave a thiadiazole instead of the anticipated thiosemicarbazone. Moreover, in the absence of acetic acid a dithiourea is formed.

Thiosemicarbazones produced by us continue to show excellent in vitro antibacterial and in vivo antiherpes simplex virus activity in further testing by collaborating laboratories.

Some of our thiosemicarbazones have been found by the U.S. Department of Agriculture, Beltsville, MD, to have a profound effect on juvenile hormone production in certain insects.

The determination of primaquine in spiked blood by high performance liquid chromatography was extended into the submicrogram per milliliter level. Samples of unknowns in the 50 to 200 ng/mL range were assayed successfully using reversed phase hplc and tetramethylammonium chloride as modifier in the mobile phase. With butylamine phosphate as a modifier, the useful lower limit for assay was about 90 ng/mL.

The hplc system for primaquine was usefully demonstrated for aqueous solutions of WR 006,026 (the 6-diethylaminohexylamino analog of primaquine), showing linearity of response and increased sensitivity of detection over primaquine.

Work Unit 156 Synthesis of Antiparasitic Drugs

Papers Published

1. Klayman, D.L., Scovill, J.P., Bartosevich, J.F., and Mason, C.J. 2-Acetylpyridine Thiosemicarbazones. 3. Selenium Analogs as Potential Antimalarial Agents, Eur. J. of Medicinal Chem., 16, 317 (1981).

2. Scovill, J.P., and Klayman, D.L., Synthesis of 2-amino-1,3,4-thiadiazol-2-yl Hydrazones. Presentation at 28th Congress, International Union of Pure and Applied Chemistry, Vancouver, B.C., August 16, 1981.

3. Shipman, C., Smith, S.H., Drach, J.C., and Klayman, D.L., Antiviral Activity of 2-Acetylpyridine Thiosemicarbazones Against Herpes Simplex Virus, Antimicrob. Agents and Chemotherapy, 19, 682 (1981).

4. Scovill, J.P., and Silverton, J.V., Unusually Facile Ring-Opening Reaction in the Pyridine System, J. Org. Chem., 45, 4372 (1980).

Papers Submitted for Publication or in Preparation

1. Klayman, D.L., and Copeland, E., "Radioprotective Agents," Kirk-Othmer Encycloped. of Chemical Technology, John Wiley and Sons, 1982.

2. Collins, F.M., Klayman, D.L., and Morrison, N.E., Activity of 2-acetylpyridine and 2-acetylquinoline Thiosemicarbazones tested in vitro in combination with other antituberculous drugs, Am. Rev. of Respiratory Diseases, Jan. 1982.

3. Scovill, J.P., Klayman, D.L., and Francino, C., 2-Acetylpyridine Thiosemicarbazones. 4. Metal Complexes as Antimalarial and Antileukemic Agents, in preparation.

4. Collins, F.M., Klayman, D.L., and Morrison, N.E., Correlation between structure and antimycobacterial activity in a series of 2-acetylpyridine thiosemicarbazones, J. General Microbiology, in press.

5. Lambros, C., Childs, G.E., Notsch, J.D., Scovill, J.P., Klayman, D.L., and Davidson, D.E., In vitro Assessment of 2-Acetylpyridine Thiosemicarbazones as Potential Antimalarial Agents Against Chloroquine Resistant Plasmodium falciparum, in preparation.

6. Scovill, J.P., and Klayman, D.L., 2-Acetylpyridine Thiosemicarbazones. 5. 2-Acetylpyridine Hydrazine Derivatives of 1-Amino-2,5-dithiobiurea, in preparation.

7. Wysor, M., and Scovill, J.P., Antiparasitic Action of Silver Salts of Sulphonamides, in press.

8. Bavoso, A., Silverton, J.V., Scovill, J.P., and Klayman, D.L., The Zwitterionic Structure of 3-Azabicyclo[3.2.2] nonane-3-thiocarboxylic acid 2-[1-(2-pyridyl)ethylidene]-hydrazide, an antimalarial thiosemicarbazone, and its Ni(II) complex, in preparation.

9. Dobek, A.S., Klayman, D.L., Dickson, E.T., and Scovill, J.P., Inhibition of Clinically-Significant Bacterial Organisms in vitro by 2-Acetylpyridine, 2-Acetylquinoline, and 1- and 3-Acetylisoquinoline Thiosemicarbazones, in preparation.

10. Brown, N.D., Poon, B.T., and Chulay, J.D., Determination of Chloroquine and its De-ethylated Metabolites in Human Plasma by Ion-Pair High Performance Liquid Chromatography, J. Chromatog., in press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6535	81 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. GROUP INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	AF	157
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Experimental Drug Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

021600 Pharmacology 002600 Biology

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		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	6.9	418
	CURRENT 82	6.9	215

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Experimental Therapeutics

ADDRESS: Washington, DC 20012

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

NAME: DAVIDSON, David E., Jr., COL

TELEPHONE: (301) 427-5029

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Drug Development; (U) Antimalarials; (U) Biology; (U) Toxicology; (U) Plasmodium; (U) Malaria; (U) Chemistry; (U) Pharmacodyanamics; (U) Drug Metabolism;

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

23. (U) To design, test and develop new drugs with chemoprophylactic or chemotherapeutic activity against diseases of military importance.

24. (U) Active compounds are identified by testing candidate drugs for activity in laboratory model systems of the disease. Information is used in guiding new drug synthesis and in selecting candidate drugs for clinical trials. New laboratory test systems are developed.

25. (U) 8010-8109 Screening tests were done by in-house and contractor laboratories on approximately 3000 compounds in animal models for suppressive, causal prophylactic or radical curative antimalarial activity. Activity was found in approximately 200 compounds and approximately 100 were selected for advanced study including repository activity against P. berghei in mice and activity against human (P. falciparum) malaria in Aotus monkeys and vivax-like P. cynomolgi in rhesus monkeys. The P. falciparum in vitro culture test system was used for testing 600 compounds. Structure-activity relationships among series of 2-acetylpyridine thiosemicarbazones and 9-phenanthrene methanols were evaluated. Seventy-eight compounds were tested for efficacy as topical prophylactic anti-schistosomal compounds. Activity was found in eight compounds, three of which retained very high prophylactic activities subsequent to post-treatment water wash. Cultivation techniques for provision of metabolically active leishmania promastigotes have been optimized. Development of a semi-automated in-vitro drug screening system using these promastigotes and amastigotes of leishmania from other culture systems is in process. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

Project 3M162770A871AF PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 157 Experimental Drug Development

Investigators:

Principal:	COL David E. Davidson, Jr., VC
	LTC Larry D. Hendricks, MSC
	MAJ George E. Childs, MSC
	CPT Lyford K. Greene, MSC
	Dr. Gerald J. McCormick
Associate:	CPT Chris Lambros, MSC
	CPT Irving W. McConnell, VC
	CPT Patrick B. McGreevy, MSC

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:

Screening tests in animal models were performed by in-house and contractor laboratories on approximately 3000 candidate antimalarial compounds, including tests in suppressive, causal prophylactic or radical curative malarial models. Antimalarial activity was found in approximately 200 compounds and approximately 100 were selected for advanced study including evaluation of repository activity against *P. berghei* in mice and investigation of activity against human (*P. falciparum*) malaria in Aotus monkeys and vivax-like *P. cynomolgi* in rhesus monkeys. Approximately 600 compounds were evaluated against chloroquine-sensitive and -resistant strains of *P. falciparum* using an *in vitro* test

system. Structure-activity relationships were evaluated within series of thiosemicarbazones, 9-phenanthrenemethanols and acridinones.

Approximately 2000 compounds were screened for activity against T. rhodesiense; approximately 100 exhibited activity. Two compounds, an hydroxylamine and a novel arsenical, had exceptional activity by both subcutaneous and oral routes of administration. An in vivo system has been developed for evaluation of passage of drugs across the blood/brain barrier. This model system will be utilized to evaluate the potential utility of candidate drugs for treatment of CNS involvement of chronic African trypanosomiasis.

Seventy-eight compounds were tested for efficacy as topical prophylactic anti-schistosomal compounds. Activity against cercarial penetration was found in eight compounds. Three of the antipenetrants provided 96-100% protection even after a 30-minute water wash. One compound provided useful activity (90-95% protection) under the same conditions and four other compounds provided lesser, but definite protection. Amoscanate was tested in detail comparing application by immersion with application by wiping with a saturated gauze pad. The latter, more practical method of application afforded good protective activity (75.5% by wiping compared to 82.8% by immersion, one day before cercarial exposure).

Development of optimal in vitro cultivation techniques to provide metabolically-active leishmania promastigotes has been completed. Amastigotes derived from tissue culture and axenic cultures of amastigotes are being compared. Evaluation of the L. braziliensis/white-tailed rat (Mystromys albicaudatus) laboratory model of cutaneous leishmaniasis has continued. The efficacy of topically-applied drugs is being investigated in this model.

FUTURE OBJECTIVES:

Screening capability will be maintained to support the search for new, active classes of antiparasitic drugs and to guide synthesis of more efficacious and less toxic analogs among classes of compounds with activity against malaria, leishmaniasis, schistosomiasis and trypanosomiasis. Attempts will be made to induce resistance to mefloquine in P. falciparum in vitro to facilitate study of the phenomenon.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6765	81 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
80 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	AF	158
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Exploratory Vaccine Development Against Parasitic Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

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 81	3.0	177
	CURRENT 82	5.0	337

19. RESPONSIBLE DOD ORGANIZATION

NAME:
ADDRESS: Walter Reed Army Institute of Research
Washington, DC 20012

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

20. PERFORMING ORGANIZATION

NAME:
ADDRESS: Walter Reed Army Institute of Research, Div of CD&I, Wash, DC 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Hockmeyer, W.T., MAJ
TELEPHONE: (202) 576-3544
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Barbaro, J.F.
NAME: Jackson, P.R. POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with security classification code) (U) Parasitic Diseases; (U) Immunity; (U) Vaccines; (U) Antigens; (U) Immunoassays

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

23 (U) The objective of this work unit is to isolate and characterize antigens particularly those of malaria, trypanosomiasis and leishmaniasis and the evaluation of them as potential immunogens in experimental animals for the development of a safe and effective vaccine. These diseases impede military performance whenever troops are deployed in endemic areas emphasizing the need for a suitable vaccine to facilitate military operations.

24 (U) The approaches used in these studies are to develop techniques for the isolation of parasitic antigens; to use standard biochemical and immunochemical procedures to characterize and purify these antigens; to develop quantitative in vitro immunoassay to monitor the purity of these isolated antigens; and to determined the effectiveness as vaccines of these antigens in experimental animals.

25 (U) 80 10 - 81 09 An early part of this program has been development of new procedures to identify human leishmanial isolates for clinical and basic research purposes. This involves the isolation and subsequent fractionation of kDNA using restriction endonucleases. All of the major species have been characterized and correlate with the clinical presentation. Furthermore, it appears that with this technique we will be able to identify geographic subspecies complexes. One of these characterized strains, WR314, a L. donovani human isolate has been used to infect Balb/c mice as a first step in a hybridoma program to use monoclonal antibodies to identify relevent promastigote and amastigote antigens for vaccination, as well as for serologic and taxonomic purposes. Part of the monoclonal antibody technology is being applied to development of a serodiagnostic assay for African trypanosomiasis. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 158: Exploratory Vaccine Development Against Parasitic Diseases

Investigators:

Principals: Peter R. Jackson, Ph.D.
MAJ Wayne T. Hockmeyer, MSC

Associates: MAJ Roy G. Taylor, MSC
CPT Jackie S. Williams, MSC
E1 Laura L. Handy
Mr. William Hildreth

Problem and Objectives:

The problem under study is the development of vaccines against the human parasites which cause leishmaniasis, trypanosomiasis and 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 the development of Leishmania identification methods based on both hybridoma monoclonal antibodies (Mabs) and kinetoplast DNA (kDNA) analysis. Specific objectives are to determine if: (1) the methods differentiate species and strains and correlate with clinical presentation; (2) the extant Leishmania identification techniques produce comparable results when applied to the same isolates; (3) the strains from within a geographic area can be identified and related. Mabs also will be used to isolate antigens for evaluation as immunogens in experimental animals.

Progress:

Leishmania kDNA from human isolates is being analyzed with the restriction enzymes Hpa II and Hae III. Fragments of kDNA, differing in size, are separated by electrophoresis in linear gradient polyacrylamide gels. Species causing viseral (L. donovani, L. chagasi, L. infantum), cutaneous (L. tropica, L. mexicana, L. major, L. aethiopica), and mucocutaneous (L. braziliensis) disease in Africa, India, the Middle East, Europe, the Caribbean, Central and South America possess distinctly different kDNA electrophoretic patterns. Strains of L. donovani from India, the Sudan, Ethiopia, and Kenya can be separated in unequivocal fashion. Strains of L. donovani from Kenya have similar, but not identical, kDNA electrophoretic patterns, allowing for the detection of geographic complexes of

Leishmania. Additional strains from each species will be analyzed. Five clones of a L. donovani strain have been established, cultured and used to infect mice. Effects of long term in vitro cultivation and animal passage on kDNA restriction enyzme analysis, will be determined. Production of leishmania-specific Mabs is beginning. BALB/c mice, which will be the source of the antibody secreting lymphocytes, have been infected with a Kenyan strain of L. donovani.

Recommendations:

Restriction endonuclease analysis of kDNA for leishmania identification is progressing well. Additional species and strains and all clones will be analyzed. A literature search will be conducted to compare kDNA analysis results with extant Leishmania identification methods. Because kDNA electrophoretic patterns are often complex, an automated detector, such as an image digitizer, analyzer and computer with proper software, would be useful for determining: (1) If characteristic kDNA electrophoretic patterns exist between known species and strains; and (2) if an unknown isolate has a kDNA electrophoretic pattern similar to that of a known isolate. Additional restriction enzymes (Roberts, 1980) may be required to differentiate Leishmania kDNA refractory to digestion with Hpa II or Hae III. Amastigote-specific Mabs will be developed. Although amastigotes are technically difficult to study, they are of great immunological and pathological importance. An indirect fluorescent antibody screening system for amastigote-specific Mab detection will be developed. We expect the Mabs to be useful for Leishmania species, strain and life stage differentiation and for isolation of antigens to be tested as immunogens in experimental animals (Mitchell 1981; WHO, 1980).

References cited:

1. Mitchell, G.F. 1981. Hybridoma antibodies in immunodiagnosis of parasitic infection. Immunology Today 2:140-142.

2. Roberts, R.J. 1980. Restriction and modification enzymes and their recognition sequences. Nucleic Acids Research 8:r63-r80.

3. World Health Organization. 1980. Hybridoma Technology with Special Reference to Parasitic Diseases.

Publications:

1) Jackson, P.R., Jackson, J.E., Raney, D.E. 1981. A sterile leakproof plastic vial for cell cryopreservation in liquid nitrogen: application to parasitic protozoa. Cryobiology 18: (In press).

D TECHNOLOGY WORK UNIT SUMMARY			1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
			DA 0G 6759	81 10 01	DD-DR&E(AR)636
IND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8a DISP'N INSTR'N	8b SPECIFIC DATA-CONTRACTOR ACCESS	9 LEVEL OF SUM
D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT
IOGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
62770A	3M162770A871		AH	159		
TOG 80-7.2:P						

ity Classification Code)*

n and Treatment of Military Important Diseases in the Tropics

LOGICAL AREAS*

al Medicine 010100 Microbiology

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

		18 RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
EXPIRATION	FISCAL YEAR PRECEDING	81	0.2	38
d. AMOUNT	FISCAL YEAR CURRENT	82	8.5	418
f. CUM. AMT.				

NIZATION			20 PERFORMING ORGANIZATION		
eed Army Institute of Research			NAME* U.S. Army Medical Component, AFRIMS		
ington, D.C. 20012			ADDRESS* Bangkok, Thailand		
			PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)		
			NAME* BENENSON, M.W., LTC		
Philip K., COL			TELEPHONE (02) 281-7776		
76-3551			SOCIAL SECURITY ACCOUNT NUMBER		POC:DA
			ASSOCIATE INVESTIGATORS NAME HARRISON,B.A.,LTC; GILBREATH,M.M.,CPT;USSERY,M.A.,CPT;		
lligence not considered			NAME ECHEVERRIA,P.D.,LTC;BURKE,D.S.,LTC		

with Security Classification Code)

(U) Immunology; (U) Hepatitis; (U) Dengue

19 APPROACH, 20 PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

echnical objective is to develop new approaches to the prevention and tropical diseases of military importance. Malaria and dengue are empha- of severity and for propensity to cause high attack rates shortly after nilitary operations.

aches include characterization of the cellular immune response in patients malaria, epidemiologic/ecologic studies to determine vectors and hosts in lop control methods, and studies to determine the etiologic factors of nagic fever, and the etiology of hepatitis.

-81 09 Studies have continued attempting to identify changes in the cell nse in patients naturally infected with malaria. Presently the cell is responsible for the changes are being elucidated and serum factors that ressing the immune response are being sought. Similar work is being done infected with dengue. The presence of enhancing antibody in dengue ver is being determined and the importance of a secondary dengue response dengue type 2 is being well documented. Mosquito vector behavioral een done for dengue and are being done for malaria with the hope of de- ntive strategies. A rapid means of identifying the members of the virus ing investigated. Studies of hepatitis A in cyno monkeys in KL suggest f a jungle cycle and this is being pursued. For technical report see my Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

or authorized approval

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

☆ U.S. GPO 1974-540-843/8691

PROJECT 3M162770A871 PREVENTION OF MILITARY HAZARDS
Work Unit 159: Prevention and Treatment of Military Important Diseases in the Tropics
INVESTIGATORS: LTC M.W.Benenson,MC; LTC B.A.Harrison, MSC; LTC P.E. Echeverria,MD; LTC D.S.Burke, MC; CPT M.J. Gilbreath, MSC; CPT M.A. Ussery, MSC

1. Dengue Hemorrhagic Fever in Thai Infants

PROBLEM: In Bangkok children over one year of age, DSS virtually always occurs in a patient with pre-existing immunity to flaviviruses. However, DSS and fatal illness also can occur in infants who develop a primary seroresponse to infection. This study were undertaken to determine the pathophysiologic mechanisms whereby DSS can occur during primary flavivirus infections in infants.

PROGRESS: During the calendar years 1979 and 1980, 73 infants (under the age of one year) were admitted to Bangkok Children's Hospital with an initial clinical diagnosis of hemorrhagic fever. Most cases occurred in children four to nine months old with a modal age of eight months; the youngest was 67 days old. In 55 cases serum was obtained from the infant's mother at the time of admission; in four cases the mother showed evidence of concurrent asymptomatic or minimally symptomatic flavivirus infection. Twenty-five typed dengue virus strains were isolated from the acute blood of patients: four DEN-1, and 21 DEN-2, DEN-2 was isolated from the acute blood of all three fatal cases of DHF in infants. Among 15 mothers of infants with DHF caused by DEN-2 and who had no evidence of recent flavivirus infection, there was a strong correlation between the infant's age at the time of admission and the mother's $PRNT_{50}$ anti-DEN-2 titer. All these 15 mothers' sera enhanced DEN-2 virus growth in cells of continuous cultures of murine and/or human macrophage cells when added at dilutions beyond the $PRNT_{50}$ titer.

These studies support the hypothesis that antibody dependent enhancement of dengue virus growth is an important factor in the pathogenesis of DHF due to DEN-2.

RECOMMENDATIONS:

1. Studies should be done in other dengue-endemic countries to determine if the problem of infant DHF exists, and if so, what virus type(s) is responsible.

2. Studies of immune enhancement of virus growth should be done using mothers' sera and the actual virus strain isolated from infants with DHF.

2. Activity of Selected "Lysosomal Enzyme" Activities in Serum from Patients with Dengue Hemorrhagic Fever

PROBLEM: The mechanisms whereby increased vascular permeability, intravascular coagulation ,and shock are produced in some dengue

virus infections are unknown. Previous work at AFRIMS and elsewhere has shown that monocytes and reticulo-endothelial cells, cells with strong phagocytic capacity and rich in lysosomes, are the predominant cell types infected in DHF. Recently we demonstrated that serum acid phosphatase activity (a lysosomal enzyme) is elevated in DHF. We therefore studied serum levels of other lysomal derived enzymes, and attempted to biochemically determine the cells of origin of the elevated serum acid phosphatase.

PROGRESS: Plasma samples were collected from ten patients with DHF on days 1, 2, 3, 7 and 17 after hospitalization and tested for lysozyme activity and beta-glucuronidase activity (by Dr. Canonico). Activities were normal during both the acute and convalescent stages of disease. Thus, in contrast to acid phosphatase, activity of lysozyme and B-glucuronidase activity are not elevated during DHF.

Serum specimens from another ten children with DHF were tested for acid phosphatase activity (by Drs. Yam and Lam). Acute illness specimen activities were higher by a factor of almost two fold than corresponding convalescent activities. The increased activity was tartarate resistant. Polyacrylamide gel electrophoresis of sera showed the increased activity to reside in ba and 5b. The tartarate resistant ba and 5 acid phosphatase is elevated exclusively in diseases of bone with oxteoclast activation, in "hairy cell" (leukemic reticuloendotheliosis), and gaucher cells, and in normal children with active bone growth and remodeling. The source of elevated acid phosphatase in serum of DHF cases probably derives from cells with osteoclast like properties.

RECOMMENDATIONS:

1. Experiments should be conducted in vitro to determine if acid phosphatase activity is induced by dengue virus infection.

2. Autopsy material should be stained to detect cells with strong tartarate resistant acid phosphatase activity.

3. Hepatitis A Virus Infections in Wild Monkeys

PROBLEM: In previous studies we have shown that a high percentage of captive cynomolgus monkeys have serum antibodies to hepatitis A virus (HAV), that epidemic HAV can occur among cynos in capativity, and that cynos innoculated intravenously with HAV will develop an antibody response to infection with four to six weeks. We next tried to determine if cynos became infected with

HAV before captivity in sites in nature remote from man. If evidence for a "Jungle cycle" of HAV were found, this could have important implications for ultimate control of the disease.

PROGRESS: Serum samples were obtained within three days of capture from 106 cynomolgus monkeys (Macaca fascicularis) in Malaysia. Fifty-two monkeys were trapped on the fringes of palm oil estates and 54 in dense primary jungle. Sera were tested for antibodies to hepatitis A virus (HAV) with a commercial radioimmunoassay. Twenty-four animals had detectable serum anti-HAV activity (six of 52 from palm oil estate sites and 18 of 54 from primary jungle sites). Antibody prevalence was strongly correlated with animal weight among monkeys at both sites. Overall only four of 69 monkeys weighing less than 2.0 kilograms had serum anti-HAV antibodies, while 20 of 37 weighing 2.0 kilograms or more, and six of eight weighing 4.0 kilograms or more, had serum anti-HAV antibodies.

These data suggest that wild cynomolgus monkeys in Malaysian jungles become infected with HAV or an HAV like virus at a rate at least as rapid as do humans in the same country, and raise the possibility of a sylvatic cycle for HAV.

RECOMMENDATIONS:

1. Several HAV strains transmitted from cyno to cyno should be recovered and if possible passed in vitro and submitted to analysis to determine if these viruses are identical to human HAV or represent a different "simian" serotype.

2. The cynomolgus monkey model of human HAV infection should be developed further to determine the sites of virus replication and the suitability of the model for vaccine testing.

4. Lymphocytotoxic Antibodies in Malaria

PROBLEM: This laboratory previously reported the presence of cold-reactive lymphocytotoxic antibody (ALA) in the sera of Thai adults infected with P. falciparum and P. vivax malaria (1). The present study was undertaken to further characterize malaria cold-reactive ALA. The effect of malaria ALA on autologous lymphocytes, E-rosetting, and cytotoxicity toward lymphocytes subpopulations was investigated. In addition, the ALA activity of sera collected during the acute and convalescent periods of malaria infection was compared. Sucrose density fractionation was done on high ALA activity sera to determine if ALA activity is associated with the presence of IgM, IgG-IgA subfractions.

In so far as both suppressor T-cell and helper T-cell numbers have recently been found by us to be reduced in malarious patients, ongoing studies are presently being performed to determine if ALA are directly responsible for the observed decrease in regulatory cell numbers and activity.

PROGRESS: Autolymphocytotoxic antibody was found in 9/12 P. falciparum and 7/14 P. vivax sera when tested in a lymphocyte microcytotoxicity assay at 15°C. No significant lymphocytotoxic activity was seen in assays done at 37°C. In patient sera, serially diluted, cytotoxicity is removed or severely decreased at dilutions as low as 1:16. Malaria patients plasma had no ALA activity or greatly reduced ALA activity when compared with serum from the same individual. ALA activity was always higher in sera collected during the acute stage of malaria infection as compared to sera collected 15 and 30 days later.

No significant difference was found in the percentage of E-rosettes formed by monocuclear cells incubated with high ALA sera or normal sera.

No association has been found between the presence of ALA in patients' serum and the ability of the serum to inhibit blast transformation (2), lectin induced cellular cytotoxicity, antibody dependent cellular cytotoxicity or spontaneous cellular cytotoxicity (3).

When malarious patient sera with high ALA activity was fractionated ALA activity was only found at 15°C in the fraction containing IgM. However, some fractions demonstrated ALA activity in the IgG-IgA fractions when tested at 37°C.

Lymphocytotoxic antibodies are primarily directed against B cells, however, some activity can generally be seen against both B and T cells at 4°C. A small number of sera showed ALA activity against B cells at 37°C.

RECOMMENDATIONS:

This project is nearing completion and will terminate in 1981.

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2. MacDermott, R.P., et al. Examination of Peripheral Blood Monocuclear Cells and Sera from Thai Adults Naturally Infected with Malaria in Assays of Blastogenic Responsiveness to Mitogenic Lectins and Allogeneic Cell Surface Antigens. Infect. Immunol. 30(3):781-785, 1980.

3. Gilbreath, M., et al. Deficient Spontaneous Cell Mediated Cytotoxicity and Lectin-Induced Cellular Cytotoxicity by Peripheral Blood Mononuclear Cells from Thai Adults Naturally Infected with Malaria. J. Immunol. (In review).

5. Fansidar and Human Mononuclear Cell Responsiveness In vitro

PROBLEM: In recent years a combination of the drugs sulfadoxine and pyrimethamine (S-Py), marketed as Fansidar (Roche Laboratories) has been widely used both for malarial chemotherapy in areas where Plasmodium falciparum is resistant to chroloquine, and for long term prophylaxis of individuals working in highly endemic malarious area.

Although other anti-malarial compounds such as primaquine (1), chloroquine (2), quinine (3) and mefloquine (4) have been shown to have immunosuppressive properties, little is known about the immunological consequences of antimalarial sulfa-pyrimethamine combinations on the immune system.

PROGRESS: Pyrimethamine, one of the components of the widely used antimalarial drug Fansidar, suppresses lectin induced blast transformation by peripheral blood (PB) mononuclear cells (MNC) when the drug is added to the cell cultures at a 10^{-5} molar concentration. A second component of Fansidar, sulfadoxine, has no suppressive effect on the MNC. MNC responsiveness in the mixed leukocyte reaction and cellular viability are not altered by either pyrimethamine or sulfadoxine.

When serum from individuals on Fansidar chemoprophylaxis is added to culture wells in the blast transformation assay no significant suppression is seen; however, the data do not rule out any clinically significant suppressive effect by Fansidar on human cellular immune responses.

RECOMMENDATIONS: The mechanism by which pyrimethamine influences lymphocyte functions remains to be shown. However, the present studies demonstrate that pyrimethamine, a component of the widely used anti-malarial drug Fansidar adversely effects in vitro lectin-induced blast transformation by human mononuclear cells. The drug concentration at which suppression occurs is equivalent to

the blood drug level range found in patients on malaria chemotherapy. Although the extrapolation of the in vitro findings to in vivo systems awaits careful clinical follow-up studies, the combination of suspected immunosuppressive effects, antifolate properties, and other hematological complications associated with pyrimethamine use are important considerations for individuals on long term S-Py chemoprophylaxis. The potential immunosuppressive effect of pyrimethamine may be of greater significance in situations in which an intact host immune response is desired, i.e. vaccination. Therefore, further studies are needed to elucidate the specific mode of action by pyrimethamine on human MNC and to clarify whether the abnormalities in immune function result in alteration of immunoregulating or immune effector functions. The techniques and data analysis used in this study may provide an effective method to rule out immune suppression in humans by experimental anti-malarial drugs.

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1. Thong, Y.H., Ferrante, A., Rowan-Kelley, B. Primaquine Inhibits Mitogen-Induced Lymphocyte Proliferative Responses. Transactions of the Royal Society of Tropical Medicine and Hygiene, 72(5):537-539, 1978.

2. Forsdyke, D.R. Evidence for a Relationship Between Chloroquine and Complement from Studies with Lymphocyte Mitogens: Possible Implications for the mechanism of Action of Chloroquine in Disease. Canadian Journal of Microbiology, 21:1581-1586, 1975.

3. Gold, E.F. and Ben-Efraim, S. Selective Killing of Mitogen-Induced Transformed Cells by Quinine Sulfate In Vitro. International Archives of Allergy and Applied Immunology, 57:177-182, 1978.

4. Thong, Y.H., Ferrante, A., Rowan-Delley, B., O'Keefe, D. Effect of Mefloquine on the Immune Response in Mice. Transactions of the Royal Society of Trop Med & Hyg, 73(4):388-390, 1979.

6. Suppressor Cell Activity in Malarious Patient's Blood

PROBLEM: Insofar as suppressor T-cells have been shown to be activated in other protozoan infections; e.g. trypanosomiasis, it would be of interest to determine if suppressor T-cells function normally in patients with naturally acquired malaria. Up to now there has been no convincing evidence for their involvement in malaria infections. However, recent reports that the peripheral blood T-cell population is decreased in patients with malaria (1), and the occurrence of lymphocytotoxic antibodies (2), as well as serum blastogenic inhibiting factors (3) in the

sera of a high percentage of patients with naturally acquired malaria suggest that a defect in suppressor T-cells may be one mechanism that influences or regulates the host's immune respose to malaria infection.

PROGRESS: As reported last year a comparison of Con A generated supressor cell activity in various patient and control individuals shows that the mean level of suppressor cell activity is substantially reduced in the malarious patients mononuclear cell population. Suppressor cell activity is lower in cultures stimulated with phytohemagglutin (PHA), Concanavalin A (Con A), Pokeweed mitogen (PWM) and in the mixed lymphocyte cultures (4). We have extended these studies recently to assess the activity in both an autologous and an allogeneic suppressor cell assay system. Normal levels of suppressor cell activity are found to return within 3-5 days after anti-malarial treatment is initiated. Also, we have determined that the defect in functional activity cannot be attributed solely to regulatory defects in macrophage-monocyte activity nor to a decrease in number and/or concentration of circulating T cells. Using an ox-rosetting assay (5) we have recently quantitated suppressor T-cell and helper T-cell numbers in malarious individuals and have found that both populations of regulatory cells are reduced when compared to control values.

RECOMMENDATIONS: Further studies should be directed at determining (1) if patients whose serum contains antibodies against T-cells lack the subset of suppressor cells, (2) if patients lacking suppressor cells have a significantly higher number of B cells secreting immunoglobulin, and (3) if the presence of autoantibodies is correlated with disease activity.

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3. MacDermott, R.P., et al. Examination of Peripheral Blood Mononuclear Cells and Sera from Thai Adults Naturally Infected with Malaria in Assays of Blastogenic Responsiveness to Mitogenic Lectins and Allogeneic Cell Surface Antigens. Infect. Imm. 30(3):781-785, 1980.

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5. Moretta, L., et al. Functional Analysis of Two Human T-Cell Subpopulations: Help and Suppression of B-Cell Responses by T-Cells Bearing Receptors for IgM and IgG. J. of Exp. Med. 146:184-200, 1977.

7. Deficient Spontaneous Cell Mediated Cytotoxicity and Lectin-Induced Cellular Cytotoxicity by Peripheral Blood Mononuclear Cells from Thai Adults Naturally Infected with Malaria

PROBLEM: A detailed understanding of the host immune system's response to malaria infection will result in clarification of the normal human response to parasitic infection as well as elucidation of the ways in which a parasite modifies the host immune response in order to avoid neutralization or destruction. We have therefore undertaken a series of experiments designed to determine the immunologic alterations which occur during parasitic infection with P. falciparum and P. vivax in naturally infected Thai adults. Our studies to date have demonstrated that patients with malaria have a true decrease in circulating T cells but no real change in Null or B cell numbers (1); anti-lymphocyte antibodies in their sera (2); a decrease in suppressor T cell generation capability (Gilbreath et al. submitted for publication); and serum factors capable of inhibiting normal lymphocyte blastogenesis (3). Because the studies which we have carried out to date have not assessed cellular effector functions, we chose in the present study to begin examination of peripheral blood (PB) mononuclear cell (MNC) mediated cytotoxicity, using MNC from Thai adults naturally infected with P. falciparum and P. vivax. The results of the present experiments indicate that patients with malaria have defective T cell and NK cell cytotoxic capability in some systems but do not exhibit defective K cell function. These abnormalities may be induced by the malaria parasite in order to allow continued replication.

PROGRESS: In order to assess general cytotoxic effector cell capabilities by peripheral blood (PB) mononuclear cells (MNC) from patients with active malaria infection, we examined antibody dependent cellular cytotoxicity (ADCC), spontaneous cell mediated cytotoxicity (SCMC), and lectin induced cellular cytotoxicity (LICC) using human red blood cell, chicken red blood cell, Chang cell line, and K562 cell line targets. We found that PB MNC from Thai adults naturally infected with malaria had a significant

impaired level of LICC with human red blood cell and Chang cell line targets. In addition, SCMC was deficient with K562 but not with Chang cell line targets. Finally, no change in ADCC of chicken red blood cell or Chang cell line targets was observed with fresh malarious patient PB MNC. There was no effect on cytotoxicity by normal PB MNC after incubation with malarious patients' serum. Thus, a physical or functional alteration exists in malarious patient's PB MNC subpopulations responsible for LICC and a subset of the NK cells responsible for SCMC, but not in the K cells responsible for ADCC. This observation coupled with our previous observations of a physical loss of peripheral blood T cells, the presence of lymphocytotoxic serum antibodies, and defective T suppressor cell generation in patients with malaria, indicates that major immune abnormalities occur in patients with an active malarial infection. These alterations, which are induced by the malarial parasite, may either represent the normal host immune response to malaria or the parasites' modulation of the hosts' immune system in order to achieve persistent infection.

RECOMMENDATIONS: Further studies are necessary to determine whether alterations in these cytotoxic effector cells are associated directly with parasitemia or develop secondary to the general infectious process. Longitudinal studies of individuals during both the acute and convalescent stage of malarial infection should be done to acquire the necessary basic data to address the question of a direct or indirect relationship between parasitemia and the impairment of the cytotoxic immune response.

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1. Wells, R.A., Pavanand, K., Zolyomi, S., Permpanich, B., and MacDermott, R.P. Loss of Circulating T Lymphocytes with Normal Levels of B and "Null" Lymphocytes in Thai Adults with Malaria. Clin. Exp. Immunol. 35:202, 1979.

2. Wells, R.A., Pavanand, K., Zolyomi, S., Permpanich, B., and MacDermott, R.P. Lymphocytotoxic Antibody in Sera of Thai Adults Infected with Plasmodium falciparum or Plasmodium vivax Malaria. Clin. Exp. Immunol., 39:663-667, 1980.

3. Gilbreath, M.J., et al. Deficient Spontaneous Cell Mediated Cytotoxicity and Lectin-Induced Cellular Cytotoxicity by Peripheral Blood Mononuclear Cells from Thai Adults Naturally infected with Malaria. J. Immunol. (In review).

4. MacDermott, R.P. et al. Examination of Peripheral Blood Mononuclear Cells and Sera from Thai Adults Naturally Infected with Malaria in Assays of Blastogenic Responsiveness of Mitogenic Lectins and Allogeneic Cell Surface Antigens. Infect. Imm 30(3):781-785, 1980.

8. Immunoregulation in Human Malaria

PROBLEM: Department of Immmunobiology, as part of the Army's Malaria Vaccine Program, is investigating the functional capabilities of subpopulations of peripheral blood cells and the role of serum regulatory factors in malarious individuals with the goal of providing basic data on the importance of cellular immune functions in humans response to malaria infection.

PROGRESS: To date we have characterized human peripheral blood cell alterations in malarious individuals in respect to quantitative changes in subpopulation of mononuclear cells, response to mitogenic lectins, cytotoxic activity in antibody dependent, lectin induced and spontaneous cellular cytotoxicity assays. Defective regulatory action has been found that can be associated with alterations in numbers and functional ability of suppressor T cells. Regulatory activity of lymphocytotoxic antibodies in acute and convalescent patients' sera have been characterized in respect to incidence, titer, immunoglobulin sub-class, target cell and their activity in regulating in vitro activity of target cells. The action of anti-malarial drugs on in vitro and in vivo blood cell responses has been assessed and indications of drug induced suppression have been found.

RECOMMENDATIONS: Future basic immunological studies should be designed to assess the sequential development of the host (human and primate) immune responses to malaria infection. Particular emphasis on the interaction of humoral, cellular and regulatory mechanism should be stressed. In vitro cultivation of infected erythrocytes will offer additional opportunities to determine if specific cellular responses are capable of controlling or eliminating parasites, as well as, the opportunity to assess molecular and biochemical interactions of the various afferent and efferent components of the host immune system with the parasitic organism and the overall hematological system.

9. Mosquito Cytogenetic, Electrophoretic and Cross Mating Studies

PROBLEM: To use the latest cytogenetic, cross mating and electrophoretic techniques to: (a) delineate the vector species and vector strains of mosquito species in Thailand and Southeast

Asia as a check against current morphological species concepts; (b) identify rapid and accurate techniques and discriminating characters for differentiating sibling species in vector species complexes; and (c) accurately determine genetic variation in natural population of vector species and correlate this variation with the susceptibility of the vector(s) to infection with human pathogens.

PROGRESS: Colonization efforts continued to receive major emphasis this year. Two Thailand strains of Anopheles nivipes were colonized after considerable effort. A manuscript (1) describing the materials and methods used in colonizing An. nivipes and An. philippinensis is near completion. Three colonies of members of the Leucosphyrus Complex also were initiated.

In the Maculatus Complex at least three types of Y and three types of X chromosomes have been seen. One colony (Huai Kuum) having a very short Y chromosome, also was significantly less susceptible to Plasmodium cynomolgi infections than the other two colonies having much longer Y chromosomes (2). Reciprocal cross mating studies between the IMR and Nakhon Nayok strains, and the Nakhon Nayok and Huai Kuum strains of An. maculatus have revealed complete compability, with no evidence of non-viability or sterility even in F_2 crosses.

Larval salivary polytene chromosome preparations of the Fraser's Hill form revealed that zone six on the X chromosome is considerably shorter than that seen on the X chromosome of An. dirus (3). The karyotype chromosomes of An. balabacensis Perlis form are acrocentric with a very distinct short arm beyond the centromere and are approximately the same length as those of An. dirus (4). Crossing studies are underway between An. dirus and the Fraser's Hill form, and strong healthy F_1 hybrids have been produced in both directions. However, some male F_1 hybrids have abnormal tests without sperm, or sperm that have big heads and are non-motile.

RECOMMENDATIONS: These studies are continuing, and electrophoretic studies are planned for the coming year. Electrophoretic techniques are needed to taxonomically differentiate field-collected specimens belonging to the various sibling-species complexes. Positive identification will help to elucidate the malaria vector status of these mosquitoes in endemic areas.

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10. Ecology and Epidemiology of Dengue Viruses in Din Daeng District, Bangkok

PROBLEM: The overall objectives were given in a previous annual report (1). The objectives applying to this report are: (a) to determine the population density of the wild Ae. aegypti population on a seasonal basis; and (b) to determine the seasonal availability of artificial containers and their utilization by Ae. aegypti for oviposition.

PROGRESS: Field aspects of this project ended on 19 December 1980. During the three years of field work in the Din Daeng study area a total of 20 surveys were conducted to determine the container usage and the positive container index for Aedes aegypti immatures. Larval and emergence trap surveillance surveys were conducted during 17 of those survey periods. Last year's report (1) presented a short summary of some preliminary findings resulting from the field aspects of the project. During this year emphasis was placed on transcribing the large amount of data into a system suitable for computer analysis.

Data from the Din Daeng study and others indicate that dengue haemorrhagic fever (DHF) cases begin to increase during the hot dry season well in advance of the rainy season. This situation has prompted several studies on the influence of temperature changes on the DHF case rates (2) and dengue virus replication in mosquitoes, and transmission by Ae. aegypti (3).

One major objective of the project was to develop surveillance techniques and/or equipment that will efficiently sample the relative density of Ae. aegypti. Early in this project a very efficient floating larval trap was designed and tested in the laboratory (1, 4). Trap returns in a field situation where

numbers of immatures per jar were variable, even as low as one, suggest the trap efficiency was high, as previously determined in the laboratory tests (4). A manuscript (5) describing the development and testing of the AFRIMS larval trap has been prepared and submitted.

RECOMMENDATIONS: None in the Din Daeng District, as this project has been terminated. Laboratory experiments concerning dengue virus strain-vector interactions are being designed.

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11. Ectoparasite and Rickettsia tsutsugamushi Studies In Thailand

PROBLEM: The objectives of this study are to establish and describe the chiggers and ticks that are vectors or potential vectors of human pathogens in Thailand, and to determine the geographical distribution of Rickettsia tsutsugamushi in natural populations of chiggers in Thailand.

PROGRESS: For many years Leptotrombidium (Leptotrombidium) deliense was the only chigger species from Thailand from which Rickettsia tsutsugamushi had been isolated. During the last several years, however, collaborative studies between this department and the U.S. Army Medical Research Unit, Kuala Lumpur, have been able to isolate several strains of this pathogen from at least nine different chigger species in Thailand (1, 2). These

e: Eutrombicula wichmanni, L. (L.) arvinum, L. (L.)
L. (L.) scutellare, L. (L.) sp. A (undescribed), L. (L.)
escribed), Leptotrombidium (Trombiculindus) panicularum,
icula chamlongi and Odontacarus sp. (undescribed).
of these species are very poorly known, studies to
ir biology and ecological requirements have continued.
year, 310 collection from birds, mammals, black plates
were made in four provinces of Thailand. These col-
esulted in over 21,000 specimens of chiggers, lice,
es and ticks. The specimens and ecological data from
ections currently are being analyzed. Several morpho-
riations of L. (L.) deliense have been found from
vations in Chiang Mai Province. Such variations
have not been encountered in Thailand. However, since
ely 19 new species of the subgenus Leptotrombidium
prep red for publication, these variations of deliense
rther study.

cklist of the Ticks of Thailand has been completed and
ed clearance for publication.

TIONS: These studies are continuing and are expected
e for the next year with emphasis on temporal and
lationships between the scrub typhus rickettsia and
s.

:

R.G., Tanskul, P.L., Harrison, B.A., Dohany, A.L.,
, A. Ectoparasite and Rickettsia tsutsugamushi
Thailand. AFRIMS Annual Progress Report, Oct 78-
. 32-38.

, A., Tanskul, P.L., Andre, R.G., Dohany, A.L., and
.L. Rickettsia tsutsugamushi Strains Found in Chiggers
in Thailand. Southeast Asian J. Trop. Med. Pub.
-6 .

Program Element: 62770A
Project Number: 3M162770A871
Work Unit Number: 159
Title: Prevention and Treatment of Military Important Diseases in the Tropics

Presentations:

1. Burke, D.S., Heisey, G.B., Graham, R.P. Hepatitis A Virus Antibodies in Malaysian Cynomolgus Monkeys. Seventeenth Annual Scientific Seminar of the Malaysian Society of Parasitology and Tropical Medicine, Juala Lumpur, Malaysia March 1981.

2. Harrison, B.A., Peyton, E.L., Baimai, V. Species Discrimination and Changing Vector Concepts in the Leucosphyrus Complex of Anopheles in Southeast Asia. Annual Meeting Am. Mosq. Contr. Assoc. San Antonio, Texas, March 1981.

3. Harrison, B.A., Peyton, E.L., Baimai, V., Klein, T.A. Changing Species Concepts and Distributions for Known and Potential Vectors of Human Malaria Parasites in Thailand. 1st National Malaria Conference, Haad Yai, Thailand, November 1980.

Publications:

1. Baimai, V., Harrison, B.A., Somchit, L. Karyotype Differentiation of Three Anopheline Taxa in the Balabacensis Complex of Southeast Asia (Diptera:Culicidae). Genetica (In press).

2. Baimai, V., Harrison, B.A. Evidence of Sibling Speciation in the Balabacensis Complex of Southeast Asia (Diptera:Culicidae). Abstr. 10th Int. Cong. Trop. Med. Malaria, Manila, pp 83-84, November 1980.

3. Burke, D.S., Heisey, G.B., Graham, R.R. Hepatitis A Virus Antibodies in Malaysia Cynomolgus Monkeys (Letter, submitted to the Lancet, 1981).

4. Gilbreath, M.J., Pavanand, K., Phisphumvithi, P., Permpanich, A., MacDermott, R.P. Deficient Spontaneous Cell Mediated Cytotoxicity and Lectin-Induced Cellular Cytotoxicity by Peripheral Blood Mononuclear Cells from Thai Adults Naturally Infected with Malaria. (In review).

5. Gilbreath, M.J., MacDermott, R.P., Pavanand, K., Phisphumvithi, P., Kongchareon, S., Wimonwattrawatee, T. Deficiency of Con-A-Induced Suppressor Cell Activity in Peripheral Blood Mononuclear Cells from Thai Adults Naturally Infected with Plasmodium falciparum and Plasmodium vivax. (In review).

6. Gilbreath, M.J., Groves, J., Pavanand, K., Phisphumvithi, P. Suppression of Mitogenic Lectin Induced Blast Transformation of Human Peripheral Blood Mononuclear Cells by Pyrimethamine. (In review, 1981).

7. Harrison, B.A., Callahan, M.C., Watts, D.M., Panthusiri, L. A Floating Trap for Determining Relative Densities of Immature Aedes aegypti (Submitted for clearance).

8. Peyton, E.L., Harrison. Anopheles (Cellia) takasagoensis Morishita 1946, An Additional Species in the Balabacensis Complex of Southeast Asia (Diptera: Culicidae). Mosq. Syst. 12:335-347, 1980.

9. Rattanarithikul, R. A Guide to the Genera of Mosquitoes (Diptera: Culicidae) of Thailand with Illustrated Keys, Biological Notes and Preservation and Mounting Techniques. (Submitted for clearance).

10. Tanskul, P. (L.), Stark, H.E., Inlao, I. A Checklist of Ticks of Thailand (Metastigmata: Ixodoidea) (Submitted for clearance).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6526	81 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. ENTR'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	AH	160
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7.2	2		

11. TITLE (Precede with Security Classification Code)

(U) Field Studies of Rickettsioses and Other Tropical Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

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)
PRECEDING FISCAL YEAR 81	5.0	155
CURRENT FISCAL YEAR 82	5.0	106

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: US Army Medical Research Unit Malaysia

ADDRESS: Kuala Lumpur, Malaysia

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

NAME: Groves, Michael G., LTC, VC

TELEPHONE: 984155, 984249

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Shirai, Akira, Ph.D. POC: DA

NAME: Oaks, Stanley C., Jr., MAJ MS

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Chiggers; (U) Scrub Typhus; (U) Rickettsia tsutsugamushi; (U) Malaysia

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

23.(U)1.Evaluate methods for the early diagnosis of R. tsutsugamushi infections; 2. Characterize the trombiculid mite vectors of scrub typhus; 3. Study the transmission of R. tsutsugamushi by vector mites; 4. Develop tests that measure various aspects of the cell-mediated response to infection with R. tsutsugamushi. There is military relevance in this research.

24.(U)1.Test sera obtained during the first 10 days of illness using a latex agglutination and radio-immunoassay procedure. 2. Study the isoenzymes and cytogenetics of selected chigger species. 3. Determine if genetic susceptibility of chiggers may be involved in the acquisition of rickettsiae by attempting to suprainfect chiggers with new strains. 4. Prepare a rickettsial antigen using a French pressure cell, and incorporate it into migration inhibition factor (MIF) and lymphocyte transformation (LT) tests.

25.(U) 80 10 - 81 09 1. Several antigen preparations have been made and studies on their specificity and sensitivity in the radioimmunoassay and latex agglutination are being evaluated. 2. Electrophoretic studies using phosphohexoisomerase have demonstrated 3 distinct phenotypes, and cytogenetic studies have revealed the chromosome number of male L. (L.) arenicola chiggers to be 2n = 14. 3. L. (L.) arenicola chiggers naturally infected with Karp and Karp-related strains of R. tsutsugamushi were able to acquire Gilliam strain infections after feeding on infected mice, but uninfected chiggers were not. 4. Specific LT has been observed with our rickettsial antigen and the agarose droplet technique for assaying MIF is working although titers are inconsistent. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contributors upon originator's approval

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Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 160 Field Studies of Rickettsioses and Other Tropical Diseases

Investigators:
Principals: LTC Michael G. Groves, VC; LTC Robert L. Ridgway, VC; Dr. Akira Shirai, Ph.D.; MAJ Stanley C. Oaks, Jr., MSC; MAJ John C. Twartz, RAAMC; CPT Gregory B. Heisey, VC; Dr. T.S. Lim, Ph.D.; Dr. K.P. Ng, Ph.D.; Miss Elsie Gan, B.S.

PLAQUING STUDIES

Background: Analysis by the direct fluorescent antibody technique has demonstrated that about 50% of Rickettsia tsutsugamushi isolates from Peninsular Malaysia, Thailand, Taiwan, the Philippines, Australia, and the islands of the Northern New Hebrides and Santa Cruz groups (7,23,25-28) were multiply reactive, several with as many as five different strains (26). The use of the rickettsial plaque assay (19) should allow us to determine whether this multiple reactivity is caused by a mixture of strains or is due to a single strain that exhibits a mosaic of antigenic determinants.

Progress: A large portion of the past year was taken up with locating a suitable source of gamma radiation and with obtaining approval to use the source, as well as in coordinating the financial and technical aspects of its utilization. The technicians have now been trained to do the plaque assay, and all are competent. Unfortunately, studies have initially been plagued with inconsistent results due chiefly to incubator malfunctions. These problems appear to have been resolved.

Future Objectives: We are now cloning multiply reactive isolates from both humans and chiggers using L929 cells. In addition, we hope to modify the assay to Vero cells, so it can be used to isolate and clone strains for use in a potential vaccine. Other minor modifications to the original plaque assay procedure are also planned.

RICKETTSIA TSUTSUGAMUSHI INFECTION OF HUMAN ENDOTHELIAL CELLS IN VITRO

Background: The in vivo pathological features of most rickettsial diseases are similar. The principal lesions appear to result from the destruction of vascular endothelial cells by rickettsiae (2,6). To date, the infection of human endothelial cells in vitro by R. tsutsugamushi has not been studied. Since the endothelial cell appears to be the key "target cell" in scrub typhus infections, this model system needs to be explored. Endothelial cells maintain their differentiated structural and functional attributes in cell culture (14-15,17) and, therefore, appear to be ideal for in vitro controlled studies of the growth cycle of R. tsutsugamushi and the cytopathology and immunopathology caused by the organism.

Progress: The cell cultures have been prepared by the method of Gimbrone (12) from humam umbilical cords obtained from a local hospital. Preliminary attempts were moderately successful. Initially, we obtained small numbers of endothelial cells that would only be maintained for short periods, 7-10 days. The procedure has now been greatly improved through some modifications, and we are presently preparing for our initial attempt at observing uninfected cells by scanning electron microscopy (SEM).

Future Objectives: We will examine both infected and uninfected endothelial cells and L-929 cells (for comparison) by SEM and transmission electron microscopy (TEM). The growth characteristics of R. tsutsugamushi in cultured human endothelial cells will also be studied using light microscopy in conjunction with the Giemsa staining technique. The SEM and TEM studies are being done in cooperation with the Division of Parasitology, Institute for Medical Research.

EARLY DIAGNOSIS OF RICKETTSIA TSUTSUGAMUSHI INFECTIONS

Background: Clinical signs and symptoms with subsequent serological confirmation 2-3 weeks after the onset of illness are the usual means by which scrub typhus infections are diagnosed. Other useful information includes recent travel or residence in an endemic area and exposure to chiggers, the vectors of scrub typhus. Nevertheless, recognition of clinical

disease is often difficult because scrub typhus frequently mimics other febrile illnesses that occur in the endemic areas.

A definitive diagnosis of scrub typhus by isolation of R. tsutsugamushi often requires 2-3 months, and serological tests can only be used to confirm an initial clinical impression and establish a retrospective diagnosis. The need for an early diagnostic measure is obvious.

Progress: 1) Latex Agglutination - Preliminary studies have demonstrated that agglutination between R. tsutsugamushi antigen and scrub typhus antibody carried on latex particles can occur.

2) Radioimmunoassay - Successful preliminary studies have been performed to determine the feasibility of using this assay for detection of scrub typhus antigens.

Future Objectives: In both assays, specificity and sensitivity tests will now be conducted using rickettsemic (antigen-containing) sera from human patients and experimentally infected animals.

ANTIGENIC ANALYSIS OF RICKETTSIA TSUTSUGAMUSHI STRAINS IN ENDEMIC AREAS

Background: The antigenic diversity of R. tsutsugamushi strains is a well established fact. However, studies of isolates from Peninsular Malaysia, Thailand, Taiwan, the Philippines, Hong Kong, Australia, and the islands of the Northern New Hebrides and Santa Cruz groups have demonstrated the predominance of the Karp and Karp-related (TA686, TA716, TA763) strains (11,23-28). The acquisition and study of isolates from Japan, China, Russia, and W. Pakistan are required to complete the information concerning the prevalence and geographic distribution of different strains throughout the endemic area.

Progress: Contacts have been made with scientists in China, and arrangements made for the exchange of R. tsutsugamushi isolates.

Future Objectives: Attempts to obtain isolates from Japan, Russia, and W. Pakistan are being continued. All isolates will be characterized antigenically by the direct fluorescent antibody technique.

TRANSOVARIAL TRANSMISSION OF RICKETTSIA TSUTSUGAMUSHI ACQUIRED FROM RODENTS

Background: Uninfected vector species are able to acquire R. tsutsugamushi by feeding on infected mice and to pass the organisms transtadially but not transovarially (30). However, transovarial and filial infection rates of almost 100% were observed in studies using our naturally infected Leptotrombidium arenicola and L. fletcheri colony mites (20-21).

The infected mites of our L. arenicola colony carry the Karp, TA686, TA716, TA763, and Kato strains but not Gilliam (unpublished observations). The chiggers from this colony were fed on mice experimentally infected with the Gilliam strain to determine if the ability to acquire and transovarially transmit R. tsutsugamushi is a genetic trait.

Progress: Five different familial lines of the infected L. arenicola were identified. Half of the chiggers in each line were fed on Gilliam-infected mice, and the other half were fed on normal mice. Subsequent generations were followed to determine whether the Gilliam strain was acquired and passed on to succeeding stages and generations.

Mites from one line appeared to have picked up and passed the Gilliam organisms through 3 generations. The other 4 lines and the control groups fed on normal mice have no detectable Gilliam infections. Concurrently, 5 lines of uninfected L. arenicola chiggers were fed on Gilliam-infected mice. To date, all mites have failed to demonstrate the Gilliam organism through 3 generations.

Fugure Objectives: The mites will be checked for the presence of Gilliam rickettsiae for a number of successive generations.

ELECTROPHORETIC STUDY OF TROMBICULID MITES

Background: Electrophoretic techniques to separate isoenzymes have been extensively used in recent years to study the genetics of a number of arthropod vectors of disease, primarily mosquitoes. The availability of infected and uninfected colonies of L. arenicola and L. fletcheri has enabled us to apply this procedure to mites.

Progress: Newly emerged adult chiggers were tested for the enzyme phosphohexoisomerase. In both the uninfected and infected L. arenicola, 4 to 6 electrophoretic phenotypes, each represented by a single band, were found. There appears to be no differences between sexes.

A more complex picture was observed with the L. fletcheri chiggers. At least 8 phenotypes with single bands and many with multiple bands have been found.

Future Objectives: Wild-caught L. fletcheri specimens collected by black-plating and from wild rodents will now be compared to the colony chiggers. Another enzyme system, phosphoglucomutase, will also be used to study the chiggers.

CYTOGENETICS OF TROMBICULID MITES

Background: Most studies of arthropod cytogenetics have involved mosquitoes (16). Although some information is available on mite species in some families of the Suborder Prostigmata, no chromosome data is available on the trombiculid mites. Cytogenetic studies are now being done using mites from our colonies.

Progress: The karyology of both L. fletcheri and L. arenicola uninfected males have been determined. Using newly emerged adults, each has been shown to have 7 pairs of chromosomes in cells of the reproductive tissues. However, attempts to visualize the chromosomes of adult females have been unsuccessful.

Future Objectives: Earlier stages (nymphs and larva) as well as other tissues of the female mites will be studied, and comparisons between uninfected and infected mites will be made.

EFFECT OF MOUSE AGE ON SUSCEPTIBILITY OF MICE TO RICKETTSIA TSUTSUGAMUSHI

Background: Variations in virulence between strains of R. tsutsugamushi have long been recognized. In addition, the virulence of a particular rickettsial strain may be modified by such factors as route of inoculation (3) and mouse genotype (13). However, despite the extensive use of mice in scrub typhus research and the past interest in R. tsutsugamushi virulence for mice, specific information on the effect of age on mouse susceptibility to infection is lacking. Because we use mouse virulence as a criterion to select rickettsial strains for use in potential vaccines, we tested the susceptibility of mice of varying ages to intraperitoneal (IP) infections with 8 prototype strains of R. tsutsugamushi.

Progress: Mouse age groups varying from 4 to 26 weeks were given IP dosages of virulent strains (Karp, Kato, Gilliam, TA763, and TH1817) ranging between $10^{0.5}$ and $10^{4.0}$ mouse 50% infectious doses (MID_{50}) and of strains of reduced virulence (TA678, TA686, and TA716) ranging between $10^{4.5}$ and $10^{8.0}$ MID_{50}. Susceptibility differences were noted only in the ICR mice inoculated with strains TA716 and TA678. In both instances, mice in the 12-weeks and younger age groups had lower death rates than did mice in the 21-weeks and older age groups. This study indicates that the age of mice used to test the virulence of R. tsutsugamushi strains may be an important consideration, especially when testing the IP lethality of strains of reduced virulence.

Future Objectives: The apparent difference in susceptibility of young mice (<12 weeks) and older mice (>21 weeks) to R. tsutsugamushi strains of reduced virulence will be confirmed using larger populations of mice.

ANALAYSES OF DATA FROM MAJOR EPIDEMIOLOGICAL STUDIES

Background: The probability that a diagnostic test result will discriminate between disease and non-disease depends on its sensitivity (ability to be positive when the subject has the disease) and specificity (ability to be negative when the subject does not have the disease) and on the prevalence of disease in the study population. Evaluation of the

sensitivity and specificity of a test is carried out in a group of diseased subjects definitely diagnosed by another means and in a comparable control group drawn from the same population but that have no increased risk of the disease. The approximate prevalence of disease may then be found by using the test in question. We have been systematically collecting data on febrile patients in rural Malaysia since 1975. The results of a wide range of tests done on thousands of these patients caused us to question some diagnostic criteria in common use. We have therefore attempted to assign probability values to a range of IFA and OXK agglutinin titers in the diagnosis of scrub typhus.

Progress: The sensitivity and specificity of the micro-immunofluorescent antibody (IFA) and OXK Weil-Felix tests for scrub typhus were established for a range of titers, using groups of diseased (scrub typhus) and non-diseased (other febrile illnesses) patients diagnosed by other methods. At a cut-off point of 1:400, the IFA test was 0.98 specific, and at ≥1:320, the OXK was 0.96 specific. Using either these highly specific levels of antibody or other rigorous diagnostic criteria (isolation or 4-fold rising titers), the prevalence of scrub typhus infection was determined to be 0.22 in an unselected population of febrile patients in a rural Malaysian hospital. Probability values (Pr) for the correct diagnosis of scrub typhus were then calculated from the specificity, sensitivity, and prevalence determination for a range of titers (29). The Pr for an OXK titer of ≥1:320 was 0.77, and the Pr for an IFA titer of ≥1:400 was 0.83. When both these titers were present in a single specimen, the Pr increased to 1.0.

Future Objectives: Data now stored in our computer will be analyzed and used to prepare the following manuscripts: "Febrile Illness in Malaysia - An Analysis of 1630 Hospitalized Patients" and "Double Infections Occurring in Rural Peninsular Malaysia".

PROPHYLAXIS OF SCRUB TYPHUS

Background: In 1980, we showed that weekly 200 mg doses of doxycycline would protect against scrub typhus in volunteers deliberately fed upon by chiggers infected with R. tsutsugamushi, if the antibiotic was given for 6 weeks after exposure to infection (manuscript in preparation). In the past year, we have

measured the antibody levels every month on both the doxycycline volunteers and the placebo subjects (who developed scrub typhus).

Progress: Twelve months post-exposure, all 10 placebo subjects and 8 out of 9 doxycycline subjects still have IFA antibody titer $\geq$1:50. These data do not fit the 61% calculated annual reversion rate to <1:50 of Saunders et al. (22) but may tend to support the findings of Bozeman and Elisberg (5) who found antibody 10 years after infection in 2 patients.

Future Objectives: We will continue to monitor antibody on as many volunteers as possible to further define the reversion of scrub typhus antibody.

STUDIES IN CELL MEDIATED IMMUNITY

Background: Recovery from intracellular infections involves cell mediated immune mechanisms. Mouse spleen T lymphocyte transfer has demonstrated this in R. tsutsugamushi infection. Our desire to study the course of scrub typhus immunity and predict the degree of protection from re-infection requires a suitable in vitro test. The 2 tests we have been interested in are lymphocyte transformation and migration inhibition factor production.

Progress:

Antigen. Our inability to define a suitable antigen of R. tsutsugamushi for use in CMI tests has slowed down progress in this field. The preliminary findings are presented.

Rickettsiae from infected L-929 cells are purified usually after the method of Eisemann and Osterman (10), with homogenization, low speed centrifugation to remove debris, and then high speed centrifugation to pellet the rickettsiae. On occasion, 20-45% Renografin gradients in K36 have been used, with 2 bands of rickettsiae being obtained, one containing some L-929 contamination (9).

Four crude antigen fractions are prepared and stored at -20C.

Fraction 1: whole washed rickettsiae, kept at 4C for 2 days before storage.

Fraction 2: the 12,000 x g pellet after twice disrupting washed rickettsiae in a French pressure cell at 18,000 16/sq. in.

Fraction 3: the supernate after 59,000 x g centrifugation.

Fraction 4: the supernate from Fraction 2 after 3 hours at 59,000 x g; resuspended in RPMI or PBS.

Fraction 3 should be equivalent to the R. typhi soluble antigen of Bourgeois et al. (4) and Fraction 4 should be equivalent to their membrane antigen. More recently, simple Tris extraction of Renografin-purified R. tsutsugamushi after the method of Dasch (8) has been attempted. No bands were seen on SDS-PAGE from this preparation but this may have been a matter of scale. Preparation by this method from a larger volume of tissue culture material is planned.

Lymphocyte transformation. Fraction 1 Karp antigen causes non-specific stimulation of normal mouse spleen cells, perhaps due to an LPS-like substance in the cell wall. Neither Fraction 3 or 4 appear to stimulate normal cells. These fractions do cause inconsistent transformation at concentrations of 10-100 ug/ml in 5 day cultures of immune spleen cells. One of the problems with our assay has been the variable counts of control wells (cells with no antigen) from one experiment to the next. This is not straight forwardly related to the duration of the mouse infection.

Migration inhibition factor (MIF) assay. Lymphocytes secrete non-immunoglobulin substances called lymphokines which convey cell mediated immune activity to adjacent lymphocytes or macrophages. Nacy and Meltzer (18) described macrophage activating factor activity in the supernatant of spleen cells from mice infected with R. tsutsugamushi that were incubated with heat-killed rickettsiae. We have used an indirect agarose microdroplet method (1) to assay migration inhibition factor (MIF) activity in supernates of immune spleen cells incubated with a variety of antigens.

Lymphokines are generated in a V bottom microwell containing 1 x 10^6 spleen cells and antigen in RPMI and 10% FCS, a total of 0.2 ml per well. After 30 hours incubation at 37C and 5% CO_2, the medium is removed and cells pelleted out in a microcentrifuge. Ten-fold dilutions are assayed in triplicate in the MIF assay, using oil-induced mouse peritoneal exudate cells as the indicator cells. The migration is projected onto graduated paper and the average migration distance of like wells determined after 24 hours incubation at 37C, 5% CO_2. Fraction 1 antigen at 60 and 120 ug/ml and Fraction 3 at 50 and 100 ug/ml distinguish normal from immune cells, with migration indices (MI) (test well migration ÷ migration of indicator cell in media alone) <0.7. Normal cells and antigen generally give MI ≥ 0.9. Mice infected between 10 and 35 days earlier have been tested for lymphokine production.

Future Objectives: We wish to make larger amounts of Fraction 3 and 4 antigen (the arrival of SW27 rotor will help) to more easily compare results in different groups of experiments. The assay will be tested in animals infected with a heterologous strain and also its relevance to predict immunity to re-infection.

With the MIF assay we are at the stage of choosing one particular antigen and concentration for testing in longitudinal homologous and heterologous systems.

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2. Oaks, S.C., Jr.: Human volunteer studies: Planning and conducting a chemoprophylactic drug trial. 6th Annual Scientific Meeting of the Malaysian Society of Pathologists, 15-16 August 1981, Kuala Lumpur.

3. Oaks, S.C., Jr., F.M. Hetrick and J.V. Osterman: Rickettsia tsutsugamushi plaque reduction assay. 17th Annual Scientific Seminar, Malaysian Society of Parasitology and Tropical Medicine, 13-15 March 1981; Kuala Lumpur.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6525	81 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. DOD'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	AH	161
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7,2;2			

11. TITLE (Precede with Security Classification Code)

(U) Anti-Schistosomal Drug Development and Malaria Vector Immunology and Studies

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology 002600 Biology 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	PRECEDING 81	2.0	89
	CURRENT 82	2.5	103

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, Philip K., COL
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: US Army Medical Research Unit-Brasilia

ADDRESS: Brasilia, Brazil

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: REID, Willis A., Jr, LTC
TELEPHONE: 272-4548 (Brazil)
SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

Foreign Intelligence not considered

ASSOCIATE INVESTIGATORS
NAME: Bosworth, Anthony B., MAJ
NAME: Prata, Aluizio R, MD

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Brazil; (U) Schistosomiasis; (U) Malaria; (U) Chemotherapy; (U) Immunology; (U) Epidemiology; (U) Drug Resistance; (U) Entomology

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

23. (U) Find new prophylactic and curative drugs for the prevention and cure of schistosomiasis infections and to study the clinical, epidemiologic, drug susceptibility and vector transmission patterns of falciparum malaria in the Amazon River basin of Brazil. Both are primary diseases which would be acquired by U.S. Military and DOD civilian personnel in the event of deployment to any of numerous tropical areas of the world.
24. (U) The WRAIR Anti-Schistosomal Drug Testing Program continues to submit candidate compounds for prophylactic (PMT) and curative (PCT) testing against schistosomiasis in mice. Compounds active in the primary screen are extensively reexamined for confirmation and dose response patterns. The malaria immunology studies include the testing of sera from endemic areas by the indirect fluorescent antibody test. Malaria vector transmission studies include field and laboratory analysis of morphological, behavioral physiological and DDT susceptibility patterns of Anopheles darlingi and other potential anophelene malaria vectors.

25. (U) 80 10 - 81 09.
During the reporting period,457 compounds were screened in the PCT and PMT. Of these 5 were designated confirmed or unconfirmed active and 23 were toxic. Nine compounds were tested in the SCT. Upgrading of research mouse colony facilities was begun. Mark and release studies of Anopheles darlingi at the Ituxi River Study Area are being conducted to determine dispersal patterns from possible larval breeding sites. Construction of an insectary is nearing completion at the University of Brasilia. Preliminary studies to colonize An. darlingi have been initiated.

*Available to contractors upon originator's approval

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

Project: 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit: 161 Antischistosomal Drug Development and Malaria Immunology and

Investigators: LTC Willis A. Reid, Jr., MAJ Anthony B. Bosworth and Dr. Aluizio Rosa Prata, MD.

PROBLEMS AND OBJECTIVES:

1. Schistosomiasis and malaria continue to be two of the major health problems facing many developing countries in South America, the Carribean, Africa, the Middle East and the Far East, and pose disease threats to American personnel stationed in these areas. There is currently no single drug which presents a totally satisfactory treatment for schistosomiasis. The USAMRU-Brasilia antischistosomal drug testing program is oriented to identifying compounds or classes of compounds which elicit prophylactic and curative activity against laboratory Schistosoma mansoni infections in the rodent model.

2. Malaria in the state of Amazonas, Brazil, has become increasingly difficult to control. Plasmodium falciparum, malignant tertain malaria, has become resistant to chloroquine, sulfametoxozol and trimethoprim, sulfadoxine and pyrimethamine and other drugs. Plasmodium vivax, beign tertain malaria, is increasing even though chloroquine remains effective in the treatment and chemo-prophylaxis against it. Current control measures for suspected Anopheles vectors involve the use of the residual insecticide DDT on the inner wall surfaces of houses at 6-month intervals. Adult female An. darlingi mosquitoes, the primary vectors of malaria in Brazil, appear to avoid DDT treated surfaces of houses. Therefore the applied insecticide appears to be effective as a repellent. One objective for this year was to continue with observations of behavioral resistance of An. darlingi to entry of treated houses, while at the same time to study the potential mosquito killing capability of these same DDT treated surfaces.

Other objectives were to prepare an insectary in the Center for Tropical Medicine and Nutrition (CTMN) in order to attempt colonization. In this regard, methods for shipping live An. darlingi to Brasilia were investigated along with the preliminary biological studies for handling, feeding and maintaining larvae

and adults. Investigations of the natural and artificial mating capabilities of these mosquitoes were studied.

In the field, experiments were conducted using mark, release and recapture methods to study the feasibility of evaluating flight behavior and dispersal of An. darlingi.

PROGRESS:

1. Schistosomiasis: In FY1982 we tested 457 bottle number compounds for prophylactic (247) and/or curative (170) antischistosomal activity. Of these, 70 compounds were identified as toxic and 5 showed indications of activity requiring retest verification in the PMT. In the PCT, 23 compounds were toxic and 6 were active requiring confirmation. A total of 574 mouse test groups were utilized (including both drug test animals and control animals). Since each test group requires 5 mice, this represents a utilization of 2,870 mice. The above workload data covers the period 1 October 1980 to 11 February 1981. Four SCT procedures, testing 9 bottle number compounds, have been performed since May, 1980. These compounds were selected on the basis of prior performance in the PCT and/or PMT, and expanded the efficacy data beyond that available in the primary test systems.

In January, 1981 it became necessary to curtail drug testing because of a lack of suitable mice for use as an animal model. The reasons for this were probably combinations of health, environmental, genetic and physical facility factors. In May, 1981 the University of Brasilia initiated extensive renovations of the Central Bioterio mouse facility. Such renovations will include insolated sealed breeding and animal stock rooms, sterilization and cleaning facilities, forced air ventilation system and animal ration and bedding storage facilities. Additionally, an improved colony management program is under review. Improved waste disposal methods and sterilization of filtered mouse bedding have been implemented. In July, we received a shipment of mouse stock (random bred strain CD-1) from Charles River Breeding Laboratories via the Walter Reed Army Institute of Research. These are destined to provide the nucleus stock for rederiving the Bioterio mouse colony. In early September, production breeding was initiated with the aim of a) providing animals for drug testing and b) producing F_1 offspring for a nucleus colony. These efforts have been highly successfull and drug testing was reestablished on 26 Oct 81.

The B. glabrata snail colony is fully capable of maintaining the necessary infection level for support of the drug testing program.

However, some critical fluctuations were noted in several monitored parameters, such as percent of infection success, infected snail mortality and/or snial fecundity (egg laying sucess). Methodology and seasonal/environmental factors certainly had some influence on these fluctuations, but genetic factors may also be contributory to the situation. Considering that both the parasite and the snail were established in the laboratory from wild stock in 1973-74, we returned to the same locale (Paulista, Pernambuco) in April, 1981 and collected uninfected 514 B. glabrata snails for separate laboratory rearing. All snails were returned to the Brasilia laboratory and, during 3 generations of rearing, were evaluated against the older laboratory strain for growth and susceptibility to schistosome infection. Surprisingly, the wild snails demonstrated a slower growth rate and a lower susceptibility to infection than their lab-reared counterparts and we have rejected their possibility for laboratory life cycle maintenance.

Several programs of physical renovations were accomplished in the schistosomiasis laboratory between January 1981 and the present. A new fume hood was installed in the Pharmacy. An isolation/ weighing room was also constructed. Engineering renovations were accomplished in the animal room to improve its isolation against feral pest penetration. The entire laboratory was repainted following severe fungal infestations during the rainy season. Equipment maintenance and repair was also a priority concern during this period.

2. Malaria: Mosquito studies were conducted at the Ituxi study area during the months of March-April and June-July, 1981.

Experiments of the March-April period reconfirmed the presence of behavioral resistance of An. darlingi to year-old DDT treated paper surfaces which lined an excito-repellency chamber. Mosquito adults were also exposed to year-old DDT treated wall surfaces of the experimental house. These mosquitoes were killed within 24 hours of exposure, whereas, less than 20% of the mosquitoes had died in the control house. Marked mosquitoes, which were released in treated and control houses, left the treated house faster than the control house. We are uncertain whether the mosquitoes which left the treated house were exposed to a lethal dose of insecticide. Again, bimodal mosquito biting activity was observed during biting collections made outside of the house. Adult collections made inside of the houses were inconclusive with regards to identifying peak feeding activity since population numbers of An. darlingi were low and may have contributed to incomplete data bases. Adults, first instar larvae and eggs of An. darlingi were successfully transported to the CTMN. Methods for handling, rearing and forced mating were attempted. Yeast, yeast and mouse laboratory chow, Cerophyll®, and wheat germ were tested as food for the larvae. With

50 or less larvae per pan (12 X 7 X 2 inches), wheat germ gave the best results. Efforts toward potential colonization are encouraging, but they were unsuccessful in producing a colony on the first attempt.

The second trip, June - July, 1981, was made to replace the roofs on two experimental houses, to conduct some preliminary experiments on releasing marked mosquitoes at various distances from the study area, and to continue efforts to colonize An. darlingi. During the time the roofs were being replaced 530 mosquitoes, each marked with one of seven different colors, were released at distances of 65 meters, 120 meters and 1 kilometer from the study area. Fifty-nine of the marked specimens (representing all 7 colors) were recovered during biting collections at the study area. About 3,000 eggs, larvae and adults were returned to the CTMN and successfully reared early instars resulted in about 400 adults (1:1 males to females). After examination of 20 empty female spermathecae forced copulation techniques were tried. Even though three different kinds of anesthetizing gases were tried on blood engorged and unengorged female mosquitoes, neither decapitated male mosquitoes nor normal males could successfully inseminate the females. The process of male genitalia interlocking with the female genitalia appeared normal (3-30 seconds). The cause for the lack of spermatozoan transfer is not known. An insectary, which is near completion at the CTMN, will be used to further study the complexities of colonizing this mosquito.

RECOMENDATIONS:

1. Reimplement antischistosomal drug testing with the establishment of a new mouse production colony. Place increased emphasis on secondary curative testing.

2. Continue studies to describe the behavioral morphological and physiological characteristics of selected anopheline species, particulary An. darlingi. Conduct comparative studies in various areas of the Amazon Basin.

3. Colonize An. darlingi for vector competence, behavioral and physiological studies under laboratory conditions.

4. Continue to monitor the effectiveness of house treatment with DDT or other insecticides on malaria vectors.

5. Begin preparations for studies on falciparum malaria strain distributions and immunologic specificities in the Amazon River basin.

PRESENTATIONS:

1. Bosworth, A. and Aire Barros. 1981. Entomological hazards in tropical medicine. VI Curso de Aperfeiçoamento em Medicina. 1 Sep 81 - 24 Oct 81, Faculty of Medicine, University of Brasilia.

2. Bosworth, A. 1981. Entomology laboratory for physicians, IBID.

3. Reid, W.A. 1981. Experimental Schistosomaisis. IBID.

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1. Bosworth, A. J. K. Olson and S. M. Meola. 1981. A study of the chorion of the egg of Psorophora columbiae. I. Taxonomic considerations. Submitted for publication to Mosquito Systematics.

2. Marsden, P. and W. Reid. 1981. New Transactions resists predations of the American conckrouch (Periplaneta americana). Transaction of the Royal Society of Tropical Medicine and Hygiene. 75:132.

3. Peterson, N.E. and R. H. Pine. 1981. Chave para identificar os mamíferos da região amazônica brasileira com exceção dos quirópteros e primatas. Submitted for publication to Acta Amazônica.

4. Peterson, N.E., Roberts, D.R., Llewellyn, C.H. and F.P. Pinheiro. 1981. Programa multidisciplinario de vigilancia de las enfermedades infecciosas en zonas colindantes con la Carretera Transamazonica en Brasil. I. Ecologia de la Region. Bulletin of the Pan American Health Organization. 91:137-148.

5. Prata, A. R., W. A. Reid, and M. S. L. Cunha. 1981. Tratamento da giardose com Tinidazol. Clínica Terapêutica.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 08 6530	81 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
80 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	AH	162
b. **********				
c. **********	STOC 80-7.2	2		

11. TITLE (Provide with Security Classification Code)
(U) Vaccine Development in Trypanosomiasis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
002600 Biology 010100 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. KIND OF AWARD: e. AMOUNT: f. CUM. AMT.

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
FISCAL YEAR PRECEDING 81	7.0	211
FISCAL YEAR CURRENT 82	7.0	190

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, PHILIP K., COL
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION
NAME: U.S. Army Medical Research Unit-Kenya
ADDRESS: Box 401 USAMRU-K
APO New York 09675

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic institution)
NAME: Reardon, Michael J.
TELEPHONE:
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Muriithi, I., DR.
NAME: Wellde, B.T. POC: DA

21. GENERAL USE
Foreign intelligence not considered

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Kenya; (U) Trypanosomiasis; (U) Vaccine; (U) Africa; (U) Cattle; (U) Immunity

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Provide 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. Complete immunity has been achieved against T. congolense.
25. (U) 80 10 - 81 09 During this period the investigators demonstrated that the antigenic character of the parasite population of T. rhodesiense from an endemic area was composed of perhaps as few as one serodeme which was antigenically stable over an 10 year period. They also found that immunity could be induced to blood and tsetse fly (metacyclic) forms by exposure of experimental animals to a broad spectrum of antigenic variants of the same serodeme: The sterile immunity was long lasting. Cross serodeme challenge with both blood and metacyclic forms did not result in protection and indicates that any vaccine would have to be developed for a specific area of which the antigenic composition of the trypanosome population were known. Metacyclic trypanosomes may be more homogeneous antigenically than blood forms. Techniques have been developed to isolate metacyclics from tsetse flies and immunization trials with metacyclics are under way. It is believed that these findings enhance the likelihood of immunologic control of trypanosomiasis. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 October 1980-30 September 1981.

*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 MAR 68 (FOR ARMY USE) ARE OBSOLETE. * U.S. GPO 1974 0691

PROJECT 3M162770A871 **PREVENTION OF MILITARY DISEASE HAZARDS**

WORK UNIT 162 VACCINE DEVELOPMENT IN TRYPANOSOMIASIS

INVESTIGATORS:

PRINCIPAL:	LTC M.J. REARDON, VC
ASSOCIATES:	LTC J.D. CHULAY, MC
	B.T. WELLDE, GS13
	MAJ L.W. ROBERTS, MSC
	MAJ A. WOZNIAK, MSC
	CPT R.F. BEACH, MSC
	CPT L.K. LIGHTNER, MSC

Introduction

Vaccine development remains a high priority in African trypanosomiasis research efforts. Previous studies and reports have documented the serodeme stability of T. b. rhodesiense and T. b. brucei in the Lambwe Valley, Kenya. These reports have also described the immunizing effects of irradiated metacyclic forms. The taxonomic separation of T. b. brucei and T. b. rhodesiense has been based largely on the host from which the parasite was isolated. Recent work at USAMRU-K suggests a closer relationship probably involving a species capable of infecting both man and cattle. Immediate objectives include further study of the host-reservoir relationship, continuation of studies of metacyclic or tissue culture forms as immunogens and evaluation of drugs ulitizing the intrathecal inoculation model.

ceral leishmaniasis in East Africa, although subjected
study, remains poorly understood. There is a paucity of
cerning host-parasite-drug interactions. Adequate second
l line drugs do not exist and the subject of parasite
ce vs patient non-responsiveness to therapy remains a
of debate. Vector-reservoir relationships also are
derstood. Objectives include better documentation of the
available drugs both in vivo and in vitro, better
on of "resistance", biochemical typing of both parasites
rs, and expansion of vector-reservoir field studies.

Trypanosomiasis

outbreak of human disease which started in May-June 1980
mbwe Valley continued until January 1981. To date some
rhodesiense isolates have been made and 80 T. b. brucei.
phic survey of the area is partially complete and will
a base for followup studies. Several significant obser-
ave been made during this outbreak: (1) neutralization
dicate that the T. brucei group trypanosomes isolated from
cients are antigenically similar to those found in cattle,
ts and game animals; (2) cattle infected with T. brucei
panosomes have been found to undergo a disease of the
ervous system comparable to that found in humans and it
hat this disease has been responsible for a large mortality;
apse rate of at least 27% following conventional Suramin
of patients considered to be "early" cases; (4) a large
deaths among individuals treated and presumed cured in
years. A complement fixation test has recently been
ed into the diagnostic battery and appears both sensitive
fic. Individuals undergoing relapse have an increase in CF
in the absence of detectable circulating trypanosomes.

aborative studies with UCD&I and the Kenya Trypanosomiasis
continue and preliminary results indicate that the
isolated from Lambwe Valley individuals continue to be
ally stable. Mechanical transmission studies are continu-
attempt to define the significance of this phenomenon and
ct it might have on a field vaccine.

ossina palliditpes colony has recently been established
n information previously obtained from experiments with
ns will be verified. Since G. palliditpes is the only
Kenya, mechanical transmission and cyclical transmission
es of this species should be addressed.

Visceral Leishmaniasis

Pilot studies have continued and protocols dealing with pentavalent antimonials and allopurinol have been submitted. The first study involving human subjects entitled "An Evaluation of High Dose Short Duration Sodium Stibogluconate (Pentostam[R]) Therapy of Kala Azar" has been started.

Field studies of vector-reservoir relationships are underway and one isolate of L. donovani has been obtained from a dog from the Turkana area of northern Kenya. Several studies are being carried out in a continuing evaluation of media for primary isolation, post treatment evaluation and bulk culturing.

Four species of sand flies have been established in colony and have been raised to the F_3 generation. Generation time is 51-58 days depending on the species. The F_3 generation represents new rearing records for all species. Phlebotomus martini had not previously been reared beyond F_1 and Sergentomyia schwetzi, S. africanus and S. antennatus had never been reared in colony. Transmission and taxonomy studies await the numerical increase in the colony.

RECOMMENDATIONS

African trypanosomiasis

It is recommended that the Lambwe Valley study be continued with emphasis on case followup and evaluation of the demographic data. The typing studies using VAT, isoenzymes and neutralization techniques should continue and be coupled with attempts to identify immunologically important antigens. The intrathecal inoculation model should be utilized to evaluate drug efficacy in CNS infection in the light of the reported relapse data. The use of serological testing should be expanded.

Visceral 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 sandflies become available.

Publications

1. Kager, P.A., Rees, P.H., Wellde, B.T., Hockmeyer, W.T. and Lyerly, W.H.: 1981. Allopurinol in the Treatment of Visceral Leishmaniasis. Trans Roy Soc Trop Med Hyg, 75, 556-559.

2. Roberts, L.W.: 1981. Probing by Glossina morsitans morsitans and Transmission of Trypanosoma (Nannomonas) congolense. Am J Trop Med Hyg, 30(5), 948-951.

3. Wellde, B.T., Hockmeyer, W.T., Kovatch, R.M., Bhogal, M.S. and Diggs C.L.: 1981. Trypanosoma congolense: Natural and Acquired Resistance in the Bovine, Exp Parasitol, 52(2), 219-233.

Manuscripts In Press

1. Preston, J.M., Kovatch, R.M. and Wellde, B.T.: Trypanosoma congolense: Thrombocyte Survival in Infected Steers. Exp Parasitol

2. Roberts, L.W., Boyce, W.L. and Lyerly, W.H.: Cordylobia anthropophagia (Diptera: Calliphoridae) Myiasis in an Infant and Dog and a Technique for Larval Rearing. J Med Ent

Presentations

1. Boyce, L., Wellde, B. and Reardon, M.: Trypanosoma brucei rhodesiense: Intrathecal Inoculation of Calves. 2nd Annual Medical Scientific Conference of the Kenya Medical Research Institute and Kenya Trypanosomiasis Research Institute. Nairobi, Kenya

2. Roberts, L.W.: Probing by Glossina morsitans morsitans and Transmission of Trypanosoma (Nannomonas) congolense. Entomological Society of America, Atlanta, GA.

3. Wellde, B., Chumo, D., Masaba, S., Siongok, T. Arap, Waema, D. and Oloo F.: Trypanosoma brucei rhodesiense: A Serological Study of Trypanosomes from Man and Domestic Animals in Western Kenya. 2nd Annual Medical Scientific Conference of the Kenya Medical Research Institute and Kenya Trypanosomiasis Research Institute. Nairobi, Kenya.

4. Wellde, B., Kovatch, R., Chumo, D., Adoyo, M., Mwongela, G. and Opio, E.: Trypanosoma vivax: Investigation of a Hemmorhagic Syndrome in Experimentally Infected Cattle. 2nd Annual Medical Scientific Conference of the Kenya Medical Research Institute and Kenya Trypanosomiasis Research Institute. Nairobi, Kenya.

5. Wellde, B., Chumo, D., Boyce, L., Reardon, M., Waema, D. and Olando, J.: Current Epidemiologic Patterns of Human and Animal Trypanosomiasis with Emphasis on Control Methods. Conference on Epidemiology and Economics of Trypanosomiasis Control in Selected Areas. International Laboratory for Research on Animal Diseases, Nairobi, Kenya.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6500	81 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
80 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	62770A	3M162770A871	AD	163
b. CONTRIBUTING				
c. [illegible]	[illegible]	[illegible]		

11. TITLE (Precede with Security Classification Code)

(U) Gastrointestinal Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 008800 Life Support 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 07	CONTINUED	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 81	8.0	703
CURRENT FISCAL YEAR 82	8.0	697

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, COL, MC, P.

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

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

ADDRESS: Washington, D.C. 20012

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

NAME: BOEDEKER LTC, E. C.

TELEPHONE: (202) 576-2582

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: CHENEY CPT, C. P.

NAME: REID LTC, R. A.

21. GENERAL USE

Foreign intelligence not considered

POC: DA

22. KEYWORDS (Precede each with 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

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

23 (U) Research efforts in this department continue to be directed toward Gastrointestinal diseases of military importance. Focus is on enteropathogenic bacterial diarrheal disease including pathogenic E. Coli, Salmonellosis and Shigellosis. 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 of effects of infections on intestinal transport and 4) Motility. Studies utilized preparations of intestinal membrane fraction and of bacterial adherence factors (pili), isolation and fractional characterization of intestinal **mononuclear** cells, in vivo volated intestinal loops, in vivo intestinal perfusions, Ussing chambers, voltage clamps, in vivo acute and chronic recor[illegible] of intestinal activity.

25 (U) 80 10-81 09 Mucosal Adherence Specific adherence f bacterial surface structures (pili) to intestinal mucosa has been demonstrated and the intestinal receptor for one type of pilus has been identified as a disaccharidase. Immurology The capability of lamina propria mononuclear cells outside of the Peyers patch to function as an effector lymphoid organ has been shown. Transport The ability of berberine to reverse secretions induced by cholera toxin, E. coli stable toxin and cAMP was demonstrated. Motility Effects of Salmonella and Shigella infections on intestinal motility in primates was documented. For technical report see Walter Reed Army Institute of Research, Annual Progress Report 1 Oct 80-30 Sept 81.

*Available to contractors upon originator's approval

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Project: 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 163: Gastrointestinal Diseases of Military Importance

Investigators:

Principal: LTC Edgar C. Boedeker, MC
Associates: LTC Robert H. Reid, MC
LTC Robert W. Sjogren, MC
LTC Thomas Gage, MC
MAJ Dennis R. Sinar, MC
MAJ James A. Wright, MC
CPT Christopher P. Cheney, MSC
Dr. Yuan-Heng Tai, GS-13
Mr. William G. Marnane, GS-11

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, notably enterotoxigenic E. coli. 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 attrach 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 adherence antigen be used as an effective oral vaccine against enteric infection with pathogenic E. coli?

ii. Role of Host Immune Mechanisms

To develope an antiserum specific for rabbit IgA. This serum will then be used to develope a solid phase radioimmunoassay for rabbit IgA with a sensitivity in nanograms. To determine if a population of lamina propria mononuclear cells can act in vitro as an affector lymphoid organ. To determine if the synthetic octapeptide (GLY-ASN-THR-ILE-VAL-ALA-VAL-GLU), a known rabbit antigenic site exists with conformational restriction in aqueous solution.

iii. Alterations of Intestinal Transport

What are the normal mechanisms for salt and water transport? What are the mechanisms for the intestinal secretion of water and electrolytes induced by bacterial toxins and other secretory stimuli? What is the mechanism of the secretion seen in intestinal obstruction? Do absorptive and secretory processes interact? Can pharmocologic agents reverse the salt and water secretion induced by bacterial toxins and other secretory stimuli?

iv. Alterations in Intestinal Motility

What are the mechanisms and neurohumoral pathways by which bacterial toxins change small bowel myoelectric patterns? Do luminal toxins produce similar changes in myoelectric patterns to native disease? Do toxins causing mucosal injury induce similar motility changes to those that do not cause mucosal damage? Are secretory/absorptive, myoelectric and microbiologic responses to toxins and infections related? How do myoelectric patterns correlate with radiographic peristalsis and transit time? Is colonic motility abnormal in response to fluid load, laxatives, antidiarrheals, antibiotics and infection of the small and large bowel?

PROGRESS

i. Role of Mucosal Adherence in Bacterial Colonization:

An animal model for adherence of an enteropathogenic E. coli to small intestine has been established. In vitro assays were developed to quantitate the adherence of E. coli strain RDEC-1 to rabbit ileal brush border membranes. The adherence is rapid and sensitive to pH, temperature and ionic strength. Adherence is not influenced by carbohydrates known to inhibit the adherence of E. coli to other organ systems. Evidence was obtained indicating that the adherence may involve hydrophobic interactions. Other studies validating the model established the species and tissue specificity of this adherence reaction. For the RDEC organisims, in vitro adherence ability correlated with its ability to infect and colonize the small intestine when a number of animal species were examined.

In order to identify the adhesins on RDEC-1 which promote their adherence to host cells, we gentically transfered RDEC-1 adherence ability to a non-adherent non-piliated Shigella flexneri organism. Electron microscopic examination revealed that transfer of adherence correlated with transfer of surface pili. The genetic information for the production of pili was shown to be located on a transferable plasmid. Further studies were performed to confirm the importance of pili from RDEC-1 as

adherence factors. RDEC-1 pili were isolated and their adherence ability to intestinal mucosa examined by immunofluorescent staining. We confirmed that purified RDEC-1 pili adhered to rabbit intestinal mucosa in the same species specific manner, and with the same distribution, as the whole organisms.

Using the techniques developed in the RDEC rabbit model, the adherence of the E. coli strain (H10407) isolated from patients with Traveller's diarrhea, to human brush borders, was confirmed. H10407 can synthesize two distinct types of pili, colonization factor antigen I and type I pili when grown under the appropriate conditios. When we examined the adherence of H10407 to freshly isolated human brush borders, we found that conditions which promoted predominantly CFA/I pili also promoted the adherence of the organism to human brush borders. In contrast, conditions which promoted predminantly type I did not promote adherence to human brush borders. Furthermore, adherence was abolished when H10407 organisms were grown under conditions which inhibited pili expression. Thus it appears that as in the case in the animal system, human pathogens only adhere to host cells when they express specific pili.

In order to identify the bacterial receptor on the host mucosa, the ability of purified pili to bind to, and precipitate with, proteins soluilized from the rabbit intestinal brush border membrane was examined. Immunoprecipitation of receptor activity was possible and the activity seen to reside in two high molecular weight brush borders proteins. Data obtained from entirely different experiments also suggested that there two high molecular weight proteins may serve as the receptor for RDEC. In these experiments, we compared the protein profile of intestinal brush borders obtained from a group of receptor negative rabbits to the protein profile of intestinal brush borders obtained from a group of receptor negative rabbits to the protein profile of brush borders obtained from our normal, receptor positive rabbits. Those brush borders lacking the receptor for RDEC-1 also lacked the two high molecular weight proteins which were selectively immunoprecipitated by purified RDEC-1 pili. Enzymatic analysis revealed that these two proteins comigrated with sucrase isomaltase activity.

Utilizing this information, further experiments were conducted to dertermine if the sucrase isomaltase enzyme served as the bacterial receptor. These experiments included papain digestion of the sucrase isomaltase complex off of the mucosa, isolating the enzyme and determining whether this enzyme complex possesses specific RDEC receptor activity. In these experiments we were able to recover a protein fraction which was enriched 15 fold in isomaltase but relatively devoid of sucrase activity. SDS PAGE showed one higher MW band that barely penetrated the gel. This fraction specifically agglutinated piliated RDEC but not

nonpiliated RDEC on type 1 piliated organisms as judged by phase microscopy. In summary, isomaltase appears to serve as the host receptor for RDEC.

In theory, an effective oral vaccine to prevent enteric infection by adherent, toxigenic E. coli should promote secretion into the intestinal lumen of IgA directed against pili which have been designated colonization factor antigens (CFA)s. To test whether intraluminal CFA can elicit a specitic local IgA response we purified CFA II pili from an E. coli pathogenic for man, we inoculated rabbit thiry-Vella loops with antigen and tested loop fluids for IgA and IgG antibody to CFA II using ELISA assays. In summary, mucosally applied CFA II induced a dose-related, specitic IgA response in intestinal secretion. These preliminary studies lend support for the use of purified E. coli CFAs as oral vaccines against E. coli diarrhea.

ii. Role of Host Immune Mechanisms

Antibody to rabbit IgA was produced by inoculating a goat with complete Freund's adjuvant and IgA isolated from rabbit colostrum. The appearance of antibodies in the goat's serum was detected by the use Ochterlony plates. While the antibody showed high affinity for rabbit IgA, it was also cross-reactive with rabbit IgG and IgM. Ig cross-reactivity was removed by passing the serum over a column of Sephadex beads to which rabbit IgG had been conjugated. To use the same method to remove the IgM cross-reactivity, it was first necessary to isolate a sufficient quantity of IgM. This was done by infecting rabbits with trypanosomes and collecting their serum after three weeks. The immunoglobulins were separated by ammonium sulfate precipitation and the IgM fraction enriched by cold percipitation. Passages over a Protein A and a DEAE column were then used to purify the IgM fraction. This IgM was then complexed to Sephadex beads and used to remove the anti-IgA antibodies with cross-reactivity to IgM. The passage of the anti-IgA serum over the IgM and IgG columns resulted in the elimination of all cross-reactivity. We are currently in the process of using this purified antibody to develop a RIA for rabbit IgA.

Peyer's patch has been considered the lymphoid organ esponsible for intestinal immunization. However, the rabbit can be immunized to produce secretory IgA antibodies to protein antigens thru a chronic Thiry-Vella loop not containing a Peyer's patch. This observation implies that lamina propria lymphocytes could be sensitized to protein antigens directly. We chose to prove this possibility by isolating lamina propria lymphocytes from ileum free of Peyer's patches. Lamina propria mononuclear cells (lymphocytes, monocytes, and macrophages) were isolated by collagenase digestion and elutriation into two populations. The small cell population was capable of being sensitized in vitro in

microcultures to a synthetic 13 amino acid peptide (a known rabbit immunogen). The cells were immunized for 7 days then challenged with fresh antigen and 3 days later the blast transformation response determined by tritiated thymidine uptake. The immunizing dose range was 0.002-2.0 ugm/ml for the 13 A.A. peptide and the challenge dose range 0.0030-20 ugm/ml. The results of one experiment are shown below.

Imm. Dose	Chal. Dose	Responses		
		Number	pos/total	net CPM±SD
0.002-2.0	0		0/20	0
0	0.0036-20		0/28	0
0.002-0.02	0.036		6/6	5263±1699
0.063-0.2	0.36-2.0		6/9	650±2324
2	11.2		3/3	8230±541

Challenge doses above and below doses giving positive responses gave negative responses for each immunizing dose. Low challenge doses required low immunizing doses and high challenge doses required high immunizing doses required high immunizing doses for positive responses. In conclusion, a population of lamina propria mononuclear cells can act in vitro as an affector lympoid organ with monocytes/macrophages capable of antigen to lympocytes interaction resulting in their sensitization. Uncommitted lymphocytes capable of being sensitized to a synthetic 13 A.A. peptide antigen must reside in the lamina propria.

The synthetic 13 amino acid peptide (the knwon rabbit immunogen) contains an antigenic site in the N-terminal portion. The N-terminal 1-8 synthetic peptide (glycine- asparagine - threonine - isoleucine - valine - alanine - valine - glutamic acid) is antigenic, stimulating lymphocytes to release MIF. The 1-7 synthetic peptide does not stimulate sensitized lymphocytes, yet is capable of binding to cell-bound cytophilic antibody. This observation suggests that a conformation of the antigenic site may be important for the stimulation of lymphocytes. For this reason the confirmation of the 1-8 synthetic peptide is being sought. The 600 MHz high resolution proton NMR spectrum of the pepetide in aqueous solution has been recorded and spectral assignments have been made, using standard chemical shift correlations and extensive spin-spin decoupling. All protons, with the exception of the alpha proton of asparagine (obscured by the water peak), have been observed. Rotameric populations of all side chains have been estimated and indicate that the hydrophilic part of the peptide does not have a strong preference for a particular conformation; however, the hydrophobic side chains are relatively restricted and this is compatible with a conformation in which these residues form a hydrophobic surface suitable for interaction with lipids. Tests for this hypothesis are underway.

iii. Alterations of Intestinal Transport

A theoretical study of the relationships among ion currents, membrane potential difference, and resistance of an epithelium indicates that the short-circuiting technique introduced by Ussing does not completely short-circuit the epithelium if the series resistance parallel to the cell layer between the voltage measuring electrodes is not properly compensated. The residual potential difference across the epithelial layer in the "short-circuit state" is proportional to both the measured short-circuit current and the resistance of the diffusion barriers not compensated. The effects of osmotic gradients on basal ion transport were studied. It appeared that the changes in electrical properties caused by osmotic gradients were associated with changes in anion transport mechanisms.

We have been studying the enzymatic mechanisms in mediating intestinal secretion of water and electrolytes using cholera toxin, heat-stable enterotoxin of E. coli (ECST), serotonin, and methylprednisolone. We found that stimulation of intestinal mucosal guanylate cyclase activity or cGMP concentration could induce secretion of water and electrolytes in the rat ileum. Both methylprednisolone administration and ECST stimulated electrogenic chloride secretion as well as increases in mucosal guanylate cyclase activity and cGMP concentration in the rat ileum. Exposure of the serosal surface of the rabbit ileal mucosa to serotonin caused electrolyte secretion in a concentration-dependent manner but did not alter the mucosal adenylate cyclase and guanylate cyclase activities. The action of serotonin may be regulated by serotonin induced increase in inracellular Ca^{++} concentration.

Acute elevation of the intraluminal hydrostatic pressure also caused intestinal secretion of water and electrolytes in the rabbit jejunum and ileum but did not alter the mucosal Na-K-ATPase and adenylate cyclase activities. It appeared that increased intraluminal hydrostatic pressure affected the hydrodynamics of the mucosal microcirculations to produce a driving force for passive filtration-secretion. A mathematical model for the dynamics of luminal fluid accumulation in intestinal obstruction was derived based on a luminal fluid material balance.

The interaction between absorption of glucose and alanine and electrogenic secretion of chloride was studied in cholera toxin, cAMP, or methylprednisolone-treated rat ileum. It was found that increased electrogenic Cl secretion stimulated glucose and alanine absorption in the rat ileum. The interaction between the absorptive and secretory processes is not well understood.

The alkaloid berberine was able to reverse the cholera toxin-induced secretion of water and electrolytes in a concentration-dependent manner and to reverse the ion secretion induced by cAMP, ECST, and methylprednisolone.

iv. Alterations in Intestinal Motility

In rabbit ileal loops, bacterial toxins, specifically E. coli heat labile toxin (LT) and cholera enterotoxin (CT), produce a diarrheogenic myoelectric pattern, the migrating action potential complex (MAPC). The MAPC is produced by the cholera B subunit, requires binding at the GM1 binding site and requires aggregation of B subunit components in a form more complex than the monomeric B subunit. This raises the possibility that potential B subunit vaccines may have untoward effects on intestinal motility. However, binding of the same receptor by TSH produces neither an electrical nor a fluid response (results not published). LT produces similar activity in similar concentrations as CT. The antigenic similarities between CT and LT are insufficient to block MAPC activity by preincubation of toxin with heterologous antiserum.

The lectins ricin and WGA produce MAPC responses similar to those of CT and LT in rabbit ileal loops but there are differences in fluid output. Unlike WGA, CT and LT, ricin causes mucosal destruction and induces, an addition to MAPC's, a second myeolectric pattern, repetitive bursts of action potentials (RBAP).

Reproduction of MAPC and RBAP activity in denervated loops of bowel has been reported elsewhere. The prototype of an in vitro system for suspension of a denervated loop of bowel in an oxygenated, nutrient bath has been constructed and is undergoing testing. A computer program has been developed which allows interpretation of MAPC's, RBAP's and electrical spike activity from analog digital recordings. This will allow greater precision and add the ability to interpret unpatterned electrical spike activity. This pair of developments should greatly extend the capabilities of future studies.

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 fasting and fed changes in the migrating myoelectric complex (MMC) as monitored by computer analysis of spike burst activity. Seven animals have developed clinical Shigella and five have developed clinical Salmonella infection with diarrhea and dysentery. Motility patterns become clearly abnormal with clinical disease and resolve with antibiotic treatment. Furthermore, Shigella and Salmonella produce different abnormal motility patterns (results not published).

Common laxatives, antidiarrheals and antibiotics have also been tested in this primate model. Some result in enhanced motility responses (sorbitol, MCT oil, caffeine), some in reduced motility (castor oil, PGI, PGE-2), some in unusual motility

patterns (castor oil, lactose) and some were without effect on motility (ampicillin, saline, loperamide, lomotil, codeine). These studies will serve as control studies for tests of antibiotic/antidiarrheal medications during native infections described above.

A rabbit model for recording chronic intestinal myoelecric activity from unanesthesized, restrained rabbits has also been developed. Our initial work with this model has shown that unlike the primate model fasting and fed changes in the MMC do not occur (results not published). Present studies utilizing computer analysis of spike burst activity include testing common laxatives, antidiarrheals, antibiotics and toxins as well as RDEC and clindamycin-induced infectious diarrheas in a manner analogous to that used in the primate model.

FUTURE PLANS AND RECOMMENDATIONS

i. Role of Mucosal Adherence in Bacterial Colonization

Over the next year we intend to continue to characterize the pili/host receptor interations in the RDEC-1 rabbit model in order to define substances which could inhibit or prevent the intestinal bacterial-host interactions. Inhibitor substances, introduced into the intestinal lumen, could prevent bacterial adherence and promote rapid clearance of the organisims. Thus non-toxic inhibitors might provide effective prophylaxis or therapy. Promising substances to be tested include a class of inert gels substituted with hydrophobic ligands.

Based on the confirmation of pili as important determinants of adherence for pathogenic human isolates of E. coli, and our previous demonstration that specific IgA could prevent and reverse RDEC-1 adherence in vitro, we intend to devote a major part of our effort toward preparation of a class of E. coli pili for use as an oral vaccine against forms of Traveller's diarrhea. Studies are under way to validate the effective immunogenicity of these pili, and a collaboration with investigators at the University of Maryland Center for vaccine development has been established or testing of this vaccine.

To determine the identity of the receptor on the hosts' mucosal surace for pathogenic bacteria, we first plan to examine the two high molecular weight brush border proteins previously described (progress section). The approaches we plan to take are based on our prior experimental observations that receptor activity can be solubilized with papain. We therefore plan to solubilize the recptor with papain, and using classical biochemical techniques, to isolate the two high molecular weight brush order proteins from the papain digest in a homogenous form. In order to determine if either of theses two proteins serve as the

receptor for RDEC-1 we will examine the ability of these isolated proteins to selectively agglutinate piliated RDEC (if the receptor is divalent in nature) and/or inhibit RDEC adherence to rabbit brush borders (if it is monovalent). We will subsequently prepare antisera directed against the purified brush border proteins of interest. Intact and Fab digests of the hyperimmune immunoglobulin fraction of these sera will be tested for their ability to inhibit RDEC adherence to rabbit brush borders using our established adherence assay. We also plan to follow up our previous experiments on the immunopricepitation of rabbit brush border receptors using purified RDEC-1 pili and solubilized, iodinated rabbit brush border proteins. We plan to utilize pili affinity chromatogrophy (i.e. pili attached to a sepharose matrix) in order to isolate the receptor from a detergent or papain solubilized fractions of rabbit brush border proteins. Since our laboratory now possesses the capability to produce other types of pili besides RDEC pili, we can use type 1, CFA-I and CFA-II pili affinity columns as controls. Hopefully the information derived about the receptor for RDEC will allow us to design the most advantageous experimental protocols for identifying the bacterial receptors on human intestinal mucosa.

ii. Role of Host Immune Mechanisms

The mechanism of local immunization and the regulation of local immune responses at the level of the intestine remain high priorities for study to develope better oral vaccines. Synthetic peptide immunogens are being developed for oral vaccines to give protective intestinal mucosal immunity.

The goal is to have vaccines giving long term T-lymphocyte memory without intertering secretory antibody production which would allow subsequent booster oral immunization to be effective. Peptide antigenic site modification is the approach being taken leading towards cross-reactive T-lymphocyte immunization without cross-reactive antibody formation. The developed peptide analog antigen would then be conjugated to a synthetic polypeptide having a specific receptors on the enterocytes and then further conjugated to a synthetic adjuvant such as muramyl dipeptide.

The peptide antigen and immunogen conjugates are tested by immunizing and challenging rabbit lamina propria T-lymphocytes in vitro. Antibody binding is determined using a solid phase radioimmunoassay and monoclonal antibody directed towards the modified antigenic site. The immunogen conjugates would also be tested for mucosal absorption and their ability to sensitize a chronic ileal looped rabbit. Ultimately it would be determined if rabbits could be protected from enteric pathogens following primary and booster immunization with the appropriate synthetic peptide vaccines.

Alterations of Intestinal Transport

Future work should attempt to obtain further information on ature of the absorptive and secretory systems, including anatomical locations, mechanisms of operation, and their ivity to various reagents, both stimulatory and inhibitory. knowledge should aid to a better understanding of the nt pathophysiology and therapeutics of diarrheal diseases. ic studies in the near future are described below.

There are several possible ways of testing directly the stion that absorption occurrs via the villous cells while ion via the crypts. First, we will make detailed nations of the effect of secretory stimulators on the otive functions and the effect of absorptive stimulators on secretory functions. For example, it would be indicative er or not cAMP or cholera toxin has any effect on the otion of D-glucose, D-galactose, and L-alanine.

If there are two totally separate transport systems, they operate on separate pools of sodium and or chloride. We will fore attempt to identify such pools by labelling them with active isotopes placed in either the mucosal or serosal ion. To a simple first approximation, an ion pool involved sorption should be labelled mainly from the mucosal solution one involved in secretion should be labelled from the al solution. If there are two separate pools for sodium or chloride), one for each transport system, they should in predictable ways when the transport systems are ed.

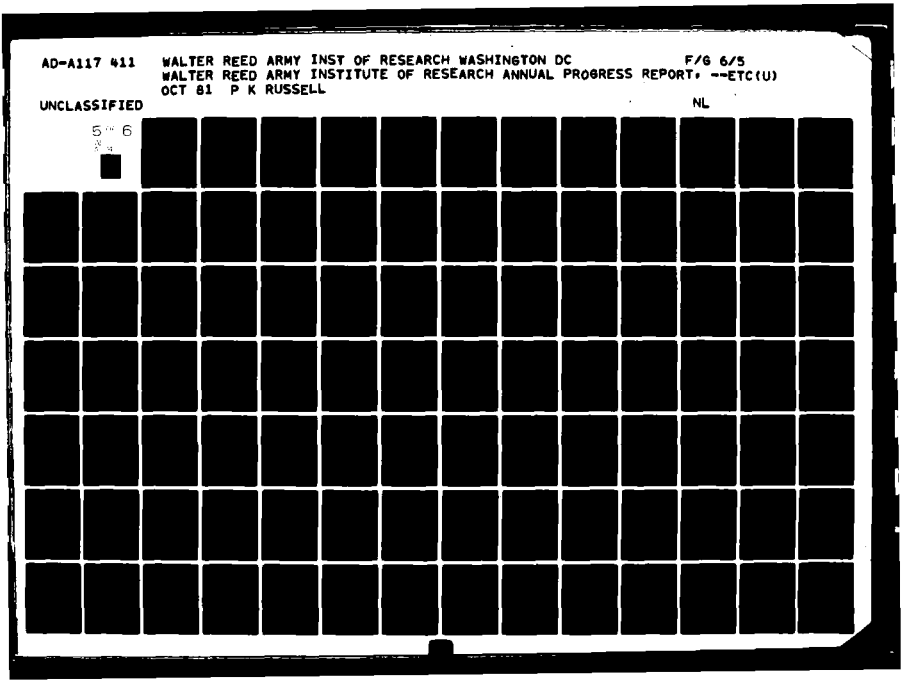
Another approach is also worth testing. If absorption and ion proceed by separate parallel pathways through the , the two paths may display significantly different es. We will explore this possibility by examining the time of approach of tracer fluxes to steady state values. The teristics of the time course for flux from mucosa to serosa the reverse direction should enable us to infer certain ties of these pathways and to determine whether they are cal or separate.

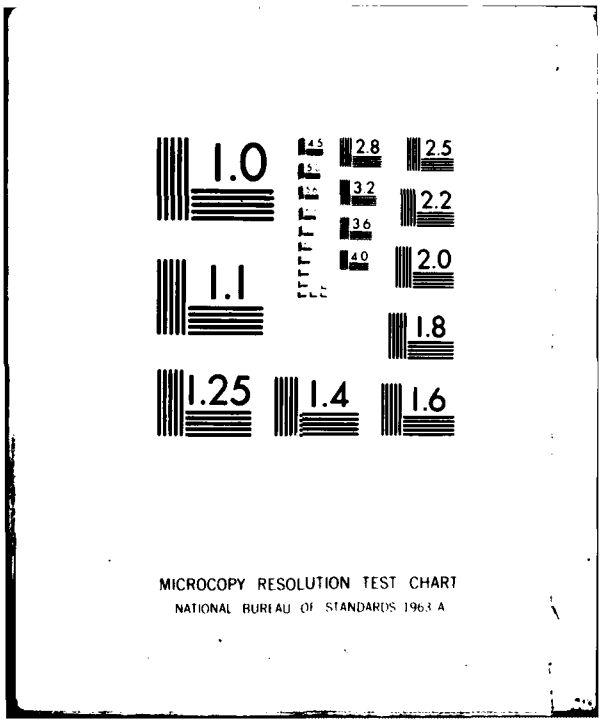
Further studies involving the anti-secretory agents berber- would include 1) the effect of berberine on the gluco- uced absorption of water and electrolytes and 2) establi- a correlation between berberine-induced changes in the orylated protein intermediats and berberine-induced changes lic nucleotide- induced electrolyte transport.

Drugs of potential use in diarrheal diseases including ial absorption stimulators, such as dopamine, fenoprofen and potential secretion inhibitors, such as trifluoper ne, chlorpromazine, fluphenazine etc., will be evaluated

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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.

iv. Alterations in Intestinal Motility

Future investigation of bacterial toxins, infections and lectins in rabbit ileal loops will utilize two approaches: in vivo ileal loops continuously perfused with C^{14} PEG to allow simultaneous determination of myoelectric activity and fluid secretion/absorption and in vitro denervated ileal loops in oxygenated nutrient bath. Utilizing these approaches the following questions will be investigated: is there a correlation between the fluid secretory/absorptive response and the motility response; what is the role of mucosal damage in fluid and motility responses; what are the neurohumoral pathways by which motility patterns are mediated and what is the effect of therapeutic agents on these pathways? Development of a computer program to analyze slow waves will enable complete computer interpretation of all measured motility events. In addition, ability to interpret slow waves will allow analysis of myoelectric motility patterns in the colon which are much more complex than those in the small bowel. Assessment of the effects of infection, toxins and pharmacologic agents on colonic motility and on secretion/absorption is essential before definitive conclusions about clinical diarrheal states and responses to therapy can be addressed (ie. are observed small bowel events compensated or exaggerated by colonic responses).

Future investigation using the primate model will compare myoelectric effects induced by purified toxins to the native infection. The temporal relationships of motility, fluid/secretory and microbiologic events in clinical diarrheal disease and their response to pharmacologic intervention will be determined. Continuation of ongoing studies using the rabbit model will allow comparison of data obtained from the rabbit ileal loop and in vitro bath to data obtained from unanesthesized rabbits with chronically implanted electrodes. Extension of the chronic rabbit model to incorporate electrodes sewn on to Thiry villa loops will enable direct instillation of toxins and microorganisms into the study loop and secretory/absorptive studies using C^{14}-PEG perfusion to be performed on unanesthesized rabbits.

Before these studies can be extended to patient populations, less invasive probes and accurate computer programs for data interpretation must be available. Eventually studies correlating myoelectric, pressure, radiographic and absorptive/secretory events should be performed. Oral pressure, myoelectric and

radiotelemetry probes need to be developed. Collaboration with development of similar probes required for intraintestinal pressure monitoring in "Blast Overpressure" studies may be rewarding.

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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	81 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 INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 10 01	D Change	U	U		NI	☒ YES ☐ NO	A. WORK UNIT

10. NO 'CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AC	181
b. CONTRIBUTING				
c. XXXXXXX	STOC 80-7.2.5			

11. TITLE (Precede 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 81	1.0	156
FISCAL YEAR CURRENT 82	1.0	195

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012
RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, COL, Phillip K.
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: FLEMING, COL, Arthur W.
TELEPHONE: (202) 576-3791
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign Intelligence not considered

POC: DA

22. KEYWORDS (Precede 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 (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23 (U) Our technical objectives are to develop both surgical and medical adjuncts for the management of blast induced injury to the lung and gastrointestinal tract. The initial goal will be to develop techniques which will allow documentation of the natural history of pulmonary injuries. The threat of exposure of American soldiers to blast waves from enemy weapon systems which may exceed established thresholds is increasing. This project proposes to respond to the threat of potential blast related problems which may be experienced by the Army in the field.

24 (U) A review of the extensive data base already available will be initiated. A rapid technic for the in vivo estimation of lung water using a double-dilution technic will be assessed in a canine model with pulmonary capillary damage to simulate blast injury.

25 (U) 80 10 - 81 09 A preliminary review of the literature from World War II to the present has been conducted. A pilot study is presently being carried out to appraise a quantitative technique for measuring extravascular lung water in vivo. Initial results suggest that this technique allows for repeated documentation of changes in lung water without an accumulative loss in blood from sampling. Following verification of these results in a canine model, we plan to carry out these experiments in sheep exposed to blast waves. For technical report see Walter Reed Army Institute of Research Annual Progress Report. 1 Oct. 80 - 30 Sept. 81

*Available to contractors upon originator's approval

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

Project 35162772A874 METHODS AND TECHNIQUES FOR COMBAT CASUALTY MANAGEMENT

Work Unit 181 Management of Military Blast Injury

Investigator:
Prinicipal: COL Arthur W. Fleming,M.C.

Problem and Objectives:

The apparent absence of massive numbers of American casualties from primary, secondary, tertiary, immersion, and solid blast injuries during the Vietnam Conflict was directly related to our air superiority and the enemy's lack of high explosives in sufficient quantaties. When blast injuries did occur during the Vietnam Conflict, the infrequent appearance, the association of multiple other injuries and the rapid evacuation of patients with the lost of direct continuity of medical care may have led to inaccurate diagnoses. Many episodes of "Wet Lung, DaNang Lung, Shock Lung, White Lung, etc," may have been secondary to blast injury.

The involvement of USAMRDC in conducting fundamental and clinical research relevant to the military medical problems of blast injury resulting from enemy weapon systems is thus a new mission which was established during FY 1981. Implementation of this research program in Research Area II - combat casualty care- is the primary responsibility of the Division of Surgery, WRAIR. This research, although intrinsically related to the research in "Blast Overpressure (BOP)" should not be confused with the Research Area III Mission- that of risk assessment of American weapon systems.

Since World War II it has been recognized that organs containing air (the lungs and the gastrointestinal tract) are the most vulnerable to blast waves. Recent clinical experience from Ireland and Israel as well as the blast demonstration for USAMRDC have confirmed these earlier findings.[1-4] Our initial efforts have focused on the early diagnosis and monitoring of pulmonary injuries.

Progress:

Following blast injury to the lungs, the predominant lesion involves exudation of edema fluid and blood into the alveoli and the interstitial space. Over a period ranging from a few minutes to several days after injury respiratory failure may develop either from

a combination of blast effects, or by fluid overload during resuscitative efforts.

Accurate measurement of extravascular lung water in patients has been attempted by a variety of methods for several years with variable success. Chest x-ray and arterial blood gas analysis are the most commonly used clinical tools for estimation of pulmonary edema, however, both may be altered by other factors and do not give either specific or quantitative information about the amount of lung water present.

A potentially accurate- and reproducible method of measuring extravascular lung water has been demonstrated by Lewis, Elings and Oppenheimer.[5-10] This technique is non-destructive and has been utilized in the quantitation of extravascular lung water (EVLW) in both experimental animals and humans. The simultaneous bolus of two indicators, cold glucose water and indocyanine green dye are employed in this technique. The cold glucose water difuses into the pulmonary interstitial fluid while the green dye remains in the intravascular space. By substracting the volume of distribution of the intravascular from the volume of distribution of the diffusible indicator, the extravascular lung water volume (ELVW) can be obtained. The use of a cold indicator as the diffusible indicator and an on-line microprocessor for computation allows for faster, easier and more reproducible measurement.

We are currently assessing this technique ("thermal-dye double indicator dilution lung water measurements)" in a canine model under controlled laboratory conditions. We are specifically comparing this technique with the currently used clinical indices of pulmonary capillary injury- i.e., the chest x-ray and blood gases - and also sacrificing the animals and measuring the lung wet-to-dry weights. This technique has particular appeal since it only requires two catheters which are routinely used for intensive care monitoring, i.e., a central venous catheter, and an arterial catheter. The ability to perform repeated measurements over an extended period of time without the continued loss of blood from sampling (measurements are made in-line) suggest that this might be a valuable instrument for assisting in establishing an early diagnosis of pulmonary injury and to monitor progress in the therapy.

Recommendations and Future Objectives:

Following verification of these results in a canine model under controlled laboratory conditions, we plan to

carry out experiments in sheep exposed to blast waves of different intensities and duration. An important aspect of these studies will be to determine if the lung water computer is a more sensitive indicator of pulmonary injury than previously used indicators such as blood gases and radiographic changes.

Our future technical objective will be to develop both surgical and medical adjuncts for the management of blast induced injury to the lung. The initial goal will be to assess techniques which will allow categorizing experimental preparations according to the following: severity of the injury; the probability of developing complications; and the overall morbidity and mortality. The institution of appropriate and successful medical and/or surgical therapy will necessarily depend upon identifying specific physiologic dysfunctions. The threat of exposure of American soldiers to blast waves from enemy weapons systems is increasing. This project proposes to respond to the threat of potential blast related problems which may be experienced by the Army in the field.

Project 35162772A874 METHODS AND TECHNIQUES FOR COMBAT CASUALTY MANAGEMENT

Work Unit 181 Management of Military Blast Injury

Literature Cited:

References:

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6769	81 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
80 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
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7 2:6			

11. TITLE (Precede 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 81	1.0	119
	CURRENT 82	1.0	72

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, COL, Phillip K.

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Insitute of Research
Division of Surgery

ADDRESS: Washingon, D.C. 20012

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

NAME: FLEMING, COL, Arthur W.

TELEPHONE: (202) 576-3791

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence not considered

22. KEYWORDS (Precede 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 (Furnish 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 intergration 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) 80 10 - 81 09 The renewed interest in the cyanoacrylate tissue adhesives as a medical devise prompted a review of the literature on the subject. In addition, a follow up was made on the first American soldiers in whom the cyanoacrylates were used in Vietnam. A protocol has been drafted to evaluate the efficacy of cyanoacrylates for potential use in future conflicts. A subsequent long-term study on the histoxicity of Butyl-cyanoacrylates is awaiting FDA recommendations. For technical report see. Walter Reed Army Institute of Research Annual Progress Report. 1 Oct 80 - 30 Sep 81.

Available to contractors upon originator's approval

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

Project 3S162772A874 METHODS AND TECHNIQUES FOR COMBAT CASUALTY MANAGEMENT

Work Unit 182 Biomedical Aspects of Medical Materiel

Investigator:
Principal: COL Arthur W. Fleming, MC

Background and Objectives:

The primary objectives are to develop and provide laboratory models for biomedical assessment of medical materiel systems. Medical materiel systems currently being developed will continue to undergo operational testing to determine if such systems are usable and useful. Our approach will be to subsequently exploit these newly developed materiels in the environment that they are designed to be used in. 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 armamentarium of the Army Medical Corps.

Progress:

Functional concepts of the working relationship between USAMBRDL and the Division of Surgery, WRAIR in regards to the biomedical aspects of medical materiels have been developed and are operative. Interest in the use of a medical device, the cyanoacrylate tissue adhesive, was renewed and influenced by the news media. This renewed interest in the cyanoacrylate tissue adhesive prompted us to review the literature on the subject. Several long-term studies to explore and investigate tissue toxicity and carcinogenic potential of cyanoacrylates have been carried out previously. The one report, however, which placed a stigma on cyanoacrylates was reported by Page, Larson and Siegmund in 1966 (Proceedings: Symposium on Physiological Adhesives, Univ. of Texas Press 11-23). Several authors have referred to other articles as demonstrating that

tissue adhesives may be carcinogenic. These references, however, tend to be circular, with each article quoting another reference as the source. For example, Goldman (Am J Gastro, 1978) stated that "Eastman 910 is markedly histotoxic and maybe carcinogenic in animals" and listed "Katon" (Gastroenterology 1976) as the source of this information. Katon, however, "did not study carcinogenesis" but rather stated that "the threat of carcinogenesis is very real, since Oppenheimer et al. (Cancer 1958) produced sarcomas in rats and mice by embedding plastic films subcutaneously in the abdominal wall." The list of articles demonstrating that cyanoacrylates are not carcinogenic are extensive and will be submitted for publication as a review article in the near future.

A protocol is currently being carried out to evaluate the efficacy of cyanoacrylates for potential use in future conflicts. A long-term study on the histotoxicity of butyl cyanoacrylate is awaiting FDA recommendations. In addition, a follow-up was made on the first American soldiers in whom the cyanoacrylates were used on in Vietnam.

Recommendations for the Future:

Full biomedical support for assessment of field medical materiels is planned for the next five (5) years. The scope and frequency of involvement, and the number of manhours expended will vary with the requirements of USAMBRDL.

Literature Cited: None

Publications: None

PROJECT 3S162772A875

MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6479	81 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
80 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	3S162772A875	AA	161
b. [redacted]				
c. [redacted]	STOG 80-7.2:1			

11. TITLE (Procede with Security Classification Code)

(U) Chemoprophylaxis of Chemical and Ionizing Radiation Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 012600 Pharmacology

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 81	2.0	232
FISCAL YEAR CURRENT 82	2.0	356

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Experimental Therapeutics

ADDRESS: Washington, DC 20012

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

NAME: DAVIDSON, David E., Jr., COL

TELEPHONE: (301) 427-5029

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign intelligence not considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Chemoprophylaxis; (U) Drug Development; (U) Ionizing Radiation; (U) Chemical Poisons; (U) Radiation Protection; (U) Chemical Defense; (U) Toxicology

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

23. (U) To find new drugs with protective activity against injury to military personnel in the event of exposure to ionizing radiation or chemical poisons.

24. (U) Candidate drugs will be tested in laboratory model systems to establish mechanisms of pharmacological effects, effects on physiological responses and pharmacokinetic characteristics. Studies will be performed in rodents, dogs, subhuman primates and in vitro.

25. (U) 8010-8109 Development of a new laboratory animal model system to be used for testing candidate antidotes for activity against chemical injury has progressed. Cyanide is used as the standard chemical agent. The 50-percent lethal dose of cyanide in adult mice has been established as 9 mg/kg by subcutaneous administration. Studies of the ability of sodium thiosulfate to antagonize the lethal effect of cyanide are underway as a test of the responsiveness of this newly-established system. Development of an analytical technique for the detection and quantitation of pyridostigmine for application to the study of the pharmacological kinetics of the compound in mice is underway in collaboration with the Department of Pharmacology. Re-establishment of the animal model system for testing candidate radio-protective compounds utilizing the new animal facilities in Building 500 is in progress. The adverse effect on survival after radiation which is due to endogeneous intestinal infections in mice is being evaluated during the development of procedures to minimize the infections. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*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 69 (FOR ARMY USE) ARE OBSOLETE

Project 3S162772A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

Work Unit 161 Chemoprophylaxis of Chemical and Ionizing Radiation Injury

Investigators:

Principal: COL David E. Davidson, Jr., VC
Associate: CPT Irving W. McConnell, VC
Dr. David Davis
Ms. Marie M. Grenan

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 could be used against us, antidotes are non-existent or unsuitable for mass administration. The development of effective defensive measures would deter use of chemical agents by an enemy, and would improve the ability of military units to perform effectively if chemical agents were used.

Chemicals are known which protect laboratory animals against ionizing radiation injury, and which have dose reduction factors of 2.0-2.7. Toxic side effects have been overcome to a great extent, but the best drugs are only effective in animal models if administered parenterally. An effort will be made to develop orally effective radioprotective drugs which could be used to protect military personnel in a nuclear environment. In-house research is complemented by and coordinated with contractor laboratory research.

PROGRESS:

Radioprotective Drugs: The re-establishment of mouse model systems for testing candidate radioprotective compounds has been undertaken utilizing the new animal facilities in Building 500. Standardization of radiation dosage and survival is in progress, but has been hampered by difficulty in obtaining mice free of intestinal infections which have adverse effect on survival after radiation. This effect is being studied critically while sources of infection-free mice are being developed. In studies to develop orally effective dosage forms of WR 2721, two approaches have been used. Three pro-drugs of WR 2721 (using the oxazaphosphorine sulfur-covering function) were

prepared and tested, but were unsuitable because of instability, toxicity and inefficacy. Three microencapsulated formulations of WR 2721 were prepared and evaluated in vitro in simulated gastric juice. The formulations prevented dephosphorylation in the acid environment for longer than 90 minutes with less than 10% loss of compound and released 70% within 15 minutes when the pH was raised above neutrality.

Chemical Antidotes: The establishment of an animal model system for screening candidate antidotes for activity against chemical injury has progressed to standardization using cyanide as the standard chemical agent. The LD_{50} of cyanide in adult mice was established as 9 mg/kg by subcutaneous administration. Studies of the ability of sodium thiosulfate to antagonize the lethal effect of cyanide are underway as a test of the responsiveness of this newly-established system. Studies of various cobalt preparations were conducted to establish tolerated dose and efficacy of antagonism of cyanide toxicity. The best-tolerated preparation was a mixture of cobalt chloride and sodium EDTA and the most efficacious compound was sodium cobaltinitrite. Studies were initiated to develop an analytical technique for the detection and quantitation of pyridostigmine for application to the study of the pharmacological kinetics of the compound in mice, in collaboration with the Department of Pharmacology.

FUTURE OBJECTIVES:

In addition to continuation of studies in development of the anti-radiation screening animal model and initiation of screening of candidate compounds in mice, further studies will be pursued with WR 2721 for the purpose of developing an orally efficacious formulation, lessening its emetic side-effect. Collaboration with the National Cancer Institute in the application of the compound as a protectant of normal tissues during irradiation of solid tumors in clinical studies will be continued. Comparisons of dose reduction factors against neutron and gamma radiations for selected compounds are planned or in progress.

The animal model for screening candidate antidotes against chemical injury will be employed with selected compounds initially, and then on a broader basis, with cyanide as the standard chemical agent. As facilities

permit, other chemical agents will be introduced as standards so that screening may be done against other known hazards. Collaborative pharmacological studies of pyridostigmine will be pursued.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6478	81 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. GRP'S INSTR'S	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	3S162772A875	AB	162
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code)

(U) The Synthesis of Antiradiation Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

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 81	2.0	156
	CURRENT 82	4.0	457

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, P., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Experimental Therapeutics

ADDRESS: Washington, DC 20012

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

NAME: Pick, Robert O., MAJ MSC

TELEPHONE: (301) 427-5422

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Klayman, D.L., Ph.D. POC: DA

NAME:

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Antiradiation Drugs; (U) Drug Development; (U) Aminoelkylthiols; (U) Aminoalkylphosphorothioates

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 manage, integrate, and provide technical direction for both a contract and in-house program to obtain potentially active antiradiation compounds for military use through rational organic syntheses.

24. (U) Necessary research areas are defined, proposed research evaluated, ongoing research guided, evaluated, and integrated with the other program elements. Technical advice is obtained through an Ad Hoc Study Group on Medicinal Chemistry. Information is exchanged by contractors through technical meetings.

25. (U) 80 10- 81 09: Compounds which might act as latentiated WR 2721 and analogs as cyclophosphamides were synthesized with difficulty. These products proved to be quite labile and further work awaits biological testing results. Biological testing on 12 no-nitrogen type antiradiation compounds has been very encouraging, with 75% of the samples exhibiting 30% or greater protection. The single adamantyl bis amidinium type synthesized showed no significant protection. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

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

Project 3S162772A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

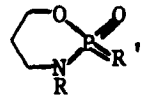
Work Unit 162 The Synthesis of Antiradiation Drugs

Investigators:

Principal: MAJ Robert O. Pick, Ph.D.
Associate: Daniel L. Klayman, Ph.D.;
William Y. Ellis, B.S.

The main thrust of this program is to design and synthesize effective antiradiation drugs that will be effective by oral administration and, at least, maintain the dose reduction factor obtained with WR 2721.

The contractual effort in latentiating WR 2721 has involved difficult problems in chemistry. The cyclophosphamide types (I)



R=H or CH_3

R'=antirad fragment such as WR 2721

I

could not be prepared with R=H, and even with R=CH_3, the samples were hydroscopic and unstable. All four of these types prepared have been considerably more toxic than WR 2721. Efficacy data is pending.

Twelve "no nitrogen" type samples have been prepared under contract, including a polysulfide, sodium sulfinate disulfides, methyl sulfinate di- and trisulfides, and disulfides without the sulfinate moiety. Of these, 75% have shown 30% or greater protection, indicating that (a) chirality has little effect, (b) higher sulfides are not better than the trisulfide, and (c) the sulfinate can be covered. These areas will be followed up in FY 82. A new work unit for defense against chemical warfare agents is planned.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6757	81 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8b. DISP'N INSTR'N	8c. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	3S162772A875	BD	163
b. XXXXXXXXX				
c. XXXXXXXXX	STOG 80-7.2:			

11. TITLE (Precede with Security Classification Code)

(U) Preclinical and Clinical Assessments of Antidotes

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 012600 Pharmacology

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 81	4.0	243
	CURRENT 82	4.0	462

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, COL P.

TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Div of Experimental Therapeutics

ADDRESS: Washington, DC 20012

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

NAME: HEIFFER, Dr. M.H.

TELEPHONE: 301-427-5393

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: VON BREDOW, MAJ J.

NAME: PAMPLIN, LTC C. POC: DA

21. GENERAL USE

Foreign intelligence not 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 (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 to support a Notice of Claimed Investigational Exemption for a New Drug (IND) for candidate antidotes 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 antidotal agents. The actual studies performed are dictated by scientific rationale and existing federal regulations and include efficacy and toxicity studies, formulation development, pharmacokinetic and metabolic studies as well as clinical tolerance and efficacy studies.

25. (U) 80 10 - 81 09 An analytical method was developed for the determination of the stability of 2-PAM chloride formulations. This method utilizes an ion suppression form of reverse phase high pressure liquid chromatography. Analysis of 8 year old aqueous formulations indicate that the 2-PAM chloride decomposes to form its corresponding acid, aldehyde and amide. During the 8 year storage period, there was a corresponding decline in the pH of the formulation from an initial value of 3.5 to a value of 0.9. Preliminary results from a safety and tolerance study of a new formulation of 2-PAM chloride, protopam chloride, were evaluated. All six volunteers who received one 2 ml dose via autoinjector of the new formulation reported mild pain at the injection site in the thigh beginning 10 to 40 minutes after injection and lasting two to four hours. However, no physical changes were detected at the site of injection. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 80 - 30 Sep 81.

* Available in restricted form on originator's approval

Project 3S162772A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

Work Unit 163 Preclinical and Clinical Assessments of Antidotes

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
Associate: MAJ J. von Bredow, CPT D. Korte, Jr., LTC C. Pamplin, Dr. L. Fleckenstein, Dr. H. Lowensohn, J. Digiovanni, SP5 C. Basamania, SP5 J. Ferri

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.

2-PAM chloride is a cholinesterase reactivator currently being developed by the department for submission to the FDA as a field antidote to nerve agents. Solutions of 2-PAM chloride are generally unstable; therefore, this compound is formulated as a freeze-dried preparation which is dissolved in sterile water shortly before intravenous administration, a concept which is readily feasible in a physician's office or at the Battalion Aid Station. However, the use of 2-PAM chloride as a field antidote in anticholinesterase intoxication requires a formulation for intramuscular injection which will remain stable in solution for prolonged periods of time. The department was asked to study the stability of 2-PAM chloride solutions that were formulated eight years ago. A technique utilizing an ion suppression form of reverse phase, high pressure liquid chromatography was developed for analysis of 2-PAM chloride and separation of its decomposition products. This investigation demonstrated the presence of 2-PAM chloride and three decomposition products in these aqueous eight year old solutions. The three decomposition products were identified as 2-carboxy-N-methylpyridinium, N-methylpyridinium-2-carboxaldehyde and 2-carboxamido-N-methylpyridinium. Concurrently there was a drop in pH from an initial value of 3.5 to 0.9 at the end of eight years.

Two clinical protocols evaluating a new formulation of 2-PAM chloride (protopam chloride) developed by Ayerst Laboratories have been approved. The protocols are "Safety and Tolerance Study of an Investigational Protopam Chloride Formulation in Autoinjector Unit" and "Protopam Chloride Bioavailability and Irritation Study." Preliminary results from the safety and tolerance study have been analyzed. Six subjects received one autoinjector (containing 2 ml of the new formulation) in their thigh. All subjects reported mild pain at the site of injection beginning 10 to 40 minutes after injection and lasting 2 to 4 hours. Transient elevations in plasma CPK levels were observed beginning on Day 2 which had returned to normal by the Day 9 close-out physical. No physical changes were detected at the site of injection.

3. Future Work.

The pharmacokinetic profile of chemical antidotes will be assayed in different animal models. Clinical studies will continue with the completion of the safety and tolerance study and the initiation of the bioavailability and irritation studies.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 8600	81 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
80 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	3S162772A875	BC	164
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2:1			

11. TITLE (Precede with Security 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 81	1.0	202
FISCAL YEAR CURRENT 82	2.0	288

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
Washington, D.C. 20012

ADDRESS:

RESPONSIBLE INDIVIDUAL

NAME: Russell, P.K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Division of Neuropsychiatry
Washington, D.C. 20012

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

NAME: Elsmore, T.F., Ph.D.

TELEPHONE: (202) 576-2483

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Hursh, S.R., MAJ

NAME: Kaufman, L.W., CPT

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH 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 of non-human primates will be developed and evaluated. The roles played by task variables and time of day in determining behavioral effects of drugs in a limited number of procedures will be investigated. The overall objective will be development of testing protocols with maximum sensitivity to behavioral effects in chemical defense agents, therapy and prophylactic compounds, and their combinations. There is military relevance in this research.

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 to determine the role of task variables in modulating drug effects. Chronopharmacological effects will be evaluated by scheduling behavioral tests at selected hours of the day and night. Methods of curve fitting and time series analysis will be used to evaluate drug effects.

25. (U) 80 10 - 81 09 Major findings: The conditioned flavor aversion paradigm was found to be relatively insensitive to organophosphate toxicity. DFP toxicity in the rat appears to be little affected by requiring daily periods of running prior to drug challenge. The toxicity of soman in the rat varies with time of day when the test is conducted. A major finding is that rats surviving soman poisoning may have extensive brain degeneration. Survivors of DFP poisoning show numerous behavioral deficits including problems in mating, escape learning and adaptation to novel environments. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 80 - 31 Sep 81.

* Available to contractors upon originator's approval

Project 3S162772A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

Work Unit 164: Behavioral Toxicology

Investigators:

Principal: Timothy F. Elsmore, Ph.D.

Associate: Sessions, MAJ G.R.; Hursh, MAJ S.R.; Kaufman, CPT L.W.; Tyner, LTC C.F.

Objectives:

The overall objective of this work unit is the development of testing methods to evaluate behavioral effects of compounds used for pretreatment or therapy (P & T) of chemical warfare agent exposure. Both the inherent toxicity of the P & T compounds and their efficacy in preventing long-term behavioral and neurological damage will be evaluated. The initial efforts in this area were directed at assessing some of the behavioral decrements to be found as a result of exposure to anticholinesterase compounds, primarily organophosphates (OPs). An additional goal is the definition of environmental variables affecting OP toxicity.

Progress:

An initial study showed that the conditioned flavor aversion procedure, a relatively low cost screening procedure for behavioral toxicity, was relatively insensitive to both reversible and irreversible cholinesterase inhibitors.

In rats the toxicity of soman, an OP chemical warfare "nerve agent", was shown to vary with time of day in rats, with lethality being lowest during the early phase of the animals' normal period of high activity. Additional studies of OP toxicity were conducted with the OP DFP, since we are at this time unable to use nerve agents themselves in our laboratories. The toxicity of DFP was found to be unaffected by requiring daily periods of running for ten weeks prior to agent challenge.

The finding that rats surviving soman exposure may suffer diffuse neuronal degeneration throughout the brain (see WU 221, Neural Mechanisms of Chemical Defense Agents and Antidotes), prompted a series of studies of behavioral deficits in rats surviving DFP poisoning. A dose-dependent deficit was found on a mating test in which male rats that had received a single dose of DFP 3 to 6 weeks earlier were placed with a receptive female rat. These

animals were also inferior on a simple task involving escape from a heated surface. A simple neurological battery revealed no abnormalities in these animals. In a second study, DFP survivors were deficient in exploring a straight alley runway, but eventually showed running speeds comparable to normals, when running was reinforced with food. These same animals also showed deficits in drinking in a novel environment. Preliminary histological results have shown no brain damage in DFP-treated animals. Taken together, these results suggest that subtle behavioral deficits in emotionality and some social behaviors may exist in untreated survivors of OP poisoning.

Future Objectives:

A number of tests will be conducted to determine the scope of behavioral deficits in survivors of OP poisoning, including tests of learning, memory, reaction time, and information processing. Effects of P & T compounds in preventing long-term deficits will be evaluated. The interactions between pyridostigmine, physostigmine and time of day will be explored in monkeys working around the clock to obtain food.

Presentations

Elsmore, T.F. Circadian susceptibility to soman poisoning. Symposium on therapy of organophosphate poisoning sponsored by USAMRDC and Society for Toxicology, San Diego, March, 1981.

Elsmore, T.F. Chronotoxicology of organophosphates. USAMRDC Chemical Defense Program Review, Ft. Detrick, June, 1981, and joint US/Israeli symposium on preventive medicine, Shoresh, Israel, July, 1981.

Publications

Elsmore, T.F. Circadian susceptibility to soman poisoning. Journal of Fundamental and Applied Toxicology. In press.

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	81 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
80 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
b. CONTRIBUTING				
XXXXXXXXX	STOG 80-7.2:4			

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	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	4.0	993
	CURRENT 82	3.0	752

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL, MC
TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Dept of Microwave Research
ADDRESS: Div of Neuropsychiatry
Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: L.E. Larsen
TELEPHONE: 202-576-3615
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: J.H. Jacobi
NAME: E.L. Hunt POC: DA

21. GENERAL USE

Foreign Intelligence Not Considered

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 (Furnish individual 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) 80 10 - 81 09 Progress includes demonstration of non-invasive microwave dosimetry using polarization transformation of forward scattered 2-4 GHz microwave radiation. A water coupled array has been developed for electronic beam steering and focusing to measure the spatial distribution of insertion loss (energy dissipation) and phase shift (energy storage) at high data rates. In situ permittivity measurements demonstrated regional differences in renal cortex and medulla as well as brain grey matter and white matter over the 2-4 GHz region. Studies of thermoacoustic expansion have demonstrated apparently greater hazard with appropriately selected pulses than with CW radiation of equal average power. Pulsed radiation may add mechanical factors to those due to heat and/or electric fields. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65

Project 3E162777A878 HEALTH HAZARDS OF MILITARY MATERIEL

Work Unit 041: Biological Interactions With and Hazards of Microwave Radiation

Investigators.
Principal: LTC Lawrence E. Larsen, M.D.
Associate: Edward L. Hunt, B.A.; John H. Jacobi, M.S.; Peter V.K. Brown, M.S.

Objectives

Five scientific areas are dosimetry, thermoacoustic expansion, behavior and dielectric relaxation. These areas are key elements to hazard assessment and prevention.

Progress and Background

a. Dosimetry measures the spatial distribution of absorbed microwave energy in biological systems. Due to the non-uniformity of absorbed energy, the target organ concept is employed to allow generality of results. Progress includes demonstration of the importance of polarization in local energy deposition for isolated renal organs and continued development of a water coupled array antenna system for rapid evaluation of absorbed dose. Data presentation is in image form.

b. Thermoacoustic expansion is a hazard mechanism which adds a mechanical pressure wave to the heat value of the applied microwave energy. This addresses the importance of modulation in safety standards. Progress includes demonstration of qualitative and quantitative differences in cellular ultrastructure of the murine ocular lens under *in vitro* exposure for pulse and continuous exposure of the same average power.

c. Behavioral studies have addressed previously reported findings of enhanced effectiveness of chlordiazepoxide when followed by pulsed microwave exposure at low power levels. Exposures conducted at both 915 and 2450 MHz in transmission lines with both fixed and variable reinforcement schedules failed to confirm the previous reports of drug action enhancement.

d. Dielectric relaxation studies relate to the constitutive properties of tissue as media for the propagation of microwave energy. Progress includes demonstration of physiologic variation of complex permittivity at microwave frequencies under conditions of changes in blood flow and various pathogenic states.

PROJECT 3E162777A878 HEALTH HAZARDS OF MILITARY MATERIEL

Work Unit 041: Biological Interactions With and Hazards of Microwave Radiation

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17. Graeber, R.C., Cuthbert, B.N., Sing, H.C., Schneider, R.J., Sessions, G.R.: Rapid transmeridial deployment: I. Use of chronobiologic countermeasures to hasten time zone adjustment in soldiers. XIV International Conference Proceedings of the International Society for Chronobiology, Il Ponte Publishing House, Milan, in press, 1981.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6472	81 10 01	DD DR&E(AR)636

3 DATE PREV SUMRY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8a CHGD'N HIST'N	8b SPECIFIC DATA CONTRACTOR ACCESS	LEVEL OF SUM
80 10 01	D.Change	U	U		NI	☒ YES ☐ NO	A WORK UNIT

10 NO /CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A878	AB	042
b. CONTRIBUTING				
c. [redacted]	STOG 80-7,2,4			

11 TITLE (Precede with Security Classification Code)
(U) Non-auditory effects of blast overpressure

12 SCIENTIFIC AND TECHNOLOGICAL AREAS
017100 Weapons Effects 013300 Protective Equipment 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 81	4.0	358
FISCAL YEAR CURRENT 82	6.0	326

19 RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, Philip K., COL, MC
TELEPHONE: 202 576-3551

20 PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Div of Med, Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: PHILLIPS, Yancy Y., MAJ, MC
TELEPHONE: 202 576-3014
SOCIAL SECURITY ACCOUNT NUMBER:

21 GENERAL USE
Foreign Intelligency Considered

ASSOCIATE INVESTIGATORS
NAME: JAEGER, James J., MAJ, MSC
NAME: HESS, JEFFREY, MAJ, VC

22 KEYWORDS (Precede each with Security Classification Code)
(U) Human Volunteers; (U) Impulse noise; (U) Blast overpressure; (U) Pulmonary Physiology; (U) M198 155mm Howitzer; (U) Combat Casualty Care

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 (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, of military importance.

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 specimens will be done. Chronic effects of repeated BOP exposure will be assessed in man and animals. Pathophysiologic events of blast injury will be monitored by implantable transducers.

25.(U) 80 10-81 09A Technical Plan was completed outlining research areas for developing generalizable Damage Risk Criteria by FY 85. Water jet impact device will be delivered by JAN 82. A building at Forest Glen was renovated to house the impactor and physiology laboratory. Contract work with Lovelace Foundation and WRAIR field studies demonstrated injurious effects of repeated blast on the larynx, intestine, and lungs. Protocols at Lovelace addressed pressure impulse, peak pressure, summated impulse, and resonance effects on injury in large animals. A workshop reviewed the state-of-the-art of modeling the thoraco-abdominal response to blast. Simple visco-elastic and finite element models are now in use. We have supported the conduct of field studies of the Viper shoulder fired antitank rocket and the M198/M203 system. Active coordination exists with companion programs in weapons effect blast injury at WRAIR and BOP auditory injury at USAARL as well as liaison with USAHEL, ARRADCOM and DARCOM. Contact has been initiated with blast research groups in the UK, France, Sweden and FRG. As a result of presentations at the May 1981 NATO panel VIII RSG-6 meeting, non-auditory effects will be included the terms of reference for the study of impulse noise. Represented US Army in casualty care of blast injuries at US/UK Navy Combat Casualty Care Workshop in Hampshire, England, 10Oct80-30Sep81

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 68 AR 70-10 (FOR ARMY USE) ARE OBSOLETE

Project: 3E162777A878 HEALTH HAZARDS OF MILITARY MATERIEL

Work Unit 042: Non-auditory effects of blast overpressure

Investigators:

Principal: Yancy Y. Phillips, MAJ, MC
Associate: James J. Jaeger, MAJ MSC; Jeffrey L. Hess, MAJ VC; Robert Hoyt, MAJ VC; Andrew Young, CPT MSC; Marvin Stein, GS-11; Earl Massey, GS-9; G.D. Ross, E-6.

The primary mission of the Department of Clinical Physiology is to define the physiologic effects and to evaluate the potential for non-auditory injury to the soldier resulting from exposure to blast overpressure (BOP) generated by the firing of Army weapons systems.

The Final Report of the data from the July 1980 Aberdeen Proving Ground study has been completed. In that study, 60 sheep were exposed to blast overpressure produced by the M198/M203 weapon system. An additional 38 animals served as controls. Animals were exposed to 50 consecutive shots with peak pressures of 4.2, 6.3, or 14.1 psi. Compared to the control population, there was an increased incidence of minor injury to the larynx of sheep exposed to 4.2 psi or greater. Minor hemorrhagic injury to the gastrointestinal tract was observed at 6.3 psi and severe injury occurred at 14.1 psi. Pulmonary injury consisted of epithelial stripping, nonspecific pleural changes, and a single pneumothorax, all at the higher blast levels.

In October 1980, a Technical Plan was completed outlining goals and research projects for FY81-85. An interim schedule-X, recognizing increased manpower requirements, was approved. A Secretarial D & F was approved and an RFQ released for selection of a contractor to assist in the characterization and predictive modelling of blast-thorax interactions and for development of implantable telemetry systems for blast measurements. Development of a farfield model of blast wave propagation from the M198/M203 and initial scoping studies on blast-thorax finite element modeling have been completed under contract. Current studies at Lovelace Foundation are investigating the effects of pressure impulse, peak pressure, summated impulse, and resonance on injury in large animals. The feasibility of using a water jet impact system as a laboratory model for blast injury has been demonstrated and such a system is scheduled for delivery in January 1982. Surgical techniques now under active development include chronic tracheostomies and thoracic lymph node cannulation

PUBLICATIONS

1. Hess, J.L., D.M. McCurnin, M.G. Riley and K.J. Koehler. Pilot study for comparison of chromic catgut suture and mechanically applied staples in enteroanastomoses. J Am Anim Hosp Assoc, 17:409, 1981.

2. Jaeger, J.J., E.C. Deal, Jr., D.E. Roberts, R.H. Ingram, Jr. and E.R. McFadden, Jr. Cold air inhalation and esophageal temperature in exercising humans. Med Sci Sports Exercise, 12:365, 1980.

and drainage. An abandoned building at Forest Glen has been renovated for use as a physiology laboratory.

The Department of Clinical Physiology provided planning support for the successful USAARL field test of hearing protection for the Viper shoulder fired anti-tank rocket (Feb 81). It is also currently playing a major role in supporting a USAARL field test of hearing protection for the M198 155 mm Howitzer firing the M203 "supercharge". Participation has included protocol preparation, logistical support, recruiting and medical screening of volunteers, and medical monitoring in the field. Liaisons in matters related to BOP exist with the following agencies: USAARL, USADARCOM, USAARRADCOM, USAHEL, USABRL, USATECOM, UNSMRDC, USAMTD (APG), and the Defense Nuclear Agency. The Department represented U.S. Army at a UK/US Navy Combat Casualty Care Workshop in Hampshire, England; NATO Panel VIII RSG-6 meeting in Saint Louis, France; and Blast Biomechanical Modeling Workshop in Albuquerque, NM. Personal contact exists with blast groups in France, the United Kingdom, Sweden, and the Federal Republic of Germany.

Further studies of the biophysical determinants of thoraco-abdominal injury are planned at Lovelace. Collaborative studies with Dr. Jonsson of Sweden on his anthropomorphic dummy are scheduled. Esophageal and gastric pressures will be measured in volunteers exposed to current operational artillery overpressure levels. A cross-sectional prevalence survey of pulmonary function in artillerymen and infantrymen is planned. Experiments with the water jet should validate this model and begin the investigation of threshold injuries.

FORMAL PRESENTATIONS

1. Phillips, Y.Y. "Blast Overpressure, an Army-Wide Problem". Presentation to Blast Biomechanical Modeling Workshop, Albuquerque, NM, December 1980.

2. Phillips, Y.Y. "Results of Field Study of Exposure of Sheep to Repeated Blasts". Presentation to NATO Panel VIII RSG-6 2nd Meeting, Saint Louis, France, May 1981.

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	81 10 01	DD-DR&E(AR)636

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
10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
MARY	62777A	3E162777A879	AA	041
[illegible]				
[illegible]	STOG 80-7.2	4		

LE (Precede with Security Classification Code)

Military Preventive Psychiatry

NTIFIC AND TECHNOLOGICAL AREAS

500 Clinical Medicine 013400 Psychology 021900 Physiology

RT DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
07	CONT	DA	C. In-House

TRACT/GRANT		17. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
ES/EFFECTIVE	EXPIRATION:			
BER		PRECEDING FISCAL YEAR 81	7.5	535
E	d. AMOUNT:			
OF AWARD	f. CUM. AMT.	CURRENT FISCAL YEAR 82	7.5	806

RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
Walter Reed Army Institute of Research Washington, DC 20012	NAME: Walter Reed Army Institute of Research Division of Neuropsychiatry ADDRESS: Washington, DC 20012
RESPONSIBLE INDIVIDUAL Russell, COL P. PHONE (202) 576-3551	PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME: Marlowe, D.H., Ph.D. TELEPHONE: (301) 427-5210 SOCIAL SECURITY ACCOUNT NUMBER:
GENERAL USE Foreign Intelligence Not Considered	ASSOCIATE INVESTIGATORS NAME: Harris, LTC, J. NAME: Knudson, CPT, K. POC: DA

KEY WORDS (Precede EACH with Security Classification Code)

(U) Psychiatric Illness; (U) Military Adjustment; Environmental Factors; (U) Social and Psychological Factors; (U) Stress

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

(U) This unit examines the dynamics of those specific factors within military organizations and environments that conduce to psychiatric illness, operate to produce psychiatric casualties and lead to the generation of dysfunctional behaviors and decrements in military performance. These studies have direct relevance for the development of programs of intervention and prevention and the development of effective techniques for the minimization of psychiatric casualties.

(U) The methods of clinical psychiatry, social and clinical psychology, social anthropology and field epidemiology are used to identify factors that generate psychiatric casualties, behavior dysfunction and performance dysfunction and decrement in order to modify such factors or the relationship between them.

(U) 80 10-81 09 Studies of the relationship between health outcomes and deployment both active duty members and military families are under way in the 82d ABN Division. Special emphasis is given to the relationship of group norms to stress responses. Studies of military communities at Ft Bragg and the relationship of community structure and family stress have been undertaken at the request of the 82d ABN Div. Preliminary studies of active duty high-frequency users of military medical care systems completed and extensive studies will be initiated in FY82. Studies to delineate the relationship between sergeant stress and trainee attrition in BT/AIT will commence (per TRADOC) in November 82. Protocols to study the impact of delayed stress syndrome on active duty military completed and studies commence FY82. Studies of the impact of military family stress on readiness and performance of active duty members developed and will be fielded in FY82. Historical studies of NP casualties in Vietnam continue. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80-30 Sep 81.

To be typed into final upon grantor's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A [illegible]

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; LTC Jesse J. Harris, MSC; LTC Jacob M. Romo, MSC; LTC Norman M. Camp, MC; MAJ Robert J. Schneider, MSC; MAJ Edwin W. Van Vranken, MSC; CPT Robert H. Stretch, MSC; CPT Linda K. Jellen, MSC; CPT Kathryn H. Knudson, MSC; CPT Darleen M. Vernon, MSC; CPT Ronald Smith, MSC; William E. Datel, Ph.D.; Joseph M. Rothberg, Ph.D.; Mady W. Segal, Ph.D.; Robert N. Dornhart, M.A.; Richard Howard, M.A.; Glenn T. Gurley, B.A.; Richard Oldakowski, SSG Edgar Marshall; SSG Rheebe Barnes; SSG Marie McCarty; SSG Mildred Hester; SSG James Hall; SSG Richard Pickle; SP5 Diane Smith; SP5 Calvin Cummings; SP5 Richard Lynk; SP5 William Rigney; SP4 Donna 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. 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, represent the central concern of this work unit. This unit 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

Field studies initiated with the 82d Airborne Division, Ft Bragg, NC, continue. These studies center on soldier and family member perceptions of health and illness and the relationship of such perceptions to patterns of deployment and soldier performance before, during, and after deployment. Particular emphasis is given to analysis of patterns of illness in these contexts. Team members have participated in a number of deployments with units of the 82d; e.g., Panama, Florida, Texas, etc, and primary data gathering continues from both active duty members and families. Studies thus far have underlined a number of potential problem areas that will be pursued throughout the course of the next fiscal year.

At the request of the 82d ABN Div, studies have been initiated of various household areas in military communities under the control of the Division's Third Brigade. Interviews of some 50 enlisted military households have taken place thus far.

Field studies attempting to define the effects, if any, of use of illicit drugs on the cohesion and performance of tank crews were carried out in USAREUR. 85 crews from an armored brigade were surveyed and data is presently being arrayed and analyzed. Preliminary results should be reported by the 2d Quarter of FY82.

Analysis of data collected in the women's study has indicated that there exists a significant population of young active duty personnel who utilize medical facilities at rates well above any anticipated norm, some 14% of a sample population comprising 35% of all sick call usage during calendar year 1980. This population did not exhibit an access of severe or chronic illness, but rather large numbers of visits for ailments usually considered to be self-manageable. The loss of duty time and the potential consequences of these patterns in terms of future deployment warrants an extended study of this population. Protocols have therefore been developed to carry out and complete this study during the ccurse of the coming fiscal year. The WRAIR women's study, as well as work carried out in the health problems of deployment study at Ft Bragg, have both demonstrated perceived patterns of well being, utilizing Dupuy's General Well Being Scale, among active duty troops, both female and male, that vary markedly from their civilian cohorts. A protocol for a follow on study to attempt to determine the source of this variance has been developed and approved and research will be completed during the course of fiscal year 82.

Negotiations were finally completed with TRADOC to commence studies designed to delineate patterns of stress and stress coping of drill sergeants and their effects on drill sergeant performance and trainee attrition. Contact officers have been appointed at the 9 Army centers and preliminary work commenced for the study which will begin in Dec 81. In addition to the above, studies designed to investigate patterns of delayed stress response among Vietnam veterans who remained in the Army, the sources of such responses and those processes that mitigated them have been developed and approved. Permission has been granted by the CO of a local post for such studies to commence in the 1st Quarter of FY 82.

Future Recommendations and Objectives

Future research will continue conducting investigation of factors in the military human environment which conduces to behavioral dysfunction performance breakdown, the possibility of breakdown in battle. It will also focus on those support systems; e.g., the military unit as a cohesive entity, and the military family, which can operate to mitigate, enhance, and degrade the combat capacity of members of the active duty Army. Further historical work will be carried out as will work dealing in greater dynamic details with the psychosocial stressors affecting health and performance of both female and male personnel. Further work is planned on the ability and need of support systems to handle perturbations centering on deployment and the impact on the health status of the soldier following combat. Further future research is in the process of development and will be designed to provide new patterns of treatment, both pharmacological and verbal and behavioral of the combat stress casualties. This research will be aimed at the provision of tools for dealing with combat stress to company and battalion level medical personnel.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 041 Military Preventive Psychiatry

Papers Presented at Scientific Meetings

1. Harris, J.J., LTC. Social Work in the Trenches. Walter Reed Social Work Symposium, 26 Jun 1981.

2. Harris, J.J., LTC. Stress and the Combat Soldier, Army Behavioral Sciences Symposium, El Paso, TX, 24 Sep 81.

3. Jones, F.D., COL. Military Psychiatry in Future Wars (with G.L. Belenky and P.A. Newhouse). 15th Anglo-American Psychiatry Symposium, Royal Army Medical College, Millbank, London, 6-9 Oct 80.

4. JONES, F.D., COL. What's Happening in the Army Now: Surgeon General's input. Family Life Symposium sponsored by Association of US Army and Officers' Wives Clubs of Greater Washington, Washington, DC. 11 Oct 80.

5. JONES, F.D., COL. Interface between Army Medical Department and the Alcohol and Drug Abuse Control and Prevention (ADAPCP) Program. EUSA ADAPCP Conference, Seoul, Korea, 13 Nov 80.

6. JONES, F.D., COL. Military Psychiatry in the 1980's. Director and Papers: (1) From Combat to Community Psychiatry (with G.L. Belenky), (2) Neuropsychiatric Casualties of Chemical Warfare with (P.A. Newhouse and G.L. Belenky), (3) Military Psychiatry in Future Wars (with G.L. Belenky and P.A. Newhouse). Annual American Psychiatric Association Convention, New Orleans, LA, 9-15 May 81.

7. JONES, F.D., COL. Combat Stress: Tripartite Model (with G.L. Belenky). Military Section, World Psychiatric Association Symposium entitled Adaptation to Military Service, Toulon, France, 25-28 May 81.

8. JONES, F.D., COL. The Prevention and Treatment of Psychiatric Casualties during Continuous Combat on the Nuclear and Chemical Battlefield (with G.L. Belenky and P.A. Newhouse). Military Section, World Psychiatric Association Symposium entitled Adaptation to Military Service, Toulon, France, 25-28 May 81.

9. KNUDSON, K.H, CPT. Enhancing the Quality of Life Through Program Support to Military Families at WRAMC. Ninth Worldwide Armed Services Education Conference, University College, University of Maryland, 9 Apr 81.

10. MARLOWE, D.H. AVF Draft and Women, Center for Philosophy and Public Policy, University of Maryland, Apr 81.

11. MARLOWE, D.H. Women in the Army, Psychosocial and Biosocial Aspects of Health, USAREUR Medical Service Corp Annual Meeting, Garmisch, Germany, May 81.

12. MARLOWE, D.H. Women in the Army, Problems and Prospects, Women's Forum, US Military Academy, West Point, NY, Aug 81.

13. SEGAL, M.W. Women in Combat: Contributions to the War of Words. Inter-University Seminar on Armed Forces and Society Meetings, Oct 80.

14. SEGAL, M.W. Paper on Policy Decisions Regarding Women in the Military, Center for Philosophy and Public Policy, University of Maryland, Jan 81.

15. SEGAL, M.W. Contemporary Problems of the Military Family - Research Findings, at the Military Family Program, US Army War College, Carlisle Barracks, PA, Mar 81.

16. SEGAL, M.W. Scientific Knowledge Affecting the Utilization of Women in the Services, at the 1981 Conference of the Committee on Women in the NATO Forces, Brussels, Belgium, May 81.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 041 Military Preventive Psychiatry

Publications

1. JONES, F.D., BELENKY, G.L., NEWHOUSE, P.A. Military psychiatry in future wars. Journal of the Royal Army Medical Corps (England), in press.

2. JONES, F.D. Combat and its aftermath: A historical view, African Journal of Psychiatry, in press.

3. JONES, F.D. Combat psychiatry in modern warfare, African Journal of Psychiatry, in press.

4. JONES, F.D. An evolutionary learning perspective on pain. Chapter in Current Concepts in the Treatment of Pain, H. Wain (Ed), Jason Aronson Pub., IL, in press.

5. JONES, F.D. Overview of pain symposium. Chapter in Current Concepts in the Treatment of Pain, H. Wain (Ed), Jason Aronson Pub., in press.

6. JONES, F.D. Film Review ("Let There Be Light") Psychiatric News, 21 Aug 81, pp. 2 & 17.

7. KNUDSON, K.H. & KAGAN, S. Relationship among affective role-taking and prosocial behavior in a sample of Anglo American and Mexican American children. Piaget and the Helping Professions, (Proceedings from the Tenth Annual Conference). University of Southern California. 1981

8. STRETCH, R.H. & FIGLEY, C. Combat and the Vietnam Veteran: Assessment of Psychosocial Adjustment. Armed Forces and Society, 27 Jul 81.

9. VAN VRANKEN, E.W., BURYK, R., & HAMLIN E., (Eds). The Army Family: Analysis & Appraisal, Proceedings of a Symposium, Washington, DC., 1980

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6454	81 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
80 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
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:4			

11. TITLE (Precede with Security Classification Code)

(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 81	5.5	329
FISCAL YEAR CURRENT 82	5.5	404

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, COL P.

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Neuropsychiatry

ADDRESS: Washington, DC 20012

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

NAME: Marlowe, D.H., Ph.D.

TELEPHONE: (301) 427-5210

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Datel, W.E., Ph.D.

NAME: Rothberg, J.M., Ph.D. POC: DA

21. GENERAL USE

Foreign Intelligence Not 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 (Furnish individual paragraphs identified by number. Precede 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) 80 10-81 09 Outpatient data continues to be collected at Ft Bragg and pilot analyses are being run on relationship of outpatient medical contact troop deployment and the utilization of mental health facilities. Analysis of soldier and family use of outpatient medical facilities is also under way. A study of disease rates in all of the armed services during the period 1970-1980 was completed and will serve as a comparative data base for future studies. Work has begun on the development of an instrument (Comprehensive Health Hazards and Protectors Inventory: Army) to be used in wide scale perspective cohort studies of health and performance outcomes of newly accessed military personnel. Work continues on studies of cohorts of female personnel and their psychosocial and career health outcomes. Analysis of the past and present patterns of psychiatric diseases, psychosomatic illness and behavioral dysfunction conditions using IPDS and other DA reporting systems and work on the epidemiology of active duty Army suicides continues. Work on the use of psychotropic medication during the Vietnam conflict continues and an instrument is being completed to survey medical personnel who served in RVN. For technical report see WRAIR Annual Progress Report, 1 Oct 80-30Sep81

*Available to contractors only upon originator's approval

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

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; LTC Jesse J. Harris, MSC; LTC Jacob M. Romo, MSC; LTC Norman M. Camp, MC; MAJ Robert J. Schneider, MSC; MAJ Edwin W. Van Vranken, MSC, CPT Robert H. Stretch, MSC; CPT Linda K. Jellen, MSC, CPT Kathryn H. Knudson, MSC; CPT Darleen M. Vernon, MSC; CPT Ronald Smith, MSC; William E. Datel, Ph.D.; Joseph M. Rothberg, Ph.D.; Mady W. Segal, Ph.D.; Robert N. Dornhart, M.A.; Richard Howard, M.A.; Glenn T. Gurley, B.A.; Richard Oldakowski; SSG Edgar Marshall; SSG Rheebe Barnes; SSG Marie McCarty; SSG Mildred Hester; SSG James Hall; SSG Richard Pickle; SP5 Diane Smith; SP5 Calvin Cummings; SP5 Richard Lynk; SP5 William Rigney; SP4 Donna 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

During the past year extensive data has been gathered at Ft Bragg, NC, of the relationship of presenting symptoms and patterns of outpatient medical contact to troop deployment. An extensive data base on outpatient symptom presentation and diagnostic classification has been and continues to be assembled. Preliminary

findings indicate that a number of relationships to deployment and field exercises appear to exist involving changes in use of the medical system. Other preliminary data at present indicates little or no relationship between personnel turbulence and sick call rate. The latter apparently is more tied to unit morale. Posting and organization of the data continues as do regular analyses. Data collecting will continue throughout the coming fiscal year.

A synoptic study of disease rates both mental and physical in all 3 armed services has been completed. This study provides baseline data for comparative and analytic purposes that has never before been available for either psychiatric or physical illness in all 3 armed services.

Cohort studies of women in the Army have demonstrated that women in non-traditional MOSs are at higher risk for pregnancy than those in traditional MOSs. These studies of a cohort accessed in 1978 continue.

Studies of the use of psychotropic medication and of forward psychiatric treatment and diagnoses continue under development. Cohort of psychiatric and other medical personnel who served in Vietnam has been generated and contacted in preparation for an extensive retrospective survey in dealing with this problem.

Work is being undertaken on the development of a series of health inventory instruments that will be utilized in future cohort studies to determine the interaction of psychosocial and health factors with retention and performance throughout the soldier's military career.

Studies of suicide among active duty Army members continue.

Members of the department continue the analyses of past and present patterns of psychiatric disease, psychosomatic illnesses, physical illnesses and behavioral dysfunctions among Army personnel. These studies are aimed at determining what indicators and specific risk factors exist in the military environment which conduce to military specific variability in disease incidence. Determinations are being made as to differentials by geographical area, post, and military occupational specialities. These studies utilize the IPDS and other DA reporting systems.

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 focus upon the completion of health and psychosocial instruments to be utilized in large scale cohort studies of soldiers. Other available instruments such as the General Well Being Scale will continue to be assessed for utility in describing perceived health status of soldiers in relationship to readiness and performance. Further work is anticipated on the relationship between unit cohesion patterns of well being and health outcome and the relationship of such data to the state of cohesiveness in the military unit. 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

Publications

1. DATEL, W.E., JONES, F.D. & ESPOSITO, M.E. Suicide in United States Army personnel, 1977-1978. Military Medicine, 1981, 146, 387-392.

2. DATEL, W.E. Book review. Voices of Death by Edwin Shneidman. Military Medicine, 1981, 146, 363.

3. DATEL, W.E. Editorial: On studying morbidity across all three armed services. Military Medicine, 1981, 146, 590.

4. DATEL, W.E. Disease rates in the military during the 1970's. Alexandria, Virginia. Defense Technical Information Center, 1981. Document AD No. 097 612.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6457	81 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
80 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	AC	043
b. CONTRIBUTING				
c. [redacted]	STOG 807.2:4			

11. TITLE (Precede with Security Classification Code)

(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. 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 81	5.0	507
	CURRENT 82	5.0	537

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, COL P.

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Neuropsychiatry

ADDRESS: Washington, DC 20012

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

NAME: Hegge, F.W. Ph.D.

TELEPHONE: 301-427-5521

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Thorne, D. Ph.D.

NAME: Taube, MAJ S. POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Stress; (U) Biological Rhythms; (U) Chronobiology; (U) Electrophysiology; (U) Psychophysiology; (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) 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) 80 10 - 81 09 Analysis of data from long term laboratory studies simulating rapid deployment across time zones reveals pronounced deficits in tasks requiring high levels of complex cognitive performance. Countermeasures as proposed have been adopted as Army Policy for minimizing the effects of circadian rhythm disruptions in military performance during deployment and have been incorporated into a circular slide rule/nomogram for distribution to Commanders to assure ease of applicability. Preliminary analysis of wrist motor activity (actigraph) data from these studies reveals in addition to circadian and ultradian rhythmicities, higher frequency components occurring in patterns that may be signatures of high stress or extreme fatigue. Biochemical analysis of urinary levels of Na, K, and cortisol during baseline performance and circadian rhythm disruption is proceeding. Studies of rhythmic aspects of mood, activation and verbal perception are revealing circadian, ultradian and fatigue related changes consistent with differential time dependent performance potential for aspects of military tasks more closely dependent upon a single cerebral hemisphere.

*Available in microfiche upon originator's approval

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

Project 3E162771A879 FACTORS LIMITING SOLDIERS' EFFECTIVENESS

Work Unit 043: Military Stress: Circadian and Ultradian Factors

Investigators:

Principal: Frederick W. Hegge, Ph.D.

Associates: MAJ R. Curtis Graeber, MSC; LTC Sander G. Genser, MC; LTC Daniel P. Redmond, MC; CPT Bruce Cuthbert, MSC; Harvey Babkoff, Ph.D. (NAS-NRC); Helen Sing, M.C.

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 adaptation 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, and the relationship between cerebral hemispheric laterality and circadian variations in military performance.

Progress

Three groups of four soldiers have been run in a series of laboratory studies of rapid deployment across time zones. These subjects were first trained to asymptote on the most recently developed form of the microcomputerized Performance Assessment Battery (PAB). Then they were placed in our isolation facility within which we have shown that it is possible to control all known time cues. The subjects were continued on U.S. time during a four day baseline period, taken through a simulated deployment flight to Germany (requiring a 6-hour advance in their daily routine) and then monitored for ten days to determine the course of their post-flight adjustment. Analysis of the cognitive performance data reveals particular deficits in the following tasks: (1) logical reasoning - the subject is shown a pair of letters on a display screen and a sentence describing the arrangement of the letters which he must identify as being true as false, e.g., "AB A does not come before B", and (2) short term memory - the subject is shown nine digits, goes through a brief waiting period and is then shown eight digits with the objective of identifying the single missing digit as rapidly as possible.

The mood-activation scale is shown as particularly sensitive to subjective dimensions of the circadian rhythm disruption accompanying the aforementioned deficits and thus may, itself, be an easily administered index of the effectiveness of the proposed regime of countermeasures.

In continuing analyses the following additional physiological measures are being explored for sensitivity to the disruptive effects of time zone (phase) shifts on performance: (1) urinary sodium, potassium, cortisol and testosterone, (2) heart rate and other aspects of cardiovascular functioning, (3) wrist motor activity (actigraph), and (4) body temperature. Preliminary analysis shows the electrolyte levels to exhibit clear circadian rhythmicity and the motor activity to exhibit as well, higher frequency components that may be markers of extreme fatigue and/or stressful events. Individual and joint analysis of these rhythms' relations to performance is continuing.

e mood-activation scale developed here and incorporated into the PAB
larly being studied to determine to what extent its aforementioned
vity to circadian rhythms disruption may be explained by disruption of
rhythms in activation patterns of the two cerebral hemispheres. Data
n collected on over twenty subjects using a lexical decision paradigm
h groups of letters that are either words or nonwords are presented
oscopically to one or the other visual hemifield. These data reveal
ected superiority of the dominant hemisphere for verbal processing.
on over time in (1) the ability of each hemisphere to perform the basic
ve task of deciding whether the letter string is a word or nonword as
(2) the site of influence of perceptual factors, e.g., location of the
within the visual hemifield are being related to the mood-activation
s associated with performance disruption.

Objectives

mediate goals include the continuation of the analysis of the
ogical and performance variables from the long term time zone shift
. The results of this analysis is expected to feed back to influence
t generation of the PAB so as to increase its usefulness for studies of
ted sleep and sleep deprivation effects on military performance.
data will be gathered on the neuropsychological substrate of the mood-
ion scale and other components of the PAB using the lexical decision
n under such stressors as high cognitive load, fragmented sleep and
eprivation. These data are to be used to converge on a PAB whose com-
are highly correlated with the pattern of performance disruption
eristically seen under stresses expected in the combat environment.
figuration of decrements on such a PAB will then permit the development
essment of specific sets of behavioral and/or pharmacological counter-
s to be used by soldiers exposed to defined subsets of stresses.

sentations and Publications

Redmond, D.P., Sing, H.C., Graeber, R.C., and Hegge, F.W.
Organization and Relationships Among Measures of Human Activity,
te, and Heart Rate Variability. The XV International Conference
nternational Society for Chronobiology, Minneapolis, Minn.
r 1981.

Graeber, R.C., Cuthbert, B.N., Sing, H.C., Schneider, R.J., &
, G.R. Rapid transmeridial deployment: I. Use of chronobiologic
easures to hasten time zone adjustment in soldiers. In XIV Inter-
Conference Proceedings of the International Society for Chronobiology.
Publishing House "Il Pointe", 1981, in press.

Cuthbert, B.N., Graeber, R.C., Sing, H.C., and Schneider, R.J.
nsmeridial deployment: II. Effects of [illegible] and counter-
under field conditions. In XIV International Conference Proceedings
ternational Society for Chronobiology, Milan: Publishing House
", 1981, in press.

Graeber, R.C. Recent studies relative to the airlifting of military
oss time zones. In L. Scheving & F. Halberg (Eds.), Chronobiology:
s and Application to Shifts in Schedules, NATO Advanced Study
Series. Alphen an den Rign: Sijthoff & Nordhoff,
. 353-369.

5. Graeber, R.C., Cuthbert, B.N., Sing, H.C., Schneider, R.J. & Sessions, G.R. Rapid transmeridian deployment: cognitive performance and chronobiologic prophylaxis for circadian dyschronism. Proc. of the 1980 Army Science Conference, USMA, West Point, N.Y., in press.

6. Graeber, R.C. Alterations in performance following rapid transmeridian flight. In F. M. Brown & R.C. Graeber (Eds.), Rhythmic Aspects of Behavior. Hillsdale, N.J.: Lawrence Erlbaum Associates, in press.

7. F.M. Brown & R.C. Graeber, (Eds.), Rhythmic Aspects of Behavior. Hillsdale, N.J.: Lawrence Erlbaum Associates, in press.

8. Graeber, R.C., Sing, H.C., Cuthbert, B.N. The Impact of Transmeridian Flight Deploying Soldiers. In L. Johnson, D. Tepas, W. P. Colguhoun and M. J. Calligan (Eds.), NIOSH Proceedings, The Twenty-four Hour Workday: Proceedings of a Symposium on Variations in Work-Sleep Schedules, DHHS (NIOSH) Publication #81-127. July 1981.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6452	81 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
80 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	AC	044
b. CONTRIBUTING				
XXXXXXXXXX NO	STOG 80-7.2:4			

11. TITLE (Precede with Security Classification Code)
(U) Neuroendocrine Response to Military Stress

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
012600 Pharmacology 002300 Biochemistry
013400 Psychology 016200 Stress Physiology 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	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 81	3.0	350
	CURRENT 82	3.0	460

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Inst. of Research
Division of Neuropsychiatry
ADDRESS: Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Meyerhoff, J.L., M.D.
TELEPHONE: (202) 576-3559
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Holaday, J.W., Ph.D.
NAME: Mougey, E.H.

21. GENERAL USE
Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Stress; (U) Transmeridian Desynchronization; (U) Neurotransmiters; (U) Hormones; (U) Peptides; (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) To examine neuroendocrine correlates of stressors specific to the military environment. Types of stress to be studied will include shock, extremes of heat and cold, psychological stress, continuous performance, and stressful social interaction.

24. (U) Laboratory and field studies will examine the neuroendocrine response to environmental and psychological stressors. These responses will be correlated with simultaneously-obtained data on performance decrement in the same subjects and with work/rest schedules. Hormonal responses will provide bases for recommendations regarding adaptation to stress, and optimization of work/rest schedules. This information is used to recommend pharmacologic and other therapies. Includes studies of physiological effects of hormones as well as assay development.

25. (U) 0 10 - 81 09 We have shown that psychological stress is sufficient to produce large increases in plasma beta-endorphin, even in the absence of physical stress such as pain. Given the marked physiological potency of this peptide, our finding that it can be markedly increased by psychological stress suggests that this response could be a contributing factor to psychiatric casualties in combat. We have also confirmed our finding that cholinergic drugs produce a large increase in plasma beta-endorphin. It was found that the removal of the adrenal medulla completely blocked the therapeutic effects of naloxone in endotoxic shock. Although naloxone reverses shock it may also potentiate traumatic pain by blocking the pain-relieving effects of endorphins. We found that TRH effectively reverses shock and improves survival in experimental models of endotoxic and hemorrhagic shock at doses which do not increase response to pain. Moreover, TRH is more efficacious than naloxone in preventing paralysis following experimental spinal cord injury. For technical report, see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

Reverse upon originator's signature

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 044 Neuroendocrine Response to Military Stress

Investigators:

Principal: Meyerhoff, J.L., M.D.

Associate: Holaday, J.W., Ph.D., Belenky, G.L., M.D., LTC, MC, Mougey, E.H., M.S., Pennington, L.L, B.S.

Objectives:

The study of neuroendocrine responses to stressors typical of combat and the military environment in order to identify conditions and processes leading to physical and/or psychiatric breakdown in combat. Types of stressors studied will include physical as well as psychological stressors.

Progress:

We have shown that psychological stress is sufficient to produce large increases in plasma beta-endorphin, even in the absence of physical stress such as pain. Given the marked physiological potency of this peptide, our finding that it can be markedly increased by psychological stress suggests that this response could be a contributing factor to psychiatric casualties in combat. We have also confirmed our finding that cholinergic drugs produce a large increase in plasma beta-endorphin. The importance of the autonomic nervous system in mediating the therapeutic effects of naloxone in experimental models of shock was determined. The finding that either vagotomy or cholinergic antagonists block the therapeutic effects of central naloxone injections in spinal shock confirms the importance of the parasympathetic-cholinergic system in mediating the cardiovascular pathophysiology of this type of shock. The sympathetic nervous system may be of predominant importance in other forms of shock. It was found that the removal of the adrenal medulla completely blocked the therapeutic effects of naloxone in endotoxic shock. Although naloxone reverses shock it may also potentiate traumatic pain by blocking the pain-relieving effects of endorphins. We found that TRH effectively reverses shock and improves survival in experimental models of endotoxic and hemorrhagic shock, at doses which do not increase response to pain. Moreover, TRH is more efficacious than naloxone in preventing paralysis following experimental spinal cord injury.

Future Objectives:

Studies will be directed toward characterizing the beta-endorphin response to psychological stress. We will attempt to determine the nature of the neural regulation of this response and to discover suitable means of preventing or limiting the response. We hope to extend these findings directly to clinical studies of stress in particular to study the hormonal responses of soldiers facing promotion boards. Such studies will include measures of plasma beta-endorphin and urinary cortisol and testosterone. With the Univ. of Georgia, we plan to study the neuroendocrine responses of monkeys to changes in social organization. Studies on cholinergic regulation of beta-endorphin secretion will continue. We will explore the relationship between stress and immune systems.

Presentations

1. Emurian, H.H., Brady, J.V., Meyerhoff, J.L., and Mougey, E.H. "Behavioral and Biological Interactions with Confined Microsocieties in a Programmed Environment," The Fifth Princeton/American Institute of Aeronautics and Astronautics/Space Studies Institute Conference on Space Manufacturing, Princeton, NJ, May 1981.

2. Holaday, J.W. American College of Neuropsychopharmacology, San Juan, Puerto Rico - invited participant in symposium on "Endorphins and Enkephalins," Dec 1980.

3. Holaday, J.W. "Naloxone Therapy in Shock," Military Psychiatry Symposium, Brooke Army Medical Center, San Antonio, TX, Mar 1981.

4. Holaday, J.W. "Naloxone Therapy in Shock," Department of Surgery, University of Iowa Hospitals and Clinics, Iowa City, IA - Grand Rounds, Apr 1981.

5. Holaday, J.W. "Opiate Antagonists and Shock," Neuroendocrine Unit, University of Rochester School of Medicine, Rochester NY. Invited lectureship and consultation, May 1981.

6. Holaday, J.W. Eighth International Congress of Pharmacology, Tokyo, Japan - Invited participant and lecturer in symposium on "Opioid Peptides and Their Relevance for CNS Mechanisms," Jul 1981.

7. Holaday, J.W. Department of Physiology, Gifu University School of Medicine, Gifu, Japan - Invited participant in symposium on "Opiate Receptor Mechanisms," Jul 1981.

8. Holaday, J.W. "Regulatory Peptides: Functional and Pharmacological Aspects," invited lecture, Gardone Riviera, Italy, Aug 1981.

9. Holaday, J.W. "Thyrotropin Releasing Hormone," satellite symposium of the International Society of Neurochemistry, Nottingham, England, Sep 1981.

10. Holaday, J.W. Charing Cross Hospital, School of Medicine, University of London, invited lectureship, London, England, Sep 1981.

11. Holaday, J.W. Department of Pharmacology, University of Kentucky College of Medicine, departmental seminar, Lexington, KY, Oct 1981.

Publications

1. Faden, A.I. and Holaday, J.W. Experimental endotoxin shock: the pathophysiologic function of endorphins and treatment with opiate antagonists. J. Infectious Diseases 142:229-238 (1980).

2. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Endorphin-parasympathetic interaction in spinal shock. J. Autonomic Nerv. System 2:295-304 (1980).

3. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Opiate antagonist improves neurologic recovery after spinal injury. Science 211:493-494 (1981).

4. Holaday, J.W. and Belenky, G.L. Opiate-like effects of electroconvulsive shock in rats: a differential effect of naloxone on nociceptive measures. Life Sci. 27:1929-1938 (1980).

5. Holaday, J.W., O'Hara, M., and Faden, A.I. Hypophysectomy alters cardiorespiratory variables: central effects of pituitary endorphins in shock. Am. J. Physiol. 241 (Heart & Circ. Physiol 10):H479-H495 (1981).

6. Belenky, G.L. and Holaday, J.W. Repeated electroconvulsive shock (ECS) and morphine tolerance: demonstration of cross sensitization in the rat. Life Sci. 29:553-563 (1981).

7. Faden, A.I., Jacobs, T.P., Feuerstein, G., and Holaday, J.W. Dopamine partially mediates the cardiovascular effects of naloxone after spinal injury. Brain Res. 213:415-421 (1981).

8. Faden, A.I., Jacobs, T.P., Mougey, E.H., and Holaday, J.W. Endorphins in spinal injury: therapeutic effect of naloxone. Annals of Neurol. 10:326-332 (1981).

9. Holaday, J.W. and Loh, H.H. The neurobiology of β endorphin and related peptides. In: Hormonal Proteins and Peptides: β Endorphin, ed. by C.H. Li. Academic Press, New York, pp. 202-291 (1981).

10. Holaday, J.W. and Faden, A.I. Endorphins in shock and spinal injury: therapeutic role for opiate antagonists. Psychopharm. Bull. 17:74-76 (1981).

11. Holaday, J.W. and Faden, A.I. The pathophysiologic role of endorphins in experimental shock. J. Infect. Dis. 143:863-864 (1981).

12. Holaday, J.W., D'Amato, R.J., and Faden, A.I. Thyroropin releasing hormone improves cardiovascular function in experimental endotoxic and hemorrhagic shock. Science 213:216-218 (1981).

13. Holaday, J.W. and Faden, A.I. Naloxone treatment in shock. Lancet i:201 (1981).

14. Holaday, J.W. and Faden, A.I. Endorphins in shock and trauma. Abstr., Am. Coll. of Neuropsychopharm. p. 3, 1980 (San Juan, PR, Dec 1980).

15. Tortella, F.C., Cowan, A., Belenky, G., and Holaday, J.W. Electroconvulsive shock-induced endorphin release: additional EEG and behavioral evidence. Fed. Proc. 40:287 (1981).

16. Holaday, J.W. and Faden, A.I. Endorphins in shock and trauma. Abstr., Am. Coll. of Neuropsychopharm. p. 3, 1980 (San Juan, PR, Dec 1980).

17. Tortella, F.C., Cowan, A., Belenky, G., and Holaday, J.W. Electroconvulsive shock-induced endorphin release: additional EEG and behavioral evidence. Fed. Proc. 40:287 (1981).

18. Holaday, J.W., D'Amato, R.J., and Faden, A.I. Thyrotropin releasing hormone (TRH), which antagonizes endorphins in vivo reverses endotoxic and hemorrhagic shock hypotension in rats. Fed. Proc. 40:272 (1981).

19. Holaday, J.W., Ruvio, B.A., D'Amato, R.J., and Faden, A.I. Thyrotropin releasing hormone (TRH) improves blood pressure and survival in endotoxic shock. Circ. Shock 8:190 (1981).

20. Faden, A.I., Jacobs, T.P., Feuerstein, G., and Holaday, J.W. Dopamine partially mediates the cardiovascular effects of naloxone after spinal injury. Neurology (1981, in press) (Amer. Acad. Neurol.; Boston, MA, May 1981).

21. Holaday, J.W. and Faden, A.I. Endorphins in shock and trauma. Abstr., Eighth Int. Cong. Pharmacol., p.58 (Tokyo, Japan, Jul 1981).

22. Holaday, J.W. and Faden, A.I. Thyrotropin releasing hormone (TRH) improves cardiovascular funcion and survival in shock without altering opiate analgesia. Abstr., Eighth Int. Cong. Pharmacol., p.854 (Tokyo, Japan, Jul 1981).

23. Holaday, J.W. and Faden, A.I. Naloxone and thyrotropin releasing hormone (TRH) interactions in the treatment of endotoxic shock. Abstr., Int. Narcotic Res. Conf. p.133 (Kyoto, Japan, Jul 1981).

24. Holaday, J.W. Action of naloxone and TRH on the autonmic regulation of circulation. Reg. Peptides: Functional and Pharmacological Aspects. (Gardone Riviera, Italy, Aug 1981).

25. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Treatment with thyrotropin releasing hormone (TRH) improves functional neurologic recovery after experimental spinal injury. Reg. Peptides: Functional and Pharmacological Aspects. (Gardone Riviera, Italy, Aug 1981).

26. Holaday, J.W. and Faden, A.I. Thyrotropin releasing hormone reverses experimentla circulatory shock: central and peripheral actions of TRH and analogues. Abstr., Thyrotropin Releasing Hormone, p.53 (Nottingham, England, Sep 1981).

27. Tortella, F.C., Cowan, A., and Holaday, J.W. The role of endogenous opioid systems in ECS-induced post ictal electrogenesis and behavioral depression in rats. Soc. Neurosci. Abstr. 7:165, 1981.

28. Meyerhoff, J.L., Bunnell, B.N., and Mougey, E.H. Plasma beta-endorphin in rats is increased by psychological stress. Neuroscience Abstracts 7:166 (1981).

29. Mougey, E.H. and Meyerhoff, J.L. Effect of cholinomimetics and cholinesterase inhibitors on plasma beta-endorphin. Neuroscience Abstracts 7:153 (1981).

30. Belenky, G.L. Training in military and combat psychiatry. Journal Psychiatrie Africaine (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OC 6450	81 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
80 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	62777A	3E162777A879	AC	045
b. CONTRIBUTING				
XXXXXXXXXX	STOG 80-7.2;4			

11. TITLE (Precede with Security Classification Code)
(U) Behavioral Variables in Autonomic Function and Disease in Military Personnel

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
013400 Psychology 012900 Physiology 016200 Stress Physiology 02500 Clinical Medici

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	81 09	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 80	4.0	124
FISCAL YEAR CURRENT 81	2.0	202

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Inst. of Research
Division of Neuropsychiatry
ADDRESS: Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic institution)
NAME: Cuthbert, B.N., CPT
TELEPHONE: (202) 576-2489
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Hamilton, B.E., CPT
NAME:

21. GENERAL USE
Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Physiology; (U) Emotions; (U) Stress; (U) Autonomic Function (U) Military Psychiatry; (Conditioning)

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

23. (U) This is a multidisciplinary effort addressing the development and use of laboratory models to define and describe the organ system responses and disease states caused by stressors in the military environment.

24. (U) The techniques of operant and respondent conditioning will be employed in the production of models of both phasic and chronic psychological and emotional stress. Cardiovascular and gastrointestinal function will be monitored by electronic transducers and chronic indwelling catheters and fluid samples will be assessed for hematological and hormonal effects. Electrophysiological measurements of central and autonomic responsiveness will provide both a more accurate interpretation of similar data collected in studies with human volunteers and a source of hypotheses relevant to preventive and therapeutic intervention for cardiovascular and gastrointestinal disorders in military personnel.

25. (U) 80 10 - 81 09 Major findings: Studies of behavioral effects of endogenous opiates show morphine-like effects of beta-endorphin on analgesia, but with a shorter duration of action. A system was developed for chronic monitoring of stomach Ph in awake monkeys, and continuous monitoring for several days confirmed earlier findings of periodicities in stomach acidity with both ultradian and circadian rhythms being apparent. Work on the correlation between behavioral and cardiovascular activation confirmed consistent patterning of behavioral and cardiovascular responses to stimuli paired with either appetitive or aversive events. Termination of this work was necessitated by separation of one PI and PCS of the other. For technical report, see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 80 - 30 Sep 81.

*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 69 FOR ARMY USE ARE OBSOLETE

Project 3E162777A879 FACTORS LIMITING SOLDIERS EFFECTIVENESS

Work Unit 045: Behavioral Variables in Autonomic Function and Disease in Military Personnel

Investigators:

Principal: Cuthbert, Bruce N., CPT, MSC

Associate: Hamilton, Bruce E., CPT, MSC

Objectives:

Future wars are likely to involve sustained, high-intensity combat which has been shown in recent Mideast conflicts to produce an extremely high incidence of psychiatric casualties frequently of a psychosomatic nature. The development of techniques for the minimization and treatment of these casualties depends upon a thorough understanding of the relationships between environmental stressors, behavioral requirements, and physiological responses. The principal objective of this work unit is the development of animal models suitable for the investigation of a variety of interactions between behavior, physiology, and stress.

Progress:

Primate models have been developed for investigations of cardiovascular and gastric responses to behavioral and environmental stressors. These models allow continuous monitoring of physiological functions in animals that are trained under various behavioral requirements. Techniques of both operant and respondent conditioning are used to develop and maintain behavior, and all behavioral and physiological monitoring is computerized for ease of data collection and subsequent data analysis.

A series of studies completed this fiscal year confirmed earlier studies demonstrating that a simple "arousal" model is inadequate to account for cardiovascular responses in anticipation of stressful events such as painful electric shocks or extended experimental sessions requiring accurate timing behavior to obtain food. Rather, the organization and patterning of cardiovascular responses depends upon details of procedure, and may differ from animal to animal. Further, there is a dissociation between behavioral and cardiovascular responses. For example, behavioral suppression may be accompanied either by cardiovascular activation or depression, depending upon the procedure, the stage of training, and the animal.

A system was developed for chronic monitoring of stomach Ph in awake rhesus monkeys via an indwelling stomach Ph probe. This system was calibrated and validated by chemical analysis of gastric fluid samples. Earlier studies using the relatively stressful procedure of withdrawing stomach fluid samples periodically for six hours via a nasogastric tube had suggested the presence of ultradian (i.e. 90-minute) rhythms in stomach acidity. These results were extended by continuously monitoring stomach Ph in several monkeys for periods up to a week. Circadian rhythms in acidity were also demonstrated. Preliminary studies suggested that stressful events are accompanied by a decrease in stomach acidity rather than an increase as is popularly believed.

The endogenous opiate beta-endorphin, which is released during stress, was demonstrated to produce analgesia in primates which were trained to adjust the intensity of an electric shock by pressing on a lever. The effects were similar to those of morphine, but dissipated within 15 minutes, whereas those of morphine lasted for at least four hours.

Future Objectives:

This work unit has been terminated due to separation of the principal investigator and PCS of the associate investigator.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6470	81 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. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80 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	046
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2	4		

11. TITLE (Precede 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 81	3.0	60
FISCAL YEAR CURRENT 82	3.0	55

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
Washington, DC 20012

ADDRESS:

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, COL P.

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
US Army Medical Research Unit-Europe

ADDRESS: HQ 7th Medical Command
APO New York 09102

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

NAME: SCHNEIDER, MAJ R.

TELEPHONE: (Avn 435) 740/626

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: ROCK, CPT S.

NAME: INGRAHAM, LTC L.

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Epidemiology; (U) Stress; (U) Psychiatry; (U) Human Volunteer; (U) Soldier Effectiveness

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual 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, questionaires, field and cohort studies and observation methods are employed to develop requisite data.

25. (U) 80 10-81 09 Observational study of how allied forces are organized and promote leadership and cohesion. Preliminary results: There are fundamental differences in the structural and social relationships between officer, NCOs and enlisted ranks among the three allied armies. It is anticipated that future analyses will help suggest strategies applicable for building cohesion in small groups in the US military. Exploratory study of soldiers' attitudes toward and knowledge about psychiatric (battle stress) casualties completed. Major results: Although a majority of soldiers have some idea of what a battle stress casualty is like, few were able to specify how one would act or should be treated. Higher ranking enlisted men also indicated the greatest unwillingness to accept such casualties back into their units.
Study of the assimilation of families into a military community. Results: This is designed to learn how families are socialized as a function of housing area and to determine the long term implications (health and general well being) of this assimilation. This research is in progress.

*Available to contractors upon sponsor's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 84

Project 3E162777A879 FACTORS LIMITING SOLDIERS EFFECTIVENESS

Work Unit 046 Medical Factors Limiting Soldier Effectiveness

Investigators

Principal: Schneider, MAJ R.J.
Associate: Rock, CPT S.K., Jr.
Ingraham, LTC, L.H.

Description.

This field unit, stationed in West Germany with the US Army Europe and Seventh Army, identifies and investigates physical, psychological, social, and organizational factors bearing on the individual and unit performance and combat readiness. Initial efforts have focused on three areas identified by commanders as important concerns within the European theater.

OBSERVATIONAL STUDY IN ALLIED ARMIES

A central concern of this organization is the role of unit cohesion in maintaining readiness for combat and in maintaining forces committed in combat. The structure of an army affects relations established among officers, NCOs, and enlisted men which in turn play a central role in the development of unit cohesion. Observers were placed in British, German and French artillery units to learn about relations among soldiers in each of these armies for comparison with similar data based on American soldiers. Two observers spent about four weeks living with their assigned units, collecting observational data and interviewing members in their own language. Data collection which was directed at documenting and assessing social relationships, was completed in July 1981. Observation summaries were completed in September 1981. Preliminary results indicate that there are fundamental differences in the structure and social relations among respondents in the four armies.

'or example, the US Army generally has higher rank
ıt the NCO levels; responsibility for decision-making
:herefore tends to be pushed higher. There is con-
;iderable difference among the armies in terms of
;ocial distance between the ranks. Such differences
ıre relatively less pronounced in the French and
;erman armies which seems to allow for much closer
'elations between junior and senior enlisted personnel
ınd officer and senior enlisted personnel.

'he implications of difference in social relations a-
ıong the armies for the development of cohesion, and
.mplications for leadership and the handling of stress
.n the US Army will be the subject of future analyses.

'RELIMINARY STUDY OF ATTITUDES AND KNOWLEDGE CONCERNING 'SYCHIATRIC CASUALTIES

ı small pilot survey designed to assess the knowledge
ıbout and attitudes towards psychiatric (battle stress)
:asualties was conducted at the request of a brigade
:ommander. Few of the respondents had ever had any
.raining in the area of battle stress casualties; how-
:ver, about eighty percent were able to appropriately
escribe such a casualty. Also, the majority believed
hat they were susceptible to such "breakdown."
ost respondents would not trust a stress casualty who
eturned to duty. Higher ranking respondents were the
east likely to trust a returned stress casualty.
ew individuals had any realistic idea of how to treat
uch casualties.

he results of this study will be confirmed utilizing
ındom subject selection and a larger data base. This
ɔnfirmation has been requested by a USAREUR Division
ɔmmander and will be used to formulate decisions con-
ɔncerning training requirements.

;SIMILATION OF FAMILIES INTO A MILITARY COMMUNITY

Community Commander requested an assessment of cohe-
.on in his community, with specific interest in family
;sues. As a result of this we have initiated an ob-
:rvational and interview study of families (spouse

and active duty) with the objective of determining how cohesion (or social support) is developed. This development is being studied as a function of housing area, since it had been suggested in earlier work that this variable effects asssimilation, adjustment, and even health outcomes.

We have now developed a cohort comprising one hundred families who were interviewed within thirty to ninety days of arrival in Germany. Interviews of various community workers have also been completed. Data are currently being arrayed for analysis. Each family will be contacted after six months to assess longer term implications of its assimilation.

Project 3E612777A879

Work Unit 046 Medical factors limiting soldier effectiveness

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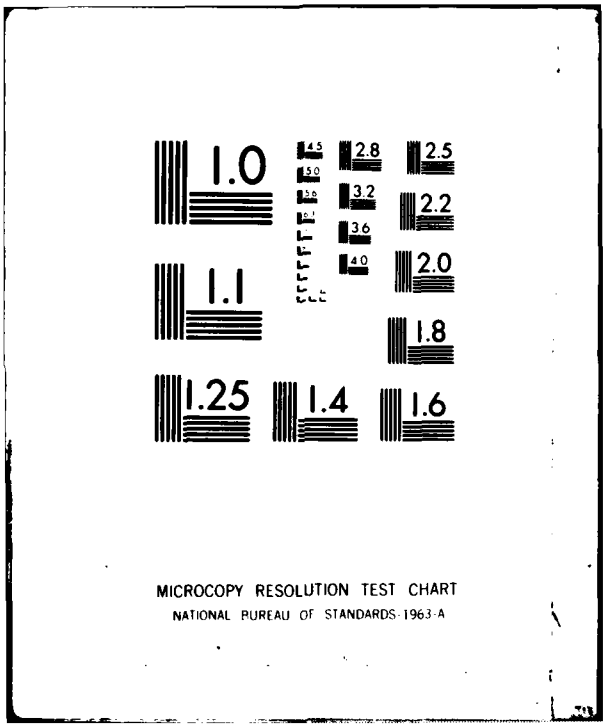
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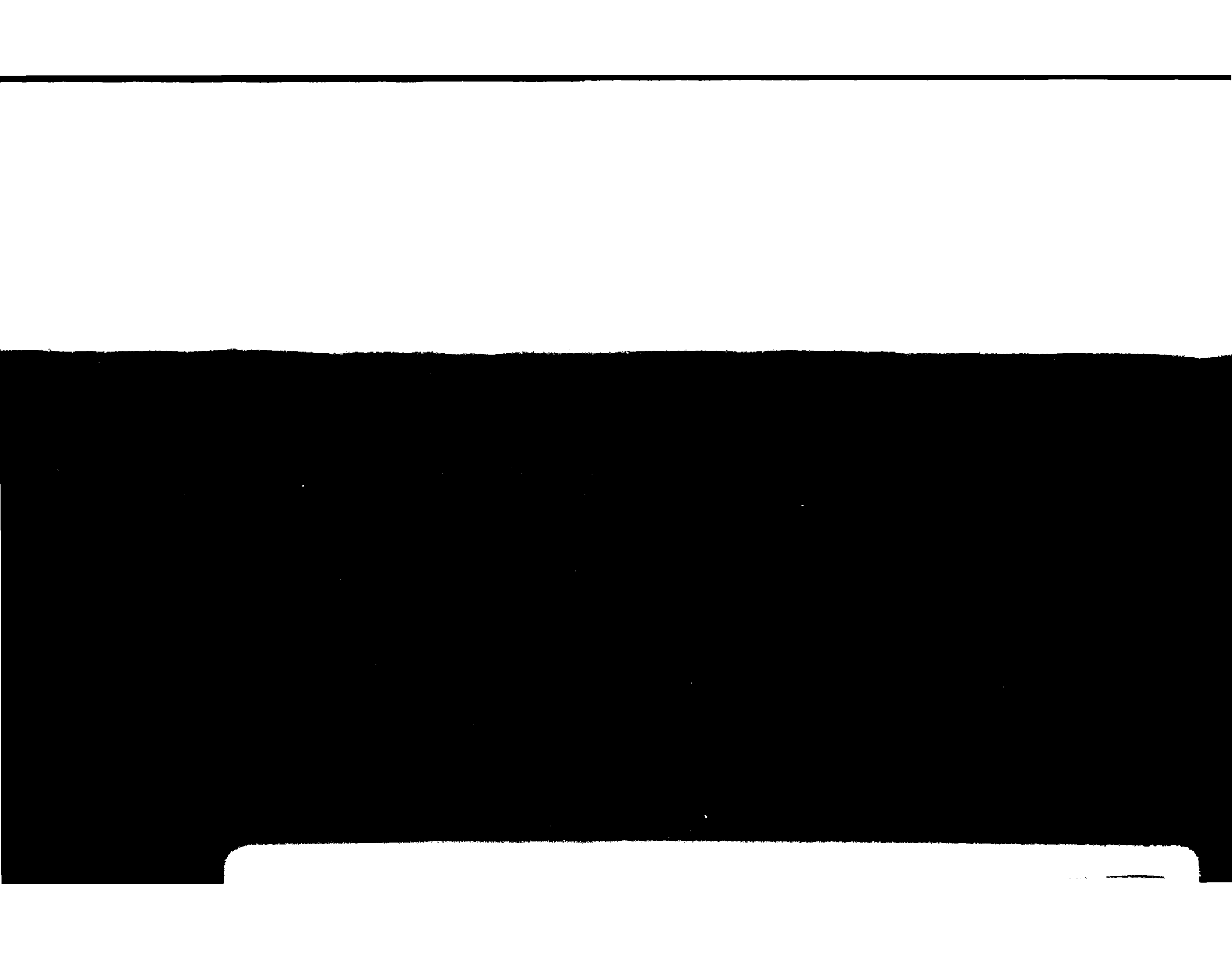
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