LEVEL II

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FISCAL YEAR 1980

(October 1979- 30 September 1980)

1 October 1980

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

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Biological Sciences	Immunology	Surgery
Medical Sciences	Internal Medicine	Veterinary Medicine
Biochemistry	Physiology	
Communicable Diseases	Psychiatry	

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

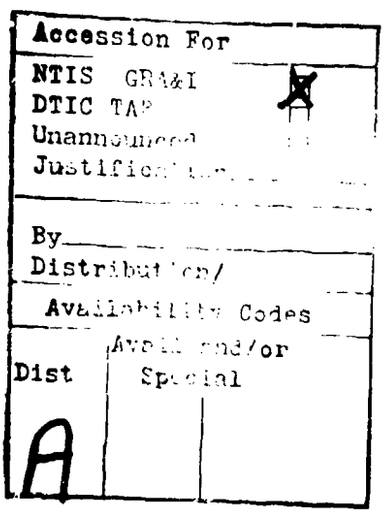
The various subjects covered in this report are listed in the Table of Contents. Abstracts of the individual investigators are included on the DD Form 1498 introducing each work unit report.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

FOREWORD

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



SUMMARY

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

FY 80 PROJECTS AND WORK UNIT NUMBERS/TITLES ARE INDICATED BY AN ASTERISK.

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PROJECT 3A161101A91C

IN-HOUSE LABORATORY INDEPENDENT RESEARCH

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6448	80 09 30	DD-DRAE(AR)636

3. DATE PREV SUM'RY	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
79 10 01	F. Term	U	U	NA	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	096
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Biochemical and Immunological Characterization of Rickettsial Proteins

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 10	30 Sept 80	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: 79	4	163
FISCAL YEAR CURRENT: 80	4	165

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: 202-576-2146

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Oaks, S. C., Jr., MAJ

NAME: Eisemann, C. S.

NAME: Havenner, J.A., 1LT

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (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) Isolate and purify the subcellular rickettsial components responsible for eliciting immunologic protection. Localize and identify the rickettsial surface proteins that affect virulence. Antigenically characterize the peripheral proteins of rickettsiae. These studies will aid in the development of inactivated vaccines capable of protecting deployed troops.

24. (U) Isolate and evaluate the peripheral rickettsial macromolecules as experimental immunogens. Analyze the rickettsial proteins using polyacrylamide gel electrophoresis and immunoelectrophoresis with specific radiolabeling by extrinsic radioiodination or sodium borohydride reduction. Evaluate the potential roles of activity for each protein isolated. Compare the composition of isolated proteins from representative strains of the different rickettsial groups. Develop the rickettsial plaque reduction assay. Determine the antigenic interrelationships among strains of scrub typhus rickettsiae using plaque reduction, complement fixation, immunofluorescent, cross-neutralization, and mouse virulence tests with specific rickettsial antibody prepared in laboratory animals.

25. (U) 79 10 - 80 09 Analysis of scrub typhus prototype strains Karp, Gilliam and Kato showed the presence of six major antigens reactive with homologous antiserum. Heterologous antisera identified two high molecular weight antigens specific to the Karp strain. However, immunization of mice with these antigens, separated by polyacrylamide gel electrophoresis, failed to protect against a homologous challenge of 100 mouse lethal doses of rickettsiae. This project has been supported by ILIR funds for three years. This funding is being terminated, and the research incorporated into other ongoing programs at WRAIR. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*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 096 Biochemical and Immunological Characterization of Rickettsial Proteins

Investigators:

Principals: Joseph V. Osterman, PhD; MAJ Stanley C. Oaks, Jr., MSC; CPT Jeffrey A. Havenner, MSC; Christine S. Eisemann, MS

Associates: SP5 Wynn Gordy; SP5 Matthew J. Nypaver; SP5 Georgiene A. Radlick

Description:

Efficacious, safe vaccines for rickettsiae are needed to preclude severe disruption of activities of deployed military troops when exposed to rickettsiae. Associated problems to attain such vaccines are : isolate and purify the subcellular rickettsial components responsible for eliciting immunologic protection, localize and identify the rickettsial surface proteins that affect virulence, and characterize antigenically the peripheral proteins of rickettsiae. Anicllary problems include development or adaptation of methods for separating and detecting rickettsial antigens and determination of an appropriate model system to evaluate protection.

Progress:

1. Analysis of Antigens of Typhus and Scrub Typhus Rickettsial Groups: Antigens of the typhus and scrub typhus rickettsial groups were analyzed using the enzyme-linked immunosorbent assay and polyacrylamide gel electrophoresis. Of the six structural proteins of typhus rickettsiae, the major group specific antigenic activity migrated with protein 4, a peripheral cell envelope protein. Analysis of scrub typhus prototype strains Karp, Gilliam, and Kato showed the presence of six major antigens reactive with homologous antiserum. Plaque purified strains of scrub typhus rickettsiae have been isolated to aid in antigenic characterization.

2. Biochemical Evaluation of Rickettsial Ribosomes: Preliminary investigations were designed to determine the feasibility of defining the biochemical characteristics of ribosomes from rickettsiae of the typhus group as compared with the ribosomes of Escherichia coli. The initial results indicate that sufficient quantities of rickettsiae can be obtained via mass culture methods to permit initiation of the basic characterization studies.

Recommendations for Future:

This project is being terminated under ILIR funding, and the research will be incorporated into other programs in the Department of Rickettsial Diseases.

Reference Cited: NONE

Presentations: NONE

Publications:

1. Anderson, G. W., Jr., and J. V. Osterman. 1980. Host defenses in experimental rickettsialpox: Resistance of C3H mouse sublines. Acta Virol. 24:294-296.

2. Oaks, S. C., Jr., F. M. Hetrick, and J. V. Osterman. 1980. A plaque reduction assay for studying antigenic relationships among strains of Rickettsia tsutsugamushi. Amer J Trop Med Hyg 29:998-1006.

3. Osterman, J. V., and C. S. Eisemann. 1980. Rickettsiae. p. 707-713. In R. Rose and H. Friedman (ed), Manual of Clinical Immunology. Amer Soc Microbiol, Washington, D.C.

Manuscripts Submitted:

1. Hechemy, K. E., J. V. Osterman, C. S. Eisemann, L. B. Elliott, and S. J. Saskowski. 1980. Detection of typhus antibodies by latex agglutination. J Clin Microbiol (Accepted).

2. Osterman, J. V., and G. Rapmund. 1980. Rickettsial infections. Medicine (Accepted).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	4. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6459	80 09 30	DD-DR&E(AR)636

2. 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
79 10 01	H. Term	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	097
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Antigenic Characterization of Sandfly Fever Viruses

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 002600 Biology 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 10	30 Sept 80	DA	

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 79	2	173
CURRENT FISCAL YEAR 80	2	175

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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Division of CD&I

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TELEPHONE: (202) 576 3757

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: BRANDT, Dr. Walter E.

NAME: DALRYMPLE, Dr. Joel M.

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Arbovirus; (U) Antigen; (U) Immunology; (U) Immunopathology

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 antigenicity and immunogenicity of the proteins of Sandfly Fever viruses, to develop rapid means of specific diagnosis, to study the immune response to infection and to develop means of reducing disability. Sandfly fever affected both American and German troops during World War II. Antigenically distinct strains of these viruses exist in the Mediterranean Basin, Central America and Asia.

24 (U) Contemporary virological and immunological methods are used to identify and compare the antigens of distinct strains of Sandfly Fever viruses. Replication of viruses in cell culture systems is studied to determine methods of producing high titered virus for biochemical analysis.

25(U)79 10-80 09.An immunoprecipitation procedure using Staphylococcus protein A to bind the Fc portion of IgG antibody was used in studies of intracellular processing of virus-specific proteins. Cells infected with Punta Toro or Karimabad virus produced viral proteins which reacted broadly with homologous and heterologous Sandfly Fever virus antibodies. Monoclonal antibodies are now being prepared to Punta Toro virus proteins to permit more specific evaluation of viral antigens. Serologic screening of 11000 human sera collected along the Transamazon Highway in Brazil is underway. Sera are tested against 5 Sandfly Fever viruses from Brazil (Candiru, Icoraci, 9556, 10344, and 213452). The prevalence of CF antibody (1 percent) and neutralizing antibody (8 percent) indicate low level transmission of Sandfly Fever viruses. Further analysis is underway. Additional studies of Sandfly Fever Viruses including Rift Valley Fever require greater biological containment than is available at the WRAIR and must be performed elsewhere. (See WRAIR Annual Progress Report 1 Oct 79 to 30 Sep 30).

*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 097 Antigenic Characterization of Sandfly Fever Viruses

Investigators:

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

Associates: Dr. Joel M. Dalrymple, Ph.D.;
Mr. Stephen Harrison;
Mr. George Onley;
SP4 Matthew Seguin;
Mr. Roger Jackson

Problem

Some 30 viruses belong to the Sandfly Fever group of the family Bunyaviridae. The recent confirmation of cross-reactions between a new world sandfly fever virus (Punta Toro) and Rift Valley fever virus (which causes epidemic disease in Africa) underscores the importance of this virus group. Little is known about the structure and replcation of SFV, the factors influencing antigenic changes or the feasibility of producing experimental immunogens. Since the genome of the virus is in 3 pieces, genetic reassortment between strains ala influenza virus is possible.

SFV are found in the Mediterranean Basin, the Middle East, Central and South America and Asia. Epidemic disease occurred in U.S. and German troops in World War II and, more recently, jungle warfare trainees in Panama. SFV infections continue to threaten the effectiveness of troops operating in endemic areas.

Progress

A broad approach is being used to study seven strains of SFV known to infect humans. The objectives include: (1) determination of the optimal conditions for virus replication in cell culture; (2) purification of viruses; (3) comparative physical and chemical analysis of viral proteins and nucleic acid; (4) preparation of specific reagents for antigenic analysis; and (5) determination of the frequency of SFV infections in residents of endemic areas. Intramural research is complemented by extramural contractors for studying SFV nucleic acid and genetics.

An immunoprecipitation procedure using Staphylococcus protein A to bind the Fc portion of IgG antibody was developed to study the intracellular processing of sandfly fever viral proteins. Cells infected with Punta Toro or Karimabad virus were found to produce viral proteins which reacted broadly with homologous and

heterologus Sandfly Fever virus antibodies. In order to improve the specificity of the analysis, monoclonal antibodies to Punta Toro virus were prepared in mouse hybridoma cells (1). It should be feasible to prepare monoclonal antibodies to other related viruses also. Additional studies of Sandfly Fever viruses including Rift Valley Fever virus, which require greater biological containment that is available at the WRAIR, will be performed elsewhere.

Over 3000 of the 11000 human sera collected from along the Transamazon Highway have been screened for CF antibody and 1500 for neutralizing antibody for five Sandfly Fever viruses from Brazil (Candiru, Icoraci, 9556, 10344, and 213452). Hemagglutination inhibition could not be used as a screening test because it was only possible to prepare hemagglutinating antigens to Candiru and Icoraci viruses. The prevalences of CF antibody (1 percent) and neutralizing antibody (8 percent) indicate a low level of Sandfly Fever virus transmission in the people tested so far. Analysis of the demographic information is pending.

Future Objectives

This work unit has achieved its purpose. Continued work on the characterization of Sandfly fever virues will be conducted along with Rift Valley Fever virus under another work unit at USAMRIID. Serologic testing of Brazilian sera will be completed under Work Unit 130, BS01, DAOA 6441, page 64.

References

1. Kennett, R.H., Denis, K.A., Tung, A.S. and Klinman, N.R. Hybrid Plasmacytoma Production: Fusion with Adult Spleen Cells, Monoclonal Spleen Fragments, Neonatal Spleen Cells and Human Spleen Cells. Current Topics in Microbiol. and Immunol. 81: 77-91, 1978.

Formal Presentation

1. Harrison, S.A., Dalrymple, J.M., and Brandt, W.E. A Description of the Structural Proteins of Some Representative Sandfly Fever Viruses. 28th Annual Meeting - The American Society of Tropical Medicine and Hygiene, 15 November 1979.

2. Dalrymple, J.M., Smith, J.F., Harrison, S.A. Bishop, D.H.L. and Ussery, M.A. Immunological and Biochemical Characterization of Phlebotomus fever Bunyaviruses. Simposio Internacional Sobre Arbovirus Dos Tropicos E Febres Hemorragicas. Belem, Brazil 14-18, April 1980.

RESEARCH A[illegible] TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOC6460	80 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
70 10 01	H. Term.	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	098
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Immunology of Plasmodium falciparum in vitro

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 10	30 Sep 80	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 79	1	155
	CURRENT 80	2	175

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Chulay, J.D., LTC: Williams J., CPT

21. GENERAL USE

Foreign Intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Immunity; (U) Malaria: (U) Tropical Medicine; (U) Antigens; (U) Antibodies

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

23 (U) The objective of this work unit is to exploit newly developed methods of culture of human malaria parasite (Plasmodium falciparum) in order to elucidate the mechanisms of immunity to the organism and explore the feasibility of developing a vaccine against this major military infectious disease problem.

24 (U) The approach is to improve culture technology to produce parasite antigens; to characterize these antigens and evaluate them as vaccine candidates in experimental animals; and to develop in vitro tests which are predictive of protective immunity.

25 (U) 79 10-80-09 Techniques to study immunity to the malaria parasite have advanced sufficiently to justify making this an ordinary work unit. Synchronization has been improved enough to allow the collection of merozoites from culture. Metabolic labelling of parasite proteins with tritiated isoleucine followed by SDS-PAGE has shown differences between trophozoite, schizonts and merozoites stages. Immunoprecipitation of solubilized merozoite antigens with surface reactive, reinvasion blocking immune monkey serum followed by SDS-PAGE shows that several high molecular weight antigens may be involved. Similar studies are underway using strain-specific reinvasion blocking immune sera. Monoclonal hybridoma antibodies have been produced which immunoprecipitate several of these antigens. Efforts continue to produce a monoclonal antibody which will block parasite reinvasion. Progress has also been made in describing non-antibody host factors which inhibit parasite growth, in describing parasite induced changes in erythrocyte membrane nutrient transport, in finding human immune sera which inhibit parasite growth, and in analyzing parasite antigenic stability in culture. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 - 30 Sep 80.

*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: 098 Immunology of Plasmodium falciparum in vitro

INVESTIGATORS: Diggs, C.L., COL, Chulay, J.D., LTC, Haynes, J.D., LTC, and Williams, J.L., CPT.

b. Problem and Objectives: The problem under study is the feasibility of developing a vaccine against the human malaria caused by Plasmodium falciparum. Malaria has been and remains a major impediment to military operations in tropical climates. The current objectives are the continued improvement of methods for collecting and stabilizing merozoites, the further development of assays which will correlate with protective immunity in experimentally immunized animals or humans, the identification of merozoite antigen(s) involved in a protective immune response, and the development of monoclonal hybridoma antibodies against this antigen(s) so that the monoclonal antibody may be used in schemes to obtain and clone parasite DNA which will allow parasite antigen production by bacteria.

c. Results: Current work is based on methods for the continuous culture of P. falciparum simultaneously developed in our laboratory and at the Rockefeller University. We have recently improved methods for synchronizing the growth of this parasite and have obtained small amounts of merozoites from culture. Parasites which have been synchronized twice with sorbitol are now routinely used for in vitro growth inhibition assays and to produce antigens. We have shown that immune serum from monkeys blocks invasion by agglutinating merozoites, causing the appearance of characteristic clusters (1).

Metabolic labelling of parasite proteins with tritiated isoleucine followed by analytical SDS-polyacrylamide gradient gel electrophoresis (SDS-PAGE) has shown differences between the trophozoite, schizont, and merozoite stages. Immunoprecipitation of solubilized merozoite antigens using merozoite surface reactive, reinvasion blocking immune monkey antibodies followed by SDS-PAGE shows that several high molecular weight merozoite-specific antigens may be involved. More definitive studies are underway using two strains of parasites and strain-specific reinvasion blocking immune sera (2).

Merozoites from culture have been used to immunize mice whose immune spleen cells have then been fused with a myeloma cell line to make hybridoma cell lines which produce monoclonal antibody against the parasite. The monoclonal antibodies have several different patterns in indirect immunofluoresecent antibody assays (IFA) with merozoites, and immunoprecipitate several of the same antigens that reinvasion blocking immune serum does. Efforts continue using a new "non-producer" myeloma cell line to make a monoclonal hybridoma antibody that will block parasite reinvasion. Prelimianry results indicate that two of these latest hybridomas produce weakly inhibitory antibody. This is an important step in selecting an antigen for which the cDNA could be cloned in bacteria or yeast to be used as antigen producers.

Progress has also been made in other in vitro assays: Non-antibody host factors (one from non-immune mononuclear cells, the other from normal serum) have been found which seem to slow growth of the parasite rather than block reinvasion the way antibody does. Stage-specific parasite induced increase in erythrocyte membrane nutrient transport has been found. And drug-free human immune sera which cause merozoite clusters and inhibit parasite growth have been found, but still require further evaluation to definatively show that this is due to antibody. Techniques have been developed (serial subculture in the presence of immune serum in microtiter plate wells, and the use of lectin attached monolayer cultures) which allow the evaluation of antigenic variation in culture - the results thus far indicate antigenic stability.

d. Recommendations and future objectives: This work is progressing well, though much still needs to be done along the lines discussed above in "Problems and Objectives". Continued improvement of parasite cultivation, purification, and antigen stabilization are still desirable. Further work should be done to collect immune sera and parasite strains from areas where malaria is endemic (eg. through our research laboratories in Thailand and Kenya) and to evaluate them for antigenic strain differences in the in vitro growth inhibition assay. This would be useful in determining how many different strains might have to be considered in developing a vaccine, and whether human antibodies to some parasite antigenic determinants might be able to transcend strain differences and provide cross-protective immunity. Further work should also be done on possible mechanisms of protective immunity. We believe that monoclonal hybridoma antibodies will provide the most important tool for identifying parasite antigen(s) involved in protective immunity. These will be indentified using the in vitro growth inhibition assay. We expect that these antibodies will be used in an extensive effort combining in-house research and extra-mural contracting to purify the antigen and the mRNA for the antigen, to produce the cDNA, to clone it in bacteria, and to put it into a bacterial or yeast system which will produce the antigen. The antigen(s) can then be evaluated as vaccine candidates in Aotus monkeys before testing in humans.

e. References:

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

(2) Chulay, J.D., J.D. Haynes, and C.L. Diggs. November 1980. Antigenic differences among strains of Plasmodium falciparum. Abstract presented at the annual meeting of the American Society for Tropical Medicine and Hygiene.

f. Formal presentations:

(1) Haynes, J.D. Co-moderator of Symposium "New Developments in Malaria Chemotherapy Using in Vitro Cultures." at the 11th ICC and 19th ICAAC sponsored by the Am. Soc. Microbiol. in Boston, Nov. 1979, at which was also presented a talk "Parasite-specific adenosine deaminase of P. falciparum: A potential target for chemotherapy."

(2) Chulay, J.D. Poster session "Inhibitory effects of immune monkey serum on synchronized P. falciparum cultures." at the 11th ICC and 19th ICAAC of the Am. Soc. Microbiol. in Boston, Nov. 1979.

(3) Haynes, J.D. Three lectures in a course "The use of nuclear methodology and techniques in the study and control of parasitic diseases of humans." sponsored by the International Atomic Energy Agency, in Bethesda, June 1980.

g. Publications:

(1) Desjardins, R.E., C.J. Canfield, J.D. Haynes, and J.D. Chulay. Dec. 1979. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents Chemother. 16:710-718.

(2) Richards, W.H.G., and J.D. Haynes (Co-moderators of Symposium), L.W. Scheibel, R. Sinden, R.E. Desjardins, and W.H. Wernsdorfer. 1980. New developments in malaria chemotherapy using in vitro cultures. Current Chemother. Infect Disease. Proceedings of the 11th ICC and the 19th ICAAC. pp. 10-13.

(3) L.H. Miller, J.G. Johnson, R. Schmidt-Ullrich, J.D. Haynes, D.F.H. Wallach, and R. Carter. 1980. Determinants on surface proteins of Plasmodium knowlesi merozoites common to Plasmodium faciparum schizonts. J. Exp. Med. 151:790-798.

(4) Chulay, J.D., M. Aikawa, C. Diggs, and J.D. Haynes. in press. Inhibitory effects of immune monkey serum on synchronized Plasmodium falciparum cultures. Am. J. Trop. Med. Hyg. (1981).

(5) Chulay, J.D., J.D. Haynes, and C. Diggs. submitted to Infect. Immunity. Antigenic differences among strains of Plasmodium falciparum.

(7 other manuscripts are in preparation).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6464	80 09 30	DD-DRS(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
79 10 01	H. Term	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

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

11. TITLE (Precede with Security Classification Code)

(U) Chemotherapy and Chemoprophylaxis of African Trypanosomiasis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 10	30 Sept 80	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 ESTIMATES		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 79	0.8	84
	CURRENT 80	0.8	92

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
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RESPONSIBLE INDIVIDUAL
NAME: Russell, Philip K., COL
TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

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Div of Experimental Therapeutics
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Davidson, D.E., Jr., COL
TELEPHONE: (202) 576-2292
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Childs, G.E., CPT
NAME: McCormick, G.J.

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Trypanosomiasis; (U) Drug Development; (U) Biology; (U) Chemistry; (U) Toxicology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number, preceded by security classification code.)

23. (U) To find new drugs with chemoprophylactic or chemotherapeutic activity against African trypanosomiasis, a debilitating and frequently fatal disease which would pose a serious threat to military personnel operating in Central Africa. Currently available drugs are neither safe to use nor fully effective in therapy. There are no adequate drugs for prophylaxis.

24. (U) Laboratory models developed in this laboratory will be utilized to test selected chemical compounds from the malaria drug inventory for activity against Trypanosoma rhodesiense: (1) in vitro, (2) in laboratory mice, (3) in cynomolgus monkeys.

25. (U) 79 10-80 09. The in vitro test system developed under this program was used for testing over 700 compounds. Studies correlating activity in vitro to that in vivo were conducted for a number of compounds and chemical classes to ascertain if activity was due to the compounds themselves or to metabolites. By contract, approximately 3000 compounds were tested for activity in vivo and studies were conducted for cross-resistance in strains of T. rhodesiense made resistant to several established drugs. Also by contract, approximately 200 compounds were tested for activity in vivo against T. cruzi; 14 had activity. This Work Unit is being terminated by reason of expiration of its three-year period. Promising studies in progress with laboratory models developed in this program, including the cynomolgous monkey model, will be continued in support of drug development efforts under other work units as appropriate. For Technical Report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79-30 Sep 80.

* Available by contractors upon originator's approval

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

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH
WORK UNIT 102 - Chemotherapy and Chemoprophylaxis of African Trypanosomiasis

Investigators:

Principle: COL David E. Davidson, Jr.
MAJ George E. Childs
Dr. Gerald J. McCormick

PROBLEM AND OBJECTIVES:

Human trypanosomiasis is endemic throughout sub-Saharan Africa, although in most areas, diligent control of the tsetse fly vector has maintained the incidence at relatively low levels in recent years. If fly control were to be disrupted by political upheaval or military operations, trypanosomiasis could be expected to reach epidemic proportions in a short time and to produce large numbers of medical casualties, particularly in non-immune military forces. New drugs are needed because those currently available are neither safe nor fully effective in therapy or prophylaxis.

PROGRESS:

The *in vitro* test system which was developed in this program was used for testing over 700 compounds. Studies correlating activity *in vitro* to that *in vivo* were conducted for a number of compounds and chemical classes to determine if activity was due to the compounds themselves or to their metabolites. Under contract, approximately 3000 compounds were tested for activity *in vivo* against *T. rhodesiense* in mice. Activity was found in 105 compounds. In addition, activity was found in 14 of approximately 200 compounds tested against *T. cruzi* infections in mice. Special studies are being conducted to investigate cross-resistance to candidate drugs in strains of *T. rhodesiense* made resistant to several established drugs (diminazene, suramin, melarsoprol, stilbamidine and pentamidine).

FUTURE OBJECTIVES:

This work unit has been terminated by expiration of its three-year period as a 91C project. Promising studies in progress with laboratory models developed under this work unit, including the cynomolgus monkey model, will be continued as appropriate in support of drug development efforts under other work units.

WORK UNIT 102 - Chemotherapy and Chemoprophylaxis of African Trypanosomiasis

PUBLICATIONS:

1. Casero, R.A., D.L. Klayman, G.E. Childs, J.P. Scovill, and R.E. Desjardins. 1980. Activity of 2-acetylpyridine thiosemicarbazones against Trypanosoma rhodesiense in vitro. Antimicrobial Agents and Chemotherapy. 18 317-322.

2. Desjardins, D.E., R.A. Casero, G.P. Willet, G.E. Childs and C.J. Canfield. 1980. Trypanosoma rhodesiense: A semiautomated microtest system for quantitative assessment of antitrypanosomal activity in vitro. Experimental Parasitology 50; 260-271.

3. Kinnamon, K.E., E.A. Steck, and D.S. Rane. 1979. Activity of antitumor drugs against African trypanosomes. Antimicrobial Agents and Chemotherapy. 15, 157-160.

4. Steck, E.A., K.E. Kinnamon, D.E. Davidson, R.E. Duxbury, A.J. Johnson, and R.E. Masters. Evaluation of 2,5-bis-(4-guanylphenyl) furan dihydrochloride as a trypanoside. (In Press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6466	80 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. DISS'N INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. Term	U	U	NA	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	103
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Infections of Cultured Intestines by Pathogenic Microbes

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology

13A. START DATE	13B. ESTIMATED COMPLETION DATE	14. FUNDING AGENCY	15. PERFORMANCE METHOD
77 10	30 Sept 80	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: NA d. AMOUNT:

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

16. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 79	1.5	30
	CURRENT 80	1.5	35

18. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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NAME: TAKEUCHI, A., M.D.

TELEPHONE (202) 576-2024

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: CHO, H., Ph.D.

NAME:

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Organ Culture; (U) Intestine; (U) Phase Microscopy; (U) Electron Microscopy

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

23(U) 79 10 - 80 09 To clarify interactions between cultured gut mucosa and pathogenic microbes in cluding bacteria, viruses, parasites and microbial toxins. The results will provide new information which should clarify the pathogenesis of acute diarrheal diseases in military personnel.

24(U) Conventional morphologic techniques including phase contrast, light and electron microscopy, histochemistry, immunochemical, biochemical and isotope tracer methods are being used. Methods such as interference microscopy and cinematography will also be utilized.

25(U) 79 10 - 80 09 An organ culture system has been established for the long term in vitro growth and functional and structural maintenance of fetal mouse intestine. As an application study for this system, mouse adenovirus and cholera toxin have been challenged to the cultured intestines. The virus could be readily infected and multiplied in the intestinal explants. Immunofluorescence showed that cells containing the viral particles were arranged in crystal arrays within the nuclei. Cholera toxin could be able to bind the epithelium. However, neither morphological nor functional change was observed except for the elevated intracellular cyclic AMP level. Other organ culture systems for adult mouse colon and small intestine were developed. Utilizing conditional media, the adult mucosal explants were cultured. Their structures and functions were maintained for 7-20 days in an atmosphere of 95 percent oxygen and 5 percent carbon dioxide at 25 C on a rocker platform. Current efforts are directed toward the physico-biochemical characterizations of the adult explants and attachments and changes of intestinal mucosa by shigella and salmonella. This work unit will be continued as a part of the work: Histopathologic Manifestations of Military Diseases and Injuries (3M162770A802). For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 - 30 Sept 80.

* Available to contractors upon originator's approval

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

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 103 Infections of Cultured Intestines by Pathogenic Microbes

Investigators:
Principal: Akio Takeuchi, M.D.
Associate: Han Y. Cho, Ph.D.

Description

Enteric diseases are a leading cause of morbidity in military operations. Intestinal organ culture provides a less complex model than animals for the study of immediate effects of various drugs, and the responses of the intestinal mucosa to various pathogenic microbes and microbe-derived toxins, associated with acute diarrhea. Intestinal organ culture is a sensitive system for the isolation of occult viruses or bacteria that primarily infect the intestine as target organ. Moreover, the utilization of *in vitro* organ culture of biopsies obtained from patients with intestinal mucosal diseases, including tropical sprue, should permit studies not possible *in vivo*.

Problem and Progress

To clarify and quantitate interactions between cultured gut mucosa and pathogenic microbes including bacteria, viruses, parasites and microbial toxins, the Department of Experimental Pathology has developed a reliable and reproducible method for the organ culture of the small and large intestine of experimental animals and man. The results will provide new information which should clarify the pathogenesis of acute diarrheal diseases in military personnel.

Conventional morphologic techniques including phase contrast, light and electron microscopy, histochemistry, immunochemical, biochemical, and isotype tracer methods are being used. Methods such as interference microscopy and cinematography will also be added as the work progresses.

Results

An organ culture system has been established for the long-term *in vitro* growth and functional and structural maintenance of fetal mouse intestine. As an application study for this system, mouse adenovirus and cholera toxin have been used as challenges to the cultured intestines. The virus infected cultured guts and multiplied in the explants. Immunofluoresence showed that cells containing the viral antigen were only localized in the intestinal epithelium and viral particles were arranged in crystal arrays within the nuclei. Cholera toxin was also able to bind the epithelium. However, neither morphological nor functional change was observed, except for the elevated intercellular cyclic AMP level, after 3 hours treatment. These findings are in agreement with the replication of adenovirus and the effect of cholera toxin in the *in vivo*

studies. Thus this system is a suitable in vitro model for the study of pathogenesis of enteric infection. Another long term in vitro organ culture system for adult mouse colon has been established by utilizing a conditional medium and O_2-saturated gas chamber. These cultured adult colonic tissues have been applied for the study of pathogenic mechanisms of enteric shigella and cholera toxin. Previous studies for the organ culture of adult small intestine have been limited by the rapid necrosis of intestinal epithelial cells. These difficulties of the culture have been overcome. Our current study shows that adult mouse small intestine could be cultured in a Tricine buffered conditional medium containing hormones, vitamins and glucose for 7 days in an atmosphere of 95% O_2 and 5% CO_2 at 25°C on a rocker platform (10 cycles per minute). Current efforts are directed toward the physico-biochemical characterizations of the adult explant and attachments and damages to intestinal mucosa by Shigella and Salmonella. These will permit clarification of the pathogenesis of various intestinal infections undisclosed by observation in vivo. This work unit has been terminated and transferred to the other unit of the Department of Experimental Pathology, "Histopathologic Manifestation of Military Diseases and Injuries", (Project Number 3M162770A802).

Abstracts

1. Cho, H. Y., Stockhausen, G. C. and Takeuchi, A.: Response of mouse small intestine in organ culture to cholera. Fed. Proc. 38, 767, 1980.

2. Cho, H. Y. and Takeuchi, A.: The multiplication of adenovirus in fetal mouse intestinal organ culture. Annual Meeting of American Society for Microbiology, May, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OC 6468	2. DATE OF SUMMARY* 80 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 79 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8. DISP'N INSTR'N NL	8a. SPECIFIC DATA CONTRACTOR ACCESS* [X] YES [ ] NO	9. LEVEL OF SUM a. WORK UNIT

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

11. TITLE (Precede with Security Classification Code)*
(U) 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	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 80	2	90
	CURRENT 81	2	179

19. RESPONSIBLE DOD ORGANIZATION
NAME:* Walter Reed Army Institute of Research
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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
NAME:* Walter Reed Army Institute of Research
ADDRESS:* Division of Medicine
Washington, D.C. 20012
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Wright, D.G., MAJ
TELEPHONE: (202) 576-3358
SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE
Foreign intelligence not considered

ASSOCIATE INVESTIGATORS
NAME: Webster, H.K.
NAME: Whaun, J.M. DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Malaria; (U) Metabolism; (U) Computer Simulation; (U) Blood Preservation

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 computer simulation model of intermediary metabolism in the erythrocyte. By constructing separate computer models of the metabolism of uninfected erythrocytes, malaria-infected erythrocytes, and free malaria parasites, one can gain a better understanding of how the parasite utilizes the metabolic machinery of the host erythrocyte. These studies are directed towards understanding parasite specific metabolic pathways that may be targets for the development of new antimalarial chemotherapy that is effective against resistant strains of malaria. Development of such chemotherapy is of major concern to the military because of the necessity to station military personnel in regions of the world where malaria is endemic.

24.(U) Laboratory studies include measurement of (1) intermediates and enzyme level of glycolysis, the pentose cycle, the tricarboxylic acid cycle, and fatty acid synthesis; and (2) intermediates and enzyme levels of the purine salvage pathways.

25.(U) 79 10 - 80 09 Studies of purine salvage pathways in normal and malaria infected RBC have shown high levels of malaria parasite-specific adenosine deaminase in infected RBC which is electrophoretically and functionally distinct from the same enzyme present in normal RBC. In vivo studies of synchronous malaria infection in monkeys demonstrated cyclic variations in purine nucleotides (e.g. ATP, UDP, GTP during infection cycles with changes correlated with different maturational stages of plasmodia in the circulating 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. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79-30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH
Work Unit 106 Red Blood Cell Metabolism

Investigators

CPT H. Kyle Webster, MSC
LTC June M. Whaun, MC
MAJ Daniel G. Wright, MC

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.

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 (African Uganda I strain) and a chloroquine-resistant form (Vietnam Smith strain) have been maintained in continuous erythrocyte culture for 18 and 8 months respectively.

B. HPLC methods were developed and refined for the systematic quantitative analysis of all major purine nucleotides, nucleosides and bases. A dual detector system was established which permits the simultaneous measurement of both concentration and radioactivity for a given purine compound separated by HPLC techniques. Through use of radiolabelled purine precursors and appropriate sampling techniques the above methods were used to map purine 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 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).

1. The specific pathways for "salvage" metabolism of hypoxanthine, adenine and guanine were identified.

2. The metabolism of adenosine and inosine were determined.

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

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 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 the first example 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 apparent only 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 aqueous 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. Hypoxanthine was found to be the major available purine base utilized for synthesis of purine nucleotides by malaria infected erythrocytes in culture.

G. A generalized metabolic response to malaria infection was found to be characteristic of the exposed erythrocyte population as a whole (uninfected and infected RBC). Addition of "spent" culture media to uninfected RBC produced a significant increase in erythrocyte [ATP] concentrations. (This finding may be relevant to erythrocyte pathology in malaria infection).

It was also observed that even at low parasitemias (∿/- 2% PRBC) total erythrocyte ATP levels are exhausted in 24 hours unless media supplementation is provided.

H. There were no detectable differences in the metabolism of purine bases by the drug-sensitive strain of P.falciparum when compared to the drug-resistant strain under conditions of continuous erythrocyte culture.

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 to leishmaniasis infections in humans.

Published Abstracts and Presentations at National and International Meetings

1. Webster, H.K., Harter, T.L., Walker, M.D., and Whaun, J.M. (1980). Purine metabolism in Human Erythrocytes Infected with Malaria in Continuous in vitro Culture. Clin. Research 28(2):326A.

2. Webster, H.K. and Whaun, J.M. (1980). "Simultaneous UV-Radioactivity Measurement of Purine Metabolites During Growth of Human Malaria Parasites in vitro." Fed. Proceeding 39(6):1062.

3. Webster, H.K. and Whaun, J.M. (1980). "Biochemistry of Purines During Continuous Erythrocyte Culture of P.falciparum: Identification of Differences in Host-Parasite Nucleotide Pathways." Fifth International Conference on Red Cell Metabolism and Function. Ann Arbor, MI.

4. Webster, H.K. and Whaun, J.M. (1980). "Selective Action of Bredinin on Purine Metabolism of Malaria-Infected Erythrocytes." 18th Congress of the International Society of Hematology. Montreal, Quebec (859A).

5. Whaun, J.M., Webster, H.K. and Haut, M.J. (1980). "Purine Metabolism in Human Erythrocytes Infected with Malaria: Identification of Parasite Specific Salvage Pathway. 18th Congress of the International Society of Hematology, Montreal, Quebec (860A).

Articles Published, In Press, or Submitted

1. Webster, H.K. and Whaun, J.M. (1980). "Purine Metabolism of P.falciparum Infected Human Erythrocytes During Continuous Culture." In Erythrocyte Structure and Function (Ed., G.J. Brewer) A.R. Liss, New York (In Press).

2. Webster, H.K. and Whaun, J.M. (Tech. Assists., M.D. Walker) (1980). "Application of Simultaneous UV-Radioactivity High Performance Liquid Chromatography to the Study of Intermediary Metabolism: I. Purine Nucleotides, Nucleosides and Bases." J. Chromatography (In Press).

3. Webster, H.K., Haut, M.J., Martin, L.K. and Hildebrandt, P.K. (1980). Studies of Nucleotide Profiles During Synchronous Malaria Infection in Monkeys: A Model for Understanding Nucleotide Requirements of Eucaryote Differentiation. International Journal of Parasitology. (In Press).

4. Webster, H.K. and Whaun, J.M. (1980). "Antimalarial Properties of Bredinin: Prediction Based on Identification of Differences in Human Host-Parasite Purine Metabolism." J. Clin. Invest. (In Review).

5. Webster, H.K. and Whaun, J.M. (1980). "Pathways of Purine Metabolism in Human Malaria Infected Erythrocytes." J. Biol. Chem. (In Review).

6. Webster, H., Lightner, L., Keenan, C., and Hansen, B. (1980). "Tryptophan Metabolism During Experimental Leishmaniasis." J. Experimental Parasitology. (In Review).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OC 6474	80 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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

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	CONT	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
	CURRENT 81	1.0	60

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Hursh, S.R., MAJ
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 interrelationships 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 simulations 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 or decreased signal resolution, or emotional disrupters.

25. (U) 79 10 - 80 09 An extensive study of the activity of cells in the sensory-motor cortex has shown that their sensitivity to whole-body stimulation can be modulated by systemic administration of several drugs. These results point to a sensory modulation system that probably involves the neurochemical GABA. Another study has provided preliminary evidence of the disruptive effects of noise stress on 24-hour response patterns and a reduction in the efficiency of food-producing responses. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 79-31 Sep 80.

*Available to contractors upon originator's approval

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

Project 3A161101A91C IN-HOUSE LABORATOPY INDEPENDENT RESEARCH

Work Unit 107 Neural and Behavioral Response to Sensory Stimulation

Investigators:
Principal: Tyner, LTC, C.F.
Associate: Raslear, CPT T.G. and Spiegelstein, Dr. M.Y.

1. Problem and objective: In this work unit we have sought to: establish models allowing exploration of the relations among sensory stimuli, brain function, and behavior; to increase our knowledge of how an individual's sensory environment controls performance capacity; and to seek explanations in terms of the vocabulary of brain function for behavioral processes such as "perception", "attention", and "vigilence".

The prospect of continuous operations warfare involving sophisticated hardware requires an increase in our 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, chemicals, and other components of extremely stressful environments.

Work to date has used two models. The first involves examining animal behavior and sensory discrimination performance in small laboratory animals exposed to controlled sensory 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.

2. Progress: Progress has been made on two projects intended to study sensory information processing in the auditory system and auditory influences on animal behavior. In the first, concerned with the effects of noise stress on circadian activity, comparisons between periods of quiet and those having 100 dB of noise showed no systematic effects of noise. The second project concerned the measurement of perceived sound duration by animals using the behavioral bisection technique. Perceptual effects similar to those found in humans were observed, suggesting that this method may be the basis for an animal model of the influences of stress, drugs, and fatigue on human perceptual processes.

In the study of brain cells involved in the control of skilled movement, the sensitivity of the cells to sensory stimuli was found to increase transiently following exposure of the animal to painful inputs; this process may represent part of the brain mechanism for "directing attention" to stressful and threatening events, and of preparing the organism for defense and escape behavior. Other results indicated that excessive increase in cell sensitivity may result in loss of specificity of cell function; this mechanism could be part of the physiological basis by which extremely stressful conditions reduces on individuals ability to execute skilled movements accurately. Finally, exposure of the experimental animals to various chemicals has been found to allow successful experimental manipulation of the level of sensitivity of many of these neurons.

3. Future objectives: In FY 81, research in this work unit will focus on extending the applicability of the bisection technique to the study of perceptual processes, and the pharmacologic manipulation of motor-sensory neuron behavior.

Publications

1. Pierrel-Sorrentino, R., and Raslear, T.G. Loudness scaling in rats and chichillas. J. Comp. and Physiol. Psychol.; 1980; 94: 757-766.

2. Raslear, T.G. Differential responding without differential reinforcement: intensity difference, continuum position and reinforcement density effects. J. Exp. Analysis of Behav.; in press.

3. Tyner, C.F., Spiegelstein, M.Y., and Howell, M.L. Pharmacologic control of receptive field size in motor-sensory cortex cats. Physiologist; 1980; 23:22.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6475	80 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
79 10 01	D-Change	U	U	NA	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 (Precede 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	CONT	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	50
FISCAL YEAR CURRENT 81	1	60

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Bates, V. MAJ
NAME: Kant, G.J.

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede 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. Precede 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 nuclectides 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) 79 10 - 80 09 Initial studies in rats kindled to 3 successive days of Stage 5 seizures revealed a significant decrease in cAMP in the stimulated amygdala with no change in the contralateral amygdala or other brain regions. In other studies kindled seizure development was shown to be independent of circadian susceptibility. Studies are in progress on the role of beta adrenergic mechanisms in kindling. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 70 - 30 Sep 80.

* Available to contractors upon originator's approval

DD FORM 1 MAR 83 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 108: Prevention of Post Traumatic Epilepsy

Investigators:
Principal: Meyerhoff, J.L., MD
Associate: Bates, V., M.D., MAJ, MC
Kant, G.J., Ph.D.
Mougey, E.H, M.S.
Collins, D.R., B.S.

Objective:

Medical Management of the soldier following penetrating missile injuries of the brain must reflect the high probability of occurrence of post-traumatic epilepsy. More than 40% of men with penetrating missile injury have post-traumatic grand-mal seizures. The problem is to understand the pathogenesis of these seizures in order to prevent this occurrence after trauma. Caviness, the leading clinical authority on this syndrome, has stressed the military-unique nature of the disorder. High risk of post-traumatic epilepsy has been associated with depth of injury, presence of infection, hypesthesia, coma, nonviable cerebral tissue and parietal location of the injury. There is a characteristic latent period from the injury to the onset of the seizure disorder. The seizures usually begin 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. Although there is a high incidence of post-traumatic epilepsy, it does not develop in all severe head injuries. Thus, it has been postulated that both head injury and an inherent susceptibility, presumably inherited, is required for the development of post-traumatic epilepsy.

Progress:

We have succeeded in setting up the "kindling" technique for producing epileptiform seizures. Kindling consists of repetitive, intermittent low intensity electrical stimulation of subcortical structures, especially the amygdala. This results in progressive changes in electrical activity and behavior, and culminates in a generalized seizure in response to an electrical stimulus which previously had produced no effect. An orderly sequence of appearance of after-discharge and inter-ictal seizure discharges appears initially only in the stimulated amygdala but then successively appears in the neostriatal system, then the midbrain reticular formation before finally causing bilateral neocortical and generalized convulsive activity.

Kindling seems a particularly good model of post-traumatic epilepsy because it permits biochemical study of seizure-prone brain tissue without the use of drugs. Moreover, the latent period seen in the kindling phenomenon is reminiscent of the characteristic delay of seizure onset in post-traumatic epilepsy. In addition to the kindling model, we are studying a strain of rats congentially sensitive to audiogenic seizures in order to assess constitutional factors in the development of post-traumatic epilepsy.

Our initial studies determined that there is no circadian variation in susceptibility to kindled seizures. We have completed a preliminary study in which we found that rats congenitally sensitive to audiogenic seizures have a shorter latency

to development of kindled seizures. Several neurotransmitters have been implicated in seizure disorder, among them norepinephreine, dopamine, taurine, gamma aminobutyric acid and enkephalin. Kindling is facilitated by lesions of noradrenergic pathways or administration of drugs which block the β-adrenergic receptor. Additionally, kindling is associated with a decrease in β-adrenergic receptors in the stimulated and contralateral amygdala. Cyclic nucleotides also modulate neuronal excitability and cyclic AMP appears to be linked to the β-adrenergic receptor. In rats kindled to 3 successive days of Stage 5 seizures, cAMP was found to be decreased in the stimulated amygdala but not in the contralateral amygdala or other brain regions. Studies currently underway are directed at determining when in the course of kindling the cAMP decrease occurs and if with further kindling this cAMP decrease will be seen in other synaptically related regions. In rats congentially sensitive to audiogenic seizures, we have shown significantly lower levels of cyclic GMP in the pineal gland. This change is unrelated to locomotor activity and may be related to differences in noradrenergic tone.

Future Objectives:

Cyclic AMP is increased by anticonvulsants in vitro and it has been postulated that cAMP may be involved in terminating convulsions. Therefore we will measure the in vivo cAMP response to administered anticonvulsants in specific brain regions. We plan further exploration of the role of noradrenergic systems in seizure-suppression. Because certain purines bind to the benzodiazepine receptor in brain, we will investigate the role of such purines in kindling. We also plan to study the role of cholinergic systems in seizure development and in post-ictal suppression of respiration.

Presentations:

Society for Neurosciences, Atlanta, Georgia, Nov. 1979. Kant, G.J., Meyerhoff, J.L., and Corcoran, M.E. "Release of norepinephrine and dopamine in vitro from brain regions of amygdaloid kindled rats."

Society for Neuroscience, Atlanta, Georgia, Nov. 1979. Meyerhoff, J.L., Kant, G.J., Hawkins, T.D., and Lenox, R.H. "Brain cyclic nucleotide levels in rats sensitive to audiogenic seizures."

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: (1980) (in press)

Bates, V.E., Meyerhoff, J.L., Kant, G.J. and Lenox, R.H. "In vivo cyclic AMP levels in rat brain regions following kindling." Neuroscience Abstracts 6:535 (1980)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 006476	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPN INSTR'N	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
79 10 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

11. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	109
b. CONTRIBUTING				
c. CONTRIBUTING				

12. TITLE (Precede with Security Classification Code)

(U) Biochemical Studies on Trypanosomiasis

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 02600 Biology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
78 10	CONT	DA	

18. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER:

c. TYPE: N/A d. AMOUNT:

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

19. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 80	4	110
FISCAL YEAR CURRENT 81	2	246

20. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligience not considered

22. PERFORMING ORGANIZATION

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

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

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

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

23. KEYWORDS (Precede EACH with Security Classification Code)

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

24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. 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 develop immunological and chemotherapeutio 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 biosynthesis of variable surface antigens and the biochemical and genetics mechanisms controlling their elaboration will be studied in order to provide a rational approach to the feasibility of immunotherapy and the development of improved chemotherapeutic measures. Immunoprecipitation methods will be employed to purify variant-specific messenger RNA (mRNA) from isolated polyribosomes and DNA complementary to the mRNA will be prepared and used as a molecular probe to define the genetic interrelationships of the variants and the mechanism of expression of variable surface antigens.

25 (U) 79 10-80 09 The charge heterogeneity of a surface glycoprotein variable antigen was characterized immunochemically by use of monoclonal antibodies with at least two different epitopic specificities. Data indicate that the charge heterogeneous components are very similary and share significant areas of primary amino acid sequence homology. Large size polyribosomes were isolated from disruption trypanosomes by means of discontinuous sucrose gradient centrifugation for Sepharose 4B column chromatography. Optimum conditions for in vitro synthesis of protein were determined. The reactions mixtures analyzed by zonal centrifugation on linear sucrose gradients showed protein to be synthesized and released from polyribosome-mRNA complexes. Specific immunoprecipitation of reactionproducts demonstrated that one of the products was variable polypeptide antigen. For technical report see Walter Reed Army Institue of Research Annual Report 10/1/79-9/30/80.

* Available to contractors upon originator's approval

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Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 109 Biochemical Studies on Trypanosomiasis

Investigators:
Principal: Bhupendra P. Doctor, Ph.D.
Associate: John G. Olenick, Ph.D., SP5 Richard W. Travis, Ph.D., Seymour Garson, Ph.D., John A. Kintzios, CPT J.A. Lyon

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 to a rational evaluation of the feasibility of immunotherapy, the application of chemotherapeutic measures, and the development of improved diagnostic procedures. The studies reported here are primarily concerned with the biochemistry and molecular biology of antigenic variation in salivarian trypanosomes.

1. Use of Monoclonal Antibody to Immunochemically Characterize Variant-Specific Surface Coat Glycoprotein from Trypanosoma rhodesiense.

2. Trypanosoma rhodesiense: Chemical and Immunological Characterization of Variant-Specific Surface Coat Glycoprotein.

1. Use of Monoclonal Antibody to Immunochemically Characterize Variant-Specific Surface Coast Glycoprotein from Trypanosoma rhodesiense.

Monoclonal antibodies derived from fused cells were employed to study the molecular basis for charge heterogeneity in variant-specific surface coat glycoprotein. Hybridomas were constructed by fusing murine plasmacytoma cells to spleen cells from mice immunized with purified surface coat glycoprotein prepared from clone CP3B4 of the Wellcome strain of Trypanosoma rhodesiense. Hybridomas secreting antibody reactive to CP3B4 glycoprotein were identified by means of immunofluorescent assays using culture supernatants and acetone-fixed smears of parasitized rat blood. Fourteen hybridomas produced CP3B4 glycoprotein-specific antibody. Culture supernatants from cloned hybrids were assayed for cross-reactivity with acetone-fixed variant antigenic types 6, 10, 12, and 13 of the Wellcome strain CP3B4 serodeme of Trypanosoma rhodesiense. At least 1000 parasites of each variant type were examined for immunofluorescent staining and no reaction of TRW 1 series monoclonal antibodies with any of these variant antigenic types was observed. No parasite heterogeneity was found in immunofluorescent assay analyses of acetone-fixed smears of

cloned CP3B4 parasites. The molecular specificities of monoclonal antibodies were evaluated by quantitatively immunoprecipitating ^{125}I-CP3B4 surface coat glycoprotein. Hyperimmune rabbit antiserum precipitated 70% of the ^{125}I-labeled material in the glycoprotein preparation. Monoclonal antibodies reacted to the extent of 40%. Monoclonal antibody was permitted to react with ^{125}I-labeled surface coat glycoprotein and the precipitated antigen was subjected to isoelectric focusing. Every monoclonal antibody tested by this method was able to selectively precipitate the three major components of the charge heterogeneous glycoprotein antigen, thus indicating that the three bands represent one basic glycoprotein since the antigenic determinants for the four monoclonal antibodies are present on each of the three bands. The four CP3B4-specific monoclonal antibodies were also tested for reaction with live trypanosomes. Only one was able to react indicating that the antigenic specificity at the determinant level differs from the specificities of the other three. Since each of the charge heterogeneous components of a surface coat glycoprotein are precipitated by monoclonal antibodies specific for at least two different epitopes, it is highly unlikely that clone CP3B4 simultaneously elaborates two or more distinct surface coat glycoproteins. Thus, although the components of purified surface coat glycoprotein from CP3B4 differ with respect to molecular charge, they are immunochemically very similar and share significant areas of primary amino acid sequence homology. It is concluded that charge heterogeneity observed in preparations of CP3B4 surface coat glycoprotein obtained from an essentially homogeneous population of trypanosomes is a manifestation of minor differences or alterations (including limited proteolysis) in the molecule that are readily resolved by electrofocusing.

2. Trypanosoma rhodesiense: Chemical and Immunological Characterization of Variant-Specific Surface Coat Glycoproteins.

Soluble surface coat glycoproteins were purified by Concanavalin A affinity chromatography from variant populations of Trypanosoma rhodesiense (Wellcome strain). Analyses of surface coat glycoprotein preparations from clone CP3B4 and the derived variants 6, 10, 12, and 13 by SDS-polyacrylamide tube gel electrophoresis under reducing conditions revealed only one component for each preparation with apparent molecular weights of 65,000, 68,000, 67,000, 59,000, and 58,000 daltons, respectively. The charge heterogeneity of the various surface coat glycoproteins was examined by isoelectric focusing in standard thin-layer polyacrylamide slab gels over a pH gradient 3.5 to 9.5. Charge heterogeneity analyses resolved from one to four closely spaced components with isoelectric points that were considerably different from variant to variant. Charge heterogeneity persisted under a variety of isolation conditions designed to eliminate or promote heterogeneity. Surface coat glycoproteins were prepared in the presence of 1mM each of the protease inhibitors, N-tosyl-L-lysyl-chloromethane and N-tosyl-L-phenylalanyl-chloromethane. Glycoprotein

isolations proceeded from trypanosomes harvested from rats at 3 or 4 days postinfection and from rats that were either irradiated or non-irradiated prior to infection. The supernatant fluid obtained from the overnight extraction of trypanosomes was incubated at 37° C to deliberately augment the activity of any released endogenous proteases. Glycoprotein preparations, dissolved in distilled H_2O at 1 mg protein per ml, were subjected to several cycles of freezing and thawing. In all instances, the protein banding pattern upon isoelectric focusing was qualitatively and quantitatively the same. Amino acid analyses revealed notable variations in amino acid compositions. The surface coat glycoproteins were found to be rich in asparate-asparageine and glytamate-glutimine pairs and in threonine, alanine, and lysine. Methionine was detected only in CP3B4. Antigenic activities of purified variant-specific surface coat glycoproteins were tested by determining if variant-specific immunoprotection against trypanosome infection could be produced in mice. Immunization of mice with purified glycoprotein protected them from homologous, but not heterologous, variant trypanosome infection. In immunoelectrophoretic or immunodiffusion tests, a single precipitin line was produced with homologous glycoproteins and hyperimmune sera raised in rabbits to purified glycoproteins; no interaction was detected in heterologous antiserum-glycoprotein combinations. The present study has delineated chemical and immunological properties of surface coat glycoproteins isolated from variants of T. rhodesiense. The results attest to the immunogenic specificity and chemical uniqueness of the individual glycoprotein preparations, which leads to the conclusion that variable antigenicity of T. rhodesiense resides in variant-specific surface coat glycoproteins.

Future investigations are intended to furnish information on the cellular and molecular synthesis of variant antigens and on the biochemical and genetic mechanisms controlling antigen synthesis. The charge heterogeneity of surface coat glycoproteins will be further characterized with a view to defining the nature of the microheterogeneity. Variant antigen synthesizing polysomes will be employed in trypanosomal amino acid translation systems and the synthesized variant protein will be characterized with an intent to demonstrate that the variant protein is a secretory protein containing a "signal" sequence. Variant antigen specific polyribosomes will be isolated from polysomes by immunoprecipitation methods and messenger RNA will be prepared from the specific polyribosomes. DNA complementary to the mRNA will be enzymatically synthesized by a reverse transcriptase and the single-stranded DNA will be used as a molecular probe to define the genetic interrelationships of the variants and the mechanism of expression of variable surface antigens.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOG 2531	80 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. OVER'N INSTR'N	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

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

11. TITLE (Precede with Security Classification Code)

(U) Genetic Basis of Virulence of Invasive Bacterial Pathogens

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: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		c. PROFESSIONAL MAN YRS	d. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 80	2.0	150
	CURRENT 81	2.0	150

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Wash. DC 20012

RESPONSIBLE INDIVIDUAL

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TELEPHONE: (202) 576-3551

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NAME: Walter Reed Army Institute of Research
Division of Communicable Diseases

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Washington D.C. 20012

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

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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

Foreign intelligence not considered

ASSOCIATE INVESTIGATORS

NAME: GEMSKI, P.

NAME:

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Genetic; (U) Virulence; (U) Antigens; (U) Plasmids; (U) Chromosomal

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

23U) The objective is to study the chromosomal and plasmid genes controlling virulence determinants of invasive enteric pathogens and to alter by genetic manipulation such virulence factors sc as to define their role in pathophysiological and invasive steps of diseases and to develop attenuated vaccines and improved methods fo 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 somatic antigens, toxins and other factors and to assess the impact of such alterations on virulence.

25(U) 79 10 - 80 09 Studies of the virulence and plasmid properties of Yersinia enterocolitica revealed a plasmid, 42.2 megadaltons in size (Vwa), which is associated with the pathogenicity and calcium dependency of this pathogen. We have also shown that Yersinia pseudotuberculosis can possess plasmids which are similar to size and function to the Vwa plasmids of Y. enterocolitica. Further investigation of these plasmids from Y. pseudotuberculosis and Y. enterocolitica with restriction endonucleases revealed significant differences in their fragementation pattern, indicating a divergence ir the micro-evolution of the virulence determinants associated with Vwa plasmids. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon submitter's approval

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT 110 Genetic Basis of Virulence of Invasive Bacterial Pathogens

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

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 the 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.

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 to the production of the V and W antigen complex, an important virulence determinant of plague bacilli. The 3 strains of Y. enterocolitica (Y7P, WA, CDC2635) chosen for study were found to have similar invasive properties. Their invasiveness could readily be demonstrated in guinea pigs using the Sereney Test for conjunctivitis. These strains also were found to be lethal to mice challenged either orallyor intraperitoneally. Oral infection of mice results in the fatal systematic disease. Within 4-14 days following infection, invasion of the liver and spleen by the challenge organism could be readily detected in moribund mice using standard bacteriological techniques. All three strains had an LD_{50} of about 100 cells, determined from the results of i.p. injection of graded doses of the pathogens. Similar animal studies were performed with non-invasive derivatives

of these strains. These derivatives failed to cause conjunctivitis in guinea pigs and a fatal infection in mice.

The plasmid content of Y7P, WA, CDC 2635 and their respective non-invasive derivatives was next examined. Purified covalently closed circular plasmid DNA was recovered from Triton-X cell lysates of Y7P by means of cesium chloride-ethidium bromide density gradient centrifugation and examined for contour length under the electron microscope to determine the molecular size of plasmids. Plasmid pSC101, with a molecular weight of 6.0 $\pm$ 0.4 megadaltons (Mdals) served as the reference standard. Two plasmid classes were distinguished in Y7P; 42.2 $\pm$ 0.9 Mdals (30 molecules measured). The plasmid profiles of various strains following electrophoretic analysis of ethanol precipitated DNA isolated from cleared bacterial lysates was also compared in WA and CDC 2635. Both, also possessed a plasmid which is 42 Mdals in size. The avirulent derivatives, however, had lost this plasmid. This plasmid class has been designated Vwa.

The availability of isogenic pairs of Y. enterocolitica which differ in their virulence properties has allowed us to initiate studies of the nature of the invasive determinant that this plasmid confers on Y. enterocolitica. Our initial efforts have addressed properties long-recognized to be related to the virulence of the yersiniae. One such property is the ability to plague bacilli to produce the V (protein) and W Lipoprotein) antigen complex. We have demonstrated, for the first time, that Y. enterocolitica produces V-W antigens and that this property is also associated with Vwa plasmids.

B. Plasmids of Yersinia pseudotuberculosis.

Although Y. pseudotuberculosis is associated classically with fatal, acute, septicemic infections of rodents it is also recognized that this pathogen can cause enteric disease in man characterized by diarrhea, mesenteric lymphadenopathy and symptoms of appendicitis. It is evident, therefore, that these clincal manifestations of infections by Y. pseudotuberculosis can in some circumstances be similar to those described for acute gastroenteritis caused by Y. enterocolitica. A consideration of these similarities as well as the knowledge that both species share the V and W virulence antigens led to the examination of the plasmid content of a virulent, invasive strain of Y. pseudotuberculosis III. A comparison of the plasmid profile of Y. pseudotuberculosis strain III with that of Y. enterocolitica strain Y7P was done. It was evident that Y. pseudotuberculosis strain III contains a plasmid of about 42 Mdals, similar to that in strain Y7P. With the finding of a plasmid in Y. pseudotuberculosis similar in size to that of Y. enterocolitica Y7P, we tested stain III for its calcium dependency when grown at 37C on magnesium oxalate agar. After overnight incubation, the growth of strain III was severly inhibited at 37C but not 26C, indicating calcium dependency and hence expression of the V and W antigens. Three clones of strain III, recovered at 37C as colonies resistant to growth inhibition on magnesium oxalate agar, were examined for their invasive and plasmid properties. All failed to provoke positive conjunctivitis and had lost Vwa. It is apparent that this virulent strain of Y. pseudotuberculosis is similar to Y. enterocolitica in that it contains a plasmid associated with pathogenicity and production of the V and W antigens.

We investigated further the similarity of the DNA from these 42 Mdal plasmids of Y. pseudotuberculosis and Y. enterocolitica by comparing the fragmentation pattern that results after their digestion by restriction endonucleases. Treatment of DNA from both plasmids with the restriction enzyme Bam Hl yields some fragments that are the same size, but in general, the fragment banding pattern is distinct for the two plasmids. Similarly, Eco Rl restriction enzyme digestion of these plasmids yields patterns which are different. Even though the plasmids from Y. pseudotuberculosis and Y. enterocolitica appear similar in size and function, the location of the specific cleavage sites of the restriction endonuclease are not found at the same positions in the plasmid molecules. Therefore, there are differences in the plasmids at the molecular level.

PUBLICATIONS

1. Gemski, P., J.R. Lazere and T. Casey. 1980. A Plasmid Associated with Pathogenicity and Calcium Dependency of Yersinia enterocolitica. Infect. Immun. 27:682-685.

2. Gemski, P., J.R. Lazere, T. Casey and J.A. Wohlhieter. 1980. Presence of a Virulence Associated Plasmid in Yersinia pseudotuberculosis. Infect. Immun. 28:1044-1047.

3. Keren, D.F., P.S. Holt, H.H. Collins, P. Gemski and S.B. Formal. 1980. Variables Affecting local Immun Response in Ileal Loops. Role of Immunization Schedule, Bacterial Flora and Postsurgical Inflammation. Infect. Immun. 28:950-956.

ABSTRACTS AND PRESENTATIONS

1. Lazere, J. and P. Gemski. 1980. Avirulent Colonial Variants of Yersinia enterocolitica. Am. Soc. Microbiol., Abst. B11.

2. Griffin, D.E. and P. Gemski. 1980. Isolation of Minicell-Producing Mutants of Shigella. Am. Soc. Microbiol., Abst. D50.

3. Gemski, P., J.R. Lazere and J.A. Wohlhieter. 1980. Plasmids Associated with Pathogenicity of the Genus Yersinia. FEMS Symposium on Microbial Envelopes. Saimmaanranta, Finland; Abst 120.

4. Keren, D.F., H.H. Collins, P.S. Holt, P. Gemski. and S.B. Formal. 1979. The Local Humoral Immune Response to Shigella flexneri Hybrids using Thiry-Vella Loops in Rabbits. Proc. 15th Joint Conference on Cholera. Bethesda, Md., p. 41.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 03 2526	2. DATE OF SUMMARY* 80 09 30		REPORT CONTROL SYMBOL DD-DR&E-AR-636
3. DATE PREV SUMRY 79 10 01	4. KIND OF SUMMARY H. Term	5. SUMMARY SCTY* U	6. CORK SECURITY* U	7. REGRADING* NA	8. DISP'N INSTR'N NL	8a. SPECIFIC DATA CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT
10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61101A	3A161101A91C		00	111		
b. CONTRIBUTING							
c. CONTRIBUTING							

11. TITLE (Precede with Security Classification Code)*
(U) Class Specific Immunoglobulin Response to Dengue Virus Infection

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	30 Sept 80	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 79	0	0
	CURRENT 80	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 Institution)
NAME:* Burke, D.S.
TELEPHONE: 281-7776
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 objectives were (a) To develop a sensitive and specific solid phase radio-immunoassay (SPRIA) for detection of immunoglobulin class specific (IgG, IgA, IgM) anti-dengue antibodies in sera of patients with acute dengue infections: (b) to develop methods for separation and purification of immunoglobulin classes from serum of patients with acute dengue infections, and assay the contribution of IgG, IgA, and IgM to the total antibody response as measured by hemagglutination inhibition (HI), plaque reduction neutralization (PRNT), complement fixation (CF), and solid phase radio-immuno[illegible] (SPRI[illegible]d (c) to examine and compare the anti-dengue immunoglobulin class response of patients with "uncomplicated" dengue infections against those with dengue hemorrhagic fever. There is a requirement for this research which will provide basic data for the design and preparation of vaccine to prevent dengue fever, a disease of significant military importance.

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

25. (U) 79 10 - 80 . Acute and convalescent phase blood specimens were obtained from patients with dengue hemorrhagic fever (DHF) and non-dengue febrile illness (NDFI). Serum 19S dengue antibodies were found by HAI or BI-ELISA in convalescent sera from 10/10 primary DHF, 8/10 secondary DHF and 0/20 NDFI patients. IgM and IgA dengue antibodies were detectable by direct ELISA in sera of 10/10 and 7/10 primary DHF, 10/10 and 9/10 secondary DHF, and 2/20 and 4/20 NDFI patients, respectively. Specific anti-dengue IgG and IgM was detected by RIA within 24 hours in microcultures containing as few as 2 X 10 3 and 2 X 10 5 mononuclear leukocytes, respectively. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sept 80.

*Available in open literature upon originator's approval

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

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 111 Class Specific Immunoglobulin Response to Dengue Virus Infection

Principal Investigator: Donald S. Burke

Problem: Dengue hemorrhagic fever (DHF), the most severe form of dengue fever, occurs more frequently in persons previously infected by a dengue virus than in persons experiencing their initial dengue infection. This project sought to determine whether the antibody response in DHF patients with second dengue infections differed from that in primary infections.

Progress: A reverse solid phase immunoassay was developed in which the solid phase is first coated with heavy chain specific anti-human immunoglobulin in contrast to conventional solid phase immunoassays in which the solid phase is first coated with antigen. In the reverse system, antibody of the desired class (IgG, IgM, or IgA) is concentrated onto a lawn covering the solid phase and competing immunoglobulins of other classes are washed from the system. Labelled antigen then adheres to the solid phase only if the bound class specific immunoglobulin has antibody specificity against that antigen. At concentrations of immunoglobulin down to 300 ng/ml this reverse immunoassay is saturated i.e. at immunoglobulin concentrations equivalent to a 10^{-5} dilution of serum for IgG or a 10^{-4} dilution for IgM. Thus, even at very low concentrations of immunoglobulins, the results are proportional to the relative concentrations (% of all immunoglobulin molecules) with anti-dengue activity, rather than to the absolute concentration of anti-dengue immunoglobulin molecules.

Acute and convalescent phase sera were obtained from patients with DHF and non-dengue febrile illnesses (NDFI). IgM and IgA dengue antibodies were detectable by direct Elisa in sera of 10/10 and 7/10 primary DHF, 10/10 and 9/10 secondary DHF, and 2/20 and 4/20 NDFI patients respectively. Specific anti-dengue IgG and IgM was detected by RIA within 24 hours in microcultures containing as few as 2×10^3 and 2×10^5 mononuclear leukocytes, respectively, obtained from individual patients.

The reverse immunoassay has proven to be an especially powerful tool to detect antibody activity in biological fluids where the absolute immunoglobulin concentration is low but the proportion of immunoglobulin molecules with specific antibody activity is high. Two such biological fluids are cerebrospinal fluid (where the system could provide a sensitive and specific assay for CSF antibody for rapid diagnosis of viral encephalitis) and in vitro cultures of peripheral blood leukocytes from patients with dengue (in which production of immunoglobulins could be detected with 24 hours for rapid diagnosis).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 3110	80 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. DISG'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. Term	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

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

11. TITLE (Precede with 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
79 10	CONT	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 79	0	0
FISCAL YEAR CURRENT 80	1.0	7

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
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TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Neuropsychiatry

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

NAME: Hursh, S.R., MAJ

TELEPHONE: (202) 576-2483

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Faden, A.I., MAJ (P)

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

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Nervous System; (U) Pharmacology; (U) Stress; (U) Injury

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

23. (U) This exploratory project will be directed at assessing simple chemical tools for the treatment of major neural and stress-related disabilities associated with military activities.

24. (U) Using the research methods of physiology and pharmacology, laboratory animal models of neural injury and stress-induced functional deficits will be standardized and replicated. A variety of pharmacologic intervention therapies will be explored in relation to these injuries, both to establish possible therapeutic modes and to elucidate the mechanisms which underlie the processes.

25. (U) 79 10 - 80 09 The opiate antagonist naloxone was administered 45 min. following spinal trauma to prevent neurological injury in experimental subjects. Bolus infusion of 2 mg/kg and continued infusion for 4 hrs. was effective in increasing mean arterial pressure, decreasing the extent of neurological damage 3 weeks after injury and decreasing necrotic pathology in grey and white matter of the spinal cord. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 79 - 30 Sep 80.

* Available to contractors upon originator's approval

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

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 112 Pharmacologic Modulation of Effects of Neural Damage and Stress

Investigators:
Principal: Hursh, S.R., MAJ
Associate: Faden, A.I., M.D.
Holaday, J.W., Ph.D.

1. Problem and Objectives: 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.

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 pressure to increase spinal injury, the elevation of peripheral blood pressure to increase spinal cord perfusion pressure and limit ischemia has promise as a therapeutic 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. We therefore investigated the possibility that naloxone would increase perfusion pressure following spinal cord injury which would then improve spinal cord blood flows and thereby reduce the area of ischemia and resulting neurological deficits.

2. Progress: Spinal trauma was induced in anesthetized experimental animals (cats) was by the method of Allen. Subjects were catheterized for monitoring cardiovascular parameters and delivering drugs. Initial experiments involved a comparison of the effects of a control solution of physiological saline with the effects of the opiate antagonist, naloxone (2mg/Kg bolus, followed by 2mg/Kg/M for 4 hours) administered 45 minutes following cord injury. Cardiovascular parameters were monitored prior to and following injury and subsequent treatment. Four hours following the initiation of treatment, experimental animals were surgically restored and allowed to recover. Neurological examinations were conducted 24 hours following injury by two Neurologists blinded as to which cats received which treatment.

The Allen procedure was shown to result in reproducible decreases in peripheral blood pressure 45 minutes following spinal cord injury. Neurologic deficits, characterized by a five point scale ranging from complete quadriparesis to normal function, were also prominent 24 hours following injury, in saline treated (control) cats, with the median animal having frequent limb movements and unable to stand. In sharp contrast to the saline treated animals, the

naloxone-treated cats showed a significant improvement in cardiovascular parameters over the 4 hour period of physiological monitoring. Moreover, at 24 hours following injury, the median naloxone-treated cats were able to stand and showed significantly less paralysis than their saline-treated counterparts. Preliminary information obtained at autopsy showed this improved neurological outcome in naloxone-treated cats were accompanied by confirmatory histopathological evidence of decreased ischemic damage (necrosis) in the spinal cords of naloxone-treated cats when compared to saline-treated control animals. Plasma levels of endogenous opiate substanced, which are believed to result in the decrease in blood pressure following spinal cord injury, were significantly elevated following spinal trauma. Thus, evidence supports the hypothesis that the opiate antagonist naloxone reverses these effects and therefore is of therapeutic value in the prevention of irreversible neurologic damage subsequent to acute spinal cord injury and trauma.

3. Future Objectives: In the next year experiments will be conducted to more precisely determine the range of effective doses of naloxone, the limits on the time between injury and naloxone treatment, and the mechanisms of action of this therapeutic modality.

Publications

1. Faden, A.I., Jacobs, T.P., and Woods, M. Cardiac contractility and the spinal sympathetic neuron. Neurol. Research 1:227-237, 1980.

2. Faden, A.I. and Holaday, J.W. Opiate antagonists: A role in the treatment of hypovolemic shock. Science 205:317-318, 1979.

3. Faden, A.I. and Holaday, J.W. Naloxone reversal of hypotension caused by spinal transection. In: Way, E.L. (Ed.), Edogenous and Exogenous Opiate Agonists and Antagonists, London, Pergamon Press, pp. 483-486, 1980.

4. Faden, A.I. and Holaday, J.W. Naloxone alteration of physiologic parameters in spinally transected animals. Trans. Amer. Neurol. Assn., 104:1-5, 1979.

5. Reynolds, D.G., Gurll, N.J., Vargish, T., Lechner, R., Faden, A.I., and Holaday, J.W. Blockade of opiate receptors with naloxone improves survival and cardiac performance in canine endotoxic shock. Circ. Shock 7:39-48, 1980.

6. Faden, A.I. and Holaday, J.W. Naloxone treatment of endotoxic shock: Stereospecificity of physiologic and pharmacologic effects in the rat. J. Pharm. Exper. Ther. 212:441-447, 1980.

7. Faden, A.I. and Holaday, J.W. A role for endorphins in the pathophysiology of endotoxin shock. J. Infectious Dis. (In press)

8. Faden, A.I. and Holaday, J.W. The role of endorphins in the pathophysiology of spinal injury. In: Martin, J.B. et al (Eds.), Neurosecretion and Brain Peptides: Implications for Brain Function and Neurologic Disease, New York, Raven Press. (In press)

9. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Endorphin-parasympathetic interaction in spinal shock. J. Autonom. Nerv. System. (In press)

10. Faden, A.I. and Mendoza, E. Peripheral sensorimotor neuropathy associated with chronic obstructive pulmonary disease: A prospective clinical and electrophysiologic study. Arch. Neurol. (In press)

11. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Opiate antagonist improves neurologic recovery after spinal injury. Science. (In press)

Presentations/Abstracts

1. Faden, A.I. and Holaday, J.W. Opiate antagonists: A role in the treatment of hypovolemic shock. Yearbook of Anesthesiology, 1980.

2. Faden, A.I. and Holaday, J.W. Naloxone reversal of hypotension caused by spinal transection. Int. Narc. Res. Conf., 1979.

3. Faden, A.I., Jacobs, T.P., and Holaday, J.W. A possible pathophysiologic role for endorphins in spinal injury. The Physiologist. (In press)

4. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Naloxone improves blood pressure and neurological recovery following cervical spinal trauma. Neurology 30:433, 1980.

5. Faden, A.I. and Holaday, J.W. A role for endorphins in the pathophysiology of spinal cord injury. Meeting on Neurosecretion and Brain Peptides, 1980.

Invited Lectures

1. Brooke Burn Shock Center

2. Presbyterian University of Pennsylvania Medical Center

3. National Institutes of Health

4. University of South Alabama

5. Indiana University

6. University of California, San Francisco

7. American Nuerologic Association

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DAOG 2532	2. DATE OF SUMMARY* 80 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 79 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N NA	8b. SPECIFIC DATA - CONTRACTOR ACCESS ☐ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

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

11. TITLE (Precede with Security Classification Code):*
(U) Immune mechanisms in leishmaniasis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
010100 Microbiology

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 80	1.0	60
	CURRENT 81	4	100

19. RESPONSIBLE DOD ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: (202) 576-3544
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Nacy, C., Ph.D.
NAME:

21. 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 and on 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) 79 10-80 09 L. tropica amastigotes replicated in suspension cultures of peritoneal macrophages: mean number of amastigotes increased from 1.3-6.8 amastigotes/infected cells in 72 hr. Increasing number of amastigotes in inoculum increased percent infected cells to a maximum of 65 percent. Treatment of macrophages with lymphokines markedly reduced the number of infected macrophages by two distinct mechanisms: (1) an initial 25-35 percent decrease in infected cells and (2) a subsequent 95 percent decrease in infected cells (intracellular killing) compared to controls. Three lymphokines have been isolated which induce intracellular killing: they are heat-labile molecules of 140,000, 50,000, and less than 10,000 M.W. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

* Available to contractors upon originator's approval

PROJECT 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 113 Immune Mechanisms in Leishmaniasis

INVESTIGATORS: Nacy, C.A.; Pappas, M.G., Henry, R.

b. Problem and Objective: The objective is to investigate leishmania macrophage interaction in vitro in an effort to isolate stages in which it is feasible 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.

c. Progress: The approaches used were to harvest peritoneal macrophages from C3H/HeN mice and to evaluate intracellular replication of leishmania amastigotes in macrophages in suspension cultures. Macrophages were activated with soluble T-lymphocyte products (lymphokines) and the effect of lymphokine activation on infection and intracellular replication of leishmania was assessed.

The mean number of Leishmania tropica amastigotes increased from 1.3 to 6.8 amastigotes per infected macrophages in 72 hours in in vitro suspension cultures of mouse peritoneal macrophages. The number of macrophages infected varied up to a maximum of 65% depending on the number of amastigotes in the inoculum. The following results were obtained when macrophages were treated with lymphokines: 1) treatment of macrophages 4 hrs prior to infection induced an initial 25 to 35% decrease in infected cells and subsequent cytostatic activity in the remaining infected macrophages; 2) pretreatment of macrophages 6 to 8 hrs induced some intracellular killing; 3) macrophages treated after infections showed a 93-95% decrease in infected cells as compared to controls. The post-infection treatment allowed analysis of intracellular killing apart from the initial effects of reduced infectivity. Three lymphokines have been isolated which induced intracellular killing of leishmania in macrophages. They are heat-labile molecules with molecular weights of 140,000, 50,000 and less than 10,000. Three mouse strains are at present being assessed as appropriate models of visceral leishmaniasis - DBA/2; C7BL/10 and B10.D2. Leishmania injected by IP, IV, or ID routes localized in the spleen, liver and bone marrow similar to the clinical picture in humans, but to date there have been no lethal infections.

d. 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.

e. Reference cited: None

f. Presentations:

1) Nacy, C.A., G. Radlick, and J.V. Osterman. Nonspecific resistance to scrub typhus. Annual Meeting of Soc. for Tropical Medicine and Hygiene, Tucson, Arizona, November , 1979.

2) Nacy, C.A., G. Radlick, and M.S. Meltzer. Role of activated macrophages in natural resistance to Rickettsia akari, 1980. International Symposium on Genetic Control of Natural Resistance to Infections and Malignancy, Montreal, Canada. March 1980.

3) Nacy, C.A., M.S. Meltzer, and D.W. Wyler. Lymphokine activation of mouse peritoneal macrophages for killing of Leishmania tropica. Annual, Meeting of Federation of American Scientists for Experimental Biology, Los Angeles, California, April 1980.

4) Nacy, C.A., E. J. Leonard, M.S. Meltzer. Characterization of lymphokines whikh induce intracellular killing in macrophages. 4th International Congress of Immunology, Paris, France, July 1980.

5) Meltzer, M.S., C.A. Nacy, E.J. Leonard. Dissociation of macrophage function during lymphokine activation. International Workshop on Heterogeneity of Mononuclear Phagocytes, Vienna, Austria, July 1980.

6) Nacy, C.A. and M.S. Meltzer. Macrophages in resistance to rickettsial infections. Metchnikoff Phagocytosis Meeting, Sicily, Italy, September 1980.

g. Publications:

1) Nacy, C.A., M.S. Meltzer, P.K. Russell, and J.V. Osterman. 1979. Immunity to Rickettsia tsu sugamushi infection induced by nonspecific activating agents. Fed. Proc. 38.

2) Nacy, C.A., G. Radlick, and M.S. Meltzer. 1980. Role of activated macrophages in natural resistance to Rickettsia akari. Symposium on Genetic Control of Natural Resistance to Infections and Malignancy, Montreal, Canada, p. 29.

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4) Nacy, C.A., E.J. Leonard, and M.S. Meltzer. 1980. Characterization of lymphokines which induce intracellular killing in macrophages. Abst. 4th Internat'l Congress of Immunology, Paris, France.

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10) M.S. Meltzer and C.A. Nacy. 1980. Macrophages in resistance to rickettsial infection: Susceptibility to lethal effects of Rickettsia akari infection in mouse strains with defective macrophage function. Cell. Immunol. 4: 487.

11) M.S. Meltzer, L.W. Wahl, E.J. Leonard, and C.A. Nacy, 1980. Macrophage activation by lymphokines: characterization of lymphokine signals for tumoricidal and microbicidal activities and for prostaglandin synthesis. In Proc. Second Internat'l Lymphokine workshop. Alan L. de Weck ed. Academic Press, p. 161.

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).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DAOG 2525	2. DATE OF SUMMARY* 80 10 01		REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 79 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61101A	3A161101A91C		00	114		
b. CONTRIBUTING							
c. CONTRIBUTING							

11. TITLE (Precede 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 80	2.0	150
FISCAL YEAR CURRENT 81	2.0	150

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME:

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Liposomes; (U) Chemoprophylaxis; (U) Drug Development (U) Chemistry; (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 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 artileishmanial 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) 79 10-80 09. Liposomes in a variety of compositions were prepared by incorporation of compounds such as palmitic acid, 24:0 galactocerebroside, 24:0 glucocerebroside, dipalmitoylphosphatidic acid, alpha-tocopherol and egg phosphatidyl choline into the lipid membrane. Preparations were tested for stability, viability and capacity for trapping drugs. Drugs used in studies included glucantime, pentostam, rifampin, lampit, and WR 6026, among others. Efficacy of liposome-encapsulated drugs was tested in 17 experiments in animal models of leishmaniasis; 15 of the experiments were performed in hamsters and two in dogs. Liposome-encapsulated glucantime had prophylactic activity and was effective when administered eight days prior to infection. WR 6026, an 8-aminoquinoline, was 700 to 1800 times more effective in liposomal preparation than was unencapsulated glucantime. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79-30 Sep 80.

*Available to contractors upon originator's approval

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

PROJECT 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH
WORK UNIT 114 - Liposomes for Treatment of Leishmaniasis

Investigators:

Principle: LTC Larry D. Hendricks
LTC Carl Alving

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:

Liposomes in a variety of compositions were prepared by incorporation of compounds such as palmitic acid, 20:0 galactocerebroside, 24:0 glucocerebroside, dipalmitoylphosphatidic acid, alpha-tocopherol and egg phosphatidyl choline into the lipid membrane. Preparations were tested for stability, viability and capacity for trapping drugs. Drugs studied included glucantime, pentostam, rifampin, lampit, and WR 6026, among others. Efficacy of liposome-encapsulated drugs was tested in 17 experiments in animal models[1] of leishmaniasis. In dogs,[2] liposome-encapsulated glucantime had prophylactic activity and was effective when administered as long as eight days prior to infection. WR 6026, an 8-aminoquinoline, was 700 to 1800 times more effective in liposomal preparations than unencapsulated glucantime.

FUTURE OBJECTIVES:

Reagent grade lipids/liposomes will be evaluated for efficacy as they are formulated. Compounds that demonstrate antileishmanial properties but which are toxic at curative dose levels will continue to be investigated using the liposome delivery system. A new cutaneous screening system in hamsters has been established. It will be determined whether the "targeting" of liposomes/drugs to cutaneous macrophages will require a different composition of lipds than that currently used with visceral leishmaniasis.

WORK UNIT 114 - Liposomes for Treatment of Leishmaniasis

REFERENCES CITED:

1. Liposomes in Leishmaniasis: Therapeutic Effects of Antimonial Drugs, 8-Aminoquinoline and Tetracycline. C. Alving, E. Steck, W. Chapman, V. Waits, L. Hendricks, G. Swartz, and W. Hanson. 1980. Life Science 26:2231-2238.

2. U.S. Army Medical Research and Development Contract #DAMD 17-75-C-5011. "Chemotherapy of Leishmaniasis," 6th Annual Progress Report.

PUBLICATIONS:

1. Liposomes in Leishmaniasis: Therapeutic Effects of Antimonial Drugs, 8-Aminoquinoline and Tetracycline. C. Alving, E. Steck, W. Chapman, V. Waits, L. Hendricks, G. Swartz, and W. Hanson. 1980. Life Science 26:2231-2238.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2221	80 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
79 10 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

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

11. TITLE (Precede with Security Classification Code)

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 80	1	50
FISCAL YEAR CURRENT 81	2	50

19. RESPONSIBLE DOD ORGANIZATION

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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 assess the effects of delivering high energy substrates in animals subjected to severe, progressive hemorrhagic shock. To determine if a radioimmunoassay method can be established for measuring prostaglandin levels in afferent and efferent pulmonary blood. To measure prostaglandin synthesis and metabolism during severe, progressive, hemorrhagic shock and to determine the effects of blockade on cardiovascular hemodynamics and survival. Severe blood loss in combat casualties accounted for 23.9% of the deaths in hospitalized patients in a survey from Vietnam during the calendar year of 1969. These studies may lead to improved methods of treating severe progressive hemorrhagic shock.

24 (U) A hemorrhagic shock model will be established where 75% to 80% of all animals expire. High energy substrates will be delivered by an extracorporeal system in the treatment groups. The radioimmunoassay measurements of prostaglandins will initially be carried out as a collaborative effort with Georgetown University. The long range goal is to establish the radioimmunoassay in our own laboratory. The blockade of prostaglandin synthesis and metabolism will be carried out with known inhibitors. Cardiovascular hemodynamics will be continually monitored and survival will be determined in each group.

25 (U) 79 10 - 80 09 Replenishing energy stores using an extracorporeal circuit with autologous red cells stored in a phosphate-dextrose solution and autologous fresh frozen plasma increased the survival time but did not prevent death in animals following 3 hours of shock. Both thromboxane B_2 and 6-Keto-Prostaglandin $F_{1\alpha}$ have been measured pre-shock and during shock in our own laboratory. Preliminary results suggest a 1 1/2 to 2 fold increase during shock. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 - 30 Sept 80.

* 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 38 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Project 3A161101A91C IN HOUSE LABORATORY INDEPENDENT RESEARCH

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

Investigator
Principal: Arthur W. Fleming, COL, MC

Description

Experimental methods to study the efficacy of various types of treatment for hemorrhagic shock have relied primarily on the use of a shock model which is created by removing blood until the blood pressure is lowered to a set value (approximately 40 mmHg) for a specific period of time (approximately 2 hours).[5] This model offers a relatively uniform mortality during a given period of time at a given institution and was used in the present study. We question, however, whether this model simulates adequately the conditions found in combat casualties where soldiers lose various volumes of blood, and have highly variable blood pressures for various period of time prior to resuscitation. 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 elucidate why severe hemorrhagic shock becomes progressive despite replacement of all the blood that is lost. Alterations in prostaglandins and depletion of energy stores may play a role in the propagation of shock.

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 loss was a contributory factor.

Progress

Hemorrhagic shock was created in a canine model by lowering the blood pressure to 40 mmHg for varying periods of time, both with and without a reservoir. Control animals were resuscitated after the prescribed period of time using their shed blood plus a volume of lactated Ringer's equal to three times the volume of shed blood. Treated animals had their energy stores replenished by an extracorporeal circuit using autologous red blood cells stored in a phosphate - dextrose solution, autologous fresh frozen plasma which was thawed, and a balanced electrolyte solution.[2,3,4] Rats were used to assess the differences between hemorrhagic shock created by maintaining the blood pressure at a specific level versus hemorrhagic shock created with a specific blood loss. Thromboxane B_2 (TXB_2) and 6-Keto-Prostaglandin $F_{1\alpha}$ were measured in our own laboratory using a newly available radioimmunoassay.

Twenty-six dogs were placed in hemorrhagic shock. Five out of 6 control dogs survived 48 hours after being bled to 40 mmHg for one hour using the reservoir technique, followed by one hour of clamping the reservoir and then resuscitation with their shed blood and lactated Ringer's. None of 8 control dogs survived for 48 hours who were kept in shock for three hours. Twelve experimental animals who also were kept in shock for 3 hours failed to be long term survivors, although initial responses were good. The rate and speed of replenishing revitalized cells, refinement of the technique employed, as well as closer monitoring during the recovery phase may all play a role in the ultimate recovery rate. Initial studies with rats have demonstrated that a removal of 37 to 40 percent of the blood volume based on an estimated blood volume of 80 ml/kg body weight is uniformly fatal. Likewise, lowering the blood pressure to 40 mmHg for 2 hours was fatal. Further documentation of the volume of blood which should be removed is required prior to the use of high energy substrates in the rat model. Both thromboxane B_2 and 6-Keto-Prostaglandin $F_{1\alpha}$ have been measured pre-shock and during shock in our own laboratory. These tests are new enough that we are not yet confident of the validity of the results. The preliminary results, however, suggest a 1-1/2 to 2 fold increase in both of these products of hydrolysis during shock.

Recommendations for the Future

The critical factor in ..et ..ining benefits from various treatments of hemorrhagic shock is to have a model that shows a high mortality with standard treatment, but not so high that massive cell death has occurred and recovery is impossible. We have insisted that standard treatment for resuscitation consist not only of the reinfusion of shed volume, but also include the administration of crystalloid, ventilatory support, and careful monitoring. As a consequence, we have had excellent survival in a group of controls treated in this manner. Some of our initial objectives must await the development of a more ideal hemorrhagic shock model. Likewise, the time course of changes in prostaglandins during hemorrhagic shock and the effects of prostaglandin blockade on survival must await confirmation of the accuracy of the radioimmunoassays for TXB_2 and 6-Keto-Prostaglandin $F_{1\alpha}$.

Project 3A161101A91C IN HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 115 The Role of High Energy Substrates and Prostaglandins on Responses to Stress & 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. Fleming, A.W., Etheridge, M.L., Jenkins, E.B.: Total coronary blood flow measurements and myocardial metabolism during cardiopulmonary bypass in a canine model. Am Surgeon 41:214-220, 1975.

3. Fleming, A.W., Green, D.C., Radcliffe, J.H., St. James, D.M., Fleming, E.W.: Development of a practical autologous blood transfusion program. Am Surgeon, 43:794-801, 1977.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2533	80 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
79 10 01	H. Term	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	116
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Purine and Tryptophan Metabolism of Leishmania

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10	CONT	DA	B. Contract

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: N/A d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING '79	0	0
FISCAL YEAR CURRENT 80	0.1	6

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Webster, H.K., CPT

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

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Leishmania; (U) Purine; (U) Tryptophan; (U) Purine Salvage; (U) De novo Synthesis

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 area: to define the relative importance of tryptophan, purine de novo and purine salvage pathways in promastigotes and emastigotes of Leishmania braziliensis panamensis (WR008) and Leishmania mexicana mexicana. The biochemical differences in the fate of these substrates between host and parasite, if fully understood, can be explored for the development of specific chemotherapy for this military important disease.

24. (U) Midlog phase amastigotes will be incubated in the presence of ^{14}C-labeled purine precursors (glycine, formate, serine, hypoxanthine, adenine, adenosine and inosine) and tryptophan for both short term (3 hr) and long term (24 hr) incubations. Following termination of the incubation period by centrifugation at 4 C, the cells and supernatant will be extracted in 1M PCA at 4 C. All samples will be stored in liquid nitrogen (-196 C) until analyzed for free pool purine bases, nucleosides, nucleotides, tryptophan and tryptophan metabolites by high pressure liquid chromatography (HPLC). Distribution of the radioactive label among these compounds will be determined by a Packard Flow Cell Scintillation Detector.

25. (U) 79 10 - 80 09 Promastigotes of Leishmania braziliensis panamensis (WR008) and Leishmania mexicana mexicana were incubated in the presence of ^{14}C-labeled precursors for 3 and 24 hr incubation periods. The purine bases hypoxanthine and adenine and the purine nucleoside inosine were readily incorporated into adenylate compounds (primarily ADP and ATP) and the nucleic acid moiety. However, the purine precursors glycine, formate and serine were not significantly incorporated into nucleic acid or other metabolites. These data suggest that promastigotes lack purine de novo synthetic capabilities but that purine salvage systems are operating.

* 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 68 AND 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

Project No.: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit: 116 Purine and Trytophan Metabolism in Leishmania

Investigators:
Principle: Brain D. Hansen, Ph.D.
Associates: SP4 Jose Perez-Arbelo, H.K. Sleeman, CPT H. Kyle Webster.

The objectives of the work unit are to define the relative importance of purine de novo synthesis and salvage pathways in promastigotes and axenic amastigotes of Leishmania braziliensis panamensis (WR008) and Leishmania mexicana mexicana and to determine the mechanism of tryptophan metabolism by promastigotes and axenic amastigotes of Leishmania sp. and how these mechanism are affected by chemical agents.

A. Purine Synthesis, Savage Pathways and Metabolism of Leishmania Sp.

1. Purine Synthesis, Savage Pathways and Metabolism of Leishmania Sp.

Leishmania is a parastic disease of medical and military importance, found predominantly in tropical regions of the world. Although chemotherapy for this disease is curatively effective, many side effects and medical complications arise from their use. Before improved chemotherapy can be intelligently disigned however, a more thorough understanding of leishmanial metabolism will be required. One potential metabolic target for direct chemotherapy are enzymes of unique parasite purine salvage pathways. Particularly promising is a demonstrated aminohydrolase activity present in both promastigote and amastigote forms, resulting in the conversion of adenine to hypoxanthine. This enzyme appears to be parasite specific and does not occur in the human host. The current objective of this study is to continue the examination of differences between host and parasite purine metabolic processess and to explore the possible inhibition of these pathways using directed chemotherapy.

The major pathways of purine intermediary metabolism for promastigotes and amastigotes of old and new world strains of the Leishmania sp. have been preliminary defined. The data indicate that under the in vitro culture conditions employed, no de novo purine synthesis occurred either in promastigotes or axenically grown amastigotes for those Leishmania species tested. However, these studies confirmed th. presence of purine salvage pathways utilized in the synthesis of purine nucleotides and the subsequent production of nucleic acid (DNA/RNA). Moreover, a specific salvage pathway unique to the parasite has been characterized: adenine hypoxanthine IMP Purine nuclotides. The aminohydrolase catalyzing the conversioin of adenine to hypoxanthine appears to be parasite specific and does not occur in the mammalian host.

Carbon 14 distribution patterns from radiolabeled purine precursors indicate distinct purine salvage pathways unique to both the leishmanial promastigote and amastigote. To confirm these findings, the catabolism of purine precursors will be examined in the presence of inhibitors specific for key enzymes of purine salvage pathways. In particular, these will include mycophenolic acid (inhibits inosinate dehydrogenase, a key enzyme in the conversion of adenylates to guanylates) and 2-deoxycoformycin, a powerful inhibitor of adenosine and adenine deaminase. Moreover, we have demonstrated a 50% decrease in serum levels of tryptophan in animals infected with Leishmania donovani as compared to uninfected control animals. This drastic reduction may indicate an essential requirement for this amino acid by the leishmanial amastigote forms. Further, allopurinol, an inhibitor of purine salvage metabolism, also acts to interfere with leishmania tryptophan metabolism. We are currently investigating these observations.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA 0G 2567	2. DATE OF SUMMARY: 80 09 30		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 80 04 01	4. KIND OF SUMMARY: H. Term	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8a. DISP'N INSTR'N: NL	8b. SPECIFIC DATA - CONTRACTOR ACCESS: [X] YES [ ] NO	9. LEVEL OF SUM: A. WORK UNIT

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

11. TITLE (Procede with Security Classification Code): (U) Molecular Biology Techniques Applied to Leishmania Identification:Restriction Endonuclease Mapping of Kinetoplast DNA

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 010 100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
1 April 80	30 Sept 80	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: 79	0	0
FISCAL YEAR CURRENT: 80	.2	10

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: Peter R. Jackson
NAME: John A. Wohlhieter

21. GENERAL USE
Foreign Intelligence not considered

22. KEYWORDS (Procede EACH with Security Classification Code)
(U) Leishmania, (U) Taxonomy, (U) Kinetoplast DNA,(U) Restriction endonuclease

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 is to develop a rapid, reliable method for identification of Leishmania parasites. Military relevance is that military personnel contract leishmaniasis in Panama and other countries. Lack of suitable Leishmania identification procedures hampers effective patient treatment and impedes the progress of military vaccine and drug development programs.

24. (U). Approach. In Leishmania and related parasites, the kinetoplast contains mitochondrial DNA. Species identification of parasites related to Leishmania has been achieved by analysis of kinetoplast DNA with restriction endonuclease enzymes. In the present work, Leishmania kinetoplast DNA was purified, radiolabelled, digested with restriction enzymes and subjected to gel electrophoresis. DNA fragments, detected by autoradiography, migrated in species specific patterns which were compiled for reference and comparison.

25.(U)80 04 -80 09. Problems with DNA isolation and electrophoresis hampered the project but a new, simple DNA isolation procedure has recently yielded adequate amounts of DNA from Leishmania donovani, Leishmania braziliensis and Leishmania tropica and the gel electrophoresis problem has been solved. Radiolabelling of DNA, digestion of DNA with restriction enzymes and autoradiography of separated DNA fragments in agarose gels have been conducted. The results are not complete but it appears that the technique will be useful in the identification of Leishmania. Thus, Leishmania donovani and Leishmania braziliensis can be separated by the DNA fragments obtained by the use of the enzyme Alu 1. The enzymes Eco R1, Alu 1, Hind III, Hha 1, Hpa II and Taq 1 are now being used to characterize the kinetoplast DNA from 3 strains of Leishmania donovani, 2 strains of Leishmania tropica and one strain of Leishmania braziliensis. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 - 30 Sep 80.

DD FORM 1498 1 MAR 66 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 117 Molecular Biology Techniques Applied to Leishmania Identification: Restriction Endonuclease Mapping of Kinetoplast DNA

Investigators:

Principal: Peter R. Jackson, Ph.D.
Associate: John A. Wohlhieter, Ph.D.

Problem: The problem is to develop a rapid, reliable method for the identification of species and strains of protozoan parasites of the genus Leishmania. U.S. military personnel are exposed to leishmaniasis but clinical treatment and military vaccine and drug development programs are hampered by inadequate parasite identification procedures. Leishmania are difficult to identify because: 1. Species are morphologically identical; 2. The pathology of an infection, which may be diagnostic, often requires months to develop; 3. Standard immunologic and biochemical identification techniques do not identify every species.

The study objective is to use Leishmania species and strain mitochrondrial DNA (also called kinetoplast DNA or KDNA) along with DNA-cutting enzymes (restriction enzymes) for the identification procedures. The KDNA is isolated from a species or strain of in vitro cultivated Leishmania, labelled with a radioactive nucleotide, then cut into fragments with several restriction enzymes. The resulting KDNA fragments are separated, by size, via polyacrylamide gel electrophoresis. The positions of the KDNA fragments in the gel are determined by autoradiography. Patterns of KDNA-fragment migration are compiled for each known species and strain of Leishmania. Different patterns are prodcued with each of many restriction enzymes. Unknown Leishmania species KDNA, is treated in a similar manner. The identity of an unknown Leishmania is determined by comparison of its KDNA fragment gel migration patterns with those of known species and strains of Leishmania. This comparison is conducted for each restriction enzyme tested.

Progress: The kinetoplast DNA of in vitro cultivated Leishmania donovani, Leishmania braziliensis, and Leishmania tropica was isolated, labelled with ^{32}P-thymidine triphosphate, then cut with the restriction enzymes ECO RI, ALU I, HIND III, HHA I, HPA II, and TAQ I. The KDNA restriction fragments, for each species and enzyme, were separated by gel electrophoresis and compared for similarities in migration. Each of the restriction enzymes tested cut the KDNA of the three Leishmania species into fragments of different sizes. These KDNA fragments migrated in species-specific patterns in polyacrylamide gels. The technique thus appears useful for the identification of these three species and may be applicable to other Leishmania species.

d. Recommendations for the future

More species and strains of known Leishmania should be analyzed by the technique in order to build a reference base which would allow for the comparison and identification, of unknown Leishmania. Different restriction enzymes should be tested in order to determine which cuts KDNA into the most useful number and size fragments. Methods should be developed which improve the yield and purity of Leishmania KDNA. Also, methods not based on radionudeotides, should be developed for detection of KDNA fragments in gels. Such methods might use the fluorescence of DNA-binding dyes, such as ethidium bromide. A method should be developed which permits the storage and retrieval of the results of the polyacrylamide gel electrophoresis of KDNA from known Leishmania species and strains. This method would simplify the comparison of known and unknown Leishmania KDNA restriction enzyme fragments.

PROJECT 3M161102BS10

RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DT OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6441	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10AA	201
b. CONTRIBUTING	61102A	3M161102BS01	00	130
c. CONTRIBUTING	STOG 80-7,2			

11. TITLE (Include 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: 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 80	4.5	459
FISCAL YEAR CURRENT 81	3.0	319

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NAME: BRANDT, Walter E.

21. GENERAL USE

Foreign intelligence not considered.

22. KEYWORDS (Include 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 are developed as needed for specific problems.

25 (U) 79-10-80 09 Arbovirus. An attenuated live dengue-2 vaccine (PR-159/S-1) was studied for reactogenicity and immunogenicity. Fifteen yellow fever immune (YFI) volunteers received graded doses of vaccine; one other received a placebo. Combined results for 19 YFI vaccines in 2 studies have revealed viremia (6 people), neutralizing antibody (10 people) and a 50 percent infectious dose of 5000 plaque forming units. No greater frequency of seroconversion was observed by giving a second dose of vaccine to 12 people or intradermal inoculation to 6 volunteers. Adherent monocytes freshly harvested from human donors have dengue virus receptors, but much greater yields of virus can be obtained if the cells are cultured in medium containing antibody. This enhancing effect of antibody can be blocked by pretreating and incubating adherent monocytes with heat aggregated IgG which reacts with Fc receptors. Oligonucleotide electrophoresis of the RNA of representative dengue-1 viruses showed the 1977 epidemic Jamaican strain to be distinctly different from strains from the Pacific and Africa. It is not known where the Caribbean strain originated. Oligonucleotide fingerprints of dengue-2 strains shows that wild parent virus and candidate attenuated vaccine virus can be distinguished by this method. For technical report, see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 to 30 Sept 80.

(Author's approval)

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Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M161102BS01 BASIC RESEARCH ON MILITARY INJURY AND DISEASE
Work Unit 201 Viral Infections of Man
* Work Unit 130 Viral Infections of Man

Investigators:

Principal: COL William H. Bancroft, MC

Associates: LTC Robert McN Scott, MC;
Dr. Walter E. Brandt, Ph.D.;
Dr. Joel M. Dalrymple, Ph.D;
Dr. Patricia Repik-Byrne, Ph.D.;
MAJ Charles H. Hoke, Jr., MC;
MAJ Bruce Booth, MC;
CPT Michael Usserv, MSC;
Mr. Jack M. McCown;
Mrs. Jeanne Burrous, M.S.;
SSG Kiran Jesrani;
SGT Wanda Williams;
Ms. Amanda Ralph;

Purposes

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 discription 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, differentiating virus strains by comparing viral RNA oligonucleotides, and production of monoclonal antibodies to viral proteins. Human volunteer studies are conducted to evaluate dengue vaccine safety and immunogenicity.

Basic research on respiratory viruses is directed toward specific identification of viruses causing acute respiratory disease (ARD) and factors affecting pathogenicity.

Progress

Very little is known about the process of dengue virus human infection, or of the replicative mechanisms that produce progeny virus. Thus, the temperature sensitive lesion(s) of the live attenuated vaccine virus is not known at the present time. Recently we found that dengue virus infection of adherent human monocytes appears to occur through either trypsin sensitive virus

receptors, or, when complexed with non-neutralizing antibody, through trypsin resistant Fc receptors. Factors affecting this process will provide information on the entry of virus into susceptible cells and provide a basis for determining differences between parent and temperature sensitive viruses at the nonpermissive temperature.

Trypsin sensitive virus receptors were regenerated on the monocytes after one to two days in culture. In support of the Fc receptor hypothesis of infection by virus-antibody complexes (immune enhancement concept of dengue virus infection), it was shown that pretreatment of monocytes with 100 ug/ml of heated-aggregated human gamma globulin resulted in a 95% reduction of virus yield. The blocking effect was dose dependent: normal yields of virus were obtained when the concentration of aggregated gamma globulin was reduced to 1.0 ug/ml. Aggregated globuin has enhanced Fc activity and binds to Fc receptors. Antibody mediated enhancement of dengue virus infection appears to be dependent on the intact IgG molecule since removal of the Fc portion eliminates the immune enhancement effect. We found that another Fc portion could be added back in the form of rabbit anti-human Fab. The concentration of the anti-Fab was critical since excess anti Fab did not result in the infection enhancement effect. A human monocyte cell line derived recently from a histocytic lymphoma obviates the requirement for processing of repeated blood samples from human donors to examine the mechanisms of antibody mediated infection (1). Preliminary work indicates that these cells do not possess virus receptors since viral replication occurs only in the presence of antibody. Thus, prior treatment with trypsin is not required to study antibody mediated infection.

Oligonucleotide electrophoresis of viral RNA was used to compare three dengue type 1 strains from Jamaica, Nigeria and Hawaii in an effort to test the hypothesis that the Caribbean dengue type 1 originated in Africa. Viral RNA was extracted from purified virions, degraded with nucleases and the resulting oligonucleotides were separated by two dimensional electrophoresis. Distinct differences were found between each of the three viruses leading to the conclusion that the Jamaican and Nigerian strains are not identical. Evaluation of the parent dengue-2 virus and the vaccine strain (PR-159/S-1) showed they were very similar, but could be distinguished by a few oligonucleotides. RNA oligonucleotide fingerprinting should be a highly sensitive method for documenting genetic changes in a virus during passage in cell culture or by mutogenesis.

The dengue type 2 (PR-159/S-1) candidate vaccine was used in the third, fourth and fifth human volunteer studies. Study #3 completed a dose response trial in 16 yellow fever immune volunteers given the following vaccine dilutions; 10^{-1} (5 people), 10^{-2} (5 people), 10^{-3} (5 people), placebo (1 person). One recipient of the 10^{-3} dilution had low levels of dengue-2

neutralizing antibody and is excluded from the analysis. Five of the 14 susceptible recipients seroconverted and two were viremic. On the basis of combined results from Study #1 (1979 Annual Report) and Study #3, the 50 percent infectious dose for yellow fever immunes is $10^{3.3}$ plaque forming units.

In Study #4, the response to reimmunization was studied in 12 volunteers who were first vaccinated in Studies #1 or 2, 4-18 months earlier (1979 Annual Report). A four-fold rise in neutralizing antibody was found in only 2 of 8 recipients who seroconverted after the first dose and 0/4 people who did not seroconvert previously. No advantage was observed from repeat immunization.

In Study #5, six yellow fever non-immune volunteers were immunized with undiluted vaccine by intradermal inoculation with a jet injection gun. Only one recipient seroconverted indicating no advantage was obtained by using an alternative means of inoculation.

Acute respiratory disease (ARD) rates on most basic training posts were reported to be equal to or less than 2.0 cases/100 men/week for most of the year. Adenovirus type 4 (ADV-4) outbreaks at Forts Wood and Knox in late summer 1979 subsided with the reinstitution of routine type 4 and 7 vaccination. As in most recent years the predominant reported adenovirus isolated was type 21; however, an experience at Fort Benning raises questions about that generalization. During a heatwave in July, ARD rates at Fort Benning rose to 2.9/100 men/week. Virus isolates were obtained from two training companies for which a shared water point was implicated in virus transmission. Nineteen virus isolates were adenovirus type 4, and six were initially identified as type 21.

The availability of convalescent sera from many soldiers provided an opportunity to determine neutralizing antibody titers to Fort Benning and reference strains of ADV. As a result, the isolates originally identified as ADV-21 were determined to be different from a reference strain of ADV-21 obtained from American Type Culture Collection (ATCC).

Adenovirus	Neutralizing Antibody Titer	
	Fort Benning (Pt. A)	ADV-21 (ATCC)
Fort Benning (Pt. A)	160	6400
ADV-21 (ATCC)	<20	6400

The Fort Benning isolate is not ADV-21 but would be identified as such by routine laboratory testing in the Adenovirus Surveillance Program. Additional identification studies are underway.

In July 1980, Wyeth Laboratories was granted product licenses

by the Bureau of Biologics to market ADV type 4 and 7 vaccines concluding over 15 years of development, testing and evaluation of these vaccines by the WRAIR.

A collaborative study with Dr. Adrian Butler at William Beaumont Hospital of 108 young people previously immunized with rubella vaccine revealed 18 (16%) lacked hemagglutination inhibiting (HAI)antibody. Reimmunization of this subgroup led to a secondary immune response in all 18 people and the conclusion that the absence of HAI antibody does not necessarily indicate susceptibility to rubella.

Future Objectives

The human monocyte cell line will be used to measure enhancing antibody produced by dengue vaccine recipients and one mechanism of viral entry into cells. Monoclonal antibodies produced to all four dengue serotypes will permit the evaluation of the role of individual viral polypeptides in infection and immunization and should lead to the development of new specific virus identification techniques.

The dengue type 2 vaccine will be tested in a larger number of recipients at Fort Bragg to further define the advantage of preliminary yellow fever vaccination.

Identification of the new adenovirus from Fort Benning will be followed by an evaluation of the contribution of this virus to the overall ARD problem currently attributed to ADV type 21.

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Formal Presentations

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Live Attenuated Dengue Type 2 Vaccine in Human Volunteers. 28th Annual Meeting - The American Society of Tropical Medicine and Hygiene, 14 November 1979.

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3. Vezza, A. C., Rosen, L., Repik, P. Dalrymple, J. and Bishop, D.H.L.
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Chemical and Antigenic Structure of Flaviviruses in The Togaviruses (ed. W. Schlesinger). Academic Press (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE	REPORT CONTROL SYMBOL
DA OB 651?	80 10	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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10AA	202
b. ~~XXXXXXXX~~	61102A	3M161102BS01	00	135
c. CONTRIBUTING	STCG 80-7.2.2			

11. TITLE (Precede with Security Classification Code)

(U) Mechanisms of Transmission of Hepatitis Viruses

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

0026 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: 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 80	2.0	133
	CURRENT 81	2.0	218

19. RESPONSIBLE DOD ORGANIZATION

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PII Redacted

21. GENERAL USE

Foreign intelligence not considered

22 KEYWORDS (Precede EACH with Security Classification Code)

(U) Viruses; (U) Hepatitis; (U) Antigen; (U) Immunology

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

23 (U) To 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) 79 10-80 09. A sensitive and specific radioimmunoassay for IgM class antibody to hepatitis B core antigen (anti-HBc) was developed with methodology previously used for hepatitis A IgM antibody. IgM anti-HBc was present in all 47 (100 percent) patients with acute hepatitis and transient hepatitis B surface antigen (HBsAg); 5 of 12 (42 percent) HBsAg chronic carriers; and only 1 of 46 (22 percent) asymptomatic people with unclassed anti-HBc. IgM anti-HBc was detected in only 4 of 143 (2.7 percent) patients with acute hepatitis A and none of 75 patients with non-A, non-B hepatitis. When the presence of HBsAg and/or anti-HBc was used as criteria for acute hepatitis B, the new assay detected 12.3 percent of cases missed by the HBsAg testing. Reanalysis of US Army hepatitis cases sampled in 1978-1979 show the following frequencies of hepatitis And B by location: Germany (HAV, 1.3 percent HBV 67 percent); Korea (HAV 14 percent, HBV 77 percent); and Fort Hood (HAV 14 percent, HBV 52 percent). The IgM anti-HBc assay is a powerful additional diagnostic test for viral hepatitis. Hepatitis A virus has been purified using a combination of differential centrifugation chloroform extraction and exclusion chromatography, in sufficient quantity to immunize mice to produce monoclonal anti-HAV. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available [illegible] with [illegible] approval

DD FORM 1498 (1 MAR) 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 & HEALTH HAZARDS
* Project 3M161102BS01 BASIC RESEARCH ON MILITARY INJURY AND DISEASE
Work Unit 202: Mechanisms of Transmission of Hepatitis Viruses
* Work Unit 135 Mechanisms of Transmission of Hepatitis Viruses

Investigators:

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

Associates: Dr. Walter E. Brandt, Ph.D.
SFC Thomas E. Simms;
SP4 Norman L. Gates;
Mr. Hubert Cannon

Problem

Over 2000 active duty U.S. Army Personnel are hospitalized annually for acute viral hepatitis, making the hepatitis viruses among the most common infectious agents responsible for serious disease among peacetime military forces today. The potential for increased transmission and epidemic spread of some forms of hepatitis, especially hepatitis A, exists during times of mobilization with possible resultant loss in combat effectiveness of troops. All forms of viral hepatitis may be prevented by interruption of virus transmission or passive and/or active immunoprophylaxis, although effective immunoprophylactic measures have not been fully developed. Current objectives within this work unit include the development of improved methods of specific virus diagnosis, characterization of hepatitis viruses, and the study of modes of virus transmission and evaluation of means of prevention of viral hepatitis.

Progress

Because many patients with acute hepatitis B lack detectable hepatitis B surface antigens (HBsAg), a radioimmunoassay specific for IgM antibody to the core antigen (IgM anti-HBc) was developed and evaluated as a diagnostic test for acute hepatitis B virus (HBV) infection. IgM anti-HBc was detected in almost all patients with acute hepatitis B (diagnostic sensitivity = 98%), and, when sought in healthy individuals with antibody to the core antigen, was highly specific for acute infection (specificity = 98%). IgM anti-HBc was the only specific marker of acute HBV infection in 12.3% of 235 hepatitis B patients, and thus appears to be useful diagnostic parameter(1). Persistent IgM anti-HBc, usually in low titer, was found in 5 of 12 chronic HBsAg carriers. Collaborative studies with Dr. Jay Hoofgnagle of the National Institutes of Health are currently in progress to determine whether persistence of IgM anti-HBc during the carrier state correlates with either evidence of active virus replication or parenchymal liver cell damage.

Studies published during the current year suggested that immune serum globulin (ISG) manufactured prior to 1972 frequently contained HBsAg bound up in immune complexes, and that such ISG might confer active immunity against HBV (2). Such a mechanism would explain the apparent protection against HBV conferred by ISG in the cooperative study of immunophylaxis conducted by the U.S. Army in Korea during 1968-1969 (3). Therefore, two remaining ISG lots employed in the Army study were examined for the presence of HBsAg after passage through acid sucrose gradients. HBsAg, but not antibody to HBsAg was detected by radioimmuneassay in both lots, suggesting that protection against HBV could have been mediated by active and not passive immunization (4). Sera collected from hepatitis cases during the Korea study have been restudied by currently available radioimmunoassay techniques. Most cases (56%) were hepatitis type A. These new data will allow a reinterpretation of the efficacy of ISG in the prevention of hepatitis A, hepatitis B, and non-A, non-B hepatitis in this Army study.

With the addition of IgM anti-HBc testing, serological analysis was completed in over 400 cases of hepatitis occurring among active duty U.S. Army personnel during 1978-1979. The majority of hepatitis worldwide was due to hepatitis B virus, even among troops stationed in the Republic of Korea. Non-A, non-B hepatitis accounted for almost a third of cases studied in the Federal Republic of Germany, but was virtually absent in Korea. In collaboration with the Division of Prevention Medicine, questionnaire data collected during this study was entered into a computer file, and final data analysis is now in progress.

An effort has been made to create murine hybridoma cells producing monoclonal antibodies to hepatitis A virus (HAV), in collaboration with the Division of Biochemistry. A procedure capable of purifying quantitative amounts of HAV from infected chimpanzee feces was developed, using a combination of differential centrifugation, chloroform extraction and exclusion chromatography. This procedure results in the removal of over 99% of contaminating protein and the final antigen produced has a titer by radioimmunoassay several-fold greater than the original starting material. Several BALB/cj mice have been immunized with this antigen and have developed antibodies to hepatitis A virus (anti-HAV). Two separate direct radioimmunoassay procedures capable of detecting murine anti-HAV have been developed and are now undergoing refinement. Cell fusion studies and efforts to detect clones producing anti-HAV are currently in progress. Monoclonal murine anti-HAV will be a research reagent of tremendous value, not only for virus strain differentiation, but also for studies of virion structure-function relationships, as a probe to detect in vitro virus replication, as a tool for rapid quantitative virus purification, and as a reagent for the development of improved diagnostic assays.

During the past year, attempts have been made to reproduce the reported successful in vitro replication of hepatitis A virus (5). Known chimpanzee-infectious inocula (see FY 1978 annual report) have been used as a source of virus, and the PLC/PKF/5 cell line (see FY 1979 annual report) as a cell substrate. Although efforts continue, viral replication has not been detected yet by either immunofluorescent or radioimmunoassay techniques.

Future recommendations and objectives.

A preliminary trial to determine the immunogenicity of an inactivated hepatitis B vaccine, administrated by vaccine injector gun, will begin in November 1980. Attempts to develop hybridoma anti-HAV antibodies will be continued as will attempts to replicate HAV in vitro. These two efforts constitute critical steps towards the development of an optimal hepatitis A vaccine. A collaborative study has been initiated with the Division of Preventive Medicine to study the long term sequelae of viral hepatitis in active duty Army personnel.

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Etiology of Viral Hepatitis Requiring Hospital Admission in US Military Personnel, USAREUR and Seventh Army Preventive Medicine Training Conference, Berchtesgaden, Federal Republic of Germany, 10 October 1979.

2. Lemon, S.M., Brown, C.D., Simms, T.E. and Bancroft, W.H. Serodiagnosis of Acute Hepatitis A Virus (HAV) Infection by Solid-Phase Radioimmunoassay for IgM Class Anti-HAV, 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2 October 1979, Boston, MA.

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IgM Antibody to Hepatitis B Core Antigen in the Diagnosis of Acute Hepatitis B Infection, 20th Interscience Conference on Antimicrobial Agents and Chemotherapy, 23 September 1980, New Orleans, LA.

5. Lemon, S.M.
Viral hepatitis in active duty U.S. Army personnel presented at the 804th Hospital Center, Fifth Medical Symposium, Boston, MA, 23 March 1980.

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Vaccine Protocol

Approval has been granted to conduct a human volunteer study of an experimental hepatitis B vaccine under protocol entitled "Immunization with Subcutaneously Administered Hepatitis B Vaccine, MSD LOT 751 (C-F271)" under IND-846 held by Merck Sharp and Dohme.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OA 6443	2. DATE SUMMARY: 80 [illegible]		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 79 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8A. DISP'N INSTR'N: NL	8B. SPECIFIC DATA CONTRACTOR ACCESS: ☐ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A		S10AB	203
b. [illegible]	61102A	3M161102BS01	00	132
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Bacterial Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15A. FUNDING AGENCY	16. PERFORMANCE METHOD
58 05	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 80	7	756
	CURRENT 81	7	909

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

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21. GENERAL USE: Foreign intelligence not considered

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

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

23 (U) Studies on the etiology, ecology, epidemiology, pathogenesis, physiological, immunological and diagnostic aspects of diseases of microbial origin which are current or potential problems to miiitary forces. Current emphasis is on control of meningococcal, gonocccal and pseudomonas infections in military forces.

24 (U) Basic studies on bacterial pathogens which will elucidate mechanisms of pathogenesis and result in future developments of prophylactic agents.

25 (U) 79 10 - 80 09 The hybridoma technique has been established to produce highly specific antibodies to bacterial antigens. Antibodies induced in volunteers by vaccination with meningococcal group B vaccines are bactericidal for group B meningococci when fresh serum is used without exogenous complement. (Meningococcal outer membrane protein is mitogenic for human peripheral blood B and T cells.) There is a correlation between the results of the gonococcal IEA test and the SPRIA test when sera from volunteers vaccinated with gonococcal pili is assayed. Pseudomonas burn wound sepsis has been prevented in the rat by vaccination with homologous but not with heterologous LPS, and by passive transfer of human hyperimmune anti-LPS serum. (For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.)

*Automatic [illegible] (Explanation required)

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

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES
Work Unit 203 Bacterial Diseases of Military Importance
* Work Unit 132 Bacterial Diseases of Military Importance

Investigators
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Associates: Herman Schneider, Ph.D.; CPT Dan McChesney, Ph.D.; Jennie Ciak; Brenda Brandt; Lynette Smith; Hazel Sidberry; Hans Hansel; Robert Mandrell; SSG Dennis Broud; SP5 Joseph O'Brien; SP4 Peter Shell; SP4 Sun Joo Yi; PVT E2 Patricia Shell; SP4 Craig Hammack

Objectives: Studies are carried out on the etiology, ecology, epidemiology, pathogenesis, physiology; immunology and diagnostic aspects of diseases of bacterial origin which are current or potential problems to military forces. Current work involves the development of a group B meningococcal vaccine, the development of a tetravalent meningococcal vaccine against serogroups A,C,Y and W-135, studies on immunity to gonococcal infections, methods to prevent or to treat by immunological procedures gram-negative infections of the blood stream, and development of techniques to improve the immune response of human beings.

Progress: Group B meningococcal vaccines which consisted of a 1:1 or a 1:3 hydrophobic complex of high molecular weight group B polysaccharide and serotype 2 outer membrane proteins was safe and immunogenic when tested in Army recruits. A tetravalent meningococcal vaccine consisting of polysaccharides of groups A,C, and W-135 has been prepared, tested in the laboratory, and injected into volunteers. The serological response is now being evaluated. A meningococcal group Y-W135-mosaic polysaccharide vaccine was prepared and tested in Army recruits. This vaccine was not as effective as a mixture of the Y and W-135 polysaccharides.

A gonococcal vaccine consisting of pili has been found to produce antibodies in volunteers which block attachment of gonococci to epithelial cells. These antibodies are not directed against LPS but do block attachment of heterologous strains containing cross-reacting pili. The vaccine has been lyophilized and has been shown to remain antigenic.

Pseudomonas burn wound sepsis has been prevented in the rat by vaccination with homologous but not with heterologous LPS and by passive transfer of human hyperimmune anti LPS serum. Chemical detoxification of P. aeruginosa LPS has resulted in a significant loss in immunogenicity but procedures have been developed to couple covalently the detoxified LPS (DLPS) to proteins. Biological studies with DLPS coupled to pseudomonas toxin A indicate that it is active in inhibiting in the opsonophagocytic test but does not exhibit satisfactory activity in mouse protection assays. While the E. coli K-1 antigen confers serum resistance on its host cell, K-1 positive organisms are killed by normal human serum in conjunction with human neutrophils. The addition of anti-K-1 serum does not appear to enhance the bactericidal activity.

The ability of thymosin fraction-5 to enhance the secretion of antibacterial antibodies by human peripheral blood lymphocytes can be mimicked by the synthetic polypeptide thymosin Alpha-1. Experiments using T-cell depleted and T-cell reconstituted populations support the concept that thymosin Alpa-1 stimulates the functional maturation of helper cells and suggest, therefore, that thymosin may be able to enhance the immunogenicity of bacterial vaccines in vivo. Meningococcal

outer membrane protein (MP) is mitogenic for human peripheral blood B-cells and T-cells. MP (especially the 28,000 M.W. fraction, pool II) can also activate human B cells to secrete antibodies directed against meningococcal polysaccharide and tetanus toxoid. These data suggest that MP is an effective vaccine component due to its immunopotentiating capabilities.

Future Plans: Basic studies to improve the immunogenicity of meningococcal group B vaccine will continue. The immune response of volunteers who received the tetravalent A,C,Y,W-135 meningococcal vaccine will be completed. Improved procedures to sterilize the gonococcal pilus vaccine will be investigated. When practical sterilization procedures are assessed, and depending upon the outcome of volunteer challenge studies, a field trial of gonococcal pilus vaccine will be conducted. Research will continue to improve the immunogenicity of detoxified pseudomonas LPS and the use of pseudomonas pili will be evaluated as vaccines in animal models. Experiments to evaluate importance of K antigens of E. coli in bacteremia will continue. Efforts will be made to improve the immune response of bacterial antigens by using cell wall proteins as adjuvants.

Work Unit 132 Bacterial Diseases of Military Importance

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29. Tramont, E.C., Ciak, J., McChesney, D.G., Boslego, J.W., Brinton, C.C. Inhibition of attachment of N. gonorrhoeae by antipilus antibodies induced by pilus vaccine. STD, Antwerp, Belgium, 1980.

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35. Lowell, G.H., L.F. Smith, D. Klein and W.D. Zollinger. 1980. Thymosin stimulates in vitro secretion of antibacterial antibodies by human peripheral blood lymphocytes. First International Conference on Immunopharmacology. (Abstract).

36. Lowell, G.H., L.F. Smith, D. Klein and W.D. Zollinger. 1980. Thymosin stimulates in vitro secretion of antibacterial antibodies by human peripheral blood lymphocytes. Clinical Research 28(2):353A. (Abstract)

37. Lowell, George H., Lynette F. Smith, David Klein and Wendell D. Zollinger. 1980. Thymosin stimulates in vitro secretion of antibacterial antibodies by human peripheral blood lymphocytes. Fourth International Congress of Immunology. (Abstract)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6514	80 10 01	DD DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10AD	205
b. ~~SUPPLEMENTARY~~	62770A	3M162770A802	00	013
c. CONTRIBUTING	STOG 80-7.2.2			

11. TITLE (Precede with Security Classification Code)

(U) Vector Transmission of Militarily Important Infectious Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: 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 80	4.3	226
FISCAL YEAR CURRENT 81	6.0	451

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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TELEPHONE: 202-576-3719
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Schneider, Dr. I.
NAME: Gingrich, CPT J.

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. Precede 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) 79 10 - 80 09 Tsetse fly infection rates can be significantly increased by infecting flies with blood form trypanosomes in a 50:50 mixture of red blood cells and culture medium. After four days starvation, mature flies are capable of developing high salivary gland infection rates. The highest percentage of gametocytes was recovered in fractions having densities of 1.101 to 1.104 grams per milliliter from discontinuous gradients composed of TC Medium 199 and a colloidal silica gel. A colony of Anopheles dirus, which is maintained by forced mating, was acquired. Eggs of the related Anopheles tagasagoensis were received and colonization is being attempted. For technical report, see Walter Reed Army Institute of Research Annual Report, 1 Oct 79 - 30 Sep 80.

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

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M162770A802 MILITARY PREVENTIVE MEDICINE AND TROPICAL DISEASES
Work Unit 205 Vector Transmission of Militarily Important Infectious Diseases
*Work Unit 013 (Same Title)

Investigators

Principal: Donald R. Roberts, MAJ, MSC

Associate: CPT John B. Gingrich, MSC; CPT Michael W. Hastriter; Ronald A. Ward, Ph.D.; Imogene Schneider, Ph.D.; Lawrence M. Macken; SP5 Megan Dowler; SP4 John F. Shaker; SP4 Michael Datkiw; SP4 Jose Ruiz; SP4 Arthur L. Butler; SP4 Sandra L. McMurray;

Objectives

The major objectives of this research are to develop physiological means of interrupting malaria transmission through an understanding of factors affecting parasite growth, invasion and infectivity in both vertebrate and invertebrate hosts; refine the current model for cyclical transmission of African trypanosomiasis to facilitate utilization of large numbers of parasites for immunological studies; assess the vector competence of viz-a-viz malaria transmission with closely related anopheline species and evaluate repellents against selected insect vectors. Attaining these objectives may lead to the prevention or control of malaria and trypanosomiasis in military troops.

Progress

African Trypanosomiasis:

Immature salivary gland infections averaging 10^3 parasites per fly can apparently develop into immature gland infections averaging 10^5 parasites/fly in as little as 4 days. Flies have also been found to extrude extra-glandular parasites from salivary probes, these forms apparently not being infective to mice. Salivary gland (SG) infections may die out, resulting in loss of fly infectivity. Mice have been infected with a single metacyclic parasite. Use of culture mediums mixed with bloodstream form parasites and red cells has yielded almost as high fly infection rates as feeding on procyclics foray in culture. Another significant finding is that mature flies (21-25 days old) become very potent transmitters of trypanosomes if starved first for 4 days. This finding gives new epidemiological significance to the role of mature flies in cyclical transmission of the disease in nature.

Malaria:

Density gradients composed of TC Medium 199 and a colloidal silica gel have been used to separate the different erythrocytic stages of Plasmodium berghei from each other and from uninfected cells. Special emphasis was placed on isolating the gametocytes from the other stages for incorporation into mosquito cell cultures. The highest percentage of gametocytes (between 23 and 31% of the cells in a single fraction) resulted from discontinuous gradients having densities between 1.090 and 1.125 g/ml. Addition of the isolated gametocytes into cell cultures resulted in variable numbers of ookinites. Immunofluorescent staining techniques are now under study as a means of identifying the early oocyst stages in culture.

A preliminary EM study (with Dr. M. Aikawa, Case Western Reserve University) was undertaken to follow the sequential events of liver penetration by P. berghei sporozoites. Mice were sacrificed 24, 42 and 49 hours post IV injection of 4.5×10^5 sporozoites. The livers of mice sacrificed after 24 hours show the presence of parasites within the Kupffercells, surrounded by a membrane but with unaltered morphology. By 42 to 49 hours after injection, the sporozoites have apparently left the Kupffer cells as some of the parasites are found in the interhepatocyte space while others are within the hepatocyte cytoplasm proper. The mechanism of transfer between the two cells is still unknown but only in the hepatocytes will the sporozoites transform into exoerythrocytic schizonts and merozoites.

To bypass the need for primate hosts in cycling P. falciparum, cultured parasites (supplied by MAJ M. McNeil, Immunology Dept.) of which a minimum are 15% gametocytes are being fed to Anopheles stephensi mosquitoes via the artificial membrane technique. Thus far, no infections have resulted in mosquitoes although exflagellation has been demonstrated on slides after removal of infected blood from the culture flasks.

Colonies of Anopheles dirus and An. tagasagoenis were introduced into the departmental insectary. Procedures are being developed for maximal yield of material for comparative studies on susceptibility to rodent and human malarial parasites.

Recommendations for Future Plans

We have evaluated the more important factors influencing tsetse fly infection rates. However, we would be better able to apply strategies to further increase infection rates if we understood how the factors operated. Hence one of the objectives is

to study the mechanisms of action of fly age and digestive enzymes in influencing infection rates. Another new area of endeavor is to develop repellent test methods for tsetse flies in the laboratory with the long-term objective of identifying repellents that are effective against tsetse flies in a field situation.

Future plans for malaria research are as follows:

1. Resume study (in collaboration with LTC C. Alving, Division of Biochemistry) using various liposome preparations to inhibit the exoerythrocytic stages of P. berghei malaria. This study has had to be suspended for 15 months due to repeated breakdowns in the various insectaries housing the anopheline mosquitoes.

2. Initiate study to determine the fate of P. berghei sporozoites in vivo prior to and upon entering the liver cell in the presence and absence of liposome preparations. The size of the inoculum should be increased to a minimum of 10^6 sporozoites to decrease time needed to locate the parasites in the fixed sections. Both conventional and electron microscopy will be utilized.

3. Refine density gradient technique to isolate and concentrate gametocytes more effectively. Test for infectivity to mosquitoes after gradient run and to rodent hosts after varying times in culture. Place emphasis on accurate identification of the young oocyst stages developing in vitro.

4. Test and assess as many factors as possible that may be required to infect anopheling mosquitoes with cultured gametocytes.

5. Complete remodeling of mosquito holding facilities so anopheline rearing can be conducted under optimal conditions.

6. Initiate study to develop a non-primate model for P. falciparum. This study is dependent upon approval of protocol using Aotus monkeys for infecting anophelines which will be used for attempted sporozoite transfer of malarial parasites to immunosuppressed mice.

7. Develop a field test for detecting sporozoites to P. falciparum in field collected anophelines. Such a test, if sufficiently specific, will have broad application in vector determination studies.

Publications

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2. Faran, M.E. and C.L. Bailey. 1980. Discovery of an over-wintering adult female Culiseta annulata in Baltimore. Mosq. News 40 (2): 284-87.

3. Gingrich, J.B., R.A. Ward, L.M. Macken and K.M. Esser. 1980. Some phenomena associated with the development of Trypanosoma brucei rhodesiense infections in tsetse flies, Glossina morsitans. Amer. Soc. Trop. Med. & Hyg. (In press).

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5. Schneider, I., and J.P. Vanderberg. 1980. Culture of the Invertebrate Stages of Plasmodia and the Culture of Mosquito Tissues. In: Malaria: Vol. 2. (J.P. Kreier, ed.). Academic Press, Inc. New York. pp. 235-270.

6. Schneider, I. 1979. Tsetse fly tissue culture and its application to the propagation of African trypanosomes in vitro. In: Practical Tissue Culture Applications. (K. Maramorosch and H. Hirumi, eds.). Academic Press, Inc. New York. pp. 373-386.

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Presentations:

"Factors Influencing Infection Rates of Trypanosoma rhodesiense in tsetse flies, Glossina morsitans". Presented to - Helminthological Society of Washington March, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				2. DA ACCESSION	3. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 6436	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY ACTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D, Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	BS10	S10AE	206
b. [illegible]	61102A	3M161102BS01	00	139
c. CONTRIBUTING				

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: 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 PREVIOUS 80	4	377
FISCAL YEAR CURRENT 81	4	508

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS NAME: Wohlhieter, J.A.

NAME: 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 (U) Recombinant DNA

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text 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 macromolecules (i.e., DNA).

25. (U) 79 10 - 80 09 The specificity of an assay system for differentiating the protective capabilities of typhoid vaccines has been improved by using a Salmonella typhimurium hybrid challenge strain differing in virulence characteristics from the formerly employed challenge strain. A previously unreported surface antigen has been discovered in Citrobacter freundii. Several bacterial plasmid genes, some of which are needed to make a bacterium virulent, have been isolated on recombinant DNA molecules for further study. The genes for S. sonnei form I surface antigen have added to the Salmonella typhi Ty21a vaccine strain. The resultant strain appears, from animal tests, to be a good bivalent vaccine candidate. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

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: 1974-540-043/8691

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

* Project 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES

Work Unit 206 Microbial Genetics and Taxonomy

* Work Unit 139 Microbial Genetics and Taxonomy

Investigators:

Principal: Louis S. Baron, Ph.D.

Associate: J.A. Wohlhieter, Ph.D.; E.M. Johnson, Ph.D.; D.J. Kopecko, Ph.D.; C.A. Life; N.J. Snellings, M.S; Kerry F. Noon, M.S.; SP5 W.C. Reid, Jr., B.S.: SP5 J.N. Coulby, B.S.

1. Problem. Fundamental research studies are carried out on pathogenic microorganisms, focusing especially on those that cause enteric diseases of military importance. The methodologies of microbial genetics, biochemistry, molecular biology, and recombinant DNA technology are employed. The objectives are:

a. To study the mechanisms by which these microorganisms are able to exchange genetic information

b. to use these genetic mechanisms to investigate the characteristics essential to an organisms ability to cause disease (e.g., cell surface antigen synthesis, toxin production)

c. to manipulate the organisms genetic makeup, allowing further examination of the steps involved in the disease process

d. to attenuate the virulence of the organism while maintaining or increasing its antigenicity so that these modified bacterial strains can be employed as vaccines

e. to isolate by recombinant DNA procedures the bacterial genes encoding protective antigens, for potential vaccine use.

2. Progress.

a. We have improved the specificity of an assay system, developed by us for differentiating the protective capabilities of vaccines against typhoid fever. This improvement was brought about by employing a newly developed Salmonella typhimurium hybrid challenge strain. The system employs Swiss Webster white mice as test animals and a mouse-virulent S. typhimurium hybrid expressing S. typhi surface antigens as the challenge organism. The hybrid used initially possessed certain cell surface characteristics that caused it to cross-react nonspecifically with immune sera stimulated by certain vaccines that should not have protected against the hybrid strain. Employment of a new S. typhimurium hybrid challenge organism with different surface characteristics has eliminated this non-specific protection and improved the ability of the system to differentiate vaccine effectiveness properly.

b. Continuing studies on the genetic basis of variable expression of the virulence (Vi) surface antigen of Citrobacter freundii have revealed the existence in this organism of a previously unreported surface antigen, designated A_{II}, whose genetic determinants appear to be linked to the determinants of the Vi antigen. However, the A_{II} antigen is expressed continuously and is not under reversible regulatory control as is the Vi antigen. In addition, we have determined that the loci controlling Vi antigen expression in C. freundii are allelic with the viaA and viaB loci controlling Vi antigen synthesis in various Salmonella species. Further genetic studies have demonstrated that the viaB locus in C. freundii contains a special genetic mechanism that is involved in regulating the variable expression of this antigen in Citrobacter. An understanding

of this novel genetic mechanism controlling surface antigen expression is of fundamental importance both in examining the disease process and in developing potential vaccine strains.

c. Shieglla sonnei is responsible for greater than two thirds of the bacillary dysentery in the U.S. and Europe. This debilitating intestinal disease is a prime concern of the military. Freshly isolated S. sonnei strains express a characteristic somatic antigen, termed form I, which is easily lost upon subculture. The results of our genetic and physical studies indicate that the determinants of form I antigen synthesis are located on a 120 Mdal extrachromosomal genetic element (i.e., plasmid). Further studies have revealed that S. sonnei cells which lose this plasmid are always avirulent. Upon reintroduction of this plasmid, the S. sonnei strains regain virulence (i.e., the ability to penetrate the intestinal epithelium).

d. The results in (c) above have been used to develope a potential bivalent vaccine against both shigellosis due to S. sonnei and typhoid fever. Recently, Swiss researchers have constructed a galE mutant of Salmonella typhi which has proven in extensive field trials to be a highly effective oral vaccine against typhoid fever. We considered that this galE strain might be used as a carrier for the form I antigen. Thus, we genetically transferred the plasmid encoding the form I antigen of S. sonnei into the galE S. typhi vaccine strain. The resulting S. typhi derivative strain maintained all of the antigenic properties of the parent plus had gained the ability to produce the form I antigen. Subsequent mouse protection tests demonstrated that this derivative vaccine strain is effective in protecting against shigellosis due to S. sonnei and against typhoid fever.

e. In collaborative studies conducted with researchers at the Mayo Clinic, we used recombinant DNA techniques to insert the Escherichia coli genes encoding lactose utilization into the ampicillin resistance transposon, Tn3. The purposes were two-fold: (1) To see if the enlarged transposable element would undergo genetic transposition; (2) to construct a transposon that would be useful in studies aimed at defining the mechanism of transposition. The recombinant transposon was transposable as an enlarged discrete unit. Furthermore, cloning into the Tn3 Bam H1 site derepressed the transposon, the transposition of which could easily be monitored on MacConkey lactose indicator agar. This derivative transposon should prove to be a valuable experimental tool for mobilizing genes and studying the mechanism of transposition.

3. Future Objectives: Future plans include (a) employment of the newly improved vaccine assay system to test the protective capabilities of genetically altered E. coli strains expressing S. typhi antigens, in comparison with those of attenuated S. typhi strains, as candidates for live, oral vaccines against typhoid fever (b) attempts will be made to characterize the Vi and A_{1} surface antigens of C. freundii by genetic and immunochemical procedures. (c) Transfer of the Vi antigen viaB locus to E. coli K-12 to examine genetically and biophysically the mechanism responsible for variable Vi antigen expression. (d) Employment of the galE S. typhi oral vaccine strain as a carrier of other antigenic determinants to protect against other intestinal diseases. (e) collaboratively conduct volunteer studies of the galE S. typhi form I derivative oral vaccine strain to test its effectiveness.

Publications:

1. Baron, L.S., D.J. Kopecko, N. Snellings, and E.M. Johnson. 1980. Transfer of the Citrobacter freundii locus controlling reversible transition of the Vi antigen to Escherichia coli K-12. Abst. Ann. Meeting, Amer. Soc. Microbiol., p. 48.

2. Diena, B.B., H. Lior, A. Ryan, P. Krol, E.M. Johnson, and L.S. Baron. 1980. Mouse protection by vaccination with Salmonella typhi galE Ty21a. Abst. Ann. Meeting, Amer. Soc. Microbiol., p. 47.

3. Gemski, P., J.R. Lazere, T. Casey, and J.A. Wohlhieter. 1980. Presence of a virulence-associated plasmid in Yersina pseudotuberculosis. Infect. Imm. 28: 1044-1047.

4. Greenblatt, J., Li, J., Adhya, S., Friedman, D.I., Baron, L.S., Redfield, B., Kung, H-F., Weissbach, H. 1980. "L actor that is Required for β-galactosidase Synthesis is the nusA Gene Product Involved in Transcription Termination" Proc. Natl. Acad. Sci. USA 77: 1991-1944.

5. Kopecko, D.J., O. Washington, and S.B. Formal. 1980. Genetic and physical evidence for plasmid control of Shigella sonnei form I cell surface antigen. Infec. Immun. 29: 207-214.

6. Kopecko, D.J. 1980. Involvement of specialized recombination in the evolution and expression of bacterial genomes. In: Stuttard and Rozee (Eds.), plasmids and transposons, pp. 165-205, Academic Press, N.Y.

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8. Manis J., D.J. Kopecko, and B. Kline. 1980. Cloning of a Lac$^+$ Bam Hl fragment into transposon Tn3 and Transposition of the Tn3 (LAC) Element. Plasmid 4: 170-174.

9. Schauer, A.T., Baron, L.S. Baumann, M.F., Masinni, E.J., Plantefaber, L.C., Strauch, M., Friedman, D.I. 1980. "Host Factors Involved in Antitermination by λ N Product: Abst. Bacteriophage Meeting, Cold Spring Harbor Lab., N.Y. p. 76.

10. Strauch, M., Zeigler, S., Bigelow, B., Baron, L.S., Friedman, D. 1980. "An E.coli Mutation Affecting the Growth of λimmP22 Hybrid Phage." Abst. Bacteriophage Meeting, Cold Spring Harbor Lab, N.Y. p. 23.

Formal Presentations:

1. D.J. Kopecko - "Bacterial Resistance to Antibiotics - A Growing Problem." Presented to Annual Symposium on Military Veterinary Medicine 19-23 May 1980, held at Walter Reed Army Institute of Research, Washington, D.C.

2. D.J. Kopecko - "Genetics of Drug Resistance" presented at Annual Meeting of American College of Veterinary Microbiologists in the Continuing Education Program entitled "Bacteria and Drug Resistance." Held in Chicago, IL. 24 November 1979.

3. D.J. Kopecko - "Role of Specialized Recombination in Gene Expression and Evolution in Bacteria." Presented in Microbiology Seminar Series at The Uniformed Services University of the Health Sciences, Bethesda, M.D. 6 December 1979.

4. D.J. Kopecko - "Bacteria are Genetic Engineers: Role of Specialized Recombination in Gene Expression and Evolution." Biological Seminar Series at Virginia Commonwealth University, Richmond, VA. 5 November 1979.

RE.. AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY A...	2. DATE OF SUMMARY	ADD/CAT CONTROL SYMBOL
DA OA	80 1 01	DD-R&E-AR(A)

3. DATE PREV. SUM.	4. KIND OF SUMMARY	5. SUMMARY SECY	6. WORK SECURITY	7. REGRADING	8a. DISPOSITION	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	BS10	S10AE	207
b. [illegible]	62770A	3M162770A802	00	002
c. CONTRIBUTING	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: 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 80	3	338
FISCAL YEAR CURRENT 81	3	455

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

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20. PERFORMING ORGANIZATION

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

TELEPHONE: (202) 576-3344

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS NAME: T. Hale, O. Washington

NAME:

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 mechanisms of penetration are investigated. Attenuated living vaccines are developed.

25. (U) 79 10 - 80 09 The form I antigen of S. sonnei is controlled by a plasmid which has been transferred to a variety of bacterial species. This antigen has been tranferred to an attenuated strain of S. typhi which has already proven to be a safe and highly effective oral vaccine. The hybrid strain expresses both typhoid and shigella antigens and will be considered as an oral vaccine. S. flexneri inhibits protein synthesis in mammalian cells following invasion. A cytotoxin has been partially purified from an E. coli strain which causes diarrhea and death in young rabbits. (For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.)

*Available in contractors own originator's approval

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

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M162770A802 MILITARY PREVENTIVE MEDICINE AND TROPICAL DISEASES
Work Unit 207 Pathogenesis of Enteric Disease
* Work Unit C02 Pathogenesis of Enteric Diseases

Investigators
Principal: Samuel B. Formal, Ph.D.
Associates: O. Washington, BS; S. Austin, BS, H. Collins, T. Hale, J. Clements, Post Docs

Objectives: The pathogenesis of bacterial infections of the intestinal tract using techniques of biochemistry, genetics, molecular biology, physiology and pathology to establish the factors and mechanisms by which disease is provoked. The current objectives of this work are to understand the interaction of enteric pathogens with the epithelial cells of the intestine and to develop vaccines to prevent disease.

Progress: A mutant typhoid strain has been isolated by workers in Switzerland which when utilized as a living oral vaccine has been highly safe and effective in protecting Egyptian children against typhoid fever. We consider this strain can be used as a carrier strain for other antigens to protect against other infections. Accordingly, a hybird has been constructed which expresses the antigens of both S. typhi and Shigella sonnei I. The hybrid strain acts no differently from the parent S. typhi mutant strain in animal safety tests.

Protein synthesis, i.e. (^{14}C)leucine incorporation into acid precipitable material, was measured in tissue culture monolayers which had been infected with shigellae. Two established cell lines of human origin served as host cells: HeLa (which is sensitive to exogenous Shiga cytotoxin) and Henle 407 (which is resistant to exogenous Shiga cytotoxin). Shigella dysenteriae 1 strain 3818T (a highly toxigenic, invasive strain) inhibited protein synthesis in both HeLa and Henle 407 cells. Strain 3818 O (a non-invasive, fully toxigenic mutant of 3818 T) inhibited protein synthesis in HeLa but not in Henle 407 cells. Inhibition of protein synthesis in infected Henle 407 cells was probably caused by cytotoxin elaborated by intracellular 3818 T, whereas cytotoxin elaborated by either strain extracellularly (or by 3818 T intracellularly) could have inhibited protein synthesis in toxin-sensitive HeLa cells. Protein synthesis was also inhibited in HeLa or Henle 407 cells which were infected with S. dysenteriae 1 strain 725 or S. flexneri 2a strain M4243. Although both these strains are invasive, they produce at least 1000-fold less cytotoxin than either 3818 T or 3818 O. These results suggest that the relatively small amounts of toxin released by intracellular shigellae are sufficient to inhibit mammalian protein synthesis. This experimental observation provides support for the suggestion that Shiga cytotoxin is an important virulence factor which contributes to destruction of the colonic mucosa during shigellosis.

An E. coli strain which causes diarrhea and death in young rabbits may cause disease by a new mechanism and the infection resembles that of infection of newborn human beings with the classical EPC E. coli serotypes. A cytotoxin from the rabbit strain has been partially purified and characterized. The cytotoxic activity is completely destroyed by heating at 55°C for 1 hr. Eighty seven percent of the activity is destroyed by heating at 42°C for 1 hr. The cytotoxin is not neutralized by S. dysenteriae 1 antitoxin, and, based on estimates obtained during gel filtration chromatography, has a molecular weight between 1000,000 and 130,000. The cytotoxin consistently caused fluid secretion in rat ileal loops but secretion was observed in only 50 percent of rabbit ileal loops.

Enteric Diseases

Future Plans: Studies on the biological activity of the hybrid typhoid S. sonnei vaccine which has been preserved by lyophilization will be conducted. The hybrid strain will then be tested for safety and antigenicity will be carried out in volunteers. Work on the interaction of enteric pathogens with epithelial cells will continue with emphasis on the mechanism of penetration of the epithelial cells by the bacterium. Research on the cytotoxin of E. coli will be carried on at the University of Rochester.

Bibliography

1. O'Brien, A.D., Scher, I., and Formal, S.B. Effect of silica on the innate resistance of inbred mice to Salmonella typhimurium infection. Infect. Immunol. 25:513-520, 1979.

2. O'Brien, A.D., Scher, G.H. Campbell, R.P. MacDermott, and S.B. Formal. Susceptibility of CBA/N mice to infection with Salmonella typhimurium: Influence of the X-linked gene controlling B-lymphocyte function. J. Immunol. 123: 720-724, 1979.

3. Liu, C.T., R.P. Sanders, J.W. Dominik, and S.B. Formal. Effects of intravenous and aerosol administration of crude shigella toxin in rhesus macaques: Preliminary study. Am. J. Vet. Res. 40:836-839, 1980.

4. Chaney, C.P., P.A. Sohad, S.B. Formal and E.C. Boedecker. Species specificity of in vitro Escherichia coli adherence to host intestinal cell membranes and its correlation with in vivo colonization and infectivity. Infect. Immun. 28:1019-1027, 1980.

5. Kopecko, D.J., O. Washington, and S.B. Formal. Genetic and physical evidence for plasmid control of Shigella sonnei form I cell surface antigen. Infect. Immun. 29:207-214, 1980.

6. O'Brien, A.D., D.L. Rosenstreich, I. Scher, G.H. Campbell, R.P. MacDermott and S.B. Formal. Genetic control of susceptibility to Salmonella typhimurium in mice. Role of the LPS gene. J. Immunol. 124:20-24, 1980.

7. Donowitz, M., A.W. Charney, R. Haynes, S.B. Formal and H. Collins. Significance of abnormal rabbit ileal histology in the pathogenesis of diarrhea. Infect. Immun. 26:380-386, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OC 6435	80 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	8a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10AF	208
b. ~~XXXXXXXX~~	61102A	3M161102BS01	00	149
c. CONTRIBUTING	STOG 80-7.2	2		

11. TITLE (Precede with Security Classification Code)

(U) Immunity in Protozoan Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: 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 80	7	672
	CURRENT 81	2	375

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence not considered

20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Haynes, J.D., LTC

NAME: McNeill, K.M., MAJ

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Antigens; (U) Protozoa; (U) Immunity; (U) Tropical Medicine; (U) Medicine

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 elucidate the protective mechanisms involved in immunity to malaria and African sleeping sickness. Malaria is a disease which has repeatedly impeded military operations and African Sleeping sickness has a high potential for doing so should there be troops in the endemic area.

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 roles 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) 79 10-80 09 Immunity to challenge has been induced with irradiated metacyclic forms of African trypanosomes. The immunity is effective against metacyclics derived from different antigen type within a serodeme. Monoclonal antibodies have been produced against metacyclics, identifying at least 3 different antigen types. Peripheral blood leukocytes from P. falciparum infected chimpanzees were active in the in vitro destruction of parasitized red blood cells. This in vitro killing, which persisted at least three months after cure, was not dependent upon the presence of host serum. This data suggest a cell rather than antibody mediated immunity. Protective immunization to P. yoeli challenge in mice was possible using multiple doses of irradiated unfractionated parasitized RBC's, but not with purified trophozoites, schizonts, ring forms or fractionated reconstituted, parasites. For technical report see Walter Reed Army Institute of Research Annual Progress Reports, 1 Oct 1979 - 30 Sep 1980.

*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 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES
WORK UNIT: 208 Immunity in Protozoan Diseases
* WORK UNIT: 149 Immunity to Protozoan Diseases

INVESTIGATORS: Diggs, C.L.; COL, Esser, K.M.; Gore, R.W.; Schoenbechler, M.; Wells, R.A.; LTC, Williams, J.S.

b. Problems and Objectives: Both malaria and African sleeping sickness pose significant health hazards to troops operating in endemic areas. Prophylaxis against infection is necessary to prevent major troop loses due to morbidity and mortality associated with these diseases. The objective of this work unit is to elucidate the protective mechanisms and antigens involved in immunity to malaria and African sleeping sickness. These studies are a crucial step in vaccine development.

c. Progress: Experimental immunization against challenge has been achieved using irradiated metacyclic forms of Trypanosoma rhodesiense. Animals immunized with three doses of metacyclics were resistant to challenge with $1X10^4$ metacyclics of homologous antigen type and also metacyclics of heterologous type derived from the same trypanosome isolate. No resistance was observed to challenge with metacyclics derived from another isolate. The immunity observed was transferable with serum and therefore appears to be antibody mediated. The metacyclic antigen types involved in eliciting the protective immunity and the antigen types present in the fly vector were analysed with monoclonal antibodies. Antibodies have been produced which identify at least three distinct metacyclic antigen types. All three types are present both in individual infected flies and in the first peak parasitemia in the fly-infected host. Some homology was found among metacyclics derived from different trypanosome isolates.

Studies on immunity to malaria were performed in both chimpanzee and mouse models. Clinical changes in chimpanzees infected with P. falciparum were unremarkable. There was little evidence of serum mediated phenomena- including no in vitro anti-parasitic effect, no serum lymphocytotoxins and no significant change in circulating malaria antibody (via IFA). Other findings included transient suppressions in T cell numbers, evidence for coombs positive anemia and enhanced phagocytosis of antibody coated sheep red cells. There was however strong evidence for cell mediated immunity. Results indicated both enhanced lectin induced blastogenesis and malaria antigen specific phagocytosis by white cells from infected animals. Immunological memory lasted at least 2-3 months after drug cure.

Studies with P. yoeli in mice demonstrated the efficacy of multiple doses of irradiated, parasitized erythrocytes for induction of protective immunity to challenge. Complete protection was achieved in mice treated with unfractionated parasites but not in mice treated with purified trophozoites, schizonts and ring forms. However, the fractionation studies were inconclusive as fractionated, reconstituted parasites were also not effective in eliciting a protective immune response.

d. Recommendations: In view of the encouraging results obtained in the experimental immunization studies with African trypanosomes, further work is indicated for the identification of antigens involved in eliciting a broad-spectrum immunity. Also, further analysis of the infective form antigen types present in the fly vector is necessary. Monoclonal antibodies should continue to be the major tool for these studies. As a protective, cell mediated immune response can be demonstrated in experimental animal models of human malaria, work should proceed on the

identification of the parasite stage involved and the discrete target antigens.

e. References cited: None

f. Presentations:

1. Use of monoclonal Hybridoma Antibodies for the Identification of Specific Antigens of Trypanosoma rhodesiense. Klaus M. Esser and Carter L. Diggs. American Society of Tropical Medicine and Hygiene, Annual Meeting, November 1979.

g. Publications:

1) Isolation and Characterization of a New Serodeme of Trypanosome rhodesiense. Campbell, G.H., Esser, K.M., Wellde, B.T., and Diggs, C.L., Am. J. Trop. Med. Hyg. 28 (6): 974-983 1979.

2) Kinetics of Peripheral Blood Leukocyte Alterations in Thai Children with Dengue Hemorrhagic Fever. Wells, R., Scott, R., Pavanand, K., Sathitsathein, V., Cheamaudon, U. and MacDermott, R. Infect. Imm. 28(2): 428-433. 1980.

3) Anti-lymphocyte antibodies in sera of Thai adults infected with Plasmodium falciparum or Plasmodium vivax. Wells, R., Pavanand, K., Zolyomi, S., Permpanich, B., and MacDermott, R. Clin. exp. Immunol. 39: 663-667. 1980.

4) Examination of Peripheral Blood Mononuclear Cells and Sera from Thai Adults Naturally Infected with Malaria in Assays of Blastogenic Responsiveness to Mitogenic Lectins and Allogenetic Cell Surface Antigens. MacDermott, R., Wells, R., Zolyomi, S., Pavanand, K., Phisphumvidhi, P., Permapaich, B., and Gilbreath, M. Infect. Imm., In press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OA 6440	80 10 01	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10AF	209
b. [illegible]	61102A	3M161102BS01	00	129
c. CONTRIBUTING	STOG 80-7.2;2			

11. TITLE (Precede with Security Classification Code)

(U) Parasitic Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

13A. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
54 09	CONT	DA	C. In-House

17. CONTRACT/GRANT — NA

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	3.0	140
	CURRENT 81	3.0	98

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign intelligence not considered.

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Parasite; (U) Schisotosomiasis; (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) 79 10-80 09 Infection of 13 strains of inbred mice with human isolates of L. braziliensis and L. mexicana manifested a series of different responses. Lymphocytes and plasma cells predominated in lesions undergoing resolution, while infected histiocytes were predominant in chronic lesions. In studies for development of regimens for treatment of T. rhodesiense, cis-diamminedichloroplatinum (II) in combination with a rescue agent, disulfiram, was curative in mice with reduced kidney toxicity at dosages far above those ordinarily tolerated. The human monocyte-derived macrophage model of leishmaniasis is being employed for evaluation in vitro of anti-leishmanial effect of experimental agents. Preliminary results indicate a number of novel nitro-imidazoles may have anti-leishmanial activity. Allopurinol appears to be less effective in this model than in other model systems of leishmaniasis. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79-30 Sep 80.

*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 & HEALTH HAZARDS
* Project 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES

Work Unit 209 Parasitic Diseases of Military Importance
* Work Unit 129 (Same Title)

Investigators:

PRINCIPAL: LTC Larry D. Hendricks
Associates: MAJ Jonathan D. Berman
MAJ George E. Childs
CPT Michael S. Wysor
Mrs. Gloria P. Willet

PROBLEM AND OBJECTIVES:

Effective management of parasitic disease problems among military personnel is dependent upon 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) evaluation of existing techniques and (c) 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:

Inoculation of 13 strains of inbred mice with human isolates of L. braziliensis and L. mexicana elicited a series of different responses. Lymphocytes and plasma cells predominated in those lesions which were undergoing resolution, while in the chronic lesions, infected histiocytes predominated.

The human monocyte-derived macrophage culture model of leishmaniasis is being employed for evaluation of anti-leishmanial effects of experimental agents. This is the only reported in vitro model in which the achievable serum levels of the major anti-leishmanial agents in clinical use are effective, eliminating more than 85% of the parasites. Preliminary results have identified a number of novel imidazoles which have potent antileishmanial activity. Allopurinol appears to be less effective in this model than in other model systems of leishmaniasis.

In studies of development of regimens for treatment of T. rhodesiense, cis-diamminedichloroplatinum (II) in combination with a rescue agent, disulfiram, and hydration, was curative in mice with reduced kidney toxicity at dosages far above those ordinarily tolerated.

FUTURE OBJECTIVES:

In addition to continuation of studies for further evaluation of therapeutic regimens exploiting the efficacy of cis-diamminedichloroplatinum (II), against T. rhodesiense, for investigation of the activity of 8-aminoquinolines against leishmaniasis in the human monocyte-derived macrophage

in vitro model, and for development of in vivo models of human leishmaniasis in mice, efforts are underway to develop a laboratory model which will allow the investigation of the ability of drugs to pass through the blood-brain barrier. This model, in inbred mice and rabbits, will aid in development of drugs active against the cerebral aspects of trypanosomiasis.

PUBLICATIONS:

1. Susceptibility of Inbred Mice to Infection with Human Isolates of Cutaneous Leishmaniasis. G. Childs, L. Lightner, L. McKinny, M. Groves, E. Price, and L. Hendricks. Submitted to International Journal of Parasitology, 1980.

2. Phototoxicity of the chemotherapeutic agents Hematoporphyrin D, meso-tetra(p-sulfophenyl)porphine and zinc-tetra(p-sulfophenyl)porphine. Grenan, M., Tsutsui, M., and Wysor, M. Res. Communs. in Chem. Path. and Pharmacology (In Press).

3. Cure of mice infected with Trypanosoma rhodesiense by cis-diammine-dichloroplatinum (II) and disulfiram rescue. Wysor, M., Zwelling, L., Sanders, J., and Grenan, M. Submitted to Proc. Natl. Acad. Sci.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA OC 6444	2. DATE OF SUMMARY: 80 10 01	REPORT CONTROL SYMBOL: DD DR&E(AR)636						
3. DATE PREV SUM: 79 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8A. DISP'N INSTR'N: NI	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	S10AH	210				
b. CONTRIBUTING	61102A	3M161102BS01	00	124				
c. CONTRIBUTING	STOG 80-7.2.2							

11. TITLE (Precede with Security Classification Code)
(U) Biochemical Research on Military Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
002300 Biochemistry 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	Cont.	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: N/A
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	8	395
	CURRENT 81	9	729

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER

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NAME: Gemski, P. Ph.D.
NAME: Wolfe, A.D. Ph.D.

21. GENERAL USE
Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Toxin; (U) Antigens; (U) DNA; (U) Hybridoma; (U) Immunoglobulin

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) 79 10 - 80 09 Purified shiga toxin has been shown to inhibit protein synthesis in HeLa cells. Shiga toxin can be activated by treatment with urea and DTT. Inhibition of peptide elongation is a primary mechanism. Clostridium difficile toxin has been known to be associated with antibiotic-induced colitis and to be toxic to HeLa cells. This has facilitated diagnosis of antibiotic-associated colitis among patients at WRAMC. Nucleotide sequence studies of enteric bacteria have established a new taxonomic group of organisms. Mouse lymphocyte hybridomas which produce monoclonal antibodies to Rindbis viral proteins and to antigens of P. falciparum have been recovered. See WRAIR Annual Progress Report 1 Oct 79 to 30 Sept 80.

*Available to contractors upon originator's approval

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

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES
Work Unit 210 Biochemical Research on Military Diseases
*Work Unit 124 Biochemical Research on Military Diseases

Investigators:

Principle: Bhupendra P. Doctor, Ph.D.

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Description:

To design and execute research programs that provide fundamental biochemical and molecular definitions of diseases and injury relevant to the military. Factors associated with disease processes such as virulence determinants of organisms, biochemical and metabolic mechanisms of parasites, and products of host responses to disease are being studied through the use of physicochemical, 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) Large Scale Purification and Characterization of Shiga Toxin from Shigella dysenteriae.

Shiga toxin has been purified to apparent homogeneity from lysates of Shigella dysenteriae 1. Purification was accomplished by high speed centrifugation, ammonium sulfate fractionation, DEAE-cellulose chromotagraphy, CM-cellulose chromatography, gel filtration chromatography, and preparative isoelectric focusing. The purified toxin, enriched 8000-fold from the cell lysate, displayed both enterotoxic activity and mouse lethality. It had an isoelectric point of pI 7.2 and displayed microheterogeneity after both electrophoresis and isoelectric focusing. The molecular weight was 60,000-70,000 daltons and the protein was composed of subunits of M_r = 30,000. Amino acid analysis indicated the presence of few methionine and cysteine residues in the protein. Monospecific antiserum has been prepared against the purified toxin protein.

(2) Inhibition of Protein Synthesis in Intact HeLa Cells by Shigella dysenteriae 1 Toxin

Shiga toxin, purified to near homogeneity from cell lysates of Shigella dysenteriae 1, inhibited protein and DNA synthesis in intact HeLa cells. Inhibition was dependent on toxin concentration and time of incubation. A minimal latent period of 30 minutes was observed with saturating doses of toxin. RNA synthesis, uptake of α-aminoisobutyric acid and maintenance of intracellular K^+ concentrations were not affected until well after maximal inhibition of protein and DNA synthesis. The inhibitory effect of toxin was sensitive to heat inactivation and was prevented by antibody neutralization. Several cytotoxic components were separated by polyacrylamide gel electrophoresis of the purified toxin preparation; all inhibited protein and DNA synthesis equally.

(3) Inhibition of Protein Synthesis by Shiga Toxin: Activation of the Toxin and Inhibition of Peptide Elongation.

Inhibition of cell-free protein synthesis by highly purified Shiga toxin was enhanced by prior activation of the protein. Inhibition of peptide elongation appeared to be the primary mechanism by which activated toxin affected protein synthesis. When toxin was pretreated with 8M urea plus 10mM DTT, a 70-fold enhancement of the ability to inhibit cell-free protein synthesis in rabbit reticulocyte lysates was observed. Incubation of toxin with 8M urea, 10mM DTT, resulted in a concomitant loss of cytotoxicity. To investigate the mechanism of inhibition by the activated toxin, the effects on kinetics of [^{3}H]leucine incorporation and on the polysome profile after inhibition were examined. Addition of toxin (about 18-fold more concentrated than 50% inhibition level) resulted in an immediate inhibition of protein synthesis. Both the toxin-treated mixture and the control sample contained numerous polysomal species with attached labeled peptidyl-tRNA. Activated Shiga toxin was assayed to determine if inhibition occurred by ADP-ribosylation of EF-2. No incorporation of [^{14}C]ADP-ribose was observed with either untreated toxin or toxin treated with DTT, urea plus DTT, SDS plus DTT, or with trypsin.

(4) Conservation of Membrane Functions in Hela Cells Treated with Shiga Toxin.

Shiga toxin, purified to apparent homogeneity from Shigella dysenteriae 1, inhibits protein and DNA synthesis in intact HeLa cells. We have now studied the effect of toxin on uptake of ^{3}H- α-aminoisobutyric acid (^{3}H- α-AIB) and on maintenance of intracellular K^+ levels to determine whether gross effects on membrane function occur. Subconfluent cell monolayers were incubated with ^{14}C-leucine without toxin for various times (zero to 150 minutes). Then ^{3}H -AIB (16mCi/mmol) was added (0.2mM final) for a 5 minute pulse. Incorporation of ^{14}C-leucine (TCA-insoluble freaction) was completely inhibited after 75 minutes by the toxin. Uptake of ^{3}H- α-AIB by toxin-treated monolayers was not significantly

different from the control values for at least 120 minutes. Maintenance of intracellular K^+ was determined by flame photometry of cell lysates following incubation of cell monolayers with toxin for up to 480 minutes. K^+ levels for toxin-treated monolayers remained identical to control values for 120 minutes although protein synthesis had completedly ceased after 75 minutes. These results demonstrate that inhibition of protein and DNA synthesis by Shiga toxin occurs prior to inhibition of amino acid uptake, depletion of cellular ATP and gross changes in membrane permeability.

(5) Characterization of Shigella dysenteriae 1 (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 filtration of ^{125}I-labeled affinity column eluates. Two chromatographic peaks were observed. The percent 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 (PAGE), but formed three bands of 33,000d, 29,000d, and 4,000-7,000d after sodium dodecyl sulfate PAGE. In addition, material estimated as 7,000d by Bio-Gel P-10 chromatography could be generated by treatment of S-200 peak I samples with 8 M area. 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 (7,000d) was 27% immunoprecipitable with antitoxin. These data indicate that three of the known biological activities of Shiga toxin are associated with a 33,000d substance which can be dissociated into 29,000 and 4,000-7,000d components.

(6) Release of Shigella dysenteriae 1 (Shiga) Toxin by Polymyxin B.

The release of Shiga toxin from Shigella dysenteriae by polymyxin B was investigated. The amount of Shiga toxin released from exponential cultures of S. dysenteriae 1 strain 3818-0 increased with both polymyxin concentration and time of incubation with polymyxin. Less than 10% of the Shiga toxin was released after incubation with polymyxin B for 2 minutes which suggests it is not a periplasmic protein. The polymyxin B release of Shiga toxin after 2 minutes more closely resembled the release of the cytoplasmic protein, inorganic pyrophosphatase, rather than the release of the periplasmic protein 5'nucleotidase. These data indicate that Shiga toxin is not a periplasmic protein, and therefore treatment with polymyxin B would not aid in the purification of Shiga toxin.

(7) Isolation and Characterization of Minicell-Producing Mutants of Shigella .

Minicells are small anucleate cells resulting from aberrant cell divisions

at the polar ends of bacilli. We have isolated minicell-producing mutant strains of S. flexneri 2a (MC-I) and S. dysenteriae 1 (MC-V) after mutagenesis with N-methyl-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 provoke keratoconjunctivitis in guinea pigs and to invade HeLa cells. Purified suspensions of minicells containing less than 1 whole cell per 10^6 minicells were obtained by a combination of differential sedimentation and density-gradient centrifugation (5-30% w/v linear sucrose gradients). Purified MC-I minicells contain about 0.005 the amount of DNA of a normal S. flexneri. MC-V minicells have about 0.003 the amount of DNA of a whole S. dysenteriae cell. Purified MCV minicells were treated with polymyxin B to release Shiga toxin. Shiga toxin was readily detected in MC-V minicells by means of the 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 the virulence.

(8) A Quantitative Microtiter Cytotoxicity Assay for Shigella Toxin.

The cytotoxic activity of Shigella dysenteriae 1 was assayed by exposing HeLa cells in microtiter cultures to dilutions of toxin. Exposure to toxin caused either failure of cells in suspension to attach or detachment of cells from established monolayers. Estimates of toxin potency were made by staining residual cells with crystal violet and visually inspecting the stained plates. Quantitation of the cytotoxic effect was made possible by eluting and spectrophotometrically measuring the stain. The dilution of toxin causing 50% cell detachment (CD_{50}), the endpoint chosen for the assay, was estimated from plots of dye absorbance vs. toxin dilution. The CD_{50} varied as a function of cell concentrations, incubation of toxin with cells in suspension or as established monolayers, and the cell line used for assay. The HeLa cell line was the most sensitive of the cell lines examined. The method was easily utilized to monitor toxin purification and to measure antitoxin neutralization of toxin activity.

B. Studies on Clostridium difficile Toxin.

(1) Cytotoxicity of Clostridium difficile Toxin in HeLa Cells.

Clostridium difficile toxin is toxic to HeLa cells. When dilutions of sterile culture filtrates and fecal filtrates from humans and guinea pigs with antibiotic-associated colitis are incubated for 16 hr at 35°C with HeLa cell monolayers in microtiter plates, a cytotoxic endpoint can be determined by visual examination of the fixed and crystal violet-stained monolayers. Decreased cell staining correlates with microscopic observations of cytotoxic effects. Cytotoxic activity is heat sensitive and can be neutralized by C. sordellii antiserum.

The effects of toxin prepared from culture filtrates on macromolecular synthesis were examined. Inhibition of ^{14}C-leucine, ^{3}H-thymidine and ^{3}H-uridine incorporation into the TCA-precipitable material occurs after 1-3 hr. exposure and is proportional to toxin concentration. After 16 hr exposure, 50% inhibition of protein and DNA synthesis occurs at the 5^{-7} dilution; however, approximately 25% residual incorporation (Leu;T) is observed at high toxin concentrations. Estimates of toxin levels by cell staining and by ^{14}C-leucine incorporation are similar. Intracellular K^{+} levels and uptake of ^{3}H- α-aminoisobutyric acid decrease concurrently with macromolecular synthesis.

(2) 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. The cytotoxicity could be neutralized by Clostridium sordellii antitoxin, but not by other clostridial antitoxins. Rabbit immmunization with toxic cecal extracts produced antibody which neutralized cytotoxicity of guinea pig cecal extracts, of stool extracts from humans with antibioticassociated colitis and of culture supernatant fluids of C. difficile. Treatment with vancomycin reduced the number of deaths and increased the survival time of penicillintreated 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 diseased animals, however, contained this cytotoxin, suggesting that C. difficile toxin is the cause of antibiotic-associated colitis in guinea pigs.

(3) C. difficile Assays for Patient Diagnosis.

The HeLa cell assay for C. difficile toxin is rapid and specific. It is useful in patient diagnosis both to confirm sigmoidoscopic observations of pseudomembranes in the colon and to provide rapid diagnosis of cases of antibiotic-associated colitis where proctoscopy findings are negative.

In the past year our laboratory has analyzed 21 patient specimens for C. difficile toxin, 10 from WRAMC, 10 from NNMC and 1 from Walson AH, Ft. Dix, NJ. Two specimens were positive; 19 were negative.

C. Nucleotide Sequence Relatedness among Enterobacteriaceae.

(1). Deoxyribonucleic Acid Relatedness in the Genus Yersinia.

A large number of Yersinia strains were characterized biochemically and by intra- and intergroup deoxyribonucleic acid (DNA) relatedness. The following conclusions were drawn from the data obtained: (a) Yersinia enterocolitica is restricted to rhamnose-negative strains belonging to one of five broad biotypes. Biotypes 1 through 4 are trehalose-positive and sucrosepositive. Biotype 5 contains trehalose-negative strains, some of which are sucrosenegative. (b) Strains positive in tests for melibiose, rhamnose, raffinose, αmethyl-Dglucoside, and Simmons' citrate belong to a new species for which the name Yersinia intermedia is proposed. (c) Strains that are rhamnosepositive only belong to a new species for which the name Yersinia frederiksenii is proposed. (d) Sucrose-negative, VogesProskauer-negative, trehalose-positive strains belong to a new species for which the name Yersinia kristensenii is proposed. (e) Additional groups belonging to the genus Yersinia, but not to any recognized species, are group X1, which is sucrose-negative, ornithine decarboxylasenegative, Voges-Proskauernegative, and trehalose-positive, and X2, which is rhamnosepositive and sucrose negative. (f) Yersinia pseudotuberculosis and Yersinia pestis are inseparable on the basis of DNA relatedness. For taxonomic purposes they should be combined as pathotypes pseudotuberculosis and pestis within the single species Y. pseudotuberculosis. the distinction between Y. pestis Y. pseudotuberculosis can and should be used for medical purposes. (g) "Yersinia" philomirangia does not belong to Yersinia.

(2). Yersinia enterocolitica sensu stricto.

The species Yersinia enterocolitica is defined sensu stricto on the bases of biochemical reactions and dexoyribonucleic acid (DNA) relatedness. Biochemically, Y. enterocolitica contains five major biotypes: 1 though 4 of Nilehn and of Wauters, and the trehalose-negative, metabolically inactive, socalled hare strains in biotype 5 of Nilehn and of Wauters. Biochemically atypical strains, including urease-negative, Simmons' citrate-positive, and lactose-, raffinose-positive strains, were shown to be Y. enterocolitica. Y. enterocolitica was distinguishable from Yersinia kristensenii on the bases of sucrose and Voges-Proskauer reactions (negative in Y. kristensenii). These species are separable by DNA relatedness, especially when thermal stability of related sequences in 60°C reactions or DNA reassociation in 75°C reactions is compared. Y. enterocolitica was also separable biochemically and by DNA relatedness from the two newly proposed rhamnose-positive species, Yersinia intermedia and Yersinia frederiksenii. Strain 161 (=ATCC 9610) is proposed as the neotype for Y. enterocolitica.

(3) Yersinia intermedia sp. nov.: a new species of Enterobacteriaceae compose of rhamnose-positive, melibiose-positive strains (formerly called Yersinia enterocolitica or Yersinia enterocolitica- like).

Yersinia intermedia sp. nov. is defined biochemically and genetically. Y. intermedia strains belong to a single deoxyribonucleic acid (DNA) relatedness group that is separable from Y. enterocolitica, Y. frederiksenii, Y. kristensenii, and Y. pseudotuberculosis. Y. intermedia is positive in reactions for melibiose, raffinose, α-methyl-D-glucoside, rhamnose, and usually Simmons'citrate. It is metabolically more active at 22°C to 28°C than at 35°C. These positive reactions serve to distinguish Y. intermedia from Y. enterocolitica and Y. kristensenii. Positive melibiose, raffinose, and α-methyl-Dglucoside reactions differentiate Y. intermedia from the other new rhamnose-positive species, Y. frederiksenii. Y. intermedia is separated from Y. pseudotuberculosis by its positive reactions for sucrose, indole, cellobiose, i-inositol, d-sorbitol, α-methyl-D-glucoside, and ornithine decarboxylase. Yersinia biotype X2, an additional rhamnosepositive, sucrose-negative, as yet unspeciated group, does not belong to Y. intermedia. Strain 3953 is proposed as the type strain for Y. intermedia.

(4) Yersinia Frederiksenii sp. nov.: a new species of Enterobacteriaceae composed of rhamnose-positive strains (formerly called atypical Yersinia enterocolitica or Yersinia enterocolitica-like).

Yersinia frederiksenii sp. nov. is defined biochemically and genetically. Y. frederiksenii strains belong to three separate deoxyribonucleic acid (DNA) relatedness groups, each of which is separable from Y. enterocolitica, Y. intermedia, Y. kristensenii, Y. pseudotuberculosis, and Yersinia biotype X2, an unspeciated, rhamnose-positive, sucrose-negative group. The three Y. frederiksenii DNA relatedness groups, 6175, 2581-77, and 867, were represented by 10, 3, and 1 strains, respectively. All three groups were phenotypically similar. Pending additional study, it was decided to retain them all in Y. frederiksenii.

The positive rhamnose reaction separates Y. frederiksenii from Y. enterocolitica and from Y. kristensenii. A positive sucrose reaction distinguishes Y. frederiksenii from the rhamnose-positive, sucrose-negative Yersinia biotype X2. Negative reactions for melibiose, raffinose, and α-methyl-D glucoside distinguish Y. frederiksenii from Y. intermedia. A negative melibiose reaction and positive reactions for ornithine decarboxylase, indole, sucrose, sorbose, sorbitol, inositol, and Voges-Proskauer separate Y. frederiksenii from Y. pseudotuberculosis. Strain 6175 is proposed as the type strain for Y. frederiksenii.

(5) Yersinia kristensenii, sp. nov.: a new species of Enterobacteriaceae composed of sucrose-negative strains (formerly called atypical Yersinia enterocolitica or Yersinia enterocolitica-like).

Yersinia kristensenii strains belong to a single deoxyribonucleic acid (DNA) relatedness group. In 60°C reactions the highest relatedness values of Y.

enterocolitica to Y. kristensenii were 70 to 79%, overlapping the lowest intraspecies Y. kristensenii values. Y. kristensenii was easily separable from Y. enterocolitica on the bases of per cent divergence within related DNA sequences in 60°C reactions and relatedness in 75°C reactions.

Y. kristensenii is trehalose-positive and negative in reactions for sucrose, Voges-Proskauer, rhamnose, raffinose, melibiose, and α-methyl-Dglucoside. Negative sucrose and Voges-Proskauer reactions differentiate Y. kristensenii from Y. enterocolitica biotypes 1 through 4. Its positive trehalose reaction, negative Voges-Proskauer reaction, and positive sorbitol and xylose reactions separate Y. kristensenii from Y. enterocolitica biotype 5. Negative rhamnose, raffinose, melibiose, and α-D-glucoside reactions, and a negative sucrose reaction distinguish Y. kristensenii from Y. intermedia. Y. frederiksenii is differentiated from Y. kristensenii on the basis of rhamnose, sucrose and Voges-Proskauer reactions. Yersinia biotypes X1 and X2 are both sucrosenegative. Positive reactions for ornithine decarboxylase, D-sorbitol, and sorbose separate Y. kristensenii biotype X1. Positive cellobiose and sorbose reactions, and negative rhamnose and Voges-Proskauer reactions separate Y. kristensenii from Yersinia biotype X2. Y. kristensenii is separated from Y. pseudotuberculosis by its positive ornithine decarboxylase, D-sorbitol, cellobiose, and sorbose reactions and negative rhamnose, melibiose, and βxylosidase reactions. Strain 105 is proposed as the type strain for Y. kristensenii.

(6) Intra-and interspecies relatedness of Yersinia pestis by DNA hybridization and its relationship to Yersinia pseudotuberculosis.

The biochemical characteristics of Yersinia pestis are presented and compared with those of Y. pseudotuberculosis. Motility at 28°C, urease, fermentation of rhamnose, and growth rate on nutrient agar are the best means of separating these organisms. Deoxyribonucleic acid (DNA) hybridization studies demonstrated that Y. pestis strains are 90% or more interrelated and that Y. pestis and Y. pseudotuberculosis are indistiguishable by DNA relatedness. On the basis of DNA data and biochemical and antigenic similarity, these organisms should be treated as two separate pathotypes of the same species. Y. pseudotuberculosis was described before Y. pestis and therefore has priority. Y. pseudotuberculosis pathotype pseudotuberculosis and Y. pseudotuberculosis pathotype pestis are recommended as new designations for Y. pseudotuberculosis and Y. pestis. For medical purposes Y. pestis and Y. pseudotuberculosis can and should continue to be used.

(7) Biochemical and Genetic Parameters of Vibrio cholerae.

Vibrio cholerae has often been studied biochemically and genetically, and divided below the species levels into biogroups, O-antigen groups, and phage types. Each strain can also be classified as toxigenic or nontoxigenic. No comprehensive study has been conducted, however, to define the phenotypic

glycol 1000. Approximately 600 microtiter well cultures were prepared for each mouse spleen, resulting in from 6-15% viable cell lines. Cultures of particular interest were cloned on soft agar, utilizing a feeder monolayer of human fibroblasts, and representative clones were frozen in liquid nitrogen. Antibody to Sindbis virus antigen(s) was detected in approximately 5-20% of all growing cultures. Experiments employing purified virion immunogens frequently produced greater numbers of growing cell cultures and an increased frequency of Sindbis virus antibody production. Hybridoma supernatants were screened for antibody production using a radioimmune assay (RIA) system that employed Sindbis-virus-infected CER cell lysates as antigen and either iodinated anti-mouse antibody or protein A from Staphylococcus. Antibody specificity was examined by RIA using purified and separated Sindbis virus nucleocapsid and each of the envelope glycoproteins. Lymphocyte hybridomas secreting antibodies to all three structural proteins were produced. Significant increases in antibody titer were observed following injection of hybridoma clones into Pristane-treated BALB/C mice and harvesting the resultant ascitic fluid.

(2) Selection of Hybridomas Producing Monoclonal Antibody to the merozotite stage of Plasmodium falciparum.

In collaboration with the Department of Immunology, DCDI program on parasitic infections, hybridoma technology was applied to research dealing with vaccine production against the merozoite stage of Plasmodium falciparum. Hybrids were constructed which neutralized in vitro parasite growth. The resulting monoclonal antibodies were assayed for their ability (1) to bind to acetone-fixed parasites using immunofluorescent assays and (2) to inhibit parasitic growth in vitro. Of 24 positive monoclonal antibodies derived from fusion, only PFC 1, an IgM secretor, was shown to be inhibitory. It seems monovalent due to heterologous chain pairing. If such is the case, these antibodies would not be expected to participate in both precipitation and agglutination-type reactions. The inhibitory properties of antibodies produced by PFC-1 may derive their being pentameric IgM. more than one antibody-combining site per molecule.

E. Mammlian Lysine-t RNA Isoacceptors in Protein Synthesis.

This study was developed to understand the relative utilization of lysine-tRNA isoacceptors during globin synthesis. These isoacceptors have been separated from one another by chromatography on RPC5 and are numbered in order of elution from the column. The present studies show that Peak II (Lys-$tRNA_4$) which is an undermodified lysine isoacceptor, is not utilized as extensively in protein synthesis as Peak I even though both recognize AAG as codon. Peak II was also utilized less extensively for globin synthesis when the [^{3}H]lys-tRNA was prepared from rabbit reticulocyte tRNA or from different preparations of rabbit liver tRNA and

the protein synthesis system was rabbit reticulocyte lysates or wheatgerm extracts. Thus, the reduced utilization of Peak II compared to Peak I is not dependent on the source or preparation of tRNA or on the protein synthesis system. It seems likely that the undermodification of lystRNA$_4$ is responsible for its reduced utilization in protein synthesis.

Peak I was utilized more frequently for globin systhesis than Peak III which recognizes AAA. Although this observation is consistent with the coding sequences for lysine in rabbit globin mRNA, it may not reflect and exact ratio of utilization of Peaks I and III for globin synthesis due to completion with endogenous lys-tRNA.

Measurements of the relative utilization of amino acyl-tRNA may elucidate which isoacceptors are required in greatest abundance for synthesis of specific proteins. Furthermore, such studies may provide information of the effects that specific modifications in the primary structures of isoacceptors have on their utilization in protein synthesis.

PROJECTED WORK

The Department of Biological Chemistry will continue biochemical and molecular studies of factors associated with diseases evoked by various organisms. Specific aims include:

1. Continuation of studies of Shiga toxin, addressing the questions of (a) receptor-mediated uptake; (b) steps of peptidyl elongations inhibited by toxin and (c) structure-function relationships of toxin subunits (binding vs active subunits).

2. Continuation of studies (a) to isolate cell hybrids which produce monoclonal antibodies against other Dengue serotypes, and against virulence components of shigella and (b) explore the use of human lymphoma cell lines for use in hybridoma fusions.

3. Continue studies of C. difficile toxins; (a) its purification and characterization (b) its genetic control.

4. Continue identification, characterization and genetic investigations of clinical and enviromental enterics such Yersiniae, Vibrio, and Kluyvera.

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7. Brown, J.E., M.A. Ussery, S.H. Leppla and S.W. Rothman. Inhibition of protein synthesis by shiga toxin: activation of toxin and inhibition of peptide elongation. Gordon Research Conference on Microbial Toxins and Pathogenesis 1980. Wolfeboro, N.H.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6744	80 10 01	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10AH	211
b. CONTRIBUTING	61102A	3M161102BS01	00	147
c. CONTRIBUTING	STOG 80-7,2;2			

11. TITLE (Precede with Security Classification Code)

(U) Immunological and Biochemical Aspects of Membranes

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: N/A

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	2	119
FISCAL YEAR CURRENT 81	3	211

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Owens, Roberta L. Ph.D.

NAME:

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) This work unit has three major objectives. First, preparation of synthetic lipid membranes (liposomes) containing entrapped drugs for treatment of leishmaniasis, exo-erythrocytic forms of malaria, or other tropical diseases. Second, preparation of liposomes containing endotoxin, bacterial exotoxins, or other substances that might serve as combined adjuvants and antigens for protection against infectious diseases. Third, identification and characterization of receptors for toxins and study of membrane-associated immunological mechanisms and complement activation.

24. (U) The approach will involve preparation of liposomes from purified lipid mixtures. Substances, such as drugs or proteins, will be trapped in the aqueous spaces separating the lipid membranes, or in the membranes themselves. Analysis of the effectiveness of the liposome-encapsulated materials will be performed by appropriate standard means, such as the cure rate of leishmania-infected, or malaria-infected, animals; or production of specific anti-microbe antibodies; or specific interactions of toxins, or antibodies, with receptors or antigens; etc.

25. (U) 79 10 - 80 09 The requirements for liposomes and entrapped drugs for studies in animals and humans for treatment of leishmaniasis have been identified and specified. A toxin attached to its receptor in liposomes is nontoxic and the immune response against the toxin in animals is increased more than 17-fold. Incorporation of the adjuvant moiety from endotoxin (lipid A) into liposomes caused more than a 600-fold stimulation of antibodies against the toxin bound to liposomes. Diphtheria toxin is a phosphate binding protein, and binds specifically to certain phospholipids. Lipid A from endotoxin causes neutropenia, but such neutropenia is eliminated by incorporation of lipid A into liposomes. For Technical Report see WRAIR Annual Progress Report 1 Oct 79 to 30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
*Project: 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES
Work Unit 211 Immunological and Biochemical Aspects of Membranes
*Work Unit 147 Immunological and Biochemical Aspects of Membranes

Investigators:
Principal: Bhupendra P. Doctor, Ph.D.
Assistant: Carl R. Alving, M.D., LTC, MC; Roberta Richards (Owens), Ph.D.; SP-4 Elizabeth Graves; PFC Diane Kovalski

The objective of this work was to investigate immunological and biochemical properties of membranes of infectious organisms. Further objectives were to use artifically prepared biological lipid membranes (liposomes) as carriers of antigens from infectious organisms (eg, proteins or lipids from viruses and parasites, or endotoxin and endotoxic lipid A from bacteria); receptors for infectious agents; and carriers of drugs. The immunologic properties of the membrane-associated substances and liposomes were investigated as adjuvants and for possible use in vaccines, or for adverse reactions that might involve the immune system.

1. Liposomes in Leishmaniasis: Therapeutic effects of Antimonial Drugs, 8-Aminoquinolines, and Tetracycline.

2. Lipid A from Endotoxin: Antigenic Activities of Purified Fractions in Liposomes.

3. Adjuvanticity of Lipid A and Lipid A Fractions in Liposomes.

4. Effects of Lipid A and Liposomes Containing Lipid A on Platelet and Fibrinogen Production in Rabbits.

5. Binding of Diphtheria Toxin to Phospholipids in Liposomes.

6. Influence of Temperature on Complement-Dependent Immune Damage to Liposomes.

1. LIPOSOMES IN LEISHMANIASIS: THERAPEUTIC EFFECTS OF ANTIMONIAL DRUGS, 8-AMINOQUINOLINES, AND TETRACYCLINE

This study investigated the role of liposomes in changing the pharmacological efficacy of anti-leishmanial drugs in hamsters infected with visceral leishmaniasis. Enhanced anti-leishmanial activity could be accounted for only by liposome encapsulated drugs. "Empty" liposomes (lacking anti-leishmanial drug) gave no therapeutic benefit by themselves, nor did they enhance the effectiveness of concurrently administered drugs. In the absence of additional drugs, empty liposomes actually resulted in a higher mortality due to endstage leishmaniasis. Mortality associated

with chronic leishmaniasis, including that induced by empty liposomes, was reduced approximately 50% by orally administered unencapsulated tetracycline. Liposome-encapsulated tetracycline, given i.c., had no antileishmanial activity, thus indicating that tetracycline did not have inherent anti-leishmanial properties, and was beneficial because of its anti-bacterial effects. Liposomes containing an antimonial drug were effective when given i.c., i.p., or i.m., but not when given s.c. or p.o. Liposome-encapsulated antimonial drug had prophylactic activity and was effective when administered 8 days prior, but not 17 days prior to infection. Unencapsulated antimonial drug had no prophylactic effect. In addition to antimonials, another class of compounds, 8-aminoquinolines, had marked anti-leishmanial activity in liposomes. One of these, WR 6026, was 700 to 1800 times more effective than an antimonial drug alone.

Table I. Relative Efficacies of Liposome-Encapsulated WR 6026.

Therapeutic Agent	Experiment No.	SD_{50}[a]	meglumine antimoniate index[b]
meglumine antimoniate	L-35	290	---
WR 6026	L-35	1.8	161
WR 6026 encapsulated in negative liposomes	L-35	0.42	690
WR 6026 encapsulated in positive liposomes	L-35	0.22	1318
WR 6026 encapsulated in neutral liposomes	L-35	0.158	1835

[a]The SD_{50} is defined as the amount of drug (WR 6026) or drug antimony (meglumine antimoniate) required to cause 50% suppression of hepatic parasites.

[b]The meglumine antimoniate index is the ratio of SD_{50} of unencapsulated meglumine antimoniate/SD_{50} of liposome-encapsulated test drug.

2. LIPID A FROM ENDOTOXIN: ANTIGENIC ACTIVITIES OF PURIFIED FRACTIONS IN LIPOSOMES

Isolation of lipid A by acid hydrolysis of Shigella flexneri lipopolysaccharide resulted in a product that consisted of a heterogeneous mixture of bands when visualized by thin layer chromatography. Differential extraction with ethyl acetate and chloroform, or extraction with EDTA, followed by chloroform-methanol-water (Bligh-Dyer extraction), or a combination of both extraction schemes, resulted in partial purification of immunologically active lipid A. Eight fractions were purified further by preparative thin layer chromatography, and each of the fractions had phosphate, carbohydrate, and esterified fatty acids. Upon incorporation into liposomes, five of the eight purified fractions reacted with antilipid A serum, but the three fractions with the most number of esterified fatty acids failed to react with anti-lipid A serum. At least one fraction that originally was unreactive with anti-lipid A serum became reactive as a hapten inhibitor upon removal of esterified fatty acids by alkaline hydrolysis. Alkali-treated fractions from "unreactive" and "reactive" lipid A had similar activities as hapten inhibitors. Our data suggest that lipid A can exist in multiple forms that differ by the number and placement, and possibly by the type, of fatty acids linked to the carbohydrate of lipid A. Highly acylated forms of lipid A do not react with antiserum against the unpurified lipid A mixture, but removal of fatty acids does expose immunoreactive groups.

TABLE II

Properties of purified lipid A fractions

TLC Band No	R_f	Phosphate[a]	Carbohydrate[b]	Esterfied Fatty Acids per Phosphate[c]
1	0.01	+	+	0.8
2	0.04	+	+	1.1
3	0.07	+	+	1.3
4	0.11	+	+	1.7
5	0.19	+	+	1.7
6	0.29	+	+	2.1
7	0.38	+	+	2.9
8	0.46	+	+	2.0

[a]Determined by spraying TLC plate with molybdenum blue, and quantified by assaying eluted fractions by the method of Gerlach and Deuticke.

[b]Determined by spraying TLC plate with orcinol reagent (0.25% orcinol in 75% sulfuric acid).

[c]Esterified fatty acids were quantified by the method of Snyder and Stephens.

TABLE III
Immune reactivities of liposomes containing purified fractions of lipid A

TLC Band No.	% of Trapped Glucose Released	
	+ Antiserum	- Antiserum
1	40	0
2	43	0
3	44	0
4	40	0
5	43	0
6	6.3	0
7	3.3	0
8	0.9	0
None	0	0

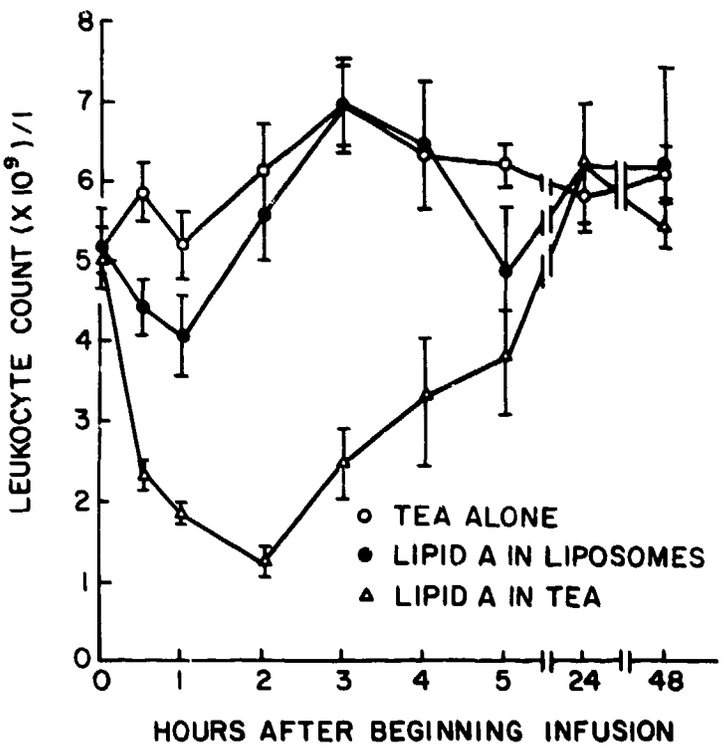
3. ADJUVANTICITY OF LIPID A AND LIPID A FRACTIONS IN LIPOSOMES In

In previous studies we reported that: 1) lipid is a chemically heterogeneous, and eight fractions that had chemical and immunologic characteristics of lipid A were isolated; and 2) injection of liposomes containing lipid A produced anti-lipid A antibodies and also produced "anti-liposome" antibodies reacting with phosphocholine, phosphatidylcholine and sphingomyelin. These observations are expanded in the present investigation, particularly by the use of a solid-phase radioimmunoassay applied to lipids. All eight lipid A fractions bound anti-lipid A antibodies, and seven of the fractions (fraction 1 excepted) were immunogenic when incorporated into liposomes and injected into rabbits. Several, but not all, of the lipid A fractions induced anti-liposome antibodies. The antiphosphatidylcholine specificity of anti-liposome antibodies was verified; low levels of anti-cholesterol and anti-dicetylphosphate antibodies also were detected. Remarkable adjuvant properties of lipid A for a liposomeassociated protein were demonstrated. The antigen consisted of cholera toxin (CT) bound to its receptor (ganglioside G_{M1}) on the surface of liposomes. The activity of anti-CT antibodies induced by the above antigen was 17 times higher than that induced by CT alone, but when lipid A was included in the liposomes the anti-CT response was 629 times higher than with CT alone.

4. EFFECTS OF LIPID A AND LIPOSOMES CONTAINING LIPID A ON PLATELET AND FIBRINOGEN PRODUCTION IN RABBITS

The effect of the lipid A moiety of endotoxin on platelet and fibrinogen production was studied in rabbits. Lipid A was infused intravenously in doses ranging from 1 to 100 µg/kg body mass; 18 hr later, selenomethionine-

^{75}Se was injected intravenously and its incorporation into fibrinogen and platelets determined. Lipid A in saline stimulated fibrinogen and platelet production, but the dose required was 50-100 times that required for an intact endotoxin. Although lipid A solubilized in triethylamine (TEA) was at least 60 times more active in the Limulus amebocyte lysate assay than was lipid A suspended in saline, the sensitivity of platelet and fibrinogen production to solubilized lipid A was increased only twofold. Incorporation of lipid A into liposomes had no effect on its Limulus activity. Lipid A in liposomes continued to stimulate platelet, but not fibrinogen, production. Leukopenia that was induced by lipid A in TEA did not occur when rabbits received the same dose of lipid A in liposomes. Lipid A, like intact endotoxin, can stimulate platelet and fibrinogen production and induce leukopenia but the doses required are high. The low solubility of lipid A in aqueous solutions may be only one factor that determines its biologic activity.



Effect of infusion of lipid A (10 μg/ml) on leukocyte counts (mean ± SEM) in rabbits. Lipid A that was in 0.5% TEA, or in liposomes, was diluted with 0.9% NaCl and infused during 1 hr into 4 rabbits. Six controls received 0.5% TEA in 0.9% NaCl.

5. BINDING OF DIPHTHERIA TOXIN TO PHOSPHOLIPIDS IN LIPOSOMES

Diphtheria toxin bound to the phosphate portion of some, but not all, phospholipids in liposomes. Liposomes consisting of dimyristoyl phosphatidylcholine and cholesterol did not bind toxin. Addition of 20 mol % (compared to dimyristoyl phosphatidylcholine) of dipalmitoyl phosphatidic acid, dicetyl phosphate, phosphatidylinositol phosphate, cardiolipin, or phosphatidylserine in the liposomes resulted in substantial binding of toxin. Inclusion of phosphatidylinositol in dimyristoyl phosphatidylcholine/cholesterol liposomes did not result in toxin binding. The calcium salt of dipalmitoyl phosphatidic acid was more effective than the sodium salt, and the highest level of binding occurred with liposomes consisting only of dipalmitoyl phosphatidic acid (calcium salt) and cholesterol. Binding of toxin to liposomes was dependent on pH, and the pattern of pH dependence varied with liposomes having different compositions. Incubation of diphtheria toxin with liposomes containing dicetyl phosphate resulted in maximal binding at pH 3.6, whereas binding to liposomes containing phosphatidylinositol phosphate was maximal above pH 7. Toxin did not bind to liposomes containing 20 mol% of a free fatty acid (palmitic acid) or a sulfated lipid (3-sulfogalactosylceramide). Toxin binding to dicetyl phosphate was inhibited by UTP, ATP, phosphocholine, or p-nitrophenyl phosphate, but not by uracil. We conclude that (a) diphtheria toxin binds specifically to the phosphate portion of certain phospholipids, (b) binding to phospholipids in liposomes is dependent on pH, but is not due only to electrostatic interaction, and (c) binding may be strongly influenced by the composition of adjacent phospholipids that do not bind toxin. We propose that a minor membrane phospholipid (such as phosphatidylinositol phosphate or phosphatidic acid), or that some other phosphorylated membrane molecule (such as a phosphoprotein) may be important in the initial binding of diphtheria toxin to cells.

6. INFLUENCE OF TEMPERATURE ON COMPLEMENT-DEPENDENT IMMUNE DAMAGE TO LIPOSOMES.

Maximal release of trapped liposomal glucose, in the presence of saturating amounts of liposomal antigen (galactocerebroside), antiserum (anti-galactocerebroside), and complement, was dependent on temperature. At lower temperatures (20-25°C), maximal glucose release was inversely related to liposomal phospholipid fatty acyl chain length (dimyristoyl phosphatidylcholine > dipalmitoyl phosphatidylcholine > distearoyl phosphatidylcholine > sphingomyelin). At higher temperatures (32-35°C) a limiting plateau of glucose release, at approx. 60%, was reached, or approached, by all preparations. Sphingomyelin liposomes still released less glucose than those prepared from other phospholipids, even at 35°C. The titers of antiserum and complement (ABL_{50} ml and CL_{50}/ml) were dependent on temperature, and differences based on liposomal phospholipid

fatty acyl chain length were observed. Analysis of antiserum and complement-dependence on temperature, and on phospholipid type, revealed that although antibody binding to galactocerebroside undoubtedly was subject to steric hindrance due to interference by surrounding phospholipids at 20-25°C, steric hindrance did not play a major role in blocking antibody binding above 32°C.

FUTURE GOALS

The future goals of this work are to continue the applications of liposomes for chemotherapy of leishmaniasis and the formulate liposome preparations that are suitable for use in humans. In the course of this work we shall explore the immunological properties of liposomes to determine whether the liposomes are immunogenic, and whether "anti-liposome" antibodies might influence the use of liposomes as drug carriers or as vehicles for vaccines. We shall also examine immunosuppressive and adjuvant properties of liposomes. The chemistry of the endotoxic portion of lipopolysaccharide (lipid A) from gram negative bacteria will be studied in much more detail, particularly with respect to structure/function relationships. The functional activities of lipid A that will be investigated in detail are the adjuvant properties of lipid A, and effects on circulating cells, such as neutrophils, and platelets, and effects on the coagulation. Effects of antibodies against phospholipids and hybridoma monoclonal anti-phospholipid antibodies on circulating cells coagulation will also be examined.

Publications

1. Alving, C.R. and Steck, E.A. Use of liposome-encapsulated drugs in treatment of leishmaniasis. Trends in Biochem. Sci. 4 N175-N177 (1979)

2. Banerji, B. and Alving, C.R. Lipid A from endotoxin: antigenic activities of purified fractions in liposomes. J. Immunol. 123 2558-2562 (1979)

3. Richards, R.L. and Alving, C.R. Immune reactivities of antibodies against glycolipids. III. Natural antibodies. in A.C.S. Symposium Series 128: "Cell Surface Glycolipids", edited by C.C. Sweeley, American Chemical Society (1980) pp. 461-473.

4. Alving, C.R., Urban, K.A. and Richards, R.L. Influence of temperature on complement-dependent immune damage to liposomes. Biochim. Biophys. Acta 600 117-125 (1980).

5. Alving, C.R., Iglewski, B., Urban, K.A., Moss, J., Richards, R.L. and Sadoff, J.C. Binding of diphtheria toxin to phospholipids in liposomes. Proc. Natl. Acad. Sci. U.S.A. 77, 1986-1990 (1980).

6. Alving, C.R., Banerji, B., Clements, J. and Richards, R.L. Immunogenic and adjuvant properties of lipid A and lipid A fractions in liposomes, in "Liposomes and Immunobiology", edited by B. H. Tom and H.R. Six, Elsevier/North-Holland. pp. 67-78 (1980).

7. Ramsey, R.B., Evatt, B.L., Alving, B.M., Finlayson, J., Alving, C.R. and Hamner, M.B. Effects of lipid A and liposomes on platelet and fibrinogen production. Blood 56 307-310 (1980).

8. Alving, C.R., Steck, E.A., Chapman, Jr., W.L., Waits, V.B., Hendricks, L.D., Swartz, Jr., G.M. and Hanson, W.L. Liposomes in leishmaniasis: therapeutic effects of antimonial drugs, 8-aminoquinolines and tetracycline. Life Sciences 26 2231-2238 (1980).

9. Saida, K., Saida, T., Alving, C.R., Brown, M.J., Silberberg, D.H. and Asbury, A.K. In vivo demyelination produced by purified antibodies to galactocerebroside. J. Neuropath. Exp. Neurol. 38, 338 (1979)

10. Banerji, B. and Alving, C.R. Activation of complement by lipid A from endotoxin. J. Immunol. 124, 1513 (1980)

11. Ramsey, R.B., Evatt, B.L., Alving, B.M., Alving, C.R. and Hamner, M.B. Platelet and fibrinogen production: effect of lipid A and liposomes. Thromb. Haemostasis. 42, 150 (1979).

PRESENTATIONS

1. Invited seminar speaker, at Navel Medical Research Institute, Bethesda, MD, 2 Oct. 79.

2. Abstract presented, VIIIth International Complement Workshop, at Key Biscayne, FL, 13-16 Oct. 79.

3. Invited speaker, Colloquia in Human Disease, at NCI-University of Maryland School of Medicine, Baltimore, MD 10 Jan 80.

4. Invited speaker, session on Macrophages, Adjuvants and Immunogenicity in National Symposium on Liposomes and Immunobiology, at University of Texas Texas Health Science Center at Houston, TX, 14-15 March 80.

5. Invited speaker, session on New Approaches to Vaccine Production, in 25th OHOLO Biological Conference on New Developments with Human and Veterinary Vaccines, at Zichron Yaacov, Israel, 24-27 March 1980.

6. Invited seminar speaker, Department of Chemical Immunology, The Weizmann Institute of Science, at Rohovot, Israel, 28 March 1980.

7. Invited seminar speaker, Fidia Research Laboratories at Abano Terme, Italy, 1 April 1980.

8. Invited speaker, in Fogarty Symposium on Cholera Toxin: From Molecular Aspects to Clinical Applications, at NIH, Bethesda, MD 16 April 1980.

9. Chairman, session on Medical Applications of Carriers, in Gordon Research Conference on Drug Carriers in Biology and Medicine, At Plymouth State College, NH, 9-13 June 1980 (elected Vice-Chairman of next Gordon Conference).

10. Invited seminar speaker, NCI, NIH, July 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
A OC 6471	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. WORK UNIT NUMBER	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10CG	212
b. CONTRIBUTING	61102A	3E161102BS09	00	001
c. CONTRIBUTING	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

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: 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 PREVIOUS 80	2	450
FISCAL YEAR CURRENT 81	2	241

19. RESPONSIBLE DOD ORGANIZATION

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NAME: VERMA, Pritam, CPT, MSC

21. GENERAL USE

Foreign Intelligency Not Considered

22. KEYWORDS (Precede each with Security Classification Code)

(U) Blast Overpressure; (U) Pulmonary Physiology; (U) Pulmonary Biochemistry; (U) Pulmonary receptors

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

23. (U) To define the physiologic effects upon the human of blast overpressure generated by firing Army weapons systems. Of primary concern is the definition of the limits of human safety for exposure to impulse noise.

24. (U) Approach includes use of biochemical assays and monitoring of physiologic tests before and after blast and impact injury. Full hemithoracic chest wall 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. Injured pulmonary tissue will be examined histologically and assayed for hormone receptor concentrations.

25. (U) 79-10-80-07Approximately 3000 serum samples were collected from 98 animals exposed to 50 rounds of the M198 Howitzer firing the M203 charge. This serum bank will be used in the evalution of putative markers of lung injury through Fy 81 and beyond. The inflammatory mediators, bradykinin and prostaglandins, appear to be so nonspecific that they are elevated with other forms of stress and/or non-blast related damage. work has been completed on a canine model of lung injury induced by intravenous oleic acid. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 79 - 30 Sep 80.

* Available [illegible] 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 & HEALTH HAZARDS
* Project 3E161102BS09 BLAST OVERPRESSURE

Work Unit 001 Physiology of systemic effects of blast overpressure
* Work Unit 001 Medical Effects of Blast Overpressure: Basic Studies

Investigators
Principal: Yancy Y. Phillips, CPT(P), MC
Associate: James J. Jaeger, CPT(P), MSC; P. S. Verma, CPT, MSC; P. E. Lorenz, CPT, MSC; R. C. Smallridge, LTC, MC; N. E. Whorton, GS-11; K. W. Clendennen, SP5; C. Umstott, GS-5; T. J. Young, SP4

The WRAIR is tasked with establishing "a research program in the pathophysiology of blast overpressure" (BOP). The project is charged with evaluating the potential for nonauditory injury after exposure to impulse noise (BOP) generated by the firing of Army weapons systems. Investigation of the biochemical and hormonal perturbations caused by blast injury offers the possibility of identifying an easily assayed marker of blast injury and may give insight into the mechanisms and consequences of said injury.

A major area of investigation has been the search for a non-invasive index of pulmonary blast injury. We have begun the evaluation of the relationship between lung injury and blood levels of inflammatory mediators (prostaglandins, kinins, kallikrein) and the pulmonary endothelial protein angiotension converting enzyme (ACE). Blood from animals was collected after exposure to actual muzzle blast, shock tube blast simulation, and an intravenous chemical insult in a laboratory model. Early indications are that, while these markers may correlate with lung injury, they are so nonspecific that they are elevated with other forms of stress and/or non-pulmonary damage. Biochemical analysis of the blood collected during the July 1980 APG field study will be performed in the future as promising new assays are developed.

The work with the canine intravenous oleic acid model of pulmonary injury has been completed. It was found that elevations of prostaglandin degradation products and bradykinin preceded detectable pulmonary injury and correlated with the eventual extent of injury.

Future investigations will use biochemical and hormonal assays and in vivo tests of physiologic responses before and after blast and impact injury. Full hemithoracic chest wall 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. Injured pulmonary tissue will be examined histologically and assayed for hormone receptor concentrations.

Work Unit 001 Physiology of systemic effects of blast overpressure

LITERATURE CITED:

References:

1. Chen, P. H., Finite element dynamic structural model of the human thorax for chest impact response and injury studies. Aviat.-Space Environ Med 49:143, 1978.

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. Smallridge, R. C., L. Wartofsky, K. E. Ward, and K. D. Burman, Dissociation of 5'-deiodinase activities for T4 and 3',5'T2 in the fasted rat: Evidence for multiple enzymes and their subcellular locations. Presented at the American Federation for Clinical Research, New Orleans, LA, January 1980.

2. Verma, P. S., P. E. Lorenz, and G. E. Sander. An improved radio-immunoassay for bradykinin in human plasma. Presented at the Federation of American Societies for Experimental Biology, New Orleans, LA, April 1980.

3. Sander, G. E., P. S. Verma, and J. Jaeger. 13, 14-dihydro-15-keto PG E_2 and $F_{2\alpha}$ levels in arterial blood as indicators of acute hemorrhagic lung injury. Presented at the meeting of the American Thoracic Society, Washington, D. C., May 1980.

4. Sander, G. E., P. S. Verma, J. J. Jaeger, P. E. Lorenz, and J. L. Hess. Activation of the Kallikrein - kinin system during oleic acid induced hemorrhagic edema in the dog. Presented at the Federation of American Societies for Experimental Biology, Anaheim, CA, June 1980.

5. Smallridge, R. C., A. R. Glass, L. Wartofsky, K. E. Ward, and K. D. Burman. Investigations into the etiology of elevated serum triiodothyronine (T3) levels in protein malnourished rats. Presented at the 62nd Annual Meeting of the Endocrine Society, Washington, D. C., June, 1980.

BIBLIOGRAPHY

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fasted rat: Enzyme characteristics and evidence for two distinct 5'-deiodinases (Submitted for publication to Endocrinology).

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30. Smallridge, R. C., Glass, A. R., Wartofsky, L., Ward, K. E., Latham, K. R., and Burman, K. D. Investigations into the etiology of elevated serum T3 levels in protein-malnourished rats. (Submitted for publication to Metabolism).

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESS	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 643	80 10 01	DD-R-1498

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPOSITION INSTR'N	9. SPECIFIC DATA CONTRACTOR ACCESS	10. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

11. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10CE	213
b. CONTRIBUTING	61102A	3M161102BS01	00	127
c. CONTRIBUTING	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 06	CONT	DA	C. In-house

17. CONTRACT/GRANT
a. DATE/EFFECTIVE: N/A
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 80	7	630
	CURRENT 81	5	447

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Wylie, R.M. Ph.D.

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 (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Investigations will seek to describe the means by which the nervous system effects bodily responses to stress and injury, and to discern those combinations of physiologic parameters which collectively define the optimal conditions for effective military performance.

24. (U) Animal models of performance will be created using the techniques of operant and respondent conditioning and the role of internal factors in performance variability assessed by neurophysiologic recording of intracellular and extracellular bioelectric potentials; the descriptive and experimental neuroanatomical techniques of light and electron microscopy and histochemistry; stimulation or lesioning of discrete brain areas; and experimental modifications of hormonal status by ablation and/or administration of exogenous hormones or other drugs.

25. (U) 79 10-80 09 Major findings: Circadian changes in performance are strongly controlled by the relative density of feedback and reward but the direction of control depends on the economic context. Sudden increases in task difficulty reduce performance at night. Changes in performance due to fatigue were found to be a special problem for nerve damaged subjects, but normal subjects require conditioning to avoid fatigue induced errors as well. Initial chemical defense research has uncovered an important need for new chemical assay procedures to insure consistent concentrations and recoverable potency. Initial anatomic studies revealed widespread neural damage in animals surviving nerve agent exposure. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 79-31 Sep 80.

*Available to contractors upon originator's approval

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

Project 3M161102BS10 (FY 81) RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
*Project 3M161102BS01 (FY 80) BASIC RESEARCH ON MILITARY DISEASES
Work Unit 213 Biological Modulation of Military Performance
*Work Unit 127 Biological Modulation of Military Performance

Investigators.
Principal: Tyner, LTC C.F.
Associate: Hursh, MAJ S.R., Campbell, C.B.G., LTC, Petras J.M., Ph.D., Wylie, R.M., Ph.D., Elsmore, T.F., Ph.D., and Kaufman, L., Ph.D.

1. Problem and objectives: This basic science project seeks to define 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. Techniques and methods are drawn from a broad spectrum of neuroscience disciplines including psychology, neurophysiology, neuroanatomy and neuropharmacology.

2. Progress: Biological rhythms in performance. It was discovered last year that the relative amplitude of circadian rhythmicity in food getting behavior was inversingly related to the frequency with which the behavior produced food; frequently reinforced behavior was least rhythmic. During this year we have explored this finding further. In the first study, the various rates of food reinforcement all occurred in sequence in a single test session; a low level of performance in one phase could be compensated for in the next phase. In a second study, the various rates of reinforcement were each tested alone with no other rate available the same day. Under these conditions, just the opposite result was obtained; the amplitude of circadian rhythmicity in food getting behavior was lowest with the least frequent reinforcement. The principles of substitution and elasticity borrowed from microeconomics can adequately explain these effects. In a related experiment, it has been found that when an animal must perform a sequence of actions to obtain food, the early part of this sequence most remote from reward was most variable and showed the largest circadian rhythmicity. In a third study of circadian variation in a problem solving task, it was found that a transcient increase in rhythmicity could be induced by a sudden increase in the difficulty of the task. A performance which was very accurate and reliable even at 0200 could be made temporarily unreliable at that hour by reducing external informational cues, eventhough it could be solved at 1400. The analysis of transition states and temporary disruptions is a new and fruitful area of work and particularly relevant to performance breakdown.

The biological economy controlling performance. Previous work had shown a complementary relation between food and water reinforced performance; when one increases in frequency of reinforcement, performance for the other increased. Physiologists and nutritionists have made a similar observation that food deprivation often causes a reduction in drinking and vis versa. It has been assumed that this complementarity was mediated by physiological water balance. This year a study was completed that shows a complementary relation between food and water reinforced behavior without changes in daily consumptions of these commodities. This finding suggests that the complementary relation between food and water can be induced directly by the local rates of intake and does not require the participation of a physiological imbalance.

Recovery of motor function after limb deafferentation. In previous reports it was noted that limb deafferentation diminishes a subjects ability to avoid errors consequent to muscle fatigue in a weight lifting task. After a long period of training even deafferented subjects can minimize these errors by taking regular rests. We now discovered, using computer analysis, that even experienced subjects produce more of these errors than normal subjects. In addition, a normal subject given a 6-month vacation produced many of these same errors after reintroduction to the task. This has the important implication that sensory information related to muscle fatigue does not protect us from fatigue-induced errors, especially if the muscles involved have not undergone specific conditioning. The new compute system has revealed other differences between normal motor function and that of the deafferented limb, including greater overshoot during sudden changes in load and delayed error corretions during motor movements.

Anatomy of the autonomic nervous system. The autonomic innervation of the esophagus, stomach, and duodenum has been studied using experimental histochemical methods. Preliminary information suggests that the vagus innervates these organs in a viscerotopic manner, ie., subgroups of neurons in the brainstem supply different sections of these organs. A primary sensory autonomic center, the nucleus tractus solitarius, was found to supply the stomach with motor fibers and a previously undescribed autonomic modullospinal cell group appears to innervate the stomach and esophagus, as well.

Preliminary studies of neuro- and behavioral toxicity of chemical warfare agents and chemical defense measures. An initial investigation of the lethality of Soman (GD) was conducted in collaboration with the neuroscience staff of the Biomedical Research Laboratory at Aberdeen Proving Grounds. It had been previously shown in another laboratory that the lethality of the organophosphate paraoxon varies with the time of day of injection. In the rat, peak lethality occurred at 1900 hrs at the start of their night. A parallel study was conducted with the nerve agent Soman. A range of five doses chosen to bracket the LD 50 dose in a pilot study was administered to groups of rats at six evenly space times around the clock. Signs of toxicity and lethalities were recorded hourly for 24 hr after injection. In addition, a parallel group not receiving poison was sacraficed at the same times of the day to permit assessment of circadian variation in brain cholinesterase activity. The results of this study are still under analysis, but two findings are clear. First, the potency of the agent in this study was not as great as that predicted from the pilot data collected just two weeks before. Even the high dose expected to approximate an LD 90 did not produce an LD 50. Second, for reasons still unclear, the group dosed at 2200 hours showed no signs of poisoning at all eventhough the protocal for preparing the doses was exactly the same as for the other injection times. These two findings have lead to a review of quality assurance procedures to stabilize the potency of the agent and to rapidly assess its quality. Preliminary anatomical pathology analysis of poisoned animals has shown wide spread neural lesions in surviving animals previously unmapped with less sensitive techniques. Another study has been prepared which will be used to assess the low dose effects of organophosphates using a conditioned flavor aversion procedure with rats. A third set of studies of central respiratory control and disruption after poisoning have been planned and facilities constructed. Several baseline subjects have been studied and the literature on known anatomy and physiology of respiratory control has been reviewed.

Future objectives: Studies of behavioral rhythms will be completed in the coming year. Basic studies of integrated multiple behavior repertories will continue using economic concepts as a framework for analysis. Studies of transition states and temporary disruption will be extended to behavior motivated by avoidance of aversive effects and "coping" behavior. In this context, stress will be treated as a sudden loss of a previously adaptive "coping" response. Neurophysiological studies of motor control will be extended to eye-hand coordination in a visual tracting task, both in normal and surgically altered subjects. Neuroanatomic studies of the innervation of the viscera will continue. All chemical defense related research will be transferred to their own projects.

Publications

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA 0C6449 | 2. DATE OF SUMMARY: 80 10 01 | SYMBOL

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. UNDR INSTR'N	8b. SPECIFIC CONTRACTOR AUTH	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	X YES NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10CD	215
b. CONTRIBUTING	61102A	3M161102B S01	00	128
XXXXXXXXXXXXXX				

11. TITLE (Procede with Security Classification Code)

(U) Mechanism of Response to Military Stress

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012200 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: 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 80	2	139
CURRENT FISCAL YEAR 81	2	340

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Belenky, G., MAJ

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Stress; (U) Cyclic Nucleotides; (U) Neurotransmitters; (U) Neurochemistry; (U) Microwave Inactivation; (U) Lateralization of Cerebral Function

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede 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 designed in this laboratory. Role of dopamine in lateralization of cerebral function.

25. (U) 79 10 - 80 09

We demonstrated stress-induced pituitary cyclic AMP increases in female as well as male rats. Cyclic nucleotides did not vary with the estrus cycle but did show circadian rhythms in hypothalamus and cerebellum. Stress increases brain norepinephrine (NE) turnover; we found that isoproterenol, a NE agonist, increased levels of cyclic AMP in 21 areas of brain. We have shown that nicotinic or muscarinic agonists elevate cyclic AMP in both the interpeduncular nucleus and the pituitary. These changes are markedly attenuated by pretreatment with nicotinic or muscarinic antagonists respectively. We have shown that dopamine in the interpeduncular region is released by amphetamine but not by potassium-induced depolarization, a finding potentially relevant to the behavioral toxicity of stimulant drugs. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498B, 1 OCT 68 (FOR SPECIAL USE) ARE OBSOLETE

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES
Work Unit 215: Mechanism of Response to Military Stress
* Work Unit 128: Mechanism of Response to Military 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 neurotransmitter regulation of pituitary function in acute and chronic exposure to stressors, as well as studies of neurochemical system interactions (i.e. cholinergic-dopaminergic interactions). These studies are intended to advance our understanding of neurochemical mechanisms in adaptation to stress, as well as to provide a database for interpretation of psychoendocrine studies of combat stress.

Progress:

Neurochemical and neuroendocrine responses to acute stress. Current research has suggested that the cyclic nucleotides (cyclic AMP and cyclic GMP) function as "second messengers" in the CNS (1,2). In vitro experiments have shown that cyclic nucleotide levels in brain tissue slices increase after incubation with a variety of neurotransmitters and neuromodulators (3). Based on in vivo studies, it has been proposed that cyclic nucleotides also perform as second messengers in the pituitary where they may be involved in the release or synthesis of pituitary hormones. Pituitary cyclic AMP responses appear very promising as a variable to study in vivo to explore neuronal mechanisms mediating hormonal responses to stress. We have discovered that forced immobilization produced a significant elevation of pituitary cyclic AMP in vivo as well as increases in plasma corticosterone and prolactin. This may represent a highly useful model for assessment of in vivo biochemical responses to stress at the pituitary level. We are presently using this model to compare pituitary responses to a variety of acute and chronic stressors and to elucidate mechanisms of neuronal and neurotransmitter regulation of pituitary response. Since several putative regulators of pituitary hormone release have been shown to fluctuate during the estrus cycle in female rats (4,5,6), we have hypothesized that pituitary cyclic nucleotide levels would vary in vivo in response to these regulators as well. These varying pituitary cyclic nucleotide levels might then have functional significance with regard to pituitary hormone output during the cycle. It was also important to determine whether cyclic nucleotide levels in the pituitary varied with the estrus cycle in order to perform stress studies in female rats. Any variation in "controls" or "unstressed" female rats due to the estrus cycle would have to be known, before stress induced increases in pituitary cyclic AMP could be evaluated.

Cyclic AMP and cyclic AMP levels did not vary in any region tested as a function of the estrus cycle. However, the time of day at which the rats were sacrificed did affect levels of cyclic AMP in the hypothalamus and cerebellum and levels of cerebellar cyclic GMP. Thus, as for many other neuroactive compounds, levels of cyclic nucleotides are subject to circadian rhythms, at least in some brain regions.

Cholinergic effects on brain regional cyclic nucleotides. We have previously reported that cholinergic agonists elevate cyclic AMP and cyclic GMP in several rat brain regions in vivo. We have now compared the effects of muscarinic versus nicotinic agonists and also examined the effect of pretreatment with muscarinic or nicotinic blockers. Oxotremorine (a centrally acting muscarinic agonist) caused marked increases in cyclic AMP in the pituitary as well as in the hypothalamus, interpeduncular region and substantia nigra all of which were attenuated by pretreatment with atropine. Nicotine markedly elevated cyclic AMP in the pituitary and the interpeduncular region and caused significant increases in plasma prolactin as well. The nicotine-induced changes were attenuated by mecamylamine pretreatment. The regional pattern of increase in cyclic GMP levels in the animals receiving oxotremorine is similar to the response following locomotor activity (7) consistent with the behavioral observation of tremor. The increases in cyclic GMP in the septal region, however, were not seen following locomotor activity alone. The tremors and the brain cyclic GMP increases were attenuated by pretreatment with atropine. Nicotine lowered cyclic GMP in the cerebellum and decreased locomotor activity. These changes were attenuated by pretreatment with mecamylamine. The marked cyclic AMP responses in the pituitary suggest that studies be initiated on cholinergic-endocrine interactions.

Drug effects on brain dopamine systems. In brain regions containing dopaminergic nerve terminals but no cell bodies, we find that both potassium depolarization and amphetamine are potent releasers of dopamine. In brain regions containing dopamine neuronal cell bodies but very few nerve terminals, we find that dopamine is released by amphetamine but not by potassium depolarization. These studies may assist in understanding the behavioral disruption produced by amphetamine and other stimulant drugs.

Future Objectives:

Major emphasis will be placed on following up the finding that immobilization stress increases pituitary cyclic AMP in male and female rats. Effects of other acute and chronic stressors will be compared. The response of the pituitary in aggressive behavior will be studied. Lesion and pharmacological blocking studies will be performed to determine which neurotransmitter/neuromodulator systems regulate the response, and which hormonal responses are modulated by the pituitary cyclic AMP increases. Studies of dopaminergic cholinergic interaction will continue. We plan to begin measuring receptors for several neurotransmitters including the beta-noradrenergic receptor and the muscarinic cholinergic receptor. Once the assays are set up and validated, we will begin examining the effects of chronic stress on receptors.

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7. Meyerhoff, J.L., Lenox, R.H., Kant, G.J., Sessions, G.R., Mougey, E.H., and Pennington, L.L. Life Sciences 24:1125-1130 (1979).

Presentations

Academy of Behavioral Medicine Research Annual Meeting, Charlottesville, VA, 1980. Address to plenary session: Meyerhoff, J.L., Lenox, R.H., Kant, G.J., Sessions, G.R., Mougey, E.H., Collins, D.R., and Pennington, L.L. "Concurrent endocrine and neurochemical parameters in animals subjected to stress."

Society for Neuroscience, Atlanta, Georgia, Nov. 1979. Lenox, R.H., Kant, G.J., and Meyerhoff, J.L. "Cyclic GMP and cyclic AMP increases in specific brain regions following central cholinergic stimulation."

Society for Neuroscience, Atlanta, Georgia, Nov. 1979. Bates, V.E., Lenox, R.H., Kant, G.J., and Meyerhoff, J.L. "Behavioral supersensitivity to apomorphine without regional changes in cAMP in rat brain."

Uniformed Services University of the Health Sciences, Department of Pharmacology, March 1980. Meyerhoff, J.L. "Role of locomotor activity in drug effects on brain cyclic GMP."

Department of Behavioral Toxicology, Johns Hopkins University, June 1980. Meyerhoff, J.L. "Behavioral interactions in neurochemical studies."

Publications

Lenox, R.H., Wray, H.L., Balcom, G.J., Hawkins, T.D., and Meyerhoff, J.L. Regional levels of cyclic nucleotides, gamma aminobutyric acid and glutamate during chronic barbiturate ingestion and withdrawal. European J. Pharmacol. 55(4):367-379 (1979).

Lenox, R.H., Brown, P.V., and Meyerhoff, J.L. Microwave inactivation: a technique with promise and pitfalls. Trends in Neuroscience 2(4):106-109 (1979).

Kant, G.J., Meyerhoff, J.L., and Lenox, R.H. Dopamine diffusion after microwave fixation at 985 MHZ. Neurochemical Research 4(4):531-536 (1979).

Meyerhoff, J.L., Lenox, R.H., Brown, P.V., and Gandhi, O.P. The inactivation of rodent brain enzymes in-vivo using high-intensity microwave irradiation. I.E.E.E. Transactions on Microwave Theory and Techniques. Proceedings of the I.E.E.E. 68(1):155-159 (1980).

Kant, G.J., Lenox, R.H., and Meyerhoff, J.L. In vivo effects of apomorphine and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO 20-1724) on cyclic nucleotides in rat brain and pituitary. Biochemical Pharmacology 29: 369-373 (1980).

Lenox, R.H., Kant, G.J., and Meyerhoff, J.L. Specific hormonal and neurochemical responses to different stressors. Neurodocrinology 30: 300-308 (1980).

Kant, G.J., Muller, T.W., Lenox, R.H., and Meyerhoff, J.L. In vivo effects of pentobarbital and halothane anesthesia on levels of adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in rat brain regions and pituitary. Biochem. Pharm. 29:1891-1896 (1980).

Sessions, G.R., Meyerhoff, J.L., Kant, G.J., and Koob, G. F. Effects of lesions of the ventral medial tegmentum on locomotor activity, biogenic amines and response to amphetamine in rats. Pharmacol., Biochem. and Behavior. 12:603-608 (1980).

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).

Craves, F.B., Loh, H.H., and Meyerhoff, J.L. The effect of morphine tolerance and dependence on cell free protein synthesis. J. Neurochem. (in press).

Lenox, R.H., Wray, H.L., Balcom, G.J., Hawkins, T.C., and Meyerhoff, J.L. Regional levels of cyclic nucleotides, gamma aminobutyric acid and glutamate during chronic barbiturate ingestion and withdrawal. European J. Pharmacol. 55(4):367-379 (1979).

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 (SHR) to aminergic and cholinergic stimulation. Neuroscience Abstracts 6:163 (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. Neuroscience Abstracts 6:535 (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. Neuroscience Abstracts 6:534 (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. Neuroscience Abstracts 6:266 (1980).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6473	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	REGRADING*	6a. DISP'N INSTR'N	6b. SPECIFIC DATA-CONTRACTOR ACCESS	7. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10CD	216
b. CONTRIBUTING	61102A	3M161102BS01	00	146
c. CONTRIBUTING	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:* 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 80	4.0	231
FISCAL YEAR CURRENT 81	4.0	221

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
Division of Neuropsychiatry

ADDRESS:* Washington, D.C. 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.

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) 79 10 - 80 09 This work unit provides the technology base for Work Unit 048, Military Stress: Circadian Ultradian Factors (Accession Number DA OC 6457). The Mark I field deployable self-contained rest/activity monitoring device was shown to be usable bilaterally for assessing sleep quality as well as rhythmical changes in performance during waking periods with modifications of its sensitivity and recording period. Redesign of the second generation, or Mark II Actigraph was accomplished to permit its manufacture in field deployable quantities with existing semiconductor memory chips. A field deployable readout device for both models was developed and work is started on a compatible Weigand Effect transducer to look at hand movement during task performance. Ingestible capsules providing continuous readout of core body temperature (initially developed by Canadians) were tested and improved and in collaboration with the Dept of Medicine a similar device to monitor pressure in the gastrointestinal tract is being developed. A Performance Assessment Battery looking at reaction times, complex cognition, mood and activation was developed and set up to be administered and scored in the field using a microcomputer system. Work has continued on the measurement of affect and perception of social support as part of quantifying stress and predicting reactions to it. For Technical Report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

DD FORM 1 MAR 84 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 83 (FOR ARMY USE) ARE OBSOLETE

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* **Project 161602BS01 BASIC RESEARCH ON MILITARY DISEASES**
Work Unit 216 Military Stress: Non-Invasive Monitoring of Health & Performance
* **Work Unit 146 Military Stress: Non-invasive Monitoring of Health and Performance**

Investigators.

Principal:	**Frederick W. Hegge, Ph.D.**
Associate:	**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**

Objectives

This work unit provides the supportive technology base for Work Unit 048, Military Stress: Circadian and Ultradian Factors (Accession Number DA OC 6457), designed to address through field and laboratory studies the central psychophysiologic problems of modern combat stress. The technical goal is to exploit, refine, and apply rapidly improving techniques of physiologic data acquisition and performance assessment. Laboratory studies emphasizing technical improvements and novel data analytic approaches are coupled with field studies for validation and the development of applications minimizing the intrusion of research into realistic operations.

Progress

The Mark I field deployable self-contained rest/activity monitoring device (Actigraph) has been modified for assessing quality of sleep and patterns of bilateral wrist activity throughout the day. After increasing movement sensitivity and decreasing the sample epoch, a high correlation was found between activity patterns during hours of attempted sleep and subjective quality of sleep. Similar modifications permit discrimination between tasks requiring differential hand involvement and may provide an unobtrusive method for identifying and quantifying military task performance patterns throughout the 24-hr day. A second set of studies is using the Mark I device combined with standard psychiatric questionnaires to study manifestations of stress among testicular cancer patients receiving chemotherapy. This situation is being studied as (1) a model of coping with extreme stress, and (2) as a substrate for the development of measures to predict the kinds of failures in coping that lead to psychiatric casualties in combat.

Research using the Mark I Actigraph demonstrated the need for a device with adjustable sensitivity, wider dynamic range, greater data storage capabilities and smaller physical size. The design for such a device was completed and a contract was let to the Army's Harry Diamond Laboratories for its manufacture. Fabrication has been delayed repeatedly due to the semiconductor industry's inability to supply current demands while maintaining published specifications. Therefore the Mark II Actigraph was redesigned with some tradeoff in physical size in order to use components that are more widely available and that meet specifications. These components have now been received and fabrication of the first units has begun. Assembly is also proceeding on the prototype of a field deployable readout device which produces a printed copy of Actigraph data. Weigand effect components have been obtained and potential placements on the hand are being explored. Further technical development awaits engineering support.

Ingestible radiopills providing a continuous readout of core body temperature were tested and improved in collaboration with the Canadian Defence and Civil Institute of Environmental Medicine. In-house fabrication is proceeding for use in current laboratory experiments. Computer programs are being written to permit the linking of successive radiopill data sets into continuous temperature readouts over extended time periods.

A microcomputer automated cognitive performance test battery was developed and implemented in deployment simulation studies. Refinement of component tasks and the development of automated computer data analyses are proceeding in conjunction with data collection.

A questionnaire has been developed to measure perception of social support and social contact. Historical data suggest that soldiers least able to evoke social support are most vulnerable to becoming psychiatric casualties in combat. Results from the current instrument demonstrate that it has excellent test-retest reliability, internal consistency, and convergent and discriminant construct validity. Responses also show a high correlation with mood scale data collected from a general population of civilians and military personnel.

Future Objectives. A primary goal is to validate the field deployability of non-invasive monitoring systems, particularly the radiopill system for continuous body temperature recording, the microcomputer based cognitive test battery, and the Mark II Actigraph. Improved utilization of the radiopill requires additional efforts on miniaturizing the antenna-recording system to include solid state recording capability with on-line data reduction and storage. Collaboration with the Division of Medicine will be pursued to transfer radiopill technology to development of a similar device to monitor pressure in the gastro-intestinal tract. Animal tests will be conducted to assess the suitability of such a radiopill to detect gastrointestinal concomitants of psychosomatic stress responses. Preliminary findings from other laboratories suggest that changes in pupil diameter may provide a noninvasive physiological index of cognitive workload in humans. The potential advantages of such a measure in studies of cognitive performance recommend that its feasibility be investigated in the near future.

Presentations and Publications

1. Blaik, R., and Genser, S.G. Perception of social support and risk of depression. Amer. Psychiatric Assn. Meetings, San Francisco, CA., 4-9 May 1980.

2. Blaik, R., and Genser, S.G. Perception of social support as a risk-factor in depression. Soc. for Epid. Rsch. Meetings, Minn. MN., 18-20 June 1980.

3. Blaik, R., and Genser, S.G. Social support satisfaction: Scale development. Amer. Psychol. Assn. Meetings, Montreal, Canada, 1-5 September 1980.

4. Blaik, R., and Genser, S.G. Social support, life-events and depression. Amer. Psychol. Assn. Meetings, Montreal, Canada, 1-5 September 1980.

RESEARCH AND TECHN.	WORK UNIT SUMMARY	1. AGENCY ACCESSION* DA OB 6448	2. DATE OF SUM 80 10	REPORT CONTROL SYMBOL

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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102S10	S10AG	217
b. [illegible]	61102A	3M161102BS01	00	122
c. CONTRIBUTING	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: 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 80	3.5	212
	CURRENT 81	3.5	196

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Walter Reed Army Institute of Research

ADDRESS:* Washington, DC 20012

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20. PERFORMING ORGANIZATION

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NAME:* HEIFFER, Dr. M. H.

TELEPHONE: 301-427-5393

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: KORTE, CPT D. W. Jr.

NAME: VON BREDOW, MAJ J.

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) Research is directed toward investigating special areas of the pharmacology of potential drugs of military importance, their interactions, their mechanisms of action, and the development, characterization and improvement of animal models for defining specific applicable parameters.

24. (U) Drugs are tested in animal models specifically designed to pinpoint mechanisms of pharmacological effects and effects on physiological responses. In vitro models are being used as well. Quantitation methodology is being developed for drugs of interest.

25. (U) 79 10 - 80 09 Cardiovascular and respiratory activity of two different intravenous dosage regimens of Pentostam were compared in rabbits. The heart rate increased in control and bolus injection animals. The duration of the QTc and the respiratory rate were greater for both control and infused animals. The R wave amplitude increased for all groups while the T wave increased only in control rabbits. A recirculating biliary cannula for dogs was designed and tested. Cooperative cardiovascular studies are continuing. Analysis of 2-PAM-Cl in Combo-Pens demonstrated the presence of the subject oxime, the major decomposition product, and physiologically insignificant amounts of cyanide. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES
Work Unit 217: Basic Pharmacological Studies
* Work Unit 122 Basic pharmacological studies

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
Associate: CPT D. Korte, Jr., Dr. H. Lowensohn, MAJ J. von Bredow, Dr. R. Rozman, SP4 C. Basamania, SP5 J. Osuch

1. Description.

This year the basic research efforts of the department were directed towards two major areas. They are: the pharmacology of promising medicinal agents; and the development of analytic capability for certain antidotes used for toxic agents.

2. Progress.

Cardiovascular and respiratory activity of two different intravenous dosage regimens of Pentostam were compared in rabbits. Pentostam was administered for 10 consecutive days either as a continuous infusion or by daily injections. At the end of the ten day period, a Pentostam challenge dose was given. The effects of cardiac function were determined directly during the ten day infusion/injection treatment regimen phase of the study and indirectly during the Pentostam challenge phase of the study. An infused, restrained rabbit model was developed for this study with an 80% study completion rate for control group rabbits. Survival data indicated that only rabbits in the injection group (50%) expired because of the drug treatment. The heart rate increased in control and bolus injection animals. The duration of the QTc and the respiratory rate were greater for both control and infused animals. The R wave amplitude increased for all groups while the T wave increased only in control rabbits. The results suggest that a continuous infusion of Pentostam for 10 consecutive days produces fewer untoward effects than administration of an equivalent dose by daily injection. In addition, the infusion was associated with a direct action on cardiac function (prolongation of QTc interval) which may enable one to titrate the desired dosage regimen.

A recirculating biliary cannula model has been developed for the unanesthetized dog. This entails aseptic surgical implantation of a biliary catheter with an externally controlled valvular system. The system works acutely and will soon be tested in chronic preparations.

Initial analysis of the contents of Combo-pens has been carried out. Proposed decomposition products of 2-PAM-Cl were synthesized and compared to 2-PAM-Cl using high pressure liquid chromatography. Possible cyanide content was checked for using nitrogen purging into alkali traps. The presence of 2-PAM and of 2-carboxy-N-methyl pyridinium was demonstrated. Physiologically insignificant amounts of cyanide were found.

3. Future objectives.

Cardiovascular and cardiorespiratory studies will be carried out with other drugs. The biliary collection system will be used to study the biliary secretion and enterohepatic circulation of selected drugs. Analysis of the Combo-pen contents will continue. Analytical methods for other antidotes will be developed.

4. Publications.

1. Bellamy, R.F., and Lowensohn, H.S.: Pressure-flow relations in the canine right coronary circulation. Physiologist. 22:9, 1979.

2. Bellamy, R.F., and Lowensohn, H.S.: Effect of systole on coronary pressure-flow relations in the right ventricle of the dog. Am. J. Physiol. 238:H481-H486, 1980.

3. Bellamy, R.F., Lowensohn, H.S., Ehrlich, W., and Baer, R.W.: Effect of coronary sinus occlusion on coronary pressure-flow relations. Am. J. Physiol. 239:H57-H64, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE	3. ANY	4. FIRST CONTROL SYMB
DA OA 6449	80		DD-DR&E(AR)636

5. DATE PREV SUMRY	6. KIND OF SUMMARY	7. SUMMARY SCTY	8. WORK SECURITY	9. REGRADING	9a. DISPOSITION	10. SPECIFIC DATA - CONTRACTOR ACCESS	11. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

12. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	BS10	S10AF	218
b. ~~XXXXXXXX~~	61102A	3M161102BS01	00	134
c. CONTRIBUTING	STOG 80-7 2:2			

13. TITLE (Precede with Security Classification Code)

(U) Immunological Mechanisms in Microbial Infections

14. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 003400 Clinical Medicine

15. START DATE	16. ESTIMATED COMPLETION DATE	17. FUNDING AGENCY	18. PERFORMANCE METHOD
62 08	CONT	DA	C. In-House

19. CONTRACT/GRANT

a. DATES/EFFECTIVE: NA EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

20. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 80	2	163
FISCAL YEAR CURRENT 81	4	430

22. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

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NAME: Russell, Philip, COL

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23. PERFORMING ORGANIZATION

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TELEPHONE: (202) 576-3544

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Barbaro, J.F.

NAME: Wong, D.T.O.

24. GENERAL USE

Foreign Intelligence not considered

25. KEYWORDS (Precede EACH with Security Classification Code)

(U) Immunity; (U) Antibodies; (U) Infectious Diseases; (U) Complement Fixation; (U) Radioimmunoassay; (U) Serodiagnosis

26. TECHNICAL OBJECTIVE, 27. APPROACH, 28. 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 microbial 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 diseases are attacked. Immunological phenomena common to a variety of different diseases are also studies.

25 (U) 79 10-80-09 Experiments were done to determine optimal conditions for obtaining the surface antigen, other than Vi, responsible for agglutination of hybrids obtained by conjugal gene transfer from Citrobactor freundii to Salmonella typhi possessing a defective gene for Vi expression. Soluble antigen preparations from these cells were prepared using (1) concentrated cell-free supernatant after cell growth; (2) supernatant from heated suspensions; and (3) alcohol extracts of hybrid cells. The surface antigen in each of these preparations has been established by adsorption experiments. Immunoprecipitation analysis showed that the major C. freundii heat stable component does not share common determinants with S. typhi. Purified Vi antigen from S. typhi or C. freundii appear to be a family of anionic molecules that are electrophoretically heterogeneous but nevertheless related antigenically. The heterogeneity is evident by cross immuno-electrophoresis analysis. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
*Project 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES

Work Unit 218 Immunological Mechanisms in Microbial Infections
*WORK UNIT: 134 Immunological Mechanisms in Microbial Infections

INVESTIGATORS: Barbaro, J.F.; Ellis, B.; Sayles, P.J.; Wong, D.T.O.

b. Problem and Objective: The objective of this work unit is to elucidate the mechanisms operative in the natural and artificial induction of immunity to a variety of microbial infections of military importance. The objective includes the isolation, purification and characterization of microbial antigens and their usefulness in eliciting an immune response. The current objective is a study of the virulence (Vi) antigen variation that occurs when hybrids are produced by the transfer of Vi genes from Citrobacter freundii to Salmonella typhi.

c. Progress: Experiments were done to determine the optimal conditions for obtaining a surface antigen, other than Vi, responsible for agglutination of hybrids obtained by conjugal gene transfer from C. freundii to S. typhi possessing a defective gene for Vi expression. These cells were agglutinated by antisera to the C. freundii but showed no agglutination with antiserum against Vi antigen. Soluble antigen preparations were obtained from hybrid cells by three different methods. Concentrates of cell-free supernatant were obtained after: 1) cell growth, 2) heating a cell suspension for 60 minutes at 60 C, and 3) 15% alcohol extraction of a cell suspension for 3 hours at 37 C. These preparations were used to adsorb antisera and it was found that the active principle in these three extracts prevented bacterial agglutination. Immunodiffusion and immunoelectrophoresis showed the presence of a major heat stable component that does not share common determinants with S. typhi or Vi antigen.

Comparison of soluble extracts containing Vi antigens from different microorganisms - S. typhi, S. typhi hybrid Type I and Type II, C. freundii Type II mutant - were conducted by line immunoelectrophoresis. No observable antigenic differences could be distinguished among those tested. Rocket-line immunoelectrophoresis demonstrated fusion of rocket and line precipitin bands indicating a common antigenicity similar to the results with line immunoelectrophoresis.

Immunoelectrophoresis of purified Vi antigen and extracts containing Vi revealed a wide variance of anodic components whose mobility rates ranged from those comparable to globulin to those exceeding albumin at pH 8.6 Cross immunoelectrophoresis of several antigen preparations clearly demonstrated the Vi antigen heterogeneity. Results with alcohol extract of C. freundii showed two broad, distinct but related peaks. The major peak had a mobility rate faster than albumin and the minor peak showed mobility rates equal to β-globulins.

d. Recommendations: Purification and characterization of the surface antigen from other antigens in the crude extracts will be attempted using cetavlon-alcohol separation and cellulose or affinity chromatographic fractionation methods.

The surface antigen will be analyzed by gradient gel and isoelectric focusing electrophoresis. The isolated antigens will be used for the production of monospecific antibody. Quantitative immunoassay procedures will be developed for the surface antigen and its distribution in other microorganisms will be determined.

e. Reference cited: None

f. Presentations: None

g. Publications:

1) Schuster, D.L., Bongiovanni, B.A., Pierson, D.L., Barbaro, J.F., Wong, D.T.O. anl Levinson, A.I., Selective deficiency of a T cell subpopulation in active atopic dermatitis., J. Immunol. 124: 1662-1667, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6744	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. FRDD'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10EB	219
b. CONTRIBUTING	62770A	3M162770A803	00	093
c. CONTRIBUTING	STOG 80-7.2:1			

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: N/A
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	9	471
FISCAL YEAR — ESTIMATE: 81	11	531

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Not Considered

20. PERFORMING ORGANIZATION

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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) Technical objectives of work unit are: (1) to provide the military with a safe, effective prophylactic and 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 agent and antidotal agents; (4) to investigate metabolites and degradation products of chemical agents and antidotal agents including identification, synthesis and quantitation; and (5) to develop methodology required for studies.

24. (U) Classical biochemical, pharmacological and physiological procedures will be used to assess the effectiveness of Apropen and oximes as nerve agent antidotes. The protection of organophosphate binding sites on acetylcholinesterase by scavenger peptides of known structure will be explored. Understanding and prevention of molecular conformational in acetylcholinesterase-organophosphate complexes (aging) will be studied. The effects of organophosphates and nerve agent antidotes on biochemical mechanism will be systemically explored, including respiratory failure by acetylcholinesterase inhibitors and modification by nerve agent antidotes.

25. (U) 79 10 - 80 09 The stability of Benactyzine under various conditions of storage and packaging was completed. Benactyzine was most stable at low pH, temperature below 5°C, and packaged in glass. The addition of Propyleneglycol increased self-life. Studies on the physical properties, toxicity, adsorption, distribution, and excretion were initiated. The toxic factor in aged atropens was isolated and identified. For Technical Report see WRAIR Annual Progress Report 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
*PROJECT: 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES
Work Unit: 093 Biochemical Aspects of Medical Defense Against Chemical Agents
*WORK UNIT: 093 Chemical Defense: Research on Nerve Agent Antidotes

INVESTIGATORS:
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Associate: Nesbitt D. Brown, M.S.; SP4 Joseph L. Crockett; William S. Eck, CPT, MSC; SSG Piyush K. Gandhi; 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.; Mary P. Strickler, Ph.D.

The objective of this work unit was to provide the military with a safe, effective prophylactic and therapeutic product against nerve agent poisoning by evaluating potential nerve agent antidotes, insuring the quality control and stability of nerve agent antidote formulations, and the isolatioin and identification of possible toxic material resulting from degration products or contamination. The development of new methodology to perform the research.

1. The Stability of Benactyzine.HCl under Simulated Storage and Packaging Conditions.
2. Isolation and Identification of Toxic Substance(s) in Aged Atropens.
3. Studies on Aprophen as a Potential Nerve Agent Antidote.
4. Methodology Development and Application for the Study of Nerve Agent Antidotes.
5. Drug and Metabolite Studies by Mass Spectrometry.
6. Collaborate Research Studies on Polyamines, Brain Peptide Hormones and Hemoglobin A.

1. The Stability of Benactyzine.HCl Under Simulated Storage and Packaging Conditions.

Nerve agent antidote formulations, unlike most pharmaceutical formulations, have an unique requirement in that the potency of the active ingredients must remain stable upon prolonged storage under widely

variable conditions. Previous studies in this laboratory and by the US Army Biomedical Laboratory showed that temperature was a major contributing factor to the instability of TAB (first letters of the active ingredients, Trimedoxime or TMB-4, Atropine Sulfate and Benactyzine.HCl with preservatives Methylparaben and Propylparaben). The purpose of the present study was to assess the stability of Benactyzine and other active components of TAB under various conditions of packaging and storage in order to establish shelf-life and to determine the mechanism of degradation. The specific objectives were (a) to determine the effects of temperature on Benactyzine.HCl when packaged in glass ampules or cartridges with and without pH adjusted to 2.7 (b) to determine the effects of a plasticizer (1) on the rate of degradation of Benactyzine.HCl, (c) to determine whether the degradation products of TAB components influenced the rate of degradation of Benactyzine.HCl, and (d) to study effects of added propylene glycol as a stabilizer of Benactyzine.HCl.

Eight different formulations of Benactyzine.HCl or TAB were prepared and packaged by Survial Technology, Inc. and were refrigerated at 5°C until used in the study. These formulations were distributed into 26 different groups. Three randomly selected samples were analyzed from each group (i.e. I at 5°C, II at 25°C, III at 54°C and etc) biweekly for 6 months, except groups XIII and XX which were analyzed monthly after the initial analysis. The concentration of Bentactyzine .HCl and the TAB components, with the exception of Atropine Sulfate, was determined by High performance liquid chromatography (2,3). Atropine and the plasticizer were determined by gas chromatography (4). Ten samples from each of the eight original formulations were analyzed prior to starting the study for the purpose of establishing baseline concentrations fo the components.

The groups stored at 5°C for the entire study showed no significant change in Benactyzine.HCl content. In the groups stored at 25°C for the entire study, some degradation of Benacytzine.HCl was noted. All the groups stored at 54°C for the entire study showed Benactyzine.HCl degradation.

The stability of Benactyzine.HCl was improved by adjusting the formulation to pH 2.7 with HCl. When the pH adjusted and pH non-adjusted formulations, in glass vials were compared the stability of Benatyzine.HCl was significantly ($p<.02$) improved by adjusting the formulation to pH adjusted specimen. The time required for Benactyzine.HCl to degrade to 50 percent of the initial value was extended also at both 25°C and 54°C in the pH 2.7 adjusted formulations. Generally, the stability of Benactyzine.HCl stored in glass vials was better than when stored in cartridges. This was true whether the pH was adjusted to 2.7 or was not adjusted. In the Benactyzine.HCl formulations with no pH adjustment there was significant ($p<.01$) difference between glass vials and cartridges at 54°C. The time for Benacytzine.HCl to degrade to 50 percent

of its initial concentration was 3.1 years in glass and 2.3 years in cartridges at 25°C and at 54°C, 3.6 months in glass and 2.1 months in cartridges respectively. The addition of propylene glycol (40 percent) to the Benactyzine.HCl formulation improved the stability of Benactyzine.HCl at 25° and 54°C. At 25° (groups XV and XXII), propylene glycol extended the time required to reach 50 percent of the initial concentration of Benactyzine.HCl form 7.4 years to over 10 years and at 54°C (groups XV and XXIII), form 3.2 to 4.9 months. The use of propylene glycol would require further study if added to other compounds or combination of compounds. Benactyzine.HCl was more stable when combined in the TAB formulation than when formulated alone. This was true when packaged either in glass vials or in cartridges. The differences were not significant at 25°C but at 54°C highly significant ($p<.02$) difference were found.

This study established several facts about the stability of Benactyzine under the studied storage and packaging conditions: (a) Benactyzine.HCl alone or in TAB is stable for over 10 years at 5°C. (b) Benactyzine.HCl is stable for over 10 years at 25°C when stored in glass at pH 2.7. (c) Benactyzine.HCl or TAB is more stable packaged in glass than in cartridges. (d) The rubber septums and/or plasticizer have an adverse effect on the stability of Benactyzine.HCl and the parabens. There is evidence of a surface and/or catalytic effect. (e) Propylene glycol improves the stability of Benactyzine.HCl. (f) Other ingredients of TAB formulations, Atropine and TMB_4 are stable at normal storage temperatures for approximately 10 years when pH is adjusted to 2.7 and packaged in glass containers. The stability of Benactyzine.HCl or TAB would be maximal when packaged in glass at pH 2.7 and stored at 5°C. However, for practical purposes, the formulations stored in glass at a controlled room temperature (22 ± 5°C) appear to be stable for about 10 years.

References

1. Farshy, D.C.: Tri-butoxyethyl phosphate as a contaminant is B-D Vacutainers. Applied Microbiology 27: 300-304, 1974.

2. Brown, N.D. and Sleeman, H.K.: An ultramicro high performance liquid chromatographic method for assaying ion-pair species of benactyzine. J. Chromatography 140: 300-303, 1977.

3. Brown, N.D., Hall, L.L., Sleeman, H.K., Doctor, B.P. and Demaree, G.E.: Ion-pair high-performance liquid chromatographs separation of a multicomponent anticholinergic drug formulation. J. Chromatography 148: 453-457, 1978.

4. The United States Pharamcopeia, 18th Revisions, Bethesda, MD, 1970. p.57.

2. Isolation and Identification of Toxic Substance(s) in Aged Atropens.

In accordance with USAMRDC letter, subject "Analyses for Potentially Toxic Material(s) in Aged Atropine Sulfate Injection", date September 1979, the Department of Applied Biochemistry, Division of Biochemistry, WRAIR in collaboration with the USABML initiated research to isolate and identify the toxic substance(s) in aged Atropens. This program was precipitated by the discovery of the formulating company, Survival Technology, Inc., that aged Atropens (over 10 years old) contained substance(s) toxic to mice. Studies were initiated to isolate and identify the toxic substance(s) in aged Atropens.

Atropen both toxic and non-toxic were subjected to analyses by high performance liquid chromatograph column chromatography on DEAE-Sephacel, Sephadex G-10 and P-6, atomic absorption, IR spectrometry, NMR, and Mass-spectrometry. The toxicity was determined by HeLa cell cytotoxicity, mouse toxicity, and enzyme inhibition (Aconitase). The toxic material isolated by column chromatography in several systems were found to contain citrate, zinc and traces of Manganese. The IR spectrometry and mass spectrometry showed the presence of a polycarboxylic compound; the NMR showed that there were 3 distinct carboxyl groups. Analyses of the cytotoxic fraction from columns for citrate and zinc indicated two toxic compounds in which the citrate-zinc ratios were 2:1 and 1:1. The source of zinc in the Atropens was the butyl rubber used in the plugger and needle holder. Zinc is cytotoxic in low concentration, 30 µg/ml, but in mice gives the classic heavy metal toxicity, i.e. death occurrying after 24 hours. The toxic material isolated from the Atropen produced death in mice in less than 1 hour.

The toxicity material formed in Atropens is probably a citrate-zinc complex. The most toxic complex occurring when the ratio of citrate to zinc is 2:1. Further studies are needed to establish the structure of the citrate-zinc complex, condition for its formation, and formulations and/or packaging to prevent its formation. This information would be most useful in the preparation of and specification for future nerve agent antidotes autoinjectors.

3. Studies on Aprophen as a Potential Nerve Agent Antidote.

Aprophen (2,2-diphenylphopionic acid-2-diethyl amino ethyl ester) has undergone preliminary studies to evaluate its potential as a nerve agent antidote. This compound, an analogue of Benactyzine, has antispasmotic properties, is less toxic than Benactyzine, and penetrates the blood brain barrier more readily than Benactyzine. These properties makes it a candidate, either alone or in combination with other drugs, for the

treatment of nerve agent poisoning.

The replacement of the hydroxyl group of Benactyzine with the methyl group in Aprophen produced a decrease in the aqueous solubility of Aprophen. Aprophen.HCl was soluble in water to 0.7 mol and the solution has a pH of about 2.5. Raising the pH above pH 5.0 results in the precipitation of the compound. The pKa of the ionizable hydrogen was determined by titration to be 6.35. Several substances were used to solubilize Aprophen, 20% propylene glycol failed to increase solubility, 2% sodium dodecyl-sulfate achieve solubility but interferred with acetylcholinesterase activity, and 26 mg/ml Tween 80 achieved solubility but also interferred to some extent with acetylcholinesterase activity.

The interaction of Aprophen and Benactyzxine with rat brain acetylcholin-esterase was studied by the method of Ellman et al (Biochem. Pharmacol, 7:88, 1961). The enzyme had a maximal rate with substrate (acetylthio-choline iodide) concentration of 590 µM., so analyses were performed with substrate concentrations of 10 to 500 µ M. Aprophen and Benactyzine concentrations of 6 mM failed to inhibit rat brain acetylch linesterase. Since an alkaline pH was required for enzymatic activity, higher concen-tration could not be tested because they resulted in precipitaton of the compounds.

The toxicity of Aprophen was tested in the rat. The LD_{50} for Apropen administered orally or intramuscularly was determined by the method of moving averages (Weil. Biometrics 8:249, 1952). The intramuscular LD_{50} was 750 mg/kg, with a 95% confidence limit range of 569 to 936 mg/Kg. Orallyadministered Aprophen had a LD_{50} of 1320 mg/Kg, with a 95% confidence limit range of 1140 to 1560 mg/Kg.

The distribution of Aprophen in selected tissue of the rat was studied. Homogenates of tissue were extracted with ethyl ether at pH 7 to 8, and recoveries, based on UV spectral analysis and gas chromatography, were 95% or better. After IM administered of 2 LD_{50} of Aprophen (death in 9 minutes) the concentration (µg/g wet tissue) in the studied organs was lung, 180, kidney, 73, brain, 30, lung, 0.1 and diaphrgm, trace. In another study, when the animals were given 0.4 LD_{50} Aprophen and sacrificed at various times, Aprophen was detected in the tissues between 2.5 and 4.5 hours after administration. When Aprophen was given orally, no intact Aprophen was found in tissues for times up to 6 hours. The recovery of the intact drug from urine and feces after oral or IM administration was less than 0.1% of the dose. No Aprophen was found after 24 hours.

Studies will be continued on the distribution and excretion of Apro-phen using a ^{14}C radioactive compound. In addition, the pharmacokinetics

and metabolism of this compound will be ascertained. Following these studies, the efficacy of Aprophen, alone or in combination with other drugs, will be evaluated.

4. Methodogy Development and Application for the Study of Nerve Agent Antidotes.

a. During the past year, a series of experimental studies have been carried out in our laboratory involving a variety of biochemical projects. Using new methods and procedures developed in our laboratory. We have been able to carry out comprehensive studies to explore the pharmacokenetic and pharmocodynamic actions of several nerve agent antidotes. We recently published several arcticles (1,2) pertaining to the degradation kinetics of aprophen hydrochloride and adiphenine hydrochloride. These two synthetic analogues are related to the Benzylic acid diethylaminoethylester family. Both compounds are administered prophylactically and therapeutically as anticholinergic and antispasmodic agents. They are structurally similar to benactyzine and like benactyzine they were found to be highly unstable at certain pH values and thermal gradients. Since the therapeutic effacacy of these compounds are totally dependent upon maintaining an intact molecules, it is imperative that safeguards be taken to maintain their therapeutic effectiveness.

In these studies, we were able to develop the analytical methods required to distinguish between the parent compounds and the oxidative by-products produced during breakdown. These studies have played a big part in determining the packaging and storage of these compounds in simple and complex drug formulations.

b. The toxic factor in aged Atropens was associated invariably with citric acid, therefore, a HPLC method was developed to quantitate the citrate levels. Citric acid was separated on a reverse phase column with 0.1% aqueous phosphoric acid as the mobile phase. The eluate was monitored at 210nm, and the citrate quantitated by peak height measurements. Fractions of the toxic compound obtained from DEAE, G-10, and Bio-Gel P-6 column contained citrate in μM levels. In some toxic fractions, a peak was found which preceeding the citric acid peak. Studies on these toxic fractions are being continued.

c. Studies were initiated to develop a model system for the micro determination of protein structure and to isolate active site of enzymes in polypeptide fractions. The methodology will be applied to the study of the nerve agents and nerve agent antidotes on acetylcholinesterase activity. Preliminary studies were performed on prostatic acid phosphatase which had been purified previously in this laboratory. Protein (approximately 1 mg) was reacted with Iodoacetamide and urea to break (alkylate) the SH bounds, then was rapidly treated by gel filtration to remove the unreacted

reagents. The protein fraction was concentrated by evaporation under nitrogen and then digested by cyanogen bromide. The reaction was monitored by gel filtration and reverse phase HFLC. The resulting digest contained a minimum of 15 distinct peaks under a number of different chromatographic conditions. The column eluate was monitored spectrophotometrically at 210 nM and 280 nm.

5. Drug and Metabolite Studies by Mass Spectrometry.

Despite the various techniques attempted for the study of metaproterenol and terbutaline in biological specimens (viz. gas chromatography and high performance liquid chromatography), only by selected-ion monitoring with the gas chromagraphy mass spectrometer can any reliable quantitative data be obtained. A procedure has been developed for blood and urine that can detect as little as 5 ng. of these drugs, and clinical studies are in progress tro determine dose responses.

From the data obtained thus far, there is no evidence of metabolites in the blood that are susceptible to glucuronidase and sulfatase hydrolysis. Even urine, which was originally thought to contain the sulfate metabolites of metaproterenol and terbutaline, seems devoid of metabolites of these drugs. However, unchanged metaproternol and terbutaline can be detected in the blood samples drawn an hour after infection, and urine contains unchanged drugs for at least 24 hours thereafter.

Some indications that aa steady state must be attained for drug efficacy are apparent in data comparison of various samplings from patients on metaproterenol or terbutaline therapy. For example, metaproternol concentrations in blood were consistently low (0.01-0.02 µg./ml.) in patients who received occasional single doses, whereas those on a daily regimen manifested levels as high as 0.42 µg./ml. and appeared to derive more benefits of the drug. Other patient studies are in progress to establish the dose necessary to achieve a steady state level, and to completely evaluate the effectiveness of the metaproterenol and terbutaline under these conditions.

REFERENCE

Leferink, J.G., Wagemaker-Engels, I., Maes, R.A.A., Lamont, H., Rauwels, R. and Van der Straeten, M., J. Chromatogr., 143, 299 (1977).

6. Collaborative Research Studies on Polyamine, Brain Peptide Hormones, and Hemoglobin A.

a. In collaboration with the Urological Clinic, WRAMC, studies were continued on the polyamine content in biological fluids form patients with genito-urinary neoplastic diseases. In this study the unbound-free

polyamine content present in serum was assayed. The new HPLC methodology developed for this project was extremely sensitive for characterizing and profiling abnormal pattern of pathological specimens. Levels of putrescine, spermidine and spermine which are present in small amounts or not present at all were compared to normal serum samples. Using this new procedures, we can detect small differences between normal and abnormal profile which were very difficult to do our earlier studies. At the same time, artifactual differences observed in the urinary samples are not seen in serum specimens. From these studies preliminary data show that vast difference exist between the samples from patients with proliferative diseases. The final results obtained from this study should establish new parameters for profiling various types of proliferative diseases which involves subtle changes in the polyamines levels.

Additional studies involving polyamine metabolism are also being investigated within this laboratory. They are as follows: 1. Polyamine profiling of prostatic carcinoma patients. 2. Indicative patterns of promastigotes and amastigotes in Leishmania by their polyamine content. 3. A method for determing parasitimia in trypanosome infection by polyamine profiling.

b. In collaboration with the Division of Neuropsychiatry, WRAIR studies were conducted on the purification of the brain hormones by high performance liquid chromatography (HPLC). The brain peptide hormone levels are measured usually by radioimmunoassay (RIA). This RIA approach, while capable of measuring the low levels of the peptide hormones, lacks specificity. This lack of specificity can be caused by the hetergeniety of the radioactive material used in the assay, and/or cross reactivity, since the peptide hormones have a common precursor. A HPLC method was developed, using reverse phase chromatography, to determine the purity of the peptide hormone prior to iodination and after iodination. In addition, this methodology provided a convenient way to following the completeness of the iodination as well as any subsequent degradation. A HPLC separation of the common brain peptides also was developed. Extracted plasma samples were chromatographed prior to RIA, hence eliminating the interferring compounds. Standard solution of β-endophin have been chromatographed and the fraction collected. Recovery from the column was over 90%. This approach to the measurement of β-endorphin levels in physiological fluids should improve the accuracy and reproducibility of the assay.

c. In collaboration with the Dept. of Hematology, Division of Medicine, WRAIR methology was developed to isolate the α and β chains of hemoglobin A. The treatment of sickle cell anemia with vitamin B_6 apparently modifies the structure of the β-chain of hemoglobin. In order to determine where and if this modification takes place, the α

and β-chains of the hemoglobin must be separated and analyzed. Current methology for the isolation of the α and β chains requires large amounts of hemoglobin A and tremendous expenditure of time and effort. The need for a better method for the separation of the α and β-chains led to the development of a reverse phase HPLC procedure. The HPLC separation employs a μ bondapak C_{18} column and a mobile phase of 47% acetonitril and 53% aqueous trifluoroacetic acid. Detection was at 210 nM. This approach provided both a semi-preparative technique for the isolation of the chains and an analytical method for quantiting these chains down to 500 ng. The method will be used to isolate the modified chains for subsequent tryptic digestion and amino acid analysis.

PUBLICATIONS

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2. Doctor, B.P., Brown, N.D., and Sleeman, H.K. The Stability of Benactyzine.HCl Under Simulated Storage and Packing Conditions. Proc. Army Science Conference. June 1980.

3. Sleeman, H.K., Doctor, B.P., Brown, N.D. and Gandhi, P.K. The Stability of Benactyzine.HCl in Solution Under Various Conditions of Storage, Federation Proced 39:1012, 1980.

PRESENTATION

1. Kazyak, L. Gas Chromatography-Mass Spectrometry. Spring Workshop of the Association of Official Analytical Chemist. April 1980.

REPORTS

1. The Isolation and Indentification of Toxic Substance(s) in Aged Atropens. Reports to USABML, Quarterly.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION NO.	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A6464	80-10-1	DD-DR&E/AR1430

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
79-10-1	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. PROG UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10BD	220
b. CONTRIBUTING	61102A	3M161102BS01		141
c. CONTRIBUTING	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: 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	9	509
FISCAL YEAR CURRENT 81	9	633

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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 stresses of military significance, such as acute renal failure, shock, infectious disease, heat stress, and gastrointestinal disorders, in order to provide rational basis for prevention and treatment.

24. (U) Clearance methods, dialysis, isotope dilutions, experimental models, in vivo micropuncture, in vitro renal microperfusion, membrane transport, tissue culture, radioimmunoassay, light and electron microscopy, and chromatography.

25. (U) 7910-8009 Studies have investigated the role of vasoactive amines in the maintenance of renal ischemia and induction of renal failure. Experiments evaluated the interrelations and balance of a number of renal vasoconstrictors and vasodilators including angiotensin, catecholamines, vasopressin, bradykinin and prostaglandins during ischemia. Infusion of bradykinin resulted in transient renal vasodilatation through non-prostaglandin-mediated mechanisms and the spontaneous return of renal blood flow to normal was accompanied by an increase in renin production. Partial renal ischemia elicited a rises in renin and prostaglandins but not kinins. Angiotensin converting enzyme inhibition resulted in a further increase in prostaglandins and also increased bradykinin production. In the gentamicin model of acute renal failure changes in renal function were preceeded by increases in renin and prostaglandin production. These studies suggest that counter balancing constrictor and dilator mechanisms maintain renal blood flow and that disruption of the balance may initiate renal vasoconstriction and renal failure. The cellular mechanisms of action of several compounds which were protective in experimental acute renal failure were investigated. These agents were found to prevent adenylate cyclase activation and calcium uptake and may act by preventing vasoconstrictor activation.

*Available in unclassified user with sponsor's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498-1, 1 MAR 68 IS FOR ARMY USE ONLY AND IS OBSOLETE.

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES
Work Unit 220: Pathogenesis of Renal Disease of Military Importance
* Work Unit 141: (Same title)

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 Wiesmann, MC

Problem and Objectives

The primary problem 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 to one in 200 and one in 600 in the Korean and Vietnam Conflicts, 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. 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 procedure. A similarly persistent high mortality rate has been noted in the civilian community. 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 are 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 and (2) to define means of hastening recovery of renal function.

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. 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. 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, renal sympathetic tone; and vasodilators: prostaglandins, kinins. 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 concentrated 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 were undertaken in the dog to define the effects of bradykinin infusion on renal hemodynamics as well as the interrelations of prostaglandins and angiotensin

II with the bradykinin effects. These studies demonstrated that infusion of bradykinin induced renal hyperemia which was independent of prostaglandin production, as it occurred with or without prostaglandin inhibition with meclofenamate. These studies also demonstrated that the response to bradykinin infusion was not sustained and that the return of renal blood flow to control levels was associated with increased renin secretion (and presumably angiotensin production) suggesting a counter regulatory role for renin in maintenance of renal blood flow.

Additonal studies evaluated the role of prostaglandins, kinins and renin in maintaining renal blood flow during unilateral renal ischemia produced by constriction. Induction of renal ischemia resulted in increased renin and prostaglandin production but no change in renal venous or urinary kinin levels. After angiotensin converting enzyme inhibition with SQ 20991, renal PGE continued to rise and there was a prompt increase in renal venous and urinary kinins. Plasma renin activity also continued to increase reflecting failure of conversion of angiotensin I to angiotensin II after enzyme inhibition. The systemic hypertension accompanying renal artery constriction was ameliorated by SQ 20881, most likely reflecting the combined effect of decreased angiotension II and increased prostaglandin and kinin production. These studies support the concept that vascular resistance and renal blood flow are dependent upon a balance of constrictor and dilator substances. These studies are currently being extended to assess the same parameters as well as renal and systemic catecholamine and vasopressin production in the hemorrhagic hypotension model.

The gentamicin model of acute renal failure was also studied in the rat and dog. This model results in dose dependent, reversible, non-oliguric renal failure in both species, similar to man but at a higher per kg dosage. In the rat 50 mg/kg gentamicin resulted in acute renal failure and hypostenuria. This was preceded by an increase in plasma renin activity and urinary prostaglandin excretion suggesting activation of at least these two regulators of renal vascular resistance. Studies are currently in progress to determine if prostaglandin inhibition will enhance the rate of development of gentamicin induced renal failure and to determine if this is dependent upon a relative imbalance in vasoactive amines.

Several classes of compounds other than volume expanders and mannitol have been shown to prevent the development of acute rena failure in various models of acute renal failure. Dithiothreitol, a sulfhydryl compound, has been shown to protect against uranyl nitrate and mercuric chloride induced acute renal failure and vanadate, a calcium uptake blocker, has been shown to protect against epinepherine induced acute renal failure. As both classes of compounds have been demonstrated to have inhibitory effects on hormone receptor interaction, we have investigated the mechanisms of action of these and other similar compounds. We have previously demonstrated that dithiothreitol interferes with vasopressin induced osmotic water flow and sodium transport in anuran membranes at a site proximal to cyclic AMP generation. Recent studies have demonstrated that the inhibitory effect lies at the level of the GTP stimulated regulator protein of adenylate cyclase. Studies with vanadate and other calcium blocking agents such as stellazine indicate that blockade of vasopressin and adenylate cyclase activation occurs primarily because of inhibition of the calcium uptake step. This mechanism is known to be important in angiotensin, adrenergic, cholenergic and vasopressin stimulation and suggests that the protective effects of these agents might be related to blockade of their renal vasoconstrictor function, although other possibilities have been considered.

Future Plans and Recommendations

A primary objective for FY 81 is to attempt to develop an ischemic model of acute renal failure secondary to hemorrhagic hypotension. A reliable model of this sort has not been reproducibly developed because of the high mortality associated with other end organ failure. To circumvent this we plan to try two approaches, sublethal hemorrhagic hypotension combined with partial suprarenal aortic constriction, and hemorrhagic hypotension in which survival is prolonged with naloxone. Should either of these models prove successful we will investigate the role of the above mentioned modulators of renal blood flow in its genesis as well as the protective effects of sulfhydryl compounds and calcium uptake blockers in its prevention. Studies will continue to assess the pathophysiology of gentamicin induced renal failure and predisposing causes as noted above.

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2. Duarte, C.G. and Old, C.W. Effects of DOCA on Renal Handling of Magnesium in Potassium-Depleted Rats. Gordon Research Conference on Magnesium. Plymouth, N.H., 1980.

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1. Butkus, D.E., and Schwartz, J.H.: Modulation of vasopressin responsiveness by oxidizing and reducing agents. Submitted Amer. J. Physiol.

2. William M. Barnett, Robert J. Beattie and James S. McNeil. The Effects of Halothane, Methoxyflurane, and Pentobarbital Sodium Anesthetic Regimens on Plasma Renin Levels in the Dog.

3. Daniel A. Nash, Jr., James S. McNeil, Donald E. Butkus, and William M. Barnett. Decreased Free Water Clearance in Glucocorticoid Insufficient Dogs. A Possible Role for Renal Prostaglandins.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6466	80 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. DIST'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10AI	222
b.	62770A	3M162770A802	00	003
	STOG 80-7 212			

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: 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 80	5	100
FISCAL YEAR CURRENT 81	5	530

19. RESPONSIBLE DOD ORGANIZATION

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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 (Furnish 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) 79 10-80 09 Immunopathologic studies on arthus-type of local immediate hypersensitivity reaction in the bowel of experimental animals are in progress. Immunologic and electron microscope work on gut associated lymphoid tissue (GALT) of normal and nude (T cell deficient) mice has been initiated in order to clarify the immunologic role of GALT. Studies on immunoglobulin A (IgA) allo suppression have determined that this transient immunologic phenomenon in mice is due to the activation of T cells specific for IgA expression but to the deletion of IgA precursor cells in Peyer's patches of guts. Studies on intraperitoneal infection of mice with Rickettsia tsutasugamushi have demonstrated that lymphoid cells in addition to mesothelial cells and macrophages are actively infected by rickettsiae. Cytochemical studies on microtubules which connect kinetoplast and basal granules of Trypanosoma brucei trypomastigotes have been completed. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 30 Sep 80.

* Available from author, unless otherwise approved

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. (DD FORMS 1498A 1 NOV 82 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M162770A802 MILITARY PREVENTIVE MEDICINE
Work Unit 003 Histopathologic Manifestations of Military Diseases & Injuries
* Work Unit 003 Histopathologic Manifestations of Military Diseases and Injuries

Investigators:
Principal: Akio Takeuchi, M.D.
Associates: 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 responses due to infection. 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 gonococcal, rickettsial and trypanosomal infections with other departments of the WRAIR.

I. Study of Immediate Hypersentivity Reaction (Arthus type) of the Gastrointestinal Tract.

Little is known about the acute immune reactions of the gastrointestinal tract of man. We lack information concerning immunopathologic aspects of the immediate local hypersensitivity reaction of the bowel of sensitized human subjects to immunogens. In addition, the effect of various types of active immunization with various antigens including microbial vaccines upon the digestive tract is little understood. We have produced an Arthus type of local immediate hypersensitivity reaction in the small intestine of actively immunized guinea pigs and rabbits. After three repeated subcutaneous injections of simple protein antigens, such as bovine serum albumin (BSA) or horseradish peroxidase (HRP) together with complete Freund's adjuvants into these animals, the same antigens were injected into intestinal loops constructed in the small intestine. An acute reaction began to appear 1/2 hour post injection, reached a maximum at 4 hours, and subsided thereafter. This reaction consisted of an enteritis characterized by severe inflammatory response, hemorrhage and vascular thrombosis. Electron microscope observations suggested the formation of an antigen-antibody complex within and around the vessels which appeared to be the

primary target of this lesion.

The current efforts have been directed to clarify the nature of this immune complex around vessels by cytochemistry and immunofluorence techniques.

II. Experimental Neisseria gonorrhoeae Infection

Gonorrhea is one of the most common venereal diseases in both civilian and military personnel. Yet, we have little knowledge concerning pathogenesis and immune responses of this important sexually transmitted infection.

To date, numerous investigators have tried to produce experimental gonococcal infections in various animals by different modes of infections in various animals by different modes of infection with no success. Recently, we have established experimental gonococcus infection in adult mice and guinea pigs by intranasal administration of freshly cultured Neisseria gonorrhoeae obtained from human patients.

Results

By intranasal challenge, mice were infected with 10^8 Neisseria gonorrhoeae organisms of a primary isolation culture of colony type 1 gonococci (GC) and sacrificed at 0, 3, 6, 12, 24, and 48 hr. after infection. One-half lung was examined histologically and the other half for GC organisms. $2x10^6$ to $2x10^7$ GC were recovered from mice sacrificed at 0, 3, and 6 hr. At 12 hr. GC were detected in small numbers or not at all and could not be cultured thereafter.

A paper describing the correlation between bacteriologic studies and light microscope observations on the lung infected by GC is in preparation. Transmission and scanning electron microscope observations on the GC infected lungs are in progress.

The correlation of colonial morphology of primary isolates of GC from patients with biologic parameters including infectivity and virulence, leukotactic factors and degradation of GC has been initiated.

III. Studies on Experimental Scrub Typhus Infection

Transmission electron microscope studies on cells of the peritoneal exudate following intraperitoneal inoculation of mice with the La (Leptotrombidium arenicola) strain of scrub typhus rickettsia have been completed; rickettsia actively infected macrophages, leukocytes, lymphocytes and mesothelial cells. Rickettsia within these host cells showed morphologic evidence of replication. During infections, peritoneal lymphocytes were

transformed into lymphoblasts and actively multiplied with increasing numbers of atypical lymphocytes.

IV. Studies of the Gut-associated Lymphoid Tissue (GALT)

Lymphoid cells of a variety of types are found in the intestinal mucosa. Large aggregates of lymphocytes are organized into follicles beneath the intestinal epithelium in Peyer's patches and appendix. These discreate lymphoepithelial structures have been studied for years. Yet, little is known about their significance. We have inititated fundamental studies on GALT.

Immunologic, light and electron microscope studies on GALT of conventional and nude mice (T cell deficient) have been initiated to clarify the immunologic role of GALT.

Studies on immunoglobulin A (IgA) allotype suppression have been completed; this transient immunologic phenomenon in mice is not due to the activation of T cells specific IgA suppression but to the depletion of IgA precursor cells in Peyer's patches of the guts.

Studies on T cells in relation to maturation of IgA plasma cells in the gut have been initiated to clarify the helper and suppressor functions of T cells. Both in vitro culture system and in vivo congenic lymphocyte transfer systm will be employed and T cells will be either deleted or introduced in these systems.

V. Studies on Trypanosoma brucei Infections

Cytochemical studies on microtubules which connect kinetoplast and basal granules of Trypanosoma brucei trypomastigotes have been completed.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6537	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM / A. WORK UNIT
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	S10AI	223
b. CONTRIBUTING	61102A	3M16 1102BS01	00	137
c. CONTRIBUTING	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:

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c. TYPE: NA d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	8	15
FISCAL YEAR CURRENT 81	8	70

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Pathogenesis; (U) Animal model; (U) Trypanosomiasis; (U) Leishmaniasis; (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) 79 10-80 09 Cure of mice infected with Trypanosoma rhodesiense by cis Diamminedichloroplatinum II and Disulfiram rescue is being investigated with the Div. of Exp. Therapeutics. The pathology of chronic T. rhodesiense infection in mice has been drafted for publication. The pathology including pathogenesis of cutaneous leishmaniasis in strains of mice is in draft for review with the Div. of Exp. Therapeutics. The use of the German shepherd dog as an experimental model for visceral leishmaniasis is being tabulated and analyzed for publication. An experimental study of the nephrotoxicity of gentamicin in dogs is in progress with the Dept. of Nephrology. The management of liver trauma by tissue adhesive is being studied in support of the Div. of Surgery. Evaluation of lung injury in sheep exposed to blast overpressure from artillery weapons. For technical report see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY & HEALTH HAZARDS
* Project 3M161102B501 BASIC RESEARCH ON MILITARY DISEASES
Work Unit 223 Pathologic Manifestations of Zoonotic Diseases of Military Importance
* Work Unit 137 Pathologic Manifestations of Zoonotic Diseases of Military Importance

Investigators:
Principal: Anthony J. Johnson, LTC, VC
Associates: Ralph M. Bunte, LTC, VC; Charlotte M. Keenan, CPT, VC; Kevin P. Keenan, CPT, VC; James E. Sanders, CPT, VC; Richard E. Long, CPT, VC; Charles B. Clifford, CPT, VC

Description:

To 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. A major effort has been directed toward defining the pathogenesis and fundamental mechanistic events operative at the cellular and subcellular levels during the induction of tissue injury. Studies have applied methods of macroscopic pathology, histopathology, clinical pathology, ultrastructural pathology, histochemistry, and immunohistochemistry.

Progress:

During the reporting period research activities have included: (1) Cure of mice infected with Trypanosoma rhodesiense by cis Diaminedichloroplatinum II (DDP) and disulfarim; (2) The pathology of chronic T. rhodesiense infection in mice; the susceptibility and pathology of inbred mice to infection with human isolates of cutaneous leishmaniasis; (3) The use of the German shepherd dog as an experimental model for evaluation of human isolates of visceral leishmaniasis; (4) An experimental study of the nephrotoxicity of gentamicin in dogs; (5) The management of liver trauma by tissue adhesive; (6) Morphologic evaluation of lymph nodes from dogs and rats exposed to toxic drugs; (7) Studies on the structural basis of injury to respiratory and other tissues in animals which have been exposed to blast overpressure generated by field artillery weapons and blast tubes; (8) Respiratory epithelial injury and regeneration; (9) Clinical pathology laboratory and histopathology laboratory support and collaborative studies; (10) Diagnosis and morphologic pathology of wildlife from the TransAmazon epidemiologic survey.

1. Cure of Mice Infected with Trypanosoma rhodesiense by cis-Diaminedichloroplatinum II and Disulfarim.

Interdepartmental study with the Department of Parasitic Diseases, WRAIR.

Cis-platinum II (DDP), a cancer chemotherapeutic agent, has been shown to have anti-trypanosomal activity. However, this drug has a dose-related nephrotoxicity which precludes its wide-scale use. Mannitol

diuresis or administering a sulphahydryl containing agent such as dithiocarbamate has been shown to greatly diminish the nephrotoxicity of cis-Platinum II. This study was designed to determine if a dosage regimen of cis-Platinum II, disulfarim and saline diuresis could be established which maintained anti-trypanosomal activity without the toxic effects on the kidneys.

DDP in doses limited by nephrotoxicity was shown to be curative of T. rhodesiense infection in mice. The following regimen was shown to ameliorate the nephrotoxicity while preserving the curative action of the drug: (1) DDP per os at the rate of 3 mg/Kg/day for 7 days; (2) 250 mg/Kg disulfarim per os 4 hours after each DDP dose; (3) Physiologic saline (3 ml subcutaneously) 3 times a day during the 7 day dosage regimen. The decreased toxicity was demonstrated by microscopic examination of the kidneys. The histopathologic findings in animals so treated and serially sacrificed over a 30 day post-treatment period suggested that the renal lesions were mild and resolving. The curative action of the regimen on T. rhodesiense infected mice was shown by the method of Rane et al. (Am. J. Trop. Med. Hyg. 25: 395-400).

2. Pathology Of Chronic Trypanosoma rhodesiense Infection In Mice (Strain C57BL/6J).

Interdepartmental study with the Department of Immunology, WRAIR.

The need for an inexpensive laboratory animal model to study the chronic manifestations of T. rhodesiense, i.e. meningoencephalitis, myocarditis and glomerulonephritis in man has been cited by several authors. For instance, in the well-known form of human trypanosomiasis called sleeping sickness the most important histopathologic change is meningoencephalitis. This can occur and has been observed in our laboratory in experimental infections in monkeys, however its occurrence is not predictable or guaranteed either by monkey selection or a particular trypanosome stabilate. In general, rodents experimentally infected with virulent T. rhodesiense strains develop acute disease, high terminal parasitemias of up to 10^9 trypanosomes/ml blood and die within 5 days. Rodents dying in this short span of trypanosomiasis are virtually free of histopathologic changes in the brain, heart and kidneys. Recently, the Department of Immunology, WRAIR established chronic T. rhodesiense infection in the C57BL/6J strain of mice to permit the tabulation and characterization of the pathologic changes by light microscopy, electron microscopy, immunohistochemistry and clinical pathology. This project is near completion. Meningoencephalitis, myocarditis, glomerulonephritis and chronic changes in the lymphoreticular system were consistently observed. These lesions produced in mice strain C57BL/6J with T. rhodesiense mimic in most instances those seen in the chronic disease in man.

3. 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 non-rodent 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. Other parameters were relatively normal. Histologically, there was granulomatous inflammation in multiple organs to include: all lymph nodes, spleen, liver, bone marrow, tonsil, lung and intestine.

The clinical, clinical pathologic and histopathologic findings are consistent with what is observed in visceral leishmaniasis in man.

4. Experimental Study Of The Nephrotoxicity Of Gentamicin In Dogs.

Interdepartmental study with the Department of Nephrology, WRAIR.

Aminoglycoside antibiotics, to include gentamicin, are known to be nephrotoxic in animal and man. Renal dysfunction was anticipated when gentamicin was first made available for clinical use; however, this has been reported infrequently. Also, estimation of the clinical incidence has been obscured by the case variables in which the drug is given, i.e. serious sepsis and by the insensitivity of parameters to monitor toxicity to patients. While studies of gentamicin in animals have proved the drug to be nephrotoxic, significant variability exists in the development of renal injury among various animal species. For example, in some strains

of rats, gentamicin administered in amounts far exceeding the usual human therapeutic dose failed to induce renal disease. Yet Kosek et al. demonstrated proximal tubule damage in Fisher rats given gentamicin in doses comparable to those given humans.

This study was devised to further investigate the functional changes that have been reported, as well as to characterize the mechanism of renal damage in dogs experimentally dosed with 2 mg/Kg body weight of gentamicin per day for a period of 28 days.

While functional parameters including glomerular filtration rate, sodium para-aminohippurate (PAH) secretion, renal plasma flow, sodium reabsorption, potassium excretion, urine volume and protein, osmolar clearance, plasma renin activity, plasma gentamicin levels, prostaglandin excretion, and gentamicin excretion are being studied by the Department of Nephrology; only the progress in morphopathologic renal changes will be reviewed here. The renal injury has been variable among dogs ranging from mild degenerative change involving the proximal tubule epithelial cells in some to severe renal damage with tubular epithelial cell coagulative necrosis, desquamation and lysis. In severely altered kidneys mild distal tubule epithelial injury is evident in addition to the marked damage of proximal tubule cells. Ultrastructurally, early changes are observed in both the mitochondria and rough endoplasmic reticulum of the proximal tubule epithelial cells.

5. Management Of Liver Trauma By Tissue Adhesive.

Interdivisional study with the Division of Surgery, WRAIR.

This study was designed by the Division of Surgery to evaluate a Bulgarian surgical glue for its success in controlling bleeding and bile leakage from surgically created trauma to the liver of rats. The rat is used in the initial stage of testing to determine if the glue is adequate in controlling severe liver injury. Tissue adhesives, such as this Bulgarian glue, are being evaluated to find improved methods of handling massive trauma to organs, as seen in war wounds. Such wounds with excessive bleeding are difficult or impossible to control with suture hemostasis.

Initial gross and histopathologic evaluations of rats subjected to surgical liver trauma and glue hemostasis have revealed good hemostasis and a tissue response essentially no different from that seen in rats with hepatic surgical trauma controlled with suture hemostasis.

6. Microscopic Evaluation Of Lymphoid And Hematopoietic Tissues Of Rats And Of Dogs Treated Orally with WR-171,669 or with 180,409.

Interdepartmental study with the Department of Therapeutics, WRAIR.

Four studies were performed: lymphoid and hematopoietic tissues including spleen, thymus, lymph nodes and bone marrow from 24 beagle

dogs which had been treated with various dosages of WR-171,669 once daily for 28 days were examined. It was found that 60 mg/Kg/day or 240 mg/Kg/day produced a significant deleterious effect on lymphoid tissue and bone marrow. The threshold for such effects in dogs was between 15 mg/Kg and 50 mg/Kg per day.

Lymphoid and hematopoietic tissues from 22 rats which had been treated with various dosages of WR-171,669 once daily for 28 days were examined. It was found that there were various lesions suggestive of a deleterious effect of WR-171,669 on lymphoid tissues of rats treated with 100 mg/Kg/day and 400 mg/Kg/day. The threshold for these effects was between 25 mg/Kg/day and 100 mg/Kg/day.

Lymphoid and hematopoietic tissues including spleen, thymus, lymph nodes, tonsil, and bone marrow from 24 dogs which had been treated with various dosages of WR-180,409 once daily for 28 days were examined. No dose related lesions were observed in these tissues.

Lymphoid and hematopoietic tissues from 70 rats given various dosages of WR-180,409 once daily for 28 days were examined. No dose related lesions were observed in these tissues.

WR-171,669 and WR-180,409 have demonstrated antimalarial activity which makes them promising chemotherapeutic agents against this disease. According to regulatory guidelines, subacute toxicity studies must be undertaken in one rodent and one non-rodent species prior to trials in man. Previous studies suggested that the lymphoid tissue were the target organs of toxicity of these drugs in rats and dogs. These studies were undertaken to further delineate the lymphoid toxicity previously described.

7. Evaluation Of Lung Injury In Lungs Exposed To Blast Overpressure From Artillery Weapons.

Interdivisional study with the Division of Medicine, WRAIR.

Studies directed to define and evaluate the physiologic effects upon the human of blast overpressure generated by extended range weapons, i.e. the M110, M198, and M109 firing top zone charges have been initiated by the Division of Medicine in collaborative studies with the Division of Pathology. While intensive long range studies are needed to characterize the physical factors of the pressure wave responsible for injury and the threshold for interaction between the blast wave and various body (organ) systems; the immediate effort is to insure crew safety while operating these weapons. To achieve this objective animals are being exposed to blast wave pressures and then examined and evaluated for injury.

The first experiment, involving exposure of 16 adult sheep to 50 consecutive blasts with about 3 minutes between each blast, was accomplished in November 1979 (WRAIR protocol 019-79). Eight of the sheep were exposed to an average peak pressure of 7.7 psi and 8 were exposed to 3.4 psi.

Seven control sheep were exposed only to the stresses of the handling, transportation, and sound phenomena of the 60 blasts. Gross and histopathologic examinations of sheep exposed to 7.7 and 3.4 psi revealed focal intra-alveolar hemorrhage with erythrophagocytosis in several animals. These lesions were also occasionally seen in controls. The presence or absence of the lung lesions was determined by non-parametric analysis of the quantified pathology findings. These findings suggest that minor lung injury may have occurred as a result of the blast wave pressure of 7.7 and 3.4 psi in sheep.

Based on the data obtained in the first preliminary study with sheep, a second was implemented to determine if unequivocal evidence of blast injury to lungs of lambs can be produced by exposure to 50 consecutive blasts with peak pressure in excess of 15 psi. The experimental design was carefully established to investigate two independent lines of evidence of lung injury, namely, diagnostic pathology and sequential radiography. Lambs with blast-related lung injury were to be analyzed from sequential blood samples obtained from an indwelling venous catheter of each lamb during sequential intervals of the 50 blasts. Ninety-eight lambs were used in this study with groups exposed to high and low pressure.

Gross and histopathology involving 98 complete necropsies, in excess of 400 gross and microscopic photographs, and 2200 microscopic slides were examined, and the pathologic findings were categorized and graded. Gross and histopathologic data analyses are being done.

8. Respiratory Epithelial Injury and Regeneration

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.

Regeneration of the tracheal epithelium is being studied after mechanically removing epithelium from a focal area. This method produces a reproducible lesion, whereby all cells are killed and removed rapidly and thereafter all phases of regeneration occur clearly in sequence. Only when these phases are precisely characterized, will it be possible to reconstruct regeneration phenomena occurring after chemical, infectious or immunological injury. These insults affect the entire tracheobronchial epithelium and produce a spectrum of sublethal to lethal cell alterations, and different regenerative phases occurring throughout the airway, each with a different starting point. This results in a very confusing picture of regeneration that will be best understood when compared to a well defined model such as regeneration from acute mechanical injury.

The roles of basal, mucous, ciliated and undifferentiated "indifferent cells" in regeneration of hamster trachea were studied after mechanical removal of the ventral epithelial quadrant with a stainless steel probe. By six hours the basal lamina was partially covered by one layer of flattened mucous and basal cells which had migrated from adjacent viable epithelium. The total circumference cell number (CCN) was one-half control values at six hours (6h) post wounding, but rose exponentially through 34h. The mitotic rate (MR, 4h colchicine) increased six-fold by 6h (0.2% control, to 1.2%, 6h) and rose to 15% at 24 h. In contrast, the labelling index (LI, 6h ^{3}HTdR) remained near control levels (0.7%) at 6h (0.8%) and 12h (0.4%), but rose to 17.0% by 24h, indicating that a large fraction of the early mitotic activity was in cells arrested in G2 at the time of injury. Of the total mitoses, 20% were in basal and 80% were in mucous cells around the tracheal circumference. By 48h the epithelium covering the wound was stratified, composed of 3 to 4 layers of hyperplastic polygonal cells containing tonofilaments, well developed Golgi apparatus and mucous granules; by 60h CCN was near control numbers. At this time and later LI and MR ran parallel, returning to control values at 72h onwards. Beginning at 48h indifferent cells were seen, increasing to large numbers by 72h, not only at the wound but also all around the tracheal circumference. These large pale cells, which extended to the lumen, resembled fetal cells and contained many free ribosomes but scant RER. Similar cells showed signs of early mucous and/or ciliary differentiation. We suggest that these apparently undifferentiated cells, which recapitulate the fetal state, can differentiate into all of the normal adult epithelial cell types. Normal epithelial cell morphology and numbers were restored by 120h post wounding.

This model is being studied further by continuous infusion of ^{3}HTdR via Alzet osmotic minipumps. When these data are collected we will have quantitative information defining the respective roles played by basal and mucous cells as proliferating cell populations during the regenerative process. We then plan to apply these methods to more complex pathologic processes involving tracheobronchial epithelium.

9. Clinical Pathology Laboratory And Histopathology Laboratory Support And Collaborative Studies.

The clinical pathology laboratory handled approximately 21,000 requests for hematology and 34,000 determinations for serum or plasma biochemistry during the reporting period. The histopathology laboratory processed 16,752 paraffin blocks and 21,529 microslides during the reporting period. These two laboratories support research and diagnostic pathology for the WRAIR.

10. Diagnosis And Morphologic Pathology Of Wildlife From The TransAmazon Epidemiologic Survey.

In collaboration with the USAMRU-Belem Team, WRAIR.

Reports of the histopathology and diagnoses of disease in rodents, marsupials, bats, and other miscellaneous mammals are being furnished the WRAIR Team-Belem. Of a total of 4100 cases submitted for histopathologic evaluation over 3/4 of these have been completed and typed pathologic reports furnished the team.

PROJECT 3M263750A808

DRUG AND VACCINE DEVELOPMENT

RESEARCH AND TECHNOLOGY WORK UNIT

1. AGENCY ACCESS	2. PERIOD OF SUMMARY	REPORT CONTROL
DA OC 6481	79 10 01	

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. RD SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	A. WORK UNIT
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M263750A808	808AA	001
b. [redacted]	63750A	3M363750A808	00	001
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code):
(U) Phase II Antimalarial Drug Trials

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
Clinical 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: 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) 80	2	75
FISCAL YEAR (CURR. FY) 81	3	192

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

RESPONSIBLE INDIVIDUAL
NAME: RUSSELL, COL P. K.
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: PAMPLIN, MAJ C.
NAME: COSGRIFF, LTC T.

21. GENERAL USE
Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Clinical Pharmacology; (U) Phase II Efficacy; (U) Antimalarial Drugs; (U) Human Volunteer

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

23. (U) The technical objective of this work unit is to evaluate the effectiveness 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 as an essential part of each official Investigational New Drug submission.
24. (U) Normal male volunteers are recruited from the civilian population of the greater metropolitan Washington, D.C., area by public advertisement. Each individual is then evaluated medically. A valid informed consent to participate in the study is obtained. As a study subject, the individual is admitted to an in-patient research unit, 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 free from any adverse effect from his participation in the study.
25. (U) 79 10 - 80 09 WR 171,669 was tested for efficacy against blood-induced Plasmodium falciparum Smith Strain in eleven volunteers. Doses ranged from 250 mg every six hours for three days down to 500 mg every twelve hours for one day. All were cured. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

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Project 3M263750A808 DRUG AND VACCINE DEVELOPMENT

* Project 3M362750A808 DRUG AND VACCINE DEVELOPMENT

Work Unit 001 Phase II Antimalarial Drug Trials

* Work Unit 001 Phase II Antimalarial Drug Trials

Investigators:

Principal: Melvin H. Heiffer, Ph.D.

Associate: MAJ C. Pamplin, LTC T. Cosgriff, COL C. Canfield, CPT E. Boudreau, LTC B. Doberstyn, MAJ B. Schuster

1. Description.

Phase II studies in human volunteers are concerned with evaluating efficacy of drugs in a limited number of patients. This type of study is the essential bridge between the determination of drug tolerance in humans and the wide scale study of the new drug in human patients. Phase II studies on new antimalarial compounds involve determining the drug effect on the course of induced malaria infections in volunteers.

2. Progress.

WR 171,669 was tested for efficacy against blood-induced Plasmodium falciparum Smith Strain in 11 volunteers. The oral dose levels were as follows:

WR 171,669 RX Levels	Number of Subjects
250 mg q6hrs x 3 days	3
250 mg q6hrs x 2 days	3
250 mg q6hrs x 1 day	3
500 mg q12hrs x 1 day	2

All cases were cured. Mean time to parasite clearance was 54 hours. Mean time to fever clearance was 79 hours.

WR 638 is currently being evaluated for the treatment of cystinosis in collaboration with the National Institutes of Health, Institute of Child Health and Development. One patient has been evaluated to date. The total dose was raised to 25 mg/kg q 6 hrs, which has been well tolerated. This dosage is maintaining the white blood cell cystine levels at no higher than 20% of predosing levels.

3. Future objectives.

It is intended to continue the study designed to determine the lowest dose of WR 171,669 capable of producing a 100% cure rate. At least one other antimalarial agent is expected to enter Phase II clinical studies. Expanded studies on the treatment of cystinosis are anticipated.

4. Publications.

1. Doberstyn, E.B., Phintuyothin, P., Noeypatimanondh, S., and Teerakiartkamjorn, C.: Single-dose therapy of falciparum malaria with mefloquine or pyrimethamine-sulfadoxine. Bull. Wrld. Health Org. 57:275-279 (1979).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 2527	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY TCT	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79/10/01	D. Change	U	U	NA	NL	☒ 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		3M362750A808		002
c. CONTRIBUTING	CARDS 114E			

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: 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 80	2.0	200
	CURRENT 81	6.0	850

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

NAME: US Army Medical Component, AFRIMS

ADDRESS: Bangkok, Thailand

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

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: DIXON, K.E., LTC; GILBREATH, M.J., CPT

NAME: WHITMIRE, R.F., LTC; HARRISON, B.A., MAJ

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Malaria (U) Mefloquine (U) Fansidar (U) Monkey

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 in the treatment of drug resistant falciparum malaria in hospitalized human volunteers. Lymphocytes from malaria-infected patients are isolated and their response to malarial antigens characterized.

25. (U) 79 10 - 80 09 Field studies have revealed problems in the effectiveness of existing drug therapies, particularly Fansidar, necessitating reinstitution of quinine therapy. New treatment regimens for quinine are being investigated. Phase II of final definitive testing of WR225448 was completed. Mefloquine treatment of falciparum malaria continued to provide radical cure for all patients studied. Mefloquine also cured acute attacks of vivax malaria, although it was not sporonticidal against either infection. Preliminary studies on the possible use of the cynomolgus monkey (Macaca fascicularis) as an animal model in the malaria drug development project were initiated. Studies were initiated with Peace Corps volunteers testing the use of doxycycline in preventing diarrhea. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

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Project 3M263750A808 DRUG AND VACCINE DEVELOPMENT
* Project 3M362750A808 DRUG AND VACCINE DEVELOPMENT

Work Unit 002 Evaluation of New Antiparisitic Drugs and Vaccines in the Tropics
* Work Unit 002 (Same title)

Investigators: LTC Ronald G. Williams, MC; LTC Kenneth E. Dixon, MC; LTC Richard E. Whitmire, VC; MAJ Bruce A. Harrison, MSC; CPT Michael J. Gilbreath, MSC; CPT Robert R. Graham, VC; CPT Terry A. Klein, MSC; Katcharinnee Pavanand, M.D.; Markapol Tingpalapong, VC; MAJ David E. Johnson, MC.

1. Investigation of Fansidar Therapy for Falciparum Malaria in Khmer Refugees

PROBLEM: As of 1 May 1980, the recommendation for the treatment of uncomplicated P. falciparum infections in Khmer refugees was Fansidar alone or with primaquine as a gametocytocidal agent (1, 2). This was based on relatively recent studies, performed in Thailand, that demonstrated a relatively high degree of efficacy of Fansidar alone (3, 4). It was the clinical impression of physicians working in this area of Thailand that this had become inadequate therapy and the Armed Forces Research Institute of Medical Sciences was asked by the International Committee of the Red Cross to test this recommendation. The objective was to test the therapeutic regimen for the treatment of falciparum malaria currently recommended by agencies responsible for the medical care of Khmer refugees.

PROGRESS: Nine consecutive patients with documented uncomplicated P. falciparum infections, who had not received antimalarials for a period of two weeks prior to presentation, were hospitalized at the Ban Kaeng Refugee Holding Center, and treated with Fansidar as their only antimalarial drug. Three patients, aged 10, 10 and 12 received two tablets each. The remaining six patients, aged 13 to 46, received three tablets. The nine patients included six females and three males. Initial parasite counts ranged from 606 to 47,514 parasites/mm^3 with a mean of 18,655 parasites/mm^3. Quantitative parasite counts were performed morning and evening for seven days and follow-up smears were obtained on day 14.

Of these nine patients, one sustained an RIII WHO classification (5) response (complete failure of therapy), seven sustained RII responses (significant reduction of parasitemia, but failure to clear) and one had RI response (temporary clearance of parasites). All patients were parasitemic on day 7. In view of the complete failure of Fansidar to effect a radical cure in any of these patients, the recommendation was made that supplemental quinine should be given in uncomplicated falciparum malaria cases as well as in more severe illness.

FUTURE OBJECTIVES: The failure of Fansidar to cure malaria leaves no "one dose" treatment available. Continued efforts will be directed toward monitoring the development of resistance and investigating the efficacy of various drugs and combinations.

REFERENCES:

1. International Committee of the Red Cross Malaria Recommendations, December 1979, p 2.

2. RTG/WHO Malaria Programme, A Study to Determine Appropriate Antimalaria Measures in Areas of Mass Immigration in Eastern Thailand, January 1980, p 52.

3. Doberstyn, E.B., Hall, A.P., Vervutanapibul, K., Sonkom, P. Single-Dose Therapy of Falciparum Malaria Using Pyrimethamine in Combination with Diformyldapsone or Sulfadoxine. Am. J. Trop. Med. Hyg. 25:14-19, 1976.

4. Doberstyn, E.B., Phintuyothin, P., Noeypatimanond, L.S., Teerakiartkamjorn, C. Single-Dose Therapy of Falciparum Malaria with Mefloquine or Pyrimethamine-Sulfadoxine. Bull. WHO 57:275-279, 1979.

5. WHO. Chemotherapy of Malaria and Resistance to Antimalarials. Technical Report Series 529, 1973.

2. Failure of Fansidar Treatment in Falciparum Malaria

PROBLEM: The combination of Pyrimethamine-sulfadoxine (Fansidar^{R}) has been effective in the treatment of falciparum malaria, and has become the standard chemoprophylactic agent in civilian and military populations in Thailand. The objective of this study, part of an ongoing effort in the evaluation of malaria chemotherapy regimens, is to determine the clinical efficacy of 2-3 tablet single-dose regimens of Fansidar in the treatment of naturally acquired P. falciparum infections.

PROGRESS: In Chantaburi, 17 patients were treated with two tablets of Fansidar, and all were resistant (12 RII and 5 RIII). A further eight were treated with three tablets of Fansidar, of which two could not be definitely categorized, one was sensitive and five were resistant (1 RI and 4 RII). All marines in Chantaburi are supposed to be Fansidar prophylaxis (two tablets every 15 days) and our results show that 93% of those admitted with malaria had measurable sulfa levels in their blood before treatment was started.

In Phrabuddhabat, 15 patients were treated with two tablets of Fansidar. Information was incomplete for four. Of the remaining 11, three were sensitive and eight were resistant (3 RI and 5 RII). Sixteen patients were treated with a three

tablet regimen. Three of these responded well but became parasitemic before day 28 but after spending at least a week in a malarious area. Of the remaining 13, three were sensitive and ten were resistant (1 RI, 7 RII and 2 RIII). Every one of the RII and RIII resistant cases of malaria diagnosed in Phrabuddhabat claimed to have acquired their malaria some distance away, on the Kampuchean border. At the present time, Fansidar resistant malaria in Thailand seems confined to the border area, but high rates of migration are likely to lead to further spread.

FUTURE OBJECTIVES: Developing resistance to Fansidar underscores the need to search for new therapeutic and prophylactic drugs. We plan to evaluate mefloquine prophylaxis in marines stationed at Chantaburi and to test a new therapeutic agent developed at WRAIR, WR 171669.

3. Treatment of the Acute Attack of Malaria Caused by Plasmodium falciparum: Response of Fansidar Resistant Malaria to Other Therapeutic Regimens

PROBLEM: The AFRIMS was asked to help evaluate the efficacy of antimalarial regimens being used to treat Thai Army troops at the 1st Medical Battalion. It was felt that these troops, exposed to malaria while deployed along the Thai-Khmer border, were not resonding to the usual antimalarial regimens. Initial observation documented the inadequacy of Fansidar or Chloroquine in curing cases of falciparum malaria. Study was then initiated to determine the efficacy of various treatment regimens in these troops.

PROGRESS: Random patients were treated with combinations of Fansidar and quinine or Fansidar and chloroquine. The efficacy of these drug combinations was assessed against seven day quinine therapy. The results indicated that quinine given over seven days was the most effective regimen, although there were three patients considered RII and two patients considered RI out of 15 patients treated. The shorter courses of quinine with either chloroquine or Fansidar were much less effective.

FUTURE OBJECTIVES: The data from this study has been expanded and is in manuscript form for clearance. Previous studies (1,2,3,4) have investigated the efficacy of various drugs and combinations in the treatment of resistant malaria. Plans are underway to continue to investigate these combinations to find the most efficacious and practical treatment.

REFERENCES:

1. Hall, A.P., Doberstyn, E.B., Mettaprakong, V. and Sonkom P. Falciparum Malaria Cured by Quinine Followed by Sulfadoxine-Pyrimethamine. Brit. Med. J. 2:15-17, 1975.

2. Doberstyn, E.B., Phintuyothin, P., Noeypatimanond, S., Teerakiartkamjorn, C. Single-Dose Therapy of Falciparum Malaria with Mefloquine or Pyrimethamine-Sulfadoxine. Bull. WHO 57:275-279, 1979.

3. Berman. S.J. Chloroquine-Pyrimethamine-Sulfisoxazole Therapy of Plasmodium falciparum Malaria. JAMA 207:128-130, 1969.

4. Hall, A.P., Segal, H.E., Pearlman, E.J., Phintuyothin, P., Kosakal, S. Comparison of a 9-Phenanthrene Methanol (WR33063), A 4-Quinoline Methanol (WR30090) and Quinine for Falciparum Malaria in Thailand. Trans. Roy. Soc. Trop. Med. and Hyg. 69:342-349, 1975.

4. Comparison of Single Dose Mefloquine Therapy with Quinine in Falciparum Malaria

PROBLEM: During the past year Falciparum malaria has become increasingly resistant to the standard Fansidar therapy in some areas of Thailand, and there have been presistant rumors of quinine resistance in other parts of Southeast Asia. This project is a continuation of a long term effort at AFRIMS to determine the best treatment regimens for falciparum malaria.

PROGRESS: Patients were admitted to the study if they were males at least 18 years of age who had uncomplicated disease of mild or moderate severity, and asexual parasite count between 1,000 and 100,000/cu. mm. and were willing to volunteer for hospitalization and follow-up. They were selected from patients presenting to the malaria eradication center at Phrabuddhabat, three hours drive north of Bangkok, and from marines stationed at Chantaburi, southeast of Bangkok on the Kampuchean border. Thirty-seven patients were treated with a single 1,500 mg. dose of mefloquine. Thirty-four responded to the drug with a mean parasite clearance time (PCT) of 66 hours and a mean fever clearance time (FCT) of 37 hours. One patient was lost to follow-up and one is tentatively classified as RI resistance. The latter was reported to have vomited on the day of admission and we are awaiting the results of serum mefloquine levels to

determine how much of the drug was absorbed. His fever clearance was rapid (20 hours) but his PCT of 94 hours was one of the longest in the mefloquine group.

Seven patients were treated with 650 mg. quinine tid for 10 days. One was lost to follow-up, the rest responding well. Thirty-six were treated with 650 mg. quinine tid for 7 days. Sixteen were excluded from analysis because they were either lost to follow-up or there was reason to doubt they had received a full dose of active quinine. Of the remaining 20, 13 were sensitive, one was resistant (RII) and six became parasitemic before day 28, but only after spending at least a week in an area of high malaria transmission, making it impossible to exclude re-infection. The mean PCT was 91 hours and the mean FCT was 50 hours.

In summary, both mefloquine and quinine (if those with possible re-infection are discarded) worked quite well, with only one case of resistance in each group. Mefloquine has the advantage of single dose administration and a more rapid PCT and FCT, but is not commercially available at this time.

FUTURE OBJECTIVES: Future research will include close monitoring of responses to both drugs to check for developing resistance, and studies to measure the effect of differing clinical conditions in malaria patients and of prior or concomitant administration of other antimalarial drugs on the kinetics of quinine.

5. Therapeutic Trial of Quinine Plus Either Fansidar or Tetracycline in the Treatment of Falciparum Malaria

PROBLEM: Falciparum malaria is becoming increasingly resistant to Fansidar in southeastern Thailand. Mefloquine is not yet available commercially and quinine therapy while effective, is expensive. This study is an effort to determine the efficacy of short term quinine combined with another less expensive antimalarial in areas where Fansidar resistant malaria is known to be present.

PROGRESS: Twelve patients were treated with quinine, 650 mg. tid for seven days plus tetracycline, either 250 mg. qid or 500 mg. bid for 10 days. All were cured and remained free of parasitemia until day 28.

Eight patients received quinine, 650 mg. tid for seven days plus three tablets of Fansidar. All were cured.

Four patients received quinine, 650 mg. tid until the parasite count became zero, with three tablets of Fansidar either at the beginning or at the end of the course of quinine. All showed RI resistance.

FUTURE OBJECTIVES: Future studies will include a trial of quinine 650 mg. tid for three days plus tetracycline for ten days, in an effort to further reduce the cost and increase patient acceptance of treatment regimens. We will also study the kinetics of quinine when used in combination with other drugs.

6. Evaluation of Macaca fasicularis as a Laboratory Model for Malaria

PROBLEM: The world wide shortage of rhesus monkeys (Macaca mulatta) resulting from the moritorium on the export of this species by the Indian Government has spawned the search for alternate animal species to conduct research in certain areas. The antimalarial drug development program is among those areas.

Reports in the literature indicate that the crab-eating macaque or Cynomolgus monkey (Macaca fasicularis) is not suitable as an animal model for malaria research using Plasmodium cynomolgi as the causative organism. This is based upon the fact that in the wild state, P. cynomolgi is a natural parasite of Macaca fasicularis thereby rendering these monkeys either infected or immune due to prior infection. Until now, no attempts have been made to our knowledge to infect laboratory-reared, non-exposed Macaca fasicularis monkeys with the P. cynomolgi organism.

PROGRESS: Two cynomolgus monkeys (AF-1 and AF-3) born and raised in our laboratory were inoculated I.V. with approximately 1,000,000 sporozoites of P. cynomolgi harvested from infected Anopheles dirus mosquitoes and are currently being monitored by examining daily blood smears to determine parasitemia levels. Both monkeys have developed parasitemias and attempts to infect Anopheles dirus mosquitoes by feeding them on these cynomolgus monkeys are underway at this time.

FUTURE OBJECTIVES: To pursue the development of an animal model for use in the antimalarial drug program using the Cynomolgus monkey - P. cynomolgi model. Additional, laboratory-raised, malaria-free cynomolgus monkeys will be required to fully develop this new model.

Presentations:

1. The Army's Antimalarial Drug Development Program. The Radical Curative Method Utilizing the Rhesus Monkey (Macaca mulatta) Plasmodium cynomolgi Model. Presented by Richard E. Whitmire at the Malaria Group, Bangkok, Thailand.

Publications:

1. Tingpalapong, M., Chapple, F.E., Andrews, W.K. Unilateral, Congenital Cleft Palate in a White-Handed Gibbon. Submitted to the J. of Primatology.

2. Tingpalapong, M., Watson, W.T., Whitmire, R.E., Chapple, F.E., Marshall, J.T. Jr. Reactions of Captive Gibbons to Natural Habitat and Wild Conspecifics After Release. Submitted to WRAIR for clearance.

3. Williams. R.G., Pongsupat, T. Failure of Fansidar in the Treatment of P. falciparum Malaria in Thailand. Submitted to Trans. Roy. Soc. Trop. Med. Hyg, May 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6448	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. LNGU'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NA	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3ML63750A808	808AB	003
b. [illegible]	61102A	3M161102BS01	00	136
c. CONTRIBUTING	CARDS 114F			

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: 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 80	4	423
	CURRENT 81	4	371

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Altieri, P.L.

NAME: Dubois, D.

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Biological products; (U) Dengue virus vaccine; (U) Meningococcal vaccine; (U) Pseudomonas vaccine; (U) Typhoid-Shigella hybrid vaccine; (U) Plague antigen; (U) Bioassay; (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) 79 10 - 80 09 Investigations on the development of new and improved biologicals for military use have continued. 1. Seven different meningococcal vaccines were made available for human studies. 2. Purified polysaccharides derived from Pseudomonas aeruginosa cultures by more advanced production procedures are currently being evaluated for suitability as vaccines. 3. Initial studies on producing stable, freeze-dried vaccines from cultures of a Salmonella typhosa-Shigella sonnei hybrid have resulted in a freeze-dried product with a 45-50 per cent recovery rate. 4. Growing the A-1122 strains of Yersinia pestis on E medium resulted in higher yields of the plague diagnostic antigen (Fraction 1) than those obtained previously. 5. Studies on developing a mouse potency assay for A and C meningococcal polysaccharide vaccines indicated that immunization of mice with these products alone did not protect mice against challenge. 6. Mosquito cell lines are curreatly being evaluated for suitability in the preparation of dengue vaccines and studies with dengue 3 virus preparations have indicated that the addition of different sugars to these preparations improved stability over the freeze-drying process. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*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 3M263750A808 DRUG AND VACCINE DEVELOPMENT
* Project 3M362750A808 DRUG AND VACCINE DEVELOPMENT

Work Unit 003 Advanced Vaccine Development
* Work Unit 136 Advanced Vaccine Development

Problem and Objectives: Investigations on the development of new and improved biological products have continued. Seven different meningococcal vaccines were made available for human trials. Methods for producing purified polysaccharides from Pseudomonas cultures were examined and the resultant products evaluated as potential vaccines. The effect of growth medium on yields of the plague diagnostic antigen (Fraction 1) was studied. Investigations were initiated on methods for producing stable, freeze-dried vaccines from agar grown harvests of a Salmonella typhosa - Shigella sonnei hybrid. Studies were also initiated on developing a mouse potency assay for the A and C meningococcal polysaccharide vaccines. Evaluation of mosquito cell lines for suitability in the preparation of dengue vaccines was initiated and also begun were studies on improving the stability of frozen and freeze-dried preparations of dengue-3 virus.

Progress: In this year, two meningococcal polysaccharide vaccines, a trivalent (groups A, C and 8021), a tetravalent (groups A, C, 80Y and W-135), and five group B meningococcal vaccines, each differing in protein-polysaccharide composition were prepared, tested and made available for trials in humans. Inactivation of Pseudomonas aeruginosa, Type 5 cultures with 1% Formalin, rather than with heat or phenol, resulted in a 2-3 fold increase in sediment collected on centrifugation and in subsequent yields of final product. Two lots of purified polysaccharide derived from Pseudomonas cultures were produced for evaluation as potential vaccines for human use. Initial studies on preparing a live, oral freeze-dried vaccine from agar grown cultures of a Salmonella typhosa - Shigella sonnei hybrid have resulted in a product with a 45-50 per cent recovery rate over the freezing and freeze-drying procedures. These results were observed when the organisms were suspended in a sucrose-phosphate-glutamate medium and dried over a 72 hour cycle. Processing cultures of the A-1122 strain of Yersinia pestis grown on "E" medium agar resulted in higher yields of the diagnostic antigen (Fraction 1) used for plague serology than those previously obtained. Initial studies on developing a mouse potency assay for the A and C meningococcal vaccines, currently in use in the military, have given no indication of a protective response. Immunization of mice with these polysaccharides alone, utilizing several different schemes and up to three doses of vaccines did not protect mice against challenge. A pool of mosquito cells has been prepared and a method of freezing, thawing and growing these cells devised. Tests were initiated on evaluating these cells and other mosquito cell lines for suitability in the preparation of dengue vaccines for human use. To date, these cells have passed all safety tests except that an RNA polymerase was found in the supernatant

tissue culture fluids. The significance of this finding is currently being investigated. Studies were also begun on improving the stability of frozen and freeze-dried preparations of dengue-3 virus. Initial results have shown that of five sugars tested as additives to the tissue culture fluid harvests, maltose and lactose favorably affected the stability of the virus.

Future Objectives: The direction of future work with the meningococcal vaccines will depend on the results of the current human trials. A multivalent vaccine is being considered that would include a group B protein-polysaccharide component. The Pseudomonas studies will be directed toward producing a product suitable for evaluation in man. A lot of S. typhosa - S. sonnei vaccine will be prepared for field studies along with continuing efforts on improving recovery over freeze-drying. Production will continue on providing a single large lot of Fraction 1 antigen to serve as a plague diagnostic antigen standard. A and C meningococcal polysaccharides will be coupled to proteins to determine if these combinations will induce an immune response in mice to a protective level. The evaluation of mosquito cell lines as a vaccine substrate will continue as well as the studies on enhancing the stability of dengue-3 virus preparations.

Publications:

1. Powell, C.J., Desett, C.R., Lowenthal, J.P., and Berman, S., The effect of adding iron to mucin on the enhancement of virulence for mice of Salmonella typhi strain TY2. Journal of Biological Standardization 8: 79-85, 1980.

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 OB 6489	80 10 01	DD DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPOSITION	8a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	870/AA	072
b. [illegible]	62770A	3M162770A802	00	001
c. CONTRIBUTING	STOC 80-7.2	2		

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: 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 PREVIOUS 80	3	20
FISCAL YEAR CURRENT 81	3	61

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Prier, Ronald E., CPT, MC

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. Multidisciplinary collaborative approaches are utilized and new methods developed as required.

25. (U) 79 10-80 09. Completed are: dengue in the Bahamas region 1977; oral adenovirus type 4, 7 & 21; morbidity and mortality resulting from motorcycle accidents; toxoplasmosis occurring in soldiers undergoing jungle training; outpatient morbidity reporting system for the 101st Abn Div; demographic variables of military and dependent tuberculosis cases. Analyses of the following studies are in progress: 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 risk of coccidioidomycosis at the National Training Center; evaluation of leishmaniasis skin test in returnees from jungle training; analysis of sandfly fever threat in Brazil; assessment of ARD at Fort Leonard Wood; the importance of splenectomy in troop deployment; long-term follow-up of soldiers with hepatitis B; survey of blast over-pressure effects in artillery men; development of better diagnostic methods for leishmaniasis. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79-30 Sep 80.

DD FORM 1498 1 MAR 74 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
* Project 3M162770A802 MILITARY PREVENTIVE MEDICINE

Work Unit 072 Assessment of Infectious Diseases of Military Importance
* Work Unit 001 Epidemiologic Studies of Military Diseases

Investigators.
Principal: COL Richard N. Miller, MC
Associate: LTC Michael W. Benenson, MC; LTC Kenneth E. Dixon, MC; LTC Caroline Gertz, ANC; MAJ Jung Han Park, MC; MAJ Mary K. McKenna, ANC; CPT Ronald E. Prier, 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: Two brigades training at different times of the year have undergone prospective study of spherulin skin test conversion and disease surveillance. Asymptomatic or mild infections have occurred and have been markedly associated with race and MOS. The overall rate of infection was 3.6% in exposed troops, but was 9 times greater in black than in other soldiers and 7.5 times greater in the 13B and 36K MOS than in all other MOS. A manuscript is in preparation. Another brigade will be studied in August 1981.

2. Cost-benefit Analysis of Hepatitis B Vaccine: Further analysis will have to await the final determination of optimal dosage and immunization schedules, both or which will affect cost. The benefit side of the equation is being further characterized by a long term follow-up study of hepatitis B infected soldiers for chronic hepatitis and cirrhosis.

3. Etiology of Viral Hepatitis in U.S. Military Personnel: This project, defining serologically and demographically 491 soldiers from Korea, Germany, and Fort Hood, Texas, is nearly complete and a manuscript is in preparation.

4. Evaluation of the Leishmaniasis Skin Test in Returnees from Jungle Training: An IND is being sought for a skin test material free from animal products. Testing will begin in CY 81 but will probably not be used in returnees from the Jungle Operations Training Center until next calendar year.

5. Analysis of Sandfly Fever Threat in Brazil: Serologic assay is underway. Data analysis of demographic variables will commence in third quarter FY 81.

6. Toxoplasmosis in Soldiers Undergoing Jungle Training: Additional serological studies using recently developed technology are now complete and a manuscript is in preparation.

7. Assessment of ARD at Fort Leonard Wood: The study found an artifactual basis for the higher rates at Fort Leonard Wood; the findings resulted in changes by OTSG in guidance to the field for reporting of ARD.

8. Hepatitis Antigen/Antibody Prevalence in Special Forces: Data analysis and serologic studies are complete. A manuscript is in preparation.

9. Survey of Blast Overpressure Effects in Artillerymen: This project is still in the study design phase. A cross-sectional study of pulmonary function in artillerymen and a control population is planned.

Recommendations: More resources should be devoted to this area of research so vital to the Army. Epidemiologic studies should address the assessment of risk of Korean Hemorrhagic Fever in Europe; Rift Valley Fever and leishmaniasis in Egypt, Somalia, and the Middle East; sandfly fever in the Americas and the Middle East; diarrheal diseases world-wide; toxoplasmosis, histoplasmosis, and leishmaniasis in Panama.

Formal Presentations:

1. "Febrile Illness Following Panama Jungle Training," American Society of Tropical Medicine and Hygiene, Tucson, Arizona, 13 November 1979. LTC Michael W. Benenson, M.D.

2. "The Coccidioidomycosis Threat at Fort Irwin," 804th Hospital Center Symposium, 22 March 1980, Boston, MA. COL Richard N. Miller, M.D.

3. "Hepatitis Outbreak at SHAPE, Belgium," Preventive Medicine Conference, Berchtesgaden, Germany, 9 October 1979. LTC Kenneth E. Dixon, M.D.

4. "Automated Morbidity Surveillance in a Troop Medical Clinic at Fort Campbell, KY," Annual Division Surgeons' Conference, 1980. Edgewood Arsenal, MD, February 1980. CPT Ronald E. Prier, M.D.

Bibliography:

1. Allen, A.M., Irwin, G.R., Karwacki, J.J., and Pinkerton, R.H. "Hepatitis B Surface Antigen and Antibody in a Military Community During a Hepatitis B Epidemic." Military Medicine, Vol. 144, No. 9, September 1979.

2. Benenson, M.W., Takafuji, E.T., Bancroft, W.H., Lemon, S.M., Callahan, M.C. and Leach, D.A. "A Military Community Outbreak of Hepatitis A Related to Transmission in a Child Care Facility." American Journal of Epidemiology, 112: 471-486, 1980.

3. Gaydos, J.C. and Brodkey, C. "U.S. Army Guidelines for Troop Living Space: An Historical Review." Military Medicine, Vol. 145, No. 6, 418-421, June 1980.

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	80 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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	871AA	151
b. [redacted]	62770A	3M162770A802	00	014
c. CONTRIBUTING	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: 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 80	2	226
	CURRENT 81	2	217

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Eckels, Kenneth H., Dr., Ph.D.

NAME: Summers, Peter L.

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) 79 10 - 80 09 1. Lot 1 of dengue-2 vaccine continued to be tested in groups of human volunteers. Intradermal inoculation resulted in seroconversion rates similar to those found in volunteers receiving the vaccine by the subcutaneous route. In another group of volunteers, all of whom were previously vaccinated with yellow fever vaccine, a dose response was demonstrated with varying dilutions of vaccine. As observed in the past, yellow fever immune volunteers appeared to stimulate higher levels of antibody than did non-immune recipients of the S-1 vaccine. Attempts to boost antibody levels by vaccination with a second dose of vaccine were unsuccessful. Although there was no evidence that re-vaccination caused more than a minimal reaction at the inoculation site, only 2 volunteers out of a total of 12 developed a 4-fold rise in serum neutralizing antibodies. 2. A clone of Aedes albopictus mosquito cells designated C6/36 can be used to grow dengue viruses to high titer and may be useful as a vaccine substrate. Passage of dengue-2 and 3 viruses in these cells selects for temperature sensitive virus. Tests for safety of these cells is underway. 3. Problems with contaminated cell cultures and low virus yields have impaired progress on a dengue-3 vaccine. The C6/36 mosquito cell line offers future hope for production of an acceptable dengue-3 vaccine. For technical report see WRAIR Annual Progress Report, 1 Oct 79 - 30 Sep 80.

* 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 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

* Project 3M162770A802 MILITARY PREVENTIVE MEDICINE

Work Unit 151 Characteristics of Attenuated Dengue Viruses

* Work Unit 014 Characteristics of Attenuated Dengue Viruses

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 continued to be tested in groups of human volunteers. Intradermal inoculation resulted in seroconversion rates similar to those found in volunteers receiving the vaccine by the subcutaneous route. In another group of volunteers, all of whom were previously vaccinated with yellow fever vaccine, a dose response was demonstrated with varying dilutions of vaccine. As observed in the past, yellow fever immune volunteers appeared to stimulate higher levels of dengue antibody than did non-immune recipients of the S-1 vaccine. Attempts to boost antibody levels by vaccination with a second dose of vaccine were unsuccessful. Although there was no evidence that re-vaccination caused more than a minimal reaction at the inoculation site, only 2 volunteers out of a total of 12 developed a four-fold rise in serum neutralizing antibodies. 2. Experiments to study adsorption of the dengue-2 S-1 clone and the C-5 clone of dengue-3 revealed inefficient adsorption of these viruses at temperatures between 23C and 38.5C. Both mosquito and monkey cells adsorbed less of the attenuated, temperature sensitive viruses than either of the parent viruses. Pre-treatment of cells with trypsin or polyethylene glycol increased adsorption for the vaccine viruses. Results indicate that adsorption may play a role in the attenuation characteristics of these viruses. 3. A clone of Aedes albopictus mosquito cells designated C6/36 can be used to grow dengue viruses to high titer and may be useful as a vaccine substrate. Passage of dengue-2 and 3 viruses in these cells selects for temperature sensitive virus. Tests for safety of these cells is underway. Studies to detect adventitious viruses were negative when the C6/36 cells were tested in various cell cultures and animals. Additionally, the line is not tumorigenic and karyologic tests indicate normal chromosome patterns. The discovery of an RNA-dependent RNA polymerase activity in the cell culture medium has aroused concern and is being currently evaluated. 4. Problems with contaminated cell cultures and low virus yields have impaired progress on a dengue-3 vaccine. The C6/36 mosquito cell line offers future hope for production of an acceptable dengue-3 vaccine.

Future Objectives: A human volunteer study of approximately 200 subjects will be conducted during this FY81 to determine the importance of prior yellow fever vaccination in recipients of the dengue-2 S-1 vaccine. Immune responses and clinical reactions will also be measured in a group of volunteers who have no prior history of yellow fever or other flavivirus exposure. The C6/36 mosquito cell line will be further characterized so that it may be used as a vaccine substrate. If safety tests show no evidence of adventitious agents, the S-1 vaccine virus will be passaged in these cells and will terminate in the preparation of a lot of vaccine suitable for human use. Dengue-3 virus clones including C-5 will also be passaged in the C6/36 line for the preparation of seed and vaccine lots. If these vaccines appear promising, the same passage procedure in C6/36 cells can be used for dengue-4 vaccine preparation.

References Cited: None.

Formal Presentations: Human immune response to dengue-2 vaccine measured by a solid phase radioimmunoassay, P.L. Summers, K.H. Eckels, J.M. Dalyrymple, and R. McN. Scott (Presented at the American Society of Tropical Medicine and Hygiene Annual Meeting, Tucson, Arizona, Nov, 1979).

Publications:

1. Eckels, K.H., V.R. Harrison, P.L. Summers, and P.K. Russell. 1980. Dengue-2 vaccine: Preparation from a small-plaque virus clone. Infect. Immun. 27:175-180.

2. Scott, R.McN., A. Nisalak, K.H. Eckels, M. Tingpalapong, V.R. Harrison, D.J. Gould, F.E. Chapple, and P.K. Russell. 1980. Dengue-2 vaccine: Viremia and immune responses in rhesus monkeys. Infect. Immun. 27:181-186.

Patents: A patent application #101667, filed 10 Dec 1979, by the Intellectural Property Division, Judge Advocate General Office, has been issued in the name of inventor, Venton R. Harrison, for an innovative microphotometer. Although of simple construction and relatively inexpensive, the prototype instrument has provided reliable, trouble-free service in the Plague Section, Department of Hazardous Microorganisms, WRAIR, for 2 years. To date, both a portable unit for use in the field and a bench model have been constructed. The portable unit has been field-tested in Peru and is currently undergoing field tests in the Union of South Africa to determine environmental stability.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6442	80 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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	871AC	153
b. [redacted]	62770A	3M162770A802	00	006
c. CONTRIBUTING	STOG 80-7,23			

11. TITLE (Precede 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 80	4	318
	CURRENT 81	4	348

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Stephenson, E. H., LTC

NAME: Jerrells, T. R., PhD

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 directly at development of safe, efficacious rickettsial vaccines that will protect deployed military troops, and development of accurate, sensitive 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 long lasting, broad-spectrum protection against Rickettsia tsutsugamushi infection. Analyze in vitro and in vivo correlates of lymphocyte recognition to determine the immune response. Determine the genetic basis of resistance and sensitivity of the mouse model to scrub typhus infection.

25. (U) 79 10 - 80 09 Culture parameters were determined for preparation of roller bottle cultures of primary chick embryo fibroblasts. Methodology to obtain maximal R. tsutsugamushi yields with minimal input was ascertained. Two congenic strains of mice exhibited significantly different degrees of susceptibility to infection with Gilliam strain. Inflammatory responses of susceptible mice were greater and consisted of a polymorphonuclear leukocyte influx, followed by a mononuclear cell response. Resistant mice exhibited a predominantly mononuclear cell response of a decreased magnitude. Both immune responses could be modified by treatment with indomethacin. Differences in susceptibility apparently are inate characteristics of host cells as irradiated C3H/HeDub mice reconstituted with bone marrow from C3H/RV mice were rendered resistant to infection. Recrudescence of rickettsia occurred when mice infected with R. tsutsugamushi were exposed to sublethal doses of gamma irradiation. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 - 30 Sep 80.

*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 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS
* Project 3M162770A802 MILITARY PREVENTIVE MEDICINE

Work Unit 153 Rickettsial Diseases of Military Personnel
* Work Unit 006 Rickettsial Diseases of Military Personnel

Investigators:
Principals: Joseph V. Osterman, PhD; LTC Edward H. Stephenson, VC; CPT Daryl J. Kelly, MSC; Thomas R. Jerrells, PhD
Associates: SP5 John A. Hallam; SP5 Guadalupe Stockhausen; SP5 Brian S. Weatherly; SP4 Brian L. Ermeling; SP4 Deborah A. Stewart

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:

1. Scrub Typhus Vaccine Development: Research efforts have continued to develop an efficacious vaccine against scrub typhus (1,2). Studies have emphasized the propagation of Rickettsia tsutsugamushi in primary cultures of chick embryo fibroblasts and using the rickettsiae obtained to produce gamma-irradiated immunogens. Culture parameters were determined for the routine preparation of roller bottle cultures of primary chick embryo fibroblasts, and the maintenance of such cultures throughout the 6 to 9 day time interval required to propagate R tsutsugamushi. Methodology to obtain maximal rickettsial yields with minimal input (MOI) was ascertained and verified. Studies are in progress to define standard procedures for preparing the stock rickettsial suspensions and immunogens from the tissue culture grown rickettsiae.

2. Immunologic Responses to Scrub Typhus: Studies were initiated to define immunologically the cellular composition of the inflammatory response developed by genetically susceptible and resistant strains of mice and to determine if anti-inflammatory agents can prolong survival. Influence of host genetic composition on susceptibility to scrub typhus infection was demonstrated. Two congenic strains of C3H mice, differing at the Ric gene (3), exhibited significantly different degrees of susceptibility to infection with the Gilliam strain. The inflammatory response of the susceptible C3H/HeDub mice was greater and consisted of an early polymorphonuclear leukocyte influx, followed by a mononuclear cell response. By constrast, the resistant C3H/RV mice exhibited a predominantly mononu-

clear cell response of a decreased magnitude. In both strains of mice the immune response could be modified by treatment with non-specific agents, e.g. thioglycollate and indomethacin. Additional studies showed that the inflammatory exudate of sensitive mice contained increasing numbers of thymus-derived lymphocytes (T-cells) and activated macrophages, both types of which are required for immunity (4,5). Susceptibility to infection, therefore, was not due to an inability of the animal to mobilize the appropriate cellular components. Apparently the differences in susceptibility are inate characteristics of the host cells, as lethally irradiated C3H/HeDub mice reconstituted with bone marrow from C3H/RV mice were rendered resistant to infection with Gilliam strain. Studies are in progress to assess the expression of delayed-type hypersensitivity (DTH) in progressive R tsutsugamushi infections in mice. Also, in vivo (DTH) and in vitro (lymphocyte transformation) correlates of lymphocyte recognition are being used to evaluate the antigenic composition of strains of R tsutsugamushi and to determine the recognitive range of lymphocytes obtained from animals immunized to scrub typhus rickettsiae.

3. Recrudescence of Latent Rickettsial Infection: Investigations continued to ascertain whether recrudescence of latent R tsutsugamushi infections in mice would occur following exposure to sublethal doses of gamma irradiation. A significant difference was noted in lethality between previously infected and uninfected mice subjected to various doses of gamma irradiation. Recrudescence of rickettsia occurred when mice infected with R tsutsugamushi were exposed to sublethal doses of gamma irradiation at 12 weeks and 52 weeks after inoculation.

Recommendation for Future:

1. Scrub Typhus Vaccine Development: Studies will continue to prepare 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 the subhuman primate model, to include in vitro correlates of immunity to ascertain safety and immunogenicity. If the testing in subhuman primates yields favorable results, safety and efficacy testing in human volunteers will be initiated.

2. Immunologic Responses to Scrub Typhus: The cellular nature of the genetic resistance of specific strains of mice will be explored further using the irradiation chimera model and evaluating the different anti-rickettsial effector cells that arise during the inflammatory response to infection. In vitro and in vivo correlates of cell mediated immunity will be developed and evaluated for use in monitoring vaccine effectiveness in the experimental host systems, mouse and subhuman primate. Immunity derived from active infection will be compared to immunity obtained by administration of experimental immunogen, using the in vitro and in vivo assay systems.

Reference 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-irradiated scrub typhus immunogens: Broad-spectrum immunity with combinations of rickettsial strains. Infect. Immun. 26:131-136.

3. Groves, M.G., and J. V. Osterman. 1978. Host defenses in experimental scrub typhus: Genetics of natural resistance to infection. Infect Immun. 19:583-588.

4. Nacy, C.A., and J. V. Osterman. 1979. Host defenses in experimental scrub typhus: Role of normal and activated macrophages. Infect. Immun. 26:744-750.

5. Shirai, A., P. J. Cantanzaro, S. M. Phillips, and J. V. Osterman. 1976. Host defenses in experimental scrub typhus: Role of cellular immunity in heterologous protection. Infect. Immun. 14:39-46.

Presentations:

1. Jerrells, T. R., and J. V. Osterman. 1980. Inflammatory responses of C3H mice differing at the Ric gene to infection with Rickettsia tsutsugamushi strain Gilliam. Rocky Mountain Laboratory Conference on Rickettsiae and Rickettsial Diseases, 3-5 September 1980, Hamilton, MT.

2. Osterman, J. V. 1980. The immunology of rickettsial diseases. Rocky Mountain Laboratory Conference on Rickettsiae and Rickettsial Diseases, 3-5 September 1980, Hamilton, MT.

Publications:

1. Anderson, G. W., Jr., and J. V. Osterman. 1980. Host defenses in experimental rickettsialpox: Genetics of natural resistance to infection. Infect. Immun. 23:132-136.

2. Eisenberg, G. H. G., Jr., and J. V. Osterman. 1979. Gamma-irradiated scrub typhus immunogens: Broad-spectrum immunity with combinations of rickettsial strains. Infect. Immun. 26:131-136.

3. Eisenberg, G. H. G., Jr., J. V. Osterman, and E. H. Stephenson. 1980. Gamma-irradiated scrub typhus immunogens: Analysis for residual, replicating rickettsiae. Infect. Immun. 28:295-297.

4. Groves, M. G., D. L. Rosenstreich, B. A. Taylor, and J. V. Osterman. 1980. Host defenses in experimental scrub typhus: Mapping the gene that controls natural resistance in mice. J. Immunol. 125:1395-1399.

5. Hemelt, I. E., G. E. Lewis, Jr., D. L. Huxsoll, and E. H. Stephenson. 1980. Serial propagation of Ehrlichia canis in primary canine peripheral blood monocyte cultures. Cornell Vet. 70:37-42.

6. Nacy, C.A., and J. V. Osterman. 1979. Host defenses in experimental scrub typhus: Role of normal and activated macrophages. Infect. Immun. 26:744-750.

7. Stephenson, E. H., and J. V. Osterman. 1980. Somatic cell hybrids of canine peritoneal macrophages and SV40-transformed human cells: Derivation, characterization, and infection with Ehrlichia canis. Amer J. Vet Res 41:234-240.

Manuscripts Submitted:

1. Jerrells, T. R., and J. V. Osterman. 1980. Delayed-type hypersensitivity responses of inbred mice infected with Rickettsia tsutsugamushi. Infect Immun. (Submitted).

2. Jerrells, T. R., and J. V. Osterman. 1980. Host defenses in experimental scrub typhus: Inflammatory response of congenic C3H mice differing at the Ric gene. Infect. Immun. (Submitted).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6531	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECUR'TY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	871AH	154
b. [redacted]	61102A	3M161102BS01	00	133
c. CONTRIBUTING	STOG 30-7,2:2			

11. TITLE (Precede 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: 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 80	3	275
FISCAL YEAR CURRENT 81	3	292

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: 301-427-5176 or 5110

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NAME: Williams, James E., MAJ, MSC

NAME: Harrison, Daniel N., Ph.D.

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) 79 10 - 80 09 Strains deficient in the production of F-1 antigen are lethal for rats immunized with Plague Vaccine, U.S.P. The ELISA technique using F-1 antigen for the detection of antibody has been field tested successfully in Peru. ELISA tests using murine toxin (MT) and F-1 are presently being conducted by a member of this Department in South Africa. An antigen inhibition control has been added to the test. MT detects low titers in known positive sera and other antigens may prove superior for the detection of F-1 deficient strains if these antigens are specific for Y. pestis. Nalidixic acid resistant strains have been selected to facilitate the study of the transfer of plasmids within the species. Programs have been written and a data base is being entered into the WRAIR research computer to develop a model for epidemiological forecasting. Procedures for colonization and infection of the common flea vector have been developed. Killed vaccines prepared by FDA criteria from avirulent strains of Y. pestis appear to protect against acute disease. Isogenic pairs have proven to produce diagnostic antigen more easily purified than antigens prepared from unselected strains. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79-30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

* Project 3M161102BS01 BASIC RESEARCH ON MILITARY INJURY AND DISEASE

Work Unit 154 Prevention and Treatment of Plague
* Work Unit 133 Ecology of Plague

Problem and Objectives: Protection of soldiers in a combat environment from morbidity and mortality due to plague requires a safe, effective forecasting system which would indicate a requirement for increased control or surveillance. The following objectives are being pursued as potential solutions to these problems: i) study of the present plague vaccine, USP, as it relates to chronic disease and challenge with aberrant, virulent strains, ii) development of rapid techniques applicable to a field environment to detect antigen as well as antibody in the area of operations, iii) development of computer models based on data generated over the past 80 years as accurate forecasting systems, iv) impact of intra or interspecies genetic transfer on the properties of the plague bacillus.

Progress: In a study of the ability of Fraction 1 (F-1) negative strains to produce chronic infections, a series of nine rats were infected with the F-1 negative CPS-1 strain and 45 days later challenged with the F-1 positive 195/P strain. From 4 months to over 1 year later, 7 of the 9 died of chronic plague. No uniform lesion was noted as abscesses were in the liver, spleen, pleural cavity or abdominal cavity. Without exception, the organisms isolated from these abscesses were F-1 negative. This does not necessarily indicate that they originated from the CPS-1 strain since $F1^-$ strains have been isolated in this laboratory following chronic infections produced by the 195/P strain.

Micro-ELISA plates sensitized with F-1 antigen are stable for at least 10 months when stored at ambient temperature. These plates are usable without any preliminary treatment. The murine toxin (MT) of the plague bacillus appears to be a satisfactory supplementary antigen. The purpose of this antigen would be to detect infections with strains of Y. pestis which are F-1 negative. The proposed antigen was prepared from seed pool colonies selected to be F-1 negative. This material was prepared and coated onto micro-ELISA plates using standard laboratory techniques. An officer of this Department is presently testing all components of the micro-ELISA system for plague under field conditions in South Africa. This includes the F-1 and MT coated plates, the WRAIR microphotometer, both direct and inhibition micro-ELISA tests, and affinity chromatography purified reagents.

A series of isogenic pairs of Y. pestis strains have been examined for the presence of plasmids. The majority of the strains contained one or more plasmids. Nalidixic acid resistant strains have been selected, and mating experiments are in progress to determine whether these plasmids can be transferred from one strain to another.

The techniques necessary to safely handle and account for infected vector fleas have been developed. Infant rats are used to provide a natural source of infection for the fleas. High titers of microorganisms have been difficult to reproduce in the rodent strains tested and some manipulation or change in the rat is planned during FY 81.

Since the California ground squirrel, Spermophilus beecheyi, has been implicated as the source of many human plague cases, the susceptibility to infection in relation to age was studied. While young and adult animals were equally susceptible, the disease appeared to be more acute in young individuals. Detectable antibody persisted for over 3 years when the study was terminated.

Computer programs to store and sort data on plague cases have been written in the Fortran language and are compatible with the WRAIR research computer graphics package. Data is being entered as time permits.

Recommendations and Future Objectives: The use of isogenic pairs to produce cellular fractions more easily purified by biochemical methods will be continued to yield candidate antigens for the ELISA test. Some of these antigens may prove to be potential subunit vaccines and a future objective involves a survey of their immunogenic potential. The distribution of aberrant strains in nature is unknown. The use of diagnostic antigens capable of detecting these strains is essential to determine their importance during military operations. The capacity of vector fleas to transmit aberrant strains is the other unknown variable in assessing the danger to soldiers operating in environments where these strains may be widespread.

Publications:

1. Cavanaugh, D.C., Fortier, M.K., Robinson, D.M., Williams, J.E., and Rust, J.H., Jr. Application of the ELISA technique to problems in the serologic diagnosis of plague. Bull. Pan Amer. Hlth. Organ. 13(4): 399-402, 1979.

2. Williams, J.E., Moussa, M.A., and Cavanaugh, D.C. Experimental plague in the California ground squirrel. J. Infect. Dis. 140(4): 618-621, 1979.

3. Harrison, D.N., Laird, W.J., Robinson, D.M., and Cavanaugh, D.C. Commonality of a virulence factor among Yersinia pestis. J. Infect. Dis. 141(3): p. 413, 1980 (March).

4. Laird, W.J. and Cavanaugh, D.C. Correlation of autoagglutination and virulence of Yersiniae. J. Clin. Microbiol. 11(4): 430-432, 1980 (Apr).

5. Hastriter, M.W., Robinson, D.M., and Cavanaugh, D.C. An improved apparatus for safely feeding fleas (Siphonaptera) in plague studies. J. Med. Entomol. 17(4): 387-388, 1980.

Submitted for publication:

1. Hudson, B.W., Turner, R.W., Sulianti, S. and Cavanaugh, D.C. Plague in Central Java, Indonesia. Bull. Wld. Hlth. Org. 58(3): , 1980.

2. 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. Bull. Wld. Hlth. Org. 58: , 1980.

3. Hastriter, M.W. and Cavanaugh, D.C. An improved apparatus for colonizing fleas (Siphonaptera) and collecting pupal cocoons. J. Med. Entomol.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DA 0B 6536	80 10 01	DD DR&E/AR 1616

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECUR.*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	871AF	155
b. ~~CONTRIBUTING~~	62770A	3M162770A803	00	087
c. CONTRIBUTING	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: 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	80	7.0	432
FISCAL YEAR CURRENT	81	3.0	213

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.

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) The technical objectives are to develop and exploit animal and in vitro models for the study of the pharmacodynamic and toxic effects of drugs intended for use as antimalarials in man. The intended purposes of these studies are to provide a basis for predicting human response and to fulfill requirements for submission of IND for clinical trials of new antimalarials for military personnel in malarious areas.
24. (U) The approaches are to study both the effects of antimalarial drugs on healthy animals and the fate of these drugs in healthy animals in order to predict the human tolerance to new drugs (Phase I). Development and utilization of methods to quantitate levels of these drugs in humans leads to a more rational approach to dosing.
25. (U) 79 10 - 80 09 Technical management continued for 11 contracts in pharmacology. These are in support of the 13 IND's classified in the Active status as well as those compounds being groomed for IND status. Pharmacokinetic analysis of WR 180,409 human blood levels showed essentially identical elimination kinetics after either capsules or tablets although relative bioavailability was somewhat greater for the tablet. In vitro determinations of drug-induced methemoglobin formation have been carried out on 9 8-aminoquinolines. A comparison of methemoglobin production by primaquine vs. WR 6026 was carried out in dogs. Thin layer chromatographic techniques for quantitating mefloquine, quinine and chloroquine plasma levels have been investigated. Fluorescent micromethodology for quantitating chloroquine plasma levels has also been investigated. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M162772A871 PREVENTION OF MILITARY DISEASE HAZARDS

* Project 3M162770A803 **DRUG DEVELOPMENT**
  Work Unit 155 Determination of Pharmacological Effects of Antimalarial Drugs
* Work Unit 087 Determination of pharmacological effects of antimalarial drugs

Investigators:

Principal: Melvin H. Heiffer, Ph.D.

Associate: Dr. R. Rozman, MAJ J. von Bredow, Dr. L. Fleckenstein, Dr. H. Chung, CPT V. Jimmerson, 1LT J. Anders, MAJ C. Pamplin, Dr. H. Lowensohn, CPT D. Korte, Jr., SFC J. Baker, J.H. Digiovanni, SP5 M. Abdelrahim, SP5 J. Osuch, SP5 J. Ferri

1. Description.

In support of the antimalarial drug development program, investigations carried out by the department have continued in two broad overlapping areas. One is the effect of the body or system on the drug, i.e., absorption, distribution, biotransformation and excretion. The second is the effect of the drug on the body or system, i.e., pharmacodynamics. In addition, continuation of the development and utilization of sensitive assay methods for several of the new antimalarial drugs was emphasized. Finally, the necessary in-house and contract work has continued to support the active IND's and those compounds being groomed for IND status.

2. Progress.

Pharmacokinetic studies were carried out on blood levels of WR 180,409 following oral administration of 750 mg of drug to 14 normal volunteers. Absorption half-lives were 0.096 $\pm$ 0.040 days and 0.084 $\pm$ 0.035 days for tablets and capsules, respectively. Elimination half-lives were 6.87 $\pm$ 1.91 days and 6.14 $\pm$ 1.50 days, respectively. Bioavailability parameters for tablets and capsules were, respectively: area under the curve, 3.08 $\pm$ 0.76 µg days/ml and 2.40 $\pm$ 1.09 µg days/ml; measured peak concentrations, 0.377 $\pm$ 0.99 µg/ml and 0.307 $\pm$ 0.123 µg/ml; and measured peak times, 0.46 $\pm$ 0.055 days and 0.50 $\pm$ 0.096 days.

In vitro determinations of drug-induced methemoglobin production have been carried out on 9 8-aminoquinolines using partially purified human hemoglobin solutions. Primaquine produced 50% methemoglobin at 3×10^{-3} molar concentrations; a quantitatively similar result was seen with 3 other compounds. The remaining compounds produced little or no methemoglobin under these conditions.

In vivo methemoglobin production of multiple daily oral doses of WR 6026 or of single oral doses of primaquine was investigated in dogs. WR 6026 at 4.1 mg/kg/day for 5 days caused a cumulative increase in methemoglobin levels. Single oral doses of either 3 mg/kg or 9 mg/kg of primaquine produced no significant increase in methemoglobin levels.

Thin layer chromatographic techniques for quantitating plasma levels of either mefloquine, quinine or chloroquine have been investigated. Mefloquine levels of 100 ng/ml to 1200 ng/ml can be quantitated using TLC spectrodensitometric equipment. These same detection systems have allowed quantitation of plasma quinine levels ranging from 3.2 ng/ml to 512 ng/ml and quantitation of plasma chloroquine levels ranging from 10 ng/ml to 80 ng/ml.

Using a fluorescence micromethod, chloroquine levels of 1 ng/ml to 100 ng/ml were quantitated.

3. Future objectives.

It is intended to continue the progress on methodology to quantitate plasma levels of antimalarial compounds. Investigation into methemoglobin-producing potential of compounds will be broadened to include metabolites generated in vitro. Pharmacokinetic studies will be extended to additional drugs. Fate of radiolabeled new drugs will be investigated.

4. Formal presentations.

1. Chung, H.: An in vitro method for screening methemoglobin producing potential of candidate drugs. Presented at the 64th Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, California, 13-18 April, 1980.

5. Publications.

1. Chung, H.: An in vitro method for screening methemoglobin producing potential of candidate drugs. Fed. Proc. 39:309, 1980.

2. Chung, H., Jimmerson, V.R., Sanders, J.E., Bounds, D.W., Rozman, R.S., and Thorne, J.: The disposition of DL-3-di-n-butylamino-1-[2,6-bis(4-trifluoromethylphenyl)-4-pyridyl]-propanol methanesulfonate (WR 172,435·CH_3SO_3H) in mice. Drug Metab. Disp. Accepted for publication.

3. Lee, C.C., Kintner, L.D., and Heiffer, M.H.: Subacute toxicity of primaquine in dogs, monkeys and rats. Submitted to Bull. Wld. Health Org.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6495	80 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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	871AG	156
b. [redacted]	62770A	3M162770A803	00	084
c. CONTRIBUTING	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: 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 80	5.0	377
FISCAL YEAR CURRENT 81	5.0	343

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:

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) 7910--80 09: In the acridinedione-acridinedione imine series Aotus monkey testing on the five most promising compounds has begun in order to choose a candidate for pharmacological studies and IND preparation. Comparisons of the imines without a side chain with the ring closed oxazole analogs has begun. Antimalarial activity in the thiosemicarbazones has been improved, but toxicity remains a problem. Quassinoids have shown activity in the in-vitro falciparum screen, and a proposal is currently under consideration to follow up this lead. Efforts to synthesize tissue schizonticides are continuing. In the areas of schistosomiasis, trypanosomiasis, and leishmaniasis, the synthetic effort had decreased, but recent data indicate some activity of new purines in the trypanosomiasis screen. Data processing conversion to an integrated in-house system continues. Fully integrated reports can now be produced using data which has undergone conversion. Inventory is complete, accession number verification of chemistry is complete and sample number verification will be completed in the 2nd quarter of next year. Rebuilding of old biology files has begun. Approximately 535 compounds were submitted from the synthesis program. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79--30 Sep 80.

*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 75 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS
* Project 3M162770A803 DRUG DEVELOPMENT

Work Unit 156 Synthesis of Antiparasitic Drugs
* Work Unit 084 Synthesis of Antiparasitic Drugs

Investigators:

Principal: MAJ Robert O. Pick, Ph.D.
Associates: COL Craig J. Canfield, MC; Thomas R. Sweeney, Ph.D; William Y. Ellis, BS; Bing T. Poon, Ph.D; Daniel L. Klayman, Ph.D; CPT John P. Scovill, Ph.D; Edgar A. Steck, Ph.D.

The Research Contract Chemical Synthesis Program

During this reporting period active contractual programs devoted to the synthesis of potential antiparasitic agents were divided as follows: malaria 7, leishmaniasis 1 1/2, trypanosomiasis 6 1/2. Two preparations laboratories and a radiolabel synthesis contract also supported the program.

Data on the 8-aminolepidines indicate good tissue schizonticidal activity, but methemoglobin formation appears to be a problem. This may be decreased in the 5 alkoxy series, and/or by inclusion of a methoxy in position 2.

Due either to lack of activity or to synthetic difficulties, work in the following areas has ceased or is being phased out: tetrahydropyrimidopyrimidines, diazanaphthalenes, and pteridine antifolate analogs.

New work has begun on aminodimethoxynaphthalenes, but no targets have been submitted to date. Work on amodiaquine analogs, acridinediones and acridinoneimines, has been refined and production slowed while advanced testing is done to determine which members of the series are clinical candidates. Screening results in the camoform (bialamicol) and indoloquinoline series have thus far indicated a disappointingly low level of activity.

In leishmaniasis, work on the rifampicin types and the phenazines allied to clofazimine has been halted. 6-amino- and 7-aminoquinolines bearing the WR 6026 side chain have not proven to be as active as the 8-aminoquinolines. Work in this area has also decreased.

In the antitrypanosomiasis synthetic effort, screening data on nucleocidin and tubercidin analogs were disappointing. However, recent results showing good curative activity for adenosine diglycolaldehyde are encouraging. Work in this area and in sulfamoyl nucleosides continues.

Antitrypanosomal activity of the bis s-triazines has been lower than that of the lead compound, so this effort has stopped. Difficulties have been encountered in the area of quaternary salts of various imidazo fused heterocyclic types, but work is continuing. Tetrahydrocarbazoles and related benzo-fused compounds have not shown the desired activity against T. cruzi, and work has ceased in this area. Synthetic efforts on stilbazol diamidines continue.

Data Processing

Parallel processing of the CHEMSTRUCS system on the IBM 7090/94 was discontinued early in the fiscal year. Currently, all components of the Chemical Information Retrieval System (CIRS) are operated within the WRAIR. Status is as follows:

1. Inventory

This system is fully operational and documented. Inventory is being updated every third day concomitant with the shipping cycle. Complete inventory reports on all samples are generated on tape and converted to microfilm periodically. Bottle (sample) number sequence reports are generated monthly while accession number sequence and source of sample sequence reports are generated quarterly.

2. Chemistry

Over 259,000 structures with their accession numbers are now indexed on this file. Re-input of unmatched accession numbers from the inventory file was completed ahead of schedule, and a second verification phase-sample number accounting--was begun. Nearly 27,000 samples must be re-input in FY 81.

3. Biology

The Rane file is operational in the production mode, and a microfilm copy was produced for office reference. Work is continuing on non-Rane systems with priority to reprogramming the Hanson system and rebuilding the Brazil schistosomiasis file. Work on production of a condensed Rane screen (MM) file was begun.

4. Integrated Reports

Biology results can now be included in the integrated reports. Modifications have been made to allow more than one processing stream at a time.

Acquisition of Compounds

The following table summarizes the number of various classes of compounds received during FY 80.

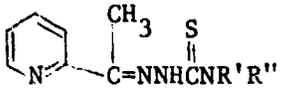
	Originals	Duplicates	Total
Purchased	26	12	38
Gifts	99	13	112
Synthesized	405	130	535
Discreet	2663	166	2829
Prep Labs	36	47	83
Total	3229	368	3597

Twenty-nine sources submitted compounds under no-dollar agreements. One of these submissions was widely active in the screens, including T. cruzi. Unfortunately, the compound is mutagenic. Analogs have been submitted for screening and subsequent structure-activity analysis. Also, a natural product received as a gift has shown antimalarial activity comparable to mefloquine in vitro. A contract proposal has been received from the submitter.

Organic Synthesis Section

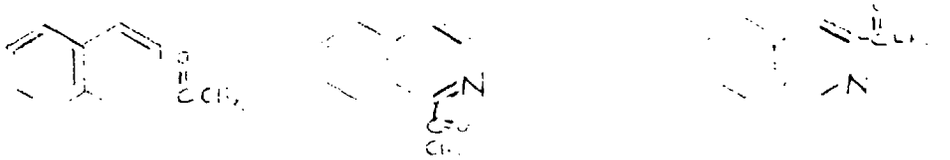
During the past year about 100 compounds were synthesized for biological testing. Most of the compounds were new and designed for their potential antimalarial activity.

In view of the good antimalarial activity achieved with certain N^4,N^4-disubstituted 2-acetylpyridine 3-thiosemicarbazones, several types of structure modifications are being investigated. The alkylidene side chain, R, of 1 has been lengthened 1-4 carbon atoms beyond R=CH_3 while retaining the R' and R" groups which



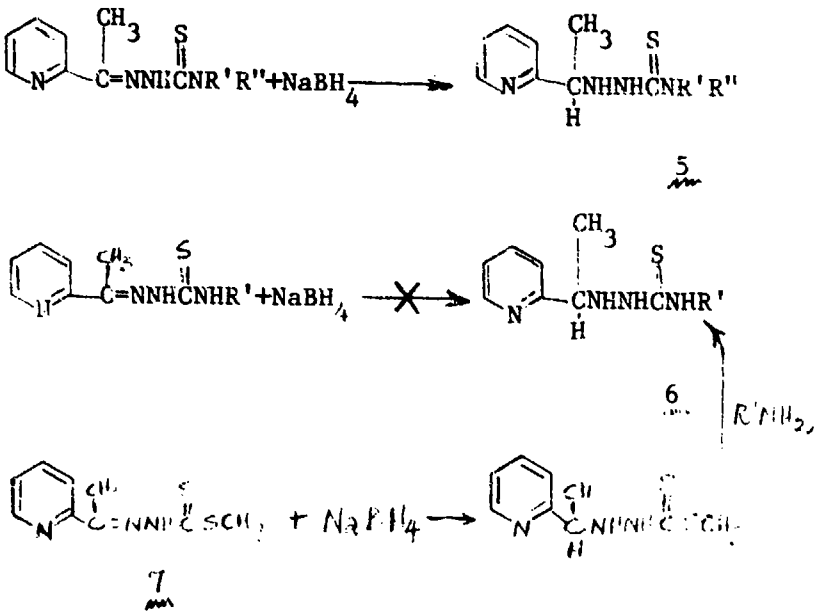
1

impart the highest antimalarial activity. This type of modification is no longer being pursued inasmuch as it has not produced compounds with superior antimalarial activity, although mouse toxicity seems to be diminished. Expansion into quinolines (i.e., benzpyridines) is now underway. Keeping the alkylidene-ring nitrogen atom relationship intact, 12 thiosemicarbazones are being made of 2-acetylquinoline (2),1-acetylisoquinoline (3),



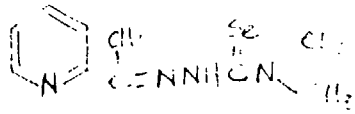
and 3-acetylisoquinoline (4). It is too early to detect antimalarial trends, although initial results from compounds derived from 2 suggest that activity is lower than the pyridine series.

Another structure modification which is being studied is the reduction of the azomethine linkage of 1. Saturation of the double bond gives compounds of types 5 and 6 which are thiosemicarbazides. Initial test results suggest that this modification will lead to an overall improvement in the basic series. The desired compounds can be made by the sodium borohydride reduction of N^4,N^4-disubstituted 2-acetylpyridine thiosemicarbazones. However, this technique fails inexplicably with N^4-monosubstituted 2-acetylpyridine thiosemicarbazones using sodium borohydride, as



well as other chemical reducing agents. Compound 6 types are made indirectly by reducing the dithiocarbazoate 7 and performing the indicated displacement with a 1° amine.

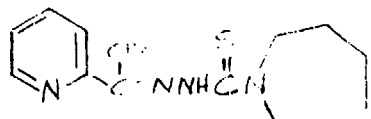
This reporting period saw the completion of the synthesis of series of 11 2-acetylpyridine 3-selenosemicarbazones and their testing as potential antimalarial agents. In general, the compounds were slightly less active than the corresponding thiosemicarbazones and appeared to be, surprisingly, less toxic. The most active compound in the selenium series, compound 8, produced "cures" at a dosage of 20 mg/kg.



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Another series of compounds which was completed consisted of 2-acetylpyridine thiosemicarbazone complexes with transition metals (Cu^{1+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, Mn^{2+}) as their Cl^{1-} and NCS^{1-} salts. Biological testing has not been fully reported for these compounds to enable an evaluation.

A preliminary study of the treatment of Mycobacterium intracellulare infected mice with thiosemicarbazone 9 has been performed by Dr. T. Kenneth McClatchy of the National Jewish Hospital



9

and Research Center. The compound was found to be remarkably effective in eliminating organisms from the spleen and lungs of the infected animals.

Dr. Arthur S. Dobek of the WRAMC Infectious Disease Service completed a study of the in vitro inhibition by 65 2-acetylpyridine thiosemicarbazones and closely related compounds using clinical isolates of 9 bacterial genera. Minimal inhibitory concentrations (MICs) of 0.002-0.062 µg/ml were obtained with 23% of

the compounds for Neisseria gonorrhoeae and 0.016-0.062 µg/ml with 17% of the compounds for N. meningitidis. Staphylococcus aureus was inhibited in the MIC range of 0.125-0.5 µg/ml by 18% of the thiosemicarbazones, whereas 26% inhibited group D enterococcus with an MIC of 0.25-2.0 µg/ml. Poor antibacterial activity was shown toward the gram-negative bacilli, i.e., Pseudomonas, Klebsiella-Enterobacter, Shigella, Escherichia coli, and Proteus. This collaboration will be renewed shortly so that recently developed compounds may also be evaluated for their antibacterial activity.

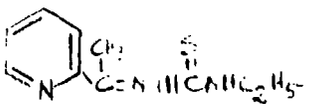
Additional biological test results

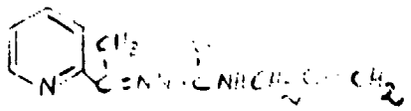
Twenty-seven 2-acetylpyridine thiosemicarbazones and analogs were tested by Robert Casero et al. for antitrypanosomal activity in an in vitro assay system. Twenty-four of the test compounds exhibited activity comparable to that found for the antitrypanosomal agent, ethidium bromide.

The antiviral activity of five 2-acetylpyridine thiosemicarbazones was determined against herpes simplex virus types 1 and 2 by Charles Shipman (University of Michigan) using a series of biochemical tests and virological assays. All compounds tested were potent inhibitors of both forms of the virus and inhibited viral replication to a greater extent than cellular DNA and protein synthesis. A test involving the application of the thiosemicarbazones to the skin of herpes simplex-infected guinea pigs is now in progress.

The influence of thiosemicarbazone 9 was determined on growth and macromolecular synthesis in Escherichia coli AT-9 by Alan D. Wolfe et al. (Division of Biochemistry, WRAIR). The compound caused bacteriostasis and a primary inhibition of ribonucleic acid synthesis. Secondary effects included inhibition of DNA and protein synthesis.

Several 2-acetylpyridine thiosemicarbazones, fed to Mycobacterium leprae-infected mice in concentrations ranging from 0.01 to 0.15% of their diet were studied in the footpad test which is being performed by Norman E. Morrison (Johns Hopkins University). After 8 months, thiosemicarbazones 10 and 11 reduced the number of organisms in the mouse footpad from 1.3×10^6 to 3-4% and 6-7% that number, respectively. Additional thiosemicarbazones are being tested in vitro and in vivo by Professor Morrison in an attempt to further diminish the M. leprae count to <1%.

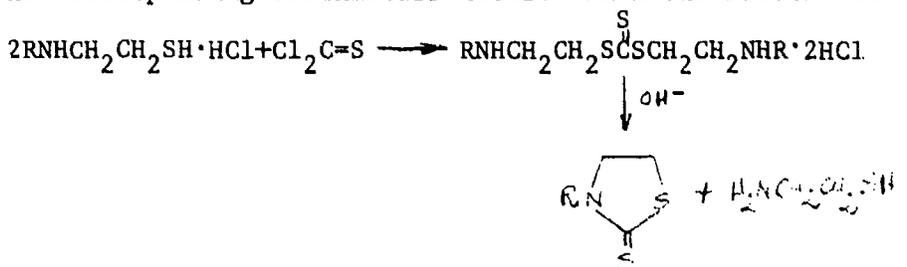




Antiradiation compounds

A new technique for the purification of the antiradiation agent, WR 638 (sodium 2-aminoethylphosphorothioate), was developed by us and was applied to several kilos of material which were over 13 years old. The highly purified compound has been administered orally to a patient suffering from cystinosis with apparently encouraging results.

The reaction of thiophosgene with aminoalkylthiols (as their hydrochloride salts) has been demonstrated by us to form the corresponding trithiocarbonates. When the latter are



converted to the free amine form, cyclizations to a thiazoline-2-thione occurs readily with the elimination of the amino-thiol. The generality of this reaction is being studied.

Drug assays in biological fluids

The assay for primaquine in biological fluids was extended to levels lower than those previously reported at 7 to 17 µg/ml using high performance liquid chromatography (HPLC). Interferences appeared below 4 µg/ml in blood and the isolation procedure was lacking near 40 ng/ml. Ongoing work is focused on changes in the methods of isolation and concentration.

In contrast, assay of spiked water with 40 to 10,000 ng/ml primaquine diphosphate gave 98.2-101.5% of theory and 0.1-14.1% CV (coefficient of variation). Tetramethylammonium chloride was found to be better than butylamine phosphate as a modifier in reversed phase HPLC. There was a linear response, correlation

coefficient r^2=0.993, with on-column charges between 20 to 5,000 nanograms.

Exploratory work on two thiosemicarbazones showed that:

2-Acetylpyridine gave a linear response, r^2=0.996, between 5 to 585 nanograms on column (MCH-10 reversed phase column).

WR 235,591 gave five peaks, linear response r^2=0.93 to 0.99, between 30 to 3,000 nanogram charge on column (MCH-10 reversed phase).

WR 242,748 Elution of peaks close to that of 2-acetylpyridine on silica gel column, and poor resolution on reversed phase MCH-10.

Papers Published

1. Klayman, D.L., Scovill, J.P., Bartosevich, J.F., and Mason, C.J. 2-Acetylpyridine Thiosemicarbazones. 2. N^4,N^4-Disubstituted Derivatives as Potential Antimalarial Agents, J. Med. Chem., 22, 1367 (1979).

2. Scovill, J.P., Klayman, D.L., Woods, T.S., and Sweeney, T.R. Primaquine Analogs: Derivatives of 4-Amino-2-methoxyacridine, J. Med. Chem., 22, 1164 (1979).

3. Dobek, A.S., Klayman, D.L., Dickson, E.T., Scovill, J. P., and Tramont, E.C. Inhibition of Clinically Significant Bacterial Organisms In Vitro by 2-Acetylpyridine Thiosemicarbazones. Antimicrob. Agents and Chemother., 18, 27 (1980).

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8. Scovill, J.P. and Silverton, J.V. Ring Cleavage of Pyridyl and Isoquinolyl Ketones by Methyl 1-Methylhydrazinecarbodithioate. A New Route to 1,2,4-Triazines. Presentation

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Book Reviews

1. Klayman, D.L. The Chemistry of Heterocyclic Compounds. Vol. 34. Thiazole and Its Derivatives. Part 1. Edited by J.V. Metzger., J. Med. Chem., 22, 1433 (1979).

2. Klayman, D.L. The Chemistry of Heterocyclic Compounds. Vol. 37. Thiazole and Its Derivatives. Part 2. Edited by J.V. Metzger, J. Med. Chem., 23, 707 (1980).

3. Klayman, D.L. The Chemistry of Heterocyclic Compounds. Vol. 34. Thiazole and its Derivatives. Part 3. Edited by J.V. Metzger, J. Med. Chem., 23, 971 (1980).

Papers Submitted for Publication or in Preparation

1. Klayman, D.L., Scovill, J.P., Bartosevich, J.F., and Mason, C.J. 2-Acetylpyridine Thiosemicarbazones. 3. Selenium Analogs as Potential Antimalarial Agents (submitted for publ.).

2. Shipman, C., Smith, S.H., Drach, J.C., Klayman, D.L. Antiviral Activity of 2-Acetylpyridine Thiosemicarbazones against Herpes Simplex Virus (submitted for publ.)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6535	80 10 01	CSO-DD-R&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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	871AF	157
b. ~~XXXXXXX~~	62770A	3M162770A803	00	086
c. CONTRIBUTING	STOG 80-7.2	2		

11. TITLE (Precede with Security Classification Code)

(U) Experimental Drug Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 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: NA
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	3.9	303
FISCAL YEAR CURRENT: 81	6.9	418

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME:
NAME:

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) Pharmacodynamics; (U) Drug Metabolism

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish 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) 79 10 - 80 09 Screening tests were done by in-house and contractor laboratories on approximately 7000 compounds in animal models or in vitro for suppressive, causal prophylactic or radical curative antimalarial activity. Activity was found in approximately 500 compounds and approximately 100 of these were selected for advanced study including testing for repository activity against P. berghei in mice, activity against human (P. falciparum) malaria in Aotus monkeys, and activity against vivax-like P. cynomolgi in rhesus monkeys. The in vitro P. falciparum culture system was used for the evaluation of 60 candidate compounds. Twenty-five were found to be active. Several compounds among a series of 2-acetylpyridine-thiosemicarbazones showed promising levels of activity. This work unit is changed from Work Unit 086, "Biological Evaluation of Antimalarial Drugs" by consolidation with Work Units 015 and 092 with title change to "Experimental Drug Development." For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available [illegible] upon originator's approval

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

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS
* Project 3M162770A803 DRUG DEVELOPMENT
Work Unit 157 Experimental Drug Development
* Work Unit 086 Biological Evaluation of Antimalarial Drugs

Investigators:

Principle: COL David E. Davidson, Jr., VC

Associate: MAJ George E. Childs, MSC
1LT Chris Lambros, MSC
Gerald J. McCormick, Ph.D.

PROBLEM AND OBJECTIVES:

In many parts of the world where falciparum malaria is endemic, resistance has developed to the drugs which are currently available for prophylaxis and treatment. Malaria control and eradication efforts are failing in many countries, with alarming increases in both falciparum and vivax malarias. The objective of this work unit is the discovery and development of new drugs for prevention and treatment of military personnel who may be required to operate in malarious areas. Efforts are being directed toward development of both blood and tissue schizonticidal drugs. In-house research is complemented by and coordinated with contractor laboratory screening and research.

PROGRESS:

Approximately 7000 compounds were screened for antimalarial activity by in-house and contractor laboratories in this fiscal year. Malaria culture systems and animal models were used to detect and characterize activity in a battery of blood schizonticidal, causal prophylactic and radical curative models. Activity was found in approximately 500 compounds and, of these, approximately 100 were selected for advanced study, including *in vitro* and *in vivo* evaluation against resistant strains of *P. falciparum*.

In collaboration with the World Health Organization, a sporozoite-induced rodent malaria model was established to screen active compounds from the U.S. Army program for repository activity. More than 500 compounds have been screened in this model and repository properties have been identified in several chemical classes. Compounds with repository activity are currently being tested at extended challenge intervals.

A patent was obtained on a new class of 4,5-disubstituted-8-aminoquinolines which have been shown to possess potent activity against persistent exoerythrocytic stages of *P. cynomolgi* malaria in rhesus monkeys. These compounds are five-times as potent as primaquine and, in addition, have potent blood schizonticidal activity against both drug-sensitive and drug-resistant parasites.

Final preclinical efficacy studies of an analog of amodiaquine (WR 228258) which is 8-20 times as potent as amodiaquine itself and is fully effective against drug-resistant strains of *P. falciparum* in Aotus monkeys are nearing completion. This compound is the most potent quinoline discovered and is the only known antimalarial with prolonged repository activity after oral administration.

The P. falciparum in vitro culture system was used for the evaluation of 60 candidate compounds. Twenty-five were found to be active. Several compounds among a series of 2-acetylpyridimethiosemicarbazones showed promising levels of activity.

FUTURE OBJECTIVES:

Limited screening capability will be maintained to continue support of the search for new classes of antimalarials and to support and guide synthesis of more efficacious and less toxic analogs among active classes. Emphasis will be on developing and characterizing efficacy of compounds which show promise of potential clinical military application. This work unit is being changed from Work Unit 086, A803, Accession No. DA0B 6535, "Biological Evaluation of Antimalarial Drugs," by consolidation with Work Units 015, A802, Accession No. DA0C 6467, page 435 and 092, A803, Accession No. DA0C 6461, page 457, with title change to "Experimental Drug Development."

PUBLICATIONS:

1. Kinnamon, K.E., and Davidson, D.E., 1980. Plasmodium Cynomolgi: Folic Acid Antagonist Combinations for Treatment of Malaria in Rhesus Monkeys. Experimental Parasitology 49, 277-280.

2. Davidson, D.E., A. Ager, J. Brown, F. Chapple, R. Whitmire and R.H. Rossan, 1981. Recent developments of tissue schizonticidal antimalarial drugs. Bulletin W.H.O., (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA GB 6526	80 10 01	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	00	160
b. CONTRIBUTING		3M162770A802		007
c. CONTRIBUTING	CARDS 114F			

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: 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 80	5	80
	CURRENT 81	5	155

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TELEPHONE: 984155, 984249

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Shirai, Akira, PhD

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) R. 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. Investigate scrub typhus in human populations; 2. Study the protection produced by "avirulent" R. tsutsugamushi to virulent strains; 3. Evaluate doxycycline as a chemoprophylactic for scrub typhus; 4. Evaluate new treatments for scrub typhus; 5. Study the basis of scrub typhus immunity; 6. Develop an animal model for scrub typhus.

24(U) 1. Compare the scrub typhus infection rates of vector chiggers, rodents, and humans in mature rubber schemes. 2. Inoculate monkeys with "avirulent" strains and sub-lethal doses of virulent strains, and compare the protection produced. 3. Treat volunteers with weekly doxycycline or placebo before and after infected chiggers are fed on them. 4. Administer single dose doxycycline in early disease. 5. Compare methods for collecting and cultivating monkey macrophages for use in CMI studies. 6. Characterize blood biochemistries in monkeys infected with R. tsutsugamushi.

25(U) 79 10 - 80 09 1. 40% (151/380) of the adult population and 37% (87/232) of the rodent population had evidence of recent (within 1½ years) scrub typhus, and 3% of the chiggers collected were infected with rickettsiae. 2. Two "avirulent" strains protected as well as virulent strains in many instances and even better than homologous strains on occasion. 3. A single, weekly 200 mg dose of doxycycline is effective in preventing scrub typhus. 4. A single, 200 mg dose of doxycycline administered on day 3 of illness is ineffective, because 7 of 10 patients relapsed. 5. A non-lethal technique for collecting peritoneal macrophages from monkeys was developed and the conditions for their culture defined. 6. Blood urea nitrogen, alkaline phosphatase, and creatinine determinations were higher in infected animals than in controls. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

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

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS
* Project 3M162770A802 MILITARY PREVENTIVE MEDICINE

Work Unit 160 Field Studies of Rickettsioses & Other Tropical Diseases

* Work Unit 007 Field Studies of Rickettsioses and Other Tropical Diseases

Investigators:
Principals: LTC Michael G. Groves, VC; Dr. Akira Shirai, Ph.D.; MAJ Stanley C. Oaks, Jr., MS; MAJ John C. Twartz, RAAMC; MAJ Alexander L. Dohany, MS; CPT Gregory B. Heisey, VC; Miss Elsie Gan, B.S.

PROPHYLAXIS OF SCRUB TYPHUS

Background: The lack of an effective vaccine for scrub typhus has caused other methods of prevention to be considered. Previously, the antibiotic chloramphenicol was shown to be an effective prophylactic agent for scrub typhus (26). However, the risk of serious side effects prevented its application to field situations. The tetracycline group of antibiotics is now the treatment of choice for scrub typhus (5). We studied the long-acting tetracycline analog, doxycycline, as a prophylactic agent, in a double-blind, placebo-controlled trial. We also studied the efficacy of single-dose, doxycycline therapy for early scrub typhus (4). We were assisted in this study by the Malaysian Armed Forces, who supplied hospital facilities and an independent physician.

Progress: Twenty male members of our staff, 16 Malaysians and 4 Americans, formed the study population. They were divided into two similar groups according to pre-existing immunofluorescent antibody (IFA) to Rickettsia tsutsugamushi (16), ethnic backgrounds, mean body weight, and age of the volunteers.

The doxycycline group was given a single weekly 200 mg dose of doxycycline (Pfizer) for 7 weeks. The other group received identical placebo capsules. Subjects were examined daily and blood specimens were drawn every 3 days. Subjects who were clinically diagnosed as having scrub typhus were hospitalized and received no further prophylaxis. A clinical diagnosis of scrub typhus was based on: (1) co-existence of two or more of the cardinal signs of eschar, generalized lymphadenopathy, hepato- and/or splenomegaly, and rash, or (2) pyrexia greater than 37.6C for 48 hours or longer. Treatment, consisting of a single 200 mg dose of doxycycline, was given on the 3rd day of illness (after 50-66 hours of illness). Because the results of laboratory tests are incomplete at this time, the following is a preliminary report based principally on clinical observations.

Three days after the first dose of prophylaxis, infected Leptotrombidium fletcheri chiggers were allowed to feed within a plastic capsule (8) on the medial aspect of the thigh of each volunteer. The time required for the chiggers to attach varied from minutes to several hours. Eventually, between 2 and 13 (mean 8.5, standard deviation (SD) 2.9) chiggers attached to each volunteer, and 2-13 (mean 7.1, SD 3.4) engorged chiggers were recovered 48 hours after attachment. Subsequent direct fluorescent antibody testing of engorged chiggers (9) confirmed all to be infected with R. tsutsugamushi.

Doxycycline group. One of the 10 subjects receiving doxycycline was treated for presumed scrub typhus, but the illness was not classical. There was fever for only one 12-hour period and no eschar formation; malaise, headache, and hepatosplenomegaly were prominent; lymphadenopathy was present but not pronounced. Several subjects had mild malaise just prior to their next dose of prophylaxis, but all remained well enough to perform normal activities. No one developed eschars, although small areas of induration and redness were evident in some subjects. After 2 weeks, most of the group had tender inguinal lymphadenopathy adjacent to the site of chigger feeding. The tenderness resolved within a week, but the nodes remained enlarged for several weeks. Hepatomegaly or left upper quadrant tenderness was found in the occasional subject. Upon cessation of prophylaxis, 6 out of the remaining 9 subjects had a recurrence of inguinal lymphadenopathy; some had mild generalized lymphadenopathy; several complained of malaise. These changes were short-lived and self-limiting. No significant side effects to doxycycline were noted.

Placebo group. Nine out of 10 members of this group were treated for presumptive scrub typhus. Onsets of fever were days 8-11 post chigger attachment. All had prodromal malaise and arthralgia/myalgia. All but one developed eschar. The untreated individual in this group had a history of scrub typhus 2 years prior to the study and developed only mild signs and symptoms.

Treatment. A single, 200 mg dose of doxycycline caused resolution of fever and severe headache in 24 hours. Rapid convalescence followed. However, 7-11 days after treatment, 7 out of the 10 treated subjects, including the one failure of doxycycline prophylaxis, clinically relapsed and were given a 7-day course of tetracycline hydrochloride 500 mg qid. There were no further illnesses.

Future Objectives: Doxycycline 200 mg in a single weekly dose, starting before exposure and continuing for 6 weeks after exposure to R. tsutsugamushi is an effective prophylaxis against scrub typhus ($p = 0.001$). Further efforts to reduce the duration of the post-exposure prophylaxis could be considered, since Smadel found a 4 week course of chloramphenicol sufficient prophylaxis (26).

STUDY OF HUMAN IgG AND IgM RESPONSES TO R. TSUTSUGAMUSHI INFECTION

Background: Rubella virus, cytomegalovirus, Toxoplasma gondii and Treponema pallidum are some of the agents known to cause transplacental infections that lead to fetal damage. Infections with the 3 latter agents characteristically result in latent or chronic disease. Coxiella burneti, a rickettsia known to produce latent infection, has been isolated from placentas of women 6 to 38 months following recovery from Q fever (28) and immunologic evidence, IgM antibodies, of fetal C. burneti infections has been reported (11). Rickettsia tsutsugamushi, the agent of scrub typhus, is another rickettsial agent that causes persistent infections following recovery from acute disease (19,25). Because such a persistence in pregnant women could lead to fetal infections, we studied the anti-R. tsutsugamushi IgM and IgG levels in the sera of mother and umbilical cord (mother/cord) pairs from a population known to have a high prevalence of scrub typhus infection.

Progress: Sera of 111 mother/cord pairs were tested for IgG and IgM antibodies to R. tsutsugamushi. Either one or both sera from 33 mother/cord pairs were positive for IgG antibody to R. tsutsugamushi. Twenty-six (79%) of the mother sera from these pairs demonstrated relatively low antibody titers (1:50 - 1:100). Six had a titer of 1:200, and one a titer of 1:400. IgG antibodies were present in both sera of 31 pairs, 25 of which had equal titers. In 3 cases, the cord sera had a 2-fold higher titer than the mother sera. Of the remaining 3 pairs, 2 of the mother sera had a 2-fold higher titer, and one had a 4-fold higher titer than the respective cord sera. The mother serum of one pair had IgG antibody, but the cord serum showed none. One mother/cord pair demonstrated the presence of IgG antibody in only the cord serum. IgM antibodies to R. tsutsugamushi were not detected in any sera.

Stiehm et al. (27) demonstrated that elevated IgM levels in neonates indicate in utero infection. Although fetal infection with R. tsutsugamushi seems feasible, we were unable to find immunologic evidence of its occurrence despite 29% of the mothers in our study showing significant antibody levels. The lack of maternal IgM antibodies was not surprising, since none of the mothers in the study suffered from an acute, febrile illness during the period of hospitalization. Also, although the low IgG titers suggested infection within the last 1-1½ years (18), they were not indicative of recent disease (6).

Future Objectives: The ability to distinguish between primary and secondary scrub typhus infection would be extremely beneficial in epidemiological studies of scrub typhus patients and in studies on the chemoprophylaxis and chemotherapy of scrub typhus. Sera from over 500 confirmed scrub typhus cases along with their complete clinical and epidemiological data are currently stored by the Unit.

The knowledge of whether a patient has had a primary or secondary infection can be correlated using our computer with hematological, biochemical, and clinical findings to furnish valuable insights into the primary and secondary disease processes. The ability to distinguish between primary and secondary scrub typhus infections is also important when assessing the results of antibiotic therapy, since the treatment of patients with second infections could give the false impression that short courses of antibiotic therapy are adequate or that relapses do not occur. Using an IFA test that distinguishes immunoglobulin classes, we propose to study the differentiation of primary and secondary scrub typhus infections by examining sequential serum samples from selected scrub typhus patients and sera from cynomolgus monkeys primarily and secondarily infected with R. tsutsugamushi.

PREVALENCE OF R. TSUTSUGAMUSHI ANTIBODIES IN SETTLERS ON A MATURE RUBBER SCHEME

Background: During investigations of large oil palm areas, a single serological survey done on a mature rubber scheme for comparison purposes revealed that 57% (64/112) of the settlers had antibodies to R. tsutsugamushi. These findings were consistent with previous surveys involving rubber workers (17) but were in marked contrast to the studies in oil palm areas where the incidence of disease declines as the trees mature (WRAIR Annual Progress Report, 1 Oct 78 - 30 Sep 79). The purpose of the present study was to compare the infectivity rates among the chigger vectors, rodents and the human populations within two mature rubber schemes, Kampong Awah and Sungei Nerek.

Progress: Table 1 presents the number (%) of humans and rodents in the 2 rubber schemes having antibodies to R. tsutsugamushi, as

Table 1. Rickettsia tsutsugamushi antibodies present in humans and rodents from 2 mature rubber schemes.

Rubber Schemes	HUMAN Positive/total (%)			RODENTS Positive/total (%)
	Settlers	Wives	Children*	
Kampong Awah	41/69 (59)	28/68(41)	18/173(10)	28/51(55)
Sungei Nerek	59/147(40)	23/96(24)	25/173(15)	59/181(33)

*<20 years old.

measured by indirect immunofluorescence. The high prevalence of antibody among the wives and children is not surprising, since field work, including tapping, weeding, etc., is shared by all members of the family. The chiggers collected by black plating were predominantly Leptotrombidium deliense. The infectivity rates, as determined by direct immunofluorescence (9) were 4.5% and 2.6% for the chiggers collected in Kampong Awah and Sungei Nerek, respectively.

Future Objectives: No additional studies are planned.

CELL-MEDIATED IMMUNITY TESTS IN SCRUB TYPHUS INFECTIONS: CYNOMOLGUS MONKEY MACROPHAGE COLLECTION AND CULTIVATION

Background: Mononuclear phagocytes (monocytes and macrophages) play an important role as effector cells in both humoral and cell mediated immunity (CMI). The in vitro cultivation of these cells has emerged as an important technique for studying the effects of the immune system on pathogenic microorganisms and tumor cells. The use of a monkey model (22,29) for scrub typhus research in our laboratory prompted us to investigate methods for the collection and cultivation of macrophage from monkeys that could be used in the development of a in vitro CMI test. Collection methods most freqeuntly described for other species either require large amounts of blood or the sacrifice of the animal, both of which are impractical when dealing with monkeys. Therefore, the purpose of this study was to develop a non-lethal technique for the collection and cultivation of peritoneal macrophages from cynomolgus monkeys.

Progress: Several techniques were compared. The peritoneal cavity was chosen as the source of macrophages because large numbers of cells were required. A multiple-holed cannula proved superior to a hypodermic needle for the collection of peritoneal washings, because it was not occluded by the omentum. With the cannula technique, 55-70% of the harvest medium could be recovered from the peritoneal cavity with a yield of 2.5-5 x 10^5 cells per ml. A closed system for injecting and collecting harvest medium was better than a syringe in preventing bacterial contamination of peritoneal washings. The closed apparatus consisted of two/250 ml bottles and a 20 cc syringe connected to the multiple-holed cannula by two/3-way stopcocks. After collection, cells were resuspended in growth medium (RPMI 1640 with 20% fetal calf serum and 4 mM glutamine) to a concentration of 5 x 10^5 cells per ml, distributed into Leighton tubes, and incubated at 36°C. After 12 hours, cultures were washed to remove non-adherent cells and refed with growth medium. After three days incubation, the macrophages began to spread on the glass slides. This technique provides a sterile, reliable method for the collection and cultivation of macrophages from monkeys.

Future Objectives: Now that a technique for collection and cultivation of macrophages from the monkey has been developed, work can be directed at developing a CMI test for scrub typhus. If successful, these assays can then be applied to human infections.

CYTOGENETICS OF TROMBICULID MITES

Background: The genetics of medically important arthropod vectors were not studied in any great depth until insecticide resistance was recognized. Most arthropod cytogenetic studies have been done in mosquitoes (12). Chromosome data are available on mite species in some families of the suborder Prostigmata; however, none of the species within the family Trombiculidae, the family containing the vectors of scrub typhus, have been studied (15). The purpose of this study is to determine if the karyotyping and study of chromosomal morphology of trombiculid mites could be used to (a) differentiate species of scrub typhus vectors, (b) differentiate between infected and uninfected chiggers within a species, and (c) explain why infected lines of laboratory-reared chiggers almost exclusively produce female offspring.

Progress: The chromosome squash technique has been perfected so that well spread chromosomes with good morphology are consistently obtained. Also, a survey of the various stages in the life cycle of the trombiculid mite has shown that the early protonymph stage yields the best chromosome preparations.

Future Objectives: Both infected and uninfected Leptotrombidium arenicola and L. fletcheri mites will now be examined.

A STUDY OF THE CROSS PROTECTION PRODUCED BY KARP-RELATED RICKETTSIA TSUTSUGAMUSHI TO VIRULENT KARP OR GILLIAM CHALLENGE IN MONKEYS

Background: A majority of R. tsutsugamushi strains isolated in the endemic scrub typhus region are Karp or Karp-related (10,20,21,23, 24). Many of the latter strains are relatively non-pathogenic for both mice and monkeys and could serve as potential vaccine strains in a gamma-irradiated immunogen or a live, avirulent vaccine. Also, if even one of the Karp-related strains can stimulate protection against the majority of strains found in the endemic region and provide some defense against all strains, the development of a multivalent vaccine may not be necessary. We, therefore, inoculated 6 groups of 8 cynomolgus monkeys each with one of the R. tsutsugamushi strains Karp, Kato, Gilliam, TA686, TA716, or TA763 and 8 control monkeys with normal yolk sac. Half of each group was challenged with the Karp strain and half with the Gilliam strain 6 months later.

Progress: The clinical observation period of the challenged monkeys has just concluded and antibody, rickettsemia and blood biochemistry studies are incomplete at this time. The Kato, TA716, and TA763 inoculated monkeys challenged with Karp were afebrile throughout the observation period. The Kato inoculated monkeys were the only group that remained afebrile when challenged with Gilliam. The TA763 group also had substantially lower mean body temperatures than the control group.

The hematology parameters analyzed included hematocrit, hemoglobin, white blood cell count, red blood cell (RBC) count, thrombocyte count, and erythrocyte sedimentation rate (ESR). All the animals challenged with Karp had decreases in their hematocrit, hemoglobin, and RBC. The greatest decreases were in the control monkeys followed by the Kato and TA763 groups. Control monkeys challenged with Gilliam have marked decreases in hematocrits, RBC, and hemoglobin. All other groups showed only slight decreases in response to Gilliam challenge.

A consistent decrease in the thrombocyte count was seen in the control groups through the first ten days after challenge with Karp or Gilliam. The Karp group of the Karp challenged animals and the TA686 and TA716 groups of the Gilliam challenged animals showed relatively little change. All other groups had moderate changes but were less affected than controls.

All groups had elevated ESR following challenge, although only the two control groups had a marked elevation. Of the Karp challenged animals, the TA716 group returned to normal first (day 13), followed by TA763 (day 22), Gilliam (day 28), TA686 (day 37) and the control group (day 40). The Karp and Kato groups continued to remain slightly elevated at day 43. With the exception of the control and Karp groups, all the groups challenged with Gilliam had normal ESR by day 28 post challenge. The Karp group returned to normal on day 40, and the control group was still slightly elevated at day 43.

In addition to fever, other clinical signs observed were skin thickening at the site of infection, eschar formation, and lymphadenopathy. The onset of skin thickening was the same for all groups. Of the Karp challenged animals, the Karp group had the earliest recovery to normal (day 26), and the TA763 and control groups the most prolonged (day 42). The Karp and Gilliam groups challenged with Gilliam were normal by day 36; other groups were normal by day 43.

Lymphadenopathy developed in all but one animal and to the inguinal lymph nodes near the infection site except in two Gilliam control animals that developed a bilateral, inguinal lymphadenopathy. Lymphadenopathy developed in two of three animals challenged with Karp in the Karp group; all animals were affected in the other groups. The lymph nodes returned to normal earliest

in the Karp, TA686, TA716, and TA763 groups following Karp challenge. The Gilliam group was the earliest among the Gilliam challenged animals.

Eschar formation was observed in most animals. Only one Karp group animal challenged with Karp developed an eschar. In the Kato and TA716 groups receiving the same challenge, three of four animals develop an eschar, monkeys in all other groups formed eschars. Two of three animals in the Gilliam group challenged with Gilliam developed an eschar. All the other animals in the Gilliam-challenged groups developed eschars.

Future Objectives: Data from this study will continue to be collated and analyzed; however, preliminary study indicates that some R. tsutsugamushi strains that are avirulent for monkeys, notably strains TA716 and TA763, appear to offer protection against R. tsutsugamushi challenge. The protection in many instances appears to be as good as that provided by virulent homologous strains. Additional studies of these strains as possible candidates for vaccines will continue. These studies will include virulence testing in laboratory animals, testing for the reversion to virulence of the strains, and cross protection studies.

RESPONSE OF MONKEYS TO EXPERIMENTAL RICKETTSIA TSUTSUGAMUSHI INFECTION: BLOOD BIOCHEMISTRIES

Background: Silvered leaf monkeys (Presbytis cristatus) were initially used as primate models for Rickettsia tsutsugamushi infection (29). However, the high morbidity and mortality of this species in captivity made studies difficult to interpret. This prompted a search for a more suitable experimental animal for use in scrub typhus studies. In a comparison study between cynomolgus monkeys (Macaca fascicularis) and silvered leaf monkeys, both species had clinical disease; however, the cynomolgus monkeys developed greater antibody responses (22). Thus the cynomolgus appears to be as good or better an animal model than the silvered leaf monkey. To further define the cynomolgus monkey as a suitable animal model, we collected blood biochemical data on these animals following inoculation with the Karp, Kato, Gilliam, TA686, TA716, or TA763 strains and with uninfected yolk sac suspensions. The biochemical assays included the following: SGPT, SGOT, BUN, bilirubin, albumin, total protein, LDH, alkaline phosphatase, creatinine, Na, and K. The results from this study will be compared with the biochemical results obtained from humans infected with scrub typhus.

Progress: The data has been collected and stored in the computer. A partial statistical analysis of the data through the first 13 days

of the 43 day study has shown little or no difference among the groups for the following parameters: SGPT, SGOT, bilirubin, protein, LDH, Na, and K.

Beginning on day 7 post inoculation, the BUN levels increased in both the Karp and Gilliam groups and continued through day 13 for the Karp group. The alkaline phosphatase levels were increased in the Karp and Gilliam groups beginning on day 4 post inoculation and continued through day 13. The increase was the highest on day 7 for the Karp group and on day 13 for the Gilliam group.

The creatinine levels for all 6 rickettsia infected groups were consistently increased when compared to the control group from day 1 through day 13. The highest creatinine levels were 1.19 mg/dl for the Kato group on day 10, 1.06 mg/dl for the Gilliam group on day 7, and 1.037 mg/dl for the Karp group on day 13.

Future Objectives: Statistical analysis of the biochemical data will continue. These results will be used as a base-line reference for the biochemical data we are beginning to obtain from cross-protection studies in monkeys. By analyzing the clinical, hematological, and biochemical results, we will be better able to assess the protection one strain of R. tsutsugamushi offers against challenge with another.

THE PREVALENCE OF RICKETTSIAL ANTIBODIES IN NEPAL

Background: Although the Nepal Health Survey of 1965-1966 (30) reported the presence of potential vectors for epidemic, endemic, tick, and scrub typhus in a wide variety of locations, little is known of the prevalence of rickettsial infections in Nepal. Therefore, we took advantage of the opportunity to collaborate with a British military physician and former member of this Unit, who is now stationed in Nepal.

Progress: Sera was collected at a small hospital in Dharan, Eastern Nepal. This is one of 2 recruiting locations for Gurkhas serving with the British Army. Venous blood specimens were collected from 100 healthy, male, Nepalis aged 17-19 years and recruited from different locations in the hills of Eastern Nepal. Samples were also taken from 88 apparently healthy, male, blood donors, aged 18-50 years, who were relatives or friends of patients requiring transfusion. The mean age of the 188 subjects was 23 years. The sera were examined for antibody to Rickettsia spp. by indirect immunofluorescence (16). As there were no discernable differences in titers between the recruits and blood donors, the results from the 2 groups have been combined.

Antibody to epidemic and murine typhus was not detected. Antibody indicative of past exposure (≥1:50) to tick typhus and scrub typhus rickettsiae was found in 56% and 10% of the subjects respectively. Additionally, the sera was tested for leptospiral antibody (13), and a high prevalence (12%) of positive (≥1:100) titers was found. These results indicate that the incidence of tick typhus may be very high in Eastern Nepal. Also, scrub typhus and leptospirosis appear to be common in the same area.

Future Objectives: No additional studies are planned. Some of these results have been submitted for publication (see manuscripts 1 and 2 in Submitted for Consideration section).

ANALYSES OF DATA FROM MAJOR EPIDEMIOLOGICAL STUDIES - ADULT FEBRILE ILLNESS IN MALAYSIA

Background: Infectious diseases are an important cause of illness in rural areas of Malaysia and other countries in Southeast Asia. Even those infectious illnesses that result in hospitalization frequently remain undiagnosed, because the diseases lack specific clinical features and there are few specialized laboratories available. There are recent reports on the etiology of febrile illness in Vietnam (7) and Indonesia (1) but the only comprehensive study reported from Malaysia was in 1957 (14). Since that time, many improvements in diagnostic techniques have occurred, as well as change to infectious disease patterns within Malaysia.

Progress: Over a period of 4 years (1975-1979), 1400 adult patients were admitted to the district hospital at Mentekab (3) in central West Malaysia for the diagnosis and treatment of febrile illness. They were investigated according to a standard protocol in support of our scrub typhus studies. Part of the data on some of the patients has already been reported (2,3,4). Demographic, clinical and laboratory data was collected and stored in the automated data processing (ADP) facility. The following laboratory investigations were performed:

(a) Hematology

A thick film was examined for malaria parasites on 3 occasions within 48 hours of admission, again 4 days later, and an additional time if fever persisted after treatment. Hemoglobin concentration and white blood cell, platelet, and differential counts were performed on admisison.

(b) Bacteriology

Throat swab, sputum, mid-stream urine, and two venous blood samples were cultured by standard techniques.

(c) Serology

Sera were collected on admission to hospital; 7 to 10 days later, if possible; and upon discharge. The sera were stored at -20°C until examined for antibody to Rickettsia tsutsugamushi, R. typhi, and R. sibericus by immunofluorescence; to Leptospira spp by the hemolytic test; to Pseudomonas pseudomallei by indirect hemagglutination, to flaviviruses by hemagglutination-inhibition and for febrile agglutinins using Salmonella group D (Widal 'O' & 'H'), Proteus OXK and OX19 (Weil-Felix), and Brucella abortus. In all instances in which apparently conflicting results were obtained, titrations were repeated for confirmation.

(d) Rickettsial and viral isolation

Isolation and identification of R. tsutsugamushi was performed by mouse inoculation and direct immunofluorescence. Flaviviruses were isolated by mosquito inoculation and cell culture plaquing of whole blood collected on admission and preserved in liquid nitrogen until transmission to AFRIMS, Bangkok, for testing.

As a result of delays in installation of the ADP equipment, the principal investigator responsible for the study departed before the analysis of this data could be carried out, and he was not replaced. When analysis was begun, errors in input, verification and programming became apparent. Lack of personnel fully conversant with the system has led to difficulty in correcting some of the errors. However, analysis of the data will start in the near future.

Future Objectives: The resignation of the locally-hired employee with prime responsibility for the ADP equipment has led to the opportunity of employing a more highly trained person. This is essential if the data that took over 4 years to collect at a cost of many thousands of dollars is to fulfill more than a small amount of its potential.

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1. Brown, G.W., M. Madasamy, P. Bernthal, and M.G. Groves: Leptospirosis in Nepal. Trans. Roy. Soc. Trop. Med. Hyg.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6525	80 10 01	DD-DR&E(AR)636

3. PRECEDING SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISPOSITION	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
PRIMARY	62770A	3M162770A871	87 AH	169
CONTRIBUTING	62770A	3M162770A802	00	009
CONTRIBUTING	81QC 80-7.2			

11. TITLE (Precede with Security Classification Code)

(U) Anti-Schistosomal Drug Development and Malaria Immunology and Vector 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

DATE/EFFECTIVE: NA EXPIRATION:

NUMBER:

TYPE: AMOUNT:

KIND OF AWARD: $ CUM. AMT.

18. RESOURCES ESTIMATE	A. PROFESSIONAL MAN YRS	B. FUNDS (In thousands)
PRECEDING FISCAL YEAR 80	2.0	60
CURRENT FISCAL YEAR 81	2.5	89

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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SUBMITTING OFFICIAL

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GENERAL USE

Foreign Intelligence not considered

20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: PRATA, Aluizio R., MD

21. KEY WORDS (Precede EACH with Security Classification Code) (U) Brazil; (U) Schistosomiasis; (U) Malaria; (U) Chemotherapy; (U) Immunology; (U) Epidemiology; (U) Drug Resistance; (U)Entomology.

22. OBJECTIVE, 23. APPROACH, 24. 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, in vitro drug susceptibility testing and creation of a cryobank of human strains of Plasmodium falciparum. 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) 79 10 - 80 09. This research is complementary to studies being conducted under DAOB 6525, work Unit 086, entitled "Chemotherapeutic Studies on Schistosomiasis". During the reporting period, 1097 compounds were screened in the PCT and PMT. Of these 17 were designated confirmed or unconfirmed active and 234 were toxic. A secondary curative test (SCT) became operational. Extensive behavioral studies of Anopheles darlingi were completed and related to mosquito resistance to house spraying with DDT. Complete seroepidemiology surveys were completed, demonstrating high antimalarial IgG and IgM titers indicative of high malaria prevalence. Ten strains of P. falciparum isolated and cryopreserved; two of these have been placed in continuous in vitro cultivation for drug resistance studies.

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 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS
* Project 3M162770A802 MILITARY PREVENTIVE MEDICINE

Work Unit 161 Anti-Schistosomal Development and Malaria Immunology and Vector Studies
* Work Unit: 009 Antischistosomal Drug Development and Malaria Immunology and Vector Studies.

Investigators: LTC Willis A. Reid, Jr., MAJ Donald R. Roberts, MAJ K. Mills McNeill and Dr. Aluizio R. Prata, MD.

PROBLEM 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 a disease threat to American military 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.

2. At the same time, major problems are surfacing for the malaria control programs in many malaria endemic areas in South America. These are complex problems involving basic questions on the status of 1) physiological and/or behavioral resistance of vector populations to DDT and 2) drug resistant strains of falciparum malaria. Anopheles darlingi is the major vector species in South America and research along the Ituxi River, in the state of Amazonas, Brazil was conducted to elucidate the parameters of host-seeking and resting behavior of natural populations of this species. An additional objective was to assess the impact of spraying houses with DDT on vector behavior. Concurrently, sero-epidemiological studies were continued to identify disease incidence and prevalence, and in vitro continuous cultivation of malaria parasite strains was instituted for drug resistance analysis and antigen production.

PROGRESS:

1. During FY1980, extensive antischistosomal Primary Curative Testing (PCT) and Primary Prophylactic (Mortality) Testing (PMT) were conducted. In the PMT, test results for 430 different compounds were compiled. Of these 150 were toxic and 6 were active. In the PCT, 667 drugs were tested, of which 84 were toxic and 11 were active. The Secondary Curative Test (SCT) was implemented to further evaluate promising compounds identified in the PCT. Two SCT runs were accomplished, evaluating 5 drugs in more detailed analyses. In all, a total of 36 PCT, PMT and SCT drug test runs were conducted during the year, identifying 17 promising compounds for further evaluation.

2. Malaria vector studies on the behavior of An. darlingi were initiated along the Ituxi River in July, 1978. Systematic collections from human bait and window traps, in combination with mark-release-recapture methods, were conducted to quantify indoor and outdoor activities in both DDT sprayed and unsprayed houses (one house each). Peak host-seeking activity occurred in the peridomiciliary environment at sunset and sunrise. Biting activity within the house was slightly greater during the first half of the night but continued unabated until sunrise, no bimodal activity patterns were found. Peak movements into the house by host-seeking populations and migration out of the house by engorged specimens occurred at sunset and sunrise, respectively. We identified the ceiling as the preferred in-house resting site of both engorged and unengorged specimens of An. darlingi. This determination resulted from repeated observations on in-house distributions of specimens marked with fluorescent powder. Since only the lower part of house ceilings are sprayed (documented by DDT residue analyses), selection of this resting site may reflect an avoidance behavior. Studies on biting activity in a house treated with DDT and in an unsprayed house revealed that populations of An. darlingi were strongly repelled by DDT treated surfaces. Ratios of attack rates per hour for the unsprayed:sprayed houses were 34:1.3 immediately after spraying and 61:2.1 eight weeks post-spraying. Marked populations released inside the DDT treated house left immediately, whereas most specimens released inside the unsprayed house remained in the house until sunrise (6-8 hrs after release). These preliminary results indicate that a significant level of protection is obtained against populations of An. darlingi by spraying houses with DDT. In this respect, the high incidence of malaria in residents along the river probably relate to the human ecological factor of poor house construction and not to any "behavioral resistance" to DDT on the part of the vector.

3. Extensive seroepidemiology testing was conducted in the Ituxi study area and on the Japurá River, also in Amazonas. Indirect Fluorescent Antibody Test (IFAT) results for anti-malaria IgM and IgG indicate very high prevalence of malaria in both areas.

Efforts with the in vitro continuous cultivation of P. falciparur have been very successful. A total of four strains have been cultured in the laboratory, three being new isolates from the state of Amazonas. Initial cultures of Camp Strain Cbl obtained from the WRAIR were succeeded by successful cultures of strain Ituxi 084 in September 1979, the latter being the first documented Brazilian isolate of P. falciparum to be placed into the system of continuous cultivation. A total of 9 additional strains have been collected and cryopreserved from patients presenting to be treatment facility at the Institute of Tropical Medicine of Manaus. Two of the 9 specimens have been placed into continuous cultivation and excellent growth has been obtained. No cultivation attempts have yet been made with the remaining 7 stabilates. In vitro drug testing has been performed on all 10 strains of P. falciparum

obtained from Amazonas. At least 2 show marked resistance to chloroquine in the preliminary tests and further study of these strains is being performed.

RECOMMENDATIONS:

1. Continue primary screening of antischistosomal curative and prophylactic compounds, especially those of related chemical classes to those compounds already identified as potentially active.

2. Continue secondary curative testing on highly potential compounds.

3. Continue studies to describe the behavioral, morphological and physiological characteristics of selected anopheline species, particularly An. darlingi. Conduct comparative studies in various areas of the Amazon Basin.

4. Assess the impact of house treatment with DDT on malaria vector behavior and population densities in various areas of the Amazon Basin.

5. Colonize An. darlingi for vector competence and behavioral studies under laboratory conditions.

6. Capitalize on the in vitro P. falciparum cultivation capabilities to thoroughly analyze the nature of malaria drug resistance in the Amazon Basin.

7. Interface the malaria immunology capabilities with the malaria vector studies, especially with regard the Recommendations 3 and 5 above.

PRESENTATIONS:

1. McNeill, K.M. 1979. "Técnicas novas para avaliação da resistência da droga em Plasmodium falciparum". Invitational presentation to the "Curso de Extensão sobre Avanços em Medicina Tropical", jointly sponsored by the University of Brasilia and the Ministry of Health, Brazil (Nov 79).

2. Roberts, D.R. 1979. "Resistência dos anofelinos ao DDT". Invitational presentation to the "Curso de Extensão sobre Avanços em Medicina Tropical", jointly sponsored by the University of Brasilia and the Ministry of Health, Brazil (Nov 79).

3. Roberts, D.R. 1979. "Comportamento das Abelhas da Tribo Euglossine atraídas pelo DDT". Invitational presentation to the Reunião Regional de Brasilia da Sociedade Brasileira de Medicina Tropical e Nutrição, Universidade de Brasilia, Brazil (5 Dec 79).

4. Roberts, D.R., J.M. Heller, S.R. Ehrhardt and A.R. Prata. 1979. "DDT como atraente de machos de abelhas Euglossinas, no Brasil"

Invitational presentation to the Academica Brasileira de Ciências, Universidade de São Paulo, São Paulo, Brasil (6 Nov 79).

5. Papers presented at the XVI Congresso da Sociedade Brasileira de Medicina Tropical, 5-8 Feb 1980, Natal, Rio Grande do Norte, Brazil (English translations of original Portuguese titles):

   a. Alecrim, W.D., M.G.C. Alecrim, K.M. McNeill, R. Araujo, A. Viana, J.A. Pires, A. Prata and P. Marsden. "Tropical splenomegaly syndrome in the region of the Ituxi River, Amazonas".

   b. Alecrim, W.D., M.G.C. Alecrim, D.R. Roberts, M.V.F. Guerra, and A.M. Tavares. "Spleen indices and malaria parasite rates in residents along the Ituxi River, Amazonas".

   c. Alecrim, W.D., K.M. McNeill, A.M. Tavares, D.R. Roberts, and J.F. Olimpio. "A serological study of malaria in a population on the Ituxi River, Amazonas".

   d. Alecrim, W.D., D.R. Roberts, K.M. McNeill, H.V. Dourado and A. Prata. "Population migration and malaria control in a malaria endemic area along the Ituxi River".

   e. McNeill, K.M., W.D. Alecrim, A.M. Tavares and D.R. Roberts. "Comparison of malarial antibody activity in filter paper samples and sera by the Indirect Fluorescent Antibody Test".

   f. McNeill, K.M., D.R. Roberts and W.D. Alecrim. "Continuous in vitro cultivation of a new strain of Plasmodium falciparum (Ituxi 084)".

   g. Roberts, D.R., W.D. Alecrim, S.R. Erhardt and J.T. Whitlaw "Euplusia purpurata, a bee attracted to DDT on sprayed house walls".

   h. Roberts, D.R., W.D. Alecrim, A.M. Tavares and K.M. McNeill. "Obervations on the behavior of Anopheles darlingi Root in a malaria endemic area of Amazonas, Brazil".

BIBLIOGRAPHY:

1. Marsden, P. and W. Reid. 1980. New Transactions resists predations of the American conckrouch (Periplaneta americana). Submitted for publication to the Transaction of the Royal Society of Tropical Medicine and Hygiene.

2. McNeill, K.M., W.D. Alecrim, A.M. Tavares on D.R. Roberts. 1980. Activity of IgG and IgM in serum and filter paper blood samples in

the Indirect Fluorescent Antibody Test for malaria. Submitted for publication to The American Journal of Tropical Medicine and Hygiene.

3. Peterson, N.E. and R.J. Izor. 1980. Notes on South American weasels. Submitted for publication to the Journal of Mammalogy.

4. Peterson, N.E., D.R. Roberts and C.H. Llewellyn. 1980. A multidisciplinary program of disease surveillance along the Transamazon Highway in Brazil. I. Area ecology. Submitted for publication to the Bulletin of the P.A.H.O.

5. Peterson, N.E. and D.R. Roberts. 1980. A multidisciplinary program of disease surveillance along the Transamazon Highway in Brazil. III. Mammalian surveillance. Submitted for publication to the Bulletin of the P.A.H.O.

6. Phillips, S.M. and W.A. Reid. 1980. Schistosoma mansoni: immune response to normal and irradiated cercariae or soluble stage-specific surface immunogens. International Journal of Nuclear Medicine and Biology, 7:173-186.

7. Phillips, S.M., W.A. Reid, B.L. Doughty and A.G. Bentley. 1980. The immunologic modulation of morbidity in schistosomiasis. Studies in athymic mice and in vitro granuloma formation. American Journal of Tropical Medicine and Hygiene, 29:820-831.

8. Prata, A., W.A. Reid and M.S.L. Cunha. 1980. Tratamento da Giardiose com Tinidazol. Submitted for publication to the Revista da Sociedade Brasileira de Medicina Tropical.

9. Roberts, D.R. and B.P. Hsi. 1979. An index of species abundance for use with mosquito surveillance data. Environmental Entomology 8:1007-1013.

10. Roberts, D.R. and J.E. Scanlon. 1979. An evaluation of morphological characters for separating females of Aedes (Ochlerotatus) atlanticus Dyar and Knab and Aedes (Ochlerotatus) tormentor Dyar and Knab (Diptera: Culicidae). Mosquito Systematic. 11:203-208.

11. Roberts, D.R., J.M. Heller, W.D. Alecrim, S.R. Ehrhardt and A. Prata. 1980. DDT- an attractant to male euglossine bees in Brazil. Submitted for publication to Science.

12. Roberts, D.R., A.L. Hoch and N.E. Peterson. 1980. A multidisciplinary program of infectious disease surveillance along the Transamazon Highway in Brazil. IV. Entomological surveillance. Submitted for publication to the Bulletin of the P.A.H.O.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 02 6530	80 10 01	DD DR&E/AR(6)6

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	871AH	162
b. CONTRIBUTING	62770A	3M162770A802	900	016
c. CONTRIBUTING	STOG 80-7.2:2			

11. TITLE (Procede 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: 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 80	4	100
FISCAL YEAR CURRENT 81	7	211

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Muriithi, I., DR.

NAME: Wellde, B.T.

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede 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. Precede text of each with Security Classification Code)

23. (U) The objective of this program is to develop an effective, practical vaccine against African trypanosomiasis, useful to both military and civilian agencies. Related benefits include acquisition of knowledge pertaining to trypanosome immunity, host response and pathology of infection. There is a requirement for these studies which should provide a basis for rational development of a vaccine for this disease which would constitute a serious hazard for military personnel operating in the endemic area.

24. (U) Experiments conducted at WRAIR and in Kenya have demonstrated that experimental animals can be successfully immunized with irradiated trypanosomes. Rodents, cattle and monkeys can be rendered completely resistant to a challenging infection of T. rhodesiense. Complete immunity has been achieved against T. congolense.

25. (U) 79 10 - 80 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 trypanosome 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 1979-30 September 1980.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE 498A 1 NOV 73

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

* Project 3M162770A802 MILITARY PREVENTIVE MEDICINE

Work Unit 162 Vaccine Development in Trypanosomiasis

* Work Unit 016 Vaccine Development in Trypanosomiasis

Principal Investigator: Wayne T. Hockmeyer, MAJ
Associates: Dr. I. Muriithi; B. T. Wellde

INTRODUCTION

Current studies on African Trypanosomiasis are designed to investigate the problem of vaccine development. Recent work at USAMRU-K and WRAIR has shown that serodeme antigenicity remains constant for at least several years in a given geographical area and further that the blood forms in the first wave of parasitemia are antigenically very similar to the metacyclic form. Previous questions posed, i.e. antigenic variability and low yield of metacyclic forms from flies, may be resolved by these observations. Immediate objectives include followup on the serodeme stability of trypanosomes isolated from individuals inhabiting the Lambwe Valley and investigation of the effectiveness of first parasitemia trypanosomes as immunizing agents.

Studies on visceral leishmaniasis are underway and are focusing on drug efficacy, and immunologic aspects of the disease, as well as vector-reservoir inter-relationships. Clear documentation of Kenyan kala azar is lacking and most published work is based on case reports or studies involving only a few individuals. Objectives include continuation of baseline immunology studies, pharmacokinetics of available drugs and establishment of sandfly and parasite colonies within the laboratory.

African trypanosomiasis

A bovine model of central nervous system disease caused by T. brucei rhodesiense has been developed. We have shown that 50 percent of the cattle infected with T.b. rhodesiense can develop a central nervous system disorder characterized by pleocytosis, increased immunoglobulin levels and the presence of trypanosomes in the cerebro-spinal fluid and meningoencephalitis which varied in severity depending on the duration of the disease. The histopathologic findings in the bovine brain were similar to those found in man (Weilde, Kovatch and Hockmeyer 1979). In an attempt to standaridize the disease process and to induce the CNS disorder in a greater percentage of animals, 6 calves were inoculated intrathecally with T. b. rhodesiense isolated from the cerebro-spinal fluid of an infected bovine. All animals developed a parasitemia in both blood and cerebro-spinal fluid which persisted. Five of six animals have died between the third and seventh month post infection. The last surviving infected animal has demonstrable trypanosomes in the CSF at 8 months post inoculation. The response of the CNS infections to suramin and melarsoprol will be studied to determine whether or not the model could be used to test the efficacy of antitrypanosomal compounds against trypanosomes in the CNS.

The stability of the Lambwe Valley serodeme is still being studied with numerous isolates collected during a large outbreak this year. Preliminary results indicate that these isolates are antigenically the same as those previously described for the area. The isolates are being characterized by both monoclonal antibody immunofluorescent techniques, in collaboration with DCD & I (WRAIR), and isoenzyme techniques, in collaboration with the Kenya Trypanosomiasis Research Institute.

Visceral Leishmaniasis

Several drug efficacy and pharmacokinetics pilot studies have been completed. The excretion of pentavalent antimonials given both intramuscularly and intravenously has been studied in a limited number of patients and healthy volunteers. Results indicate that the pentavalent antimonials are rapidly excreted with miminal accumulation. This rapid excretion with resulting low blood levels may well explain the variable response to therapy that has been observed.

Allopurinol as a part of the treatment regimen was studied in 10 patients, 6 of whom had previously failed to respond to Pentostam(R). If appears that allopurinol may be effective either following pentavalent antimonials or in conjunction with them.

A complement fixation test was evaluated for both early diagnosis and followup of visceral leishmaniasis and was adopted as a part of the diagnostic battery by the Clinical Research Centre. Baseline hematology and clinical chemistry studies were performed on the population from an endemic area. The area surveyed is programed to be a combined multidiscipline study area for visceral leishmaniasis research.

RECOMMENDATIONS

African trypanosomiasis

It is recommended that the Lambwe Valley study be continued to further document serodeme stability, to acquire new isolates and to serve as a test area for vaccines and/or candidate drugs developed by WRAIR. Specific short term goals should be: (1) analyze collected data and publish as appropriate: (2) conduct further studies to elucidate the T.b. brucei - T. b. rhodesiense relationship in Lambwe Valley: (3) pursue the bovine CNS model as a drug efficacy screening method.

Visceral leishmaniasis

Primary emphasis should be placed on: (1) followup studies of pentavalent antimonials and/or allopurinal: (2) immunologic studies addressing the question of immunosuppression of leishmaniasis - cause or result; (3) establishment of sand fly colony; (4) field studies to further explore vector - reservor relationships in an endemic area.

Publications

Rees, P.H., Keating, M.I., Kager, P.A., and Hockmeyer, W.T., Renal Clearance of Pentavalent Antimony (Sodium Stibogluconate), Lancet 2 August 1980.

Submitted for Publication

Kager, P.A., Rees, P.H., Wellde, B.T., Hockmeyer, W.T., Lyerly, W.H. Jr., Allopurinol in the Treatment of Visceral Leishmaniasis.

Roberts, L.W., Probing by Glossina morsitans morsitans and Transmission of Trypanosoma (Nannomonas) congolense. Am. J. Trop. Med. Hyg.

Wellde, B.T., Hockmeyer, W.T., Kovatch, R.M., Bhogal, M.S., Diggs, C., T. congolense Infection in Cattle - Natural and Acquired Resistance. Exp. Parasit.

Presentations

Hockmeyer, W.T., Wellde, B.T., Williams, J.S. Smith, D.H., Kager, P.A., Rees, P.H., Comparison of the CF and Micro-ELISA Techniques for the Diagnosis of Visceral Leishmaniasis in Kenya. 1st Annual Medical Scientific Conference of Kenya Medical Research Institute and Kenya Trypanosomiasis Research Institute, Nairobi, Kenya.

Kager, P.A, Rees, P.H., Wellde, B.T., Hockmeyer, W., Allopurinol in the Treatment of Visceral Leishmaniasis - Some Observations. 1st Ann Med. Sci. Cont. - KMRI and KETRI, Nairobi, Kenya

Rees, P.H., Keating, M., Kager, P.A., Hockmeyer, W., Excretion of Pentavalent Antimony. 1st Ann. Med. Sci. Conf. - KMRI and KETRI, Nairobi, Kenya

Smith, D.H., Roberts, J.M., Hockmeyer, W., Efficacy of Therapy of Visceral Leishmaniasis with Pentavalent Antimonials - A Preliminary Report. 1st Ann. Med. Sci. Conf. - KMRI and KETRI, Nairobi, Kenya

Wellde, B.T., Kovatch, R.M., Hockmeyer, W.T., Pathogenicity of Trypanosoma brucei rhodesiense for Cattle. 1st Ann. Med. Sci. Conf. - KMRI and KETRI, Nairobi, Kenya.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA0B 6500	80 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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	871AD	163
b. CONTRIBUTING	62770A	3M162770A802	00	005
c. CONTRIBUTING	STOC 80-7.2.2			

11. TITLE (Procede 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: 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 1980	8	460
FISCAL YEAR CURRENT 1981	8	703

19. RESPONSIBLE DOD ORGANIZATION

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NAME: CHENEY CPT C.P.

NAME: DUNN MAJ M.A.

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Activity; (U) Bacterial Mucosal Adherence; (U) Liver Fibrosis; (U) Schistomiasis; (U) E. Coli

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 diarrhea disease including pathogenic E. Coli, Salmonellosis and Shigellosis. These have critical military relevance because of their influence on troop mobility. Studies are also performed on the determinants of fibrosis in parasitic liver disease (schistomiasis).

24. (U) Studies of bacterial diarrhea are being conducted in 4 general areas 1) Mucosal adherence as a determinant of bacterial colonization, 2) Cellular immune response to intestinal infection, 3) Pharmacologic modification of effects of infections on intestinal transport and 4) motility. Studies utilized in vivo intestinal perfusions of rabbits and rats, rat ileal loop models, Ussing chambers, in vivo recording of intestinal myoelectric activity and in vitro agglutination of intestinal membrane fractions. Isolation and functional characterization of intestinal lymphocytes is performed.

25. (U) 79 10 - 80 09 Mucosal Adherence - The bacterial surface structures (pili) mediating adherence have been identified and isolated and intestinal receptors for pili have been identified. Immunology - Intestinal mononuclear cells have been isolated and their functions studied. A suppressor effect of these cells has been identified and substances capable of stimulating B cell proliferation sought. Transport - Anti secretory effect of the alkoloid berberine has been identified and its mechanism studied. Motility- ability of subunit of cholera toxin to cause changes in intestinal myoelectric activity has been documented. Liver Injury and Fibrosis- Mechanisms for control of limiting steps of hepatic fibrosis have been studied. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 - 30 Sept 80.

*Available to contractors upon originator's approval

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

Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS
*Project 3M162770A802 MILITARY PREVENTIVE MEDICINE

Work Unit 005 Gastrointestinal Diseases of Military Importance
*Work Unit 163 Gastrointestinal Diseases of Military Importance

Investigators
Principal: Edgar C. Boedeker, LTC, MC
Associates: Christopher P. Cheney, CPT, MSC; Michael A. Dunn, MAJ, MC; Sumner Kraft, COL, MC; Robert H. Reid, LTC, MC; Dennis R. Sinar, MAJ, MC; Robert W. Sjogren, LTC, MC; Edward A. Swabb, CPT, MC; Yuan-Heng Tai, Ph.D.; James A. Wright, CPT, MC.

Problem 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 attach to the host's mucosal cells? What are the receptors, or binding sites, for bacteria on the host's intestinal cells? What immunologic or pharmacologic means can be used to prevent or reverse the adherence of pathogenic bacteria to the intestine?

ii. Role of Host Immune Mechanisms

What are the normal immune defense mechanisms operating at the level of the intestine capable of protecting against enteric pathogens? Are the lamina propria cells (lymphocytes, macrophages, and polymorphonuclear leukocytes) capable of acting as effectors in antibody dependent cellular cytotoxicity reactions? Can systemic (splenic) T lymphocytes function as effectors in the lamina propria? How are lymphocytes effected by bacterial pathogens? Are B lymphocytes selectively stimulated by the Staphlococcal protein A mitogen? Are T lymphocytes required? How does preexisting systemic immunity influence intestinal immunization? What effect does passively administered antibody have on the development of active circulating immune responses following the chronic injection of a soluble protein antigen?

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 by which bacterial toxins change small bowel myoelectric patterns? Do luminal toxins produce similar changes in myoeletric patterns to native disease? What are the changes in myoelectric patterns induced by therapeutic maneuvers (antibiotics, antidiarrheals)?

v. Liver Injury and Repair

Can the liver recover and repair itself after tissue injury? What determines whether attempts to repair injury will lead instead to fibrosis with permanent organ damage? How can the process of fibrosis be controlled, prevented, or reversed? Can the functional integrity of liver be assessed by any quantitative tests of its metabolic response?

Progress

i. Role of Mucosal Adherence in Bacterial Colonization

An animal model for adherence of an enteropathogenic E.Coli to the 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. (1) 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. (2) 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 identity the adhesins on RDEC which promote their adherence to host cells, we gentically transfere DEC-1 adherence ability to a non-adherent Shigella flexneri organism. ectron microscopic examination revealed that transfer of adherence cor ated with transfer of surface pili. (3) 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 immiunofluorescent staining. (4,5) 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.

In order to identity the bacterial receptor on the host mucosa, the ability of purified pili to bind to, and precipitate with, protein solubilized 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 border proteins. (6) Using the techniques developed in the animal model, the adherence of a well characterized E.Coli strain (H10407) isolated from patients with Traveller's diarrhea, to human brush borders, was confirmed. (7) 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 amimal system, human pathogens only adhere to host cells when they express specific pili.

ii. Role of Host Immune Mechanisms

We studied the antibody dependent cellular cytotoxivity (ADCC) of rabbit gastrointestinal associated lymphoid tissue (GALT) consisting of the individual cell populations, Peyer's patch, mesenteric lymph node, and intestinal lamina propria as effectors and chick red blood cells as targets. No ADCC activity was found in these populations in contrast to spleen cells. The spleen ADCC activity was found to follow the neutrophiles in cell enrichment experiments. The GALT cell populations contained very few if any neutrophiles. Therefore, our observed lack of ADCC activity using rabbit GALT cells is probably due to the relative absence of the neutrophile effector cells. The rabbit neutrophile might be preferentially utilized in ADCC against other cell targets and thereby provide a useful animal model for neutrophile ADCC without interfering lymphocyte or macrophage ADCC (8).

We have found the rabbit ileal lamina propria (LP) mononuclear cells (lymphocytes, monocytes, and macrophages) isolated by collagenase digestion to suppress the autologous splenic lymphocyte response to phytohemagglutinin (PHA). In the present experiments, the LP cells have been futher separated by depleting monocytes/macrophages. The unseparated mononuclear and monocyte/macrophage depleted LP populations were tested for supprressive ability in paired repetitive experiments. The suppression of the splenic lymphocyte PHA response by the ileal LP mononuclear cells was increased by monocyte/macrophage depletion, suggesting that this suppressive capacity of intestinal LP mononuclear cells is due to the lymphocytes more than the monocytes and macrophages. A subpopulation of LP lymphocytes may function in vitro to limit participation of systemic (spleen) T lymphocytes in immunological responses in the rabbit intestine (9). Protein A from Staphylococcus aureus has been reported to be an efficient inducer of rabbit B-cell division and could be a useful mitogen for measuring the production of specific immunoglobulin (Ig) and antibody by rabbit intestinal lymphoid cells. Protein A coupled to Sepharose CL-4B (SpA-s) is a well defined solid-phase reagent known to be capable of stimulating human lymphocytes in vitro. We thus tested the mitogenicity of SpA-s against mononuclear cells isolated from normal rabbit spleen and Peyer's patches (PP) using Sephadex G-10 columns to deplete monocytes and macrophages (confirmed by nonspecific esterase staining and latex fixation), and Sephadex G-200 anti-rabbit F(ab') immunoabsorbent columns to

separate B-cell enriched populations. The SpA-s was found not to be mitognic for B cells from rabbit spleen and Peyer's patch. In contrast the SpA-s did act as a rabbit ileal mitogen. These results indicate that either the insoluble form of protein A acts differently from the soluble form or that the original was increased.(10) Immunization against pathogens and other enviromental antigens at mucosal surfaces are some of the ways mammals aquire circulating "natural" immunity. To study the effects of passive immunity on the immunologic responses to ingested antigen, adult rabbits were given hyperimmune anti-BsA i.v. 24 hrs before starting prolonged feeding of 0.1% BsA in the drinking water. The active immune responses of these animals were then compared to those of animals only ingesting the antigen. Passive immunity was found not to change the amount of circulating anti- BsA produced after BsA ingestion. These observations suggested that there is little or no effect of passively administored antibody on the developement of active circulating immune responses that follow ingestion of a soluble protein antigen. (11)

iii. Alterations of Intestinal Transport

To investigate the normal mechanisms for salt and water transport, theoretical and experimental relationships between short-circuit current and ion fluxes in the rat ileum were studied (15,19). Three transport processes for ions moving across the intestine are postulated to account for the observed [illegible] transport patterns: 1) an electrogenic Na absorptive process giving rise to the short-circuit current; 2) a neutral NaCl coupled secretory process; 3) a bicarbonate secretory - Cl absorptive exchange system. The effects of osmotic gradients on basal ion transport were studied (13,18). It appeared that the changes in electrical properties caused by osmtic 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 stimulatin 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 (17,23,24,25, 26). 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 (16). The action of serotonin may be regulated by serotonin-induced increase in intracellular Ca concentration. Choleragenoid as well as cholera toxin produced ileal electrolyte secretion in the rabbit but the mechanism of action of choleragenoid is unclear and remains to be studied (14).

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 microcirculation to produce a

driving force for passive filtration-secretion (27,28). A mathematical model for the dynamics of luminal fluid accumulation in intestinal obstruction was derived based on a luminal fluid material balance (29).

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 (22). 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 (21) and reverse the ion secretion induced by cAMP, ECST, and methylprednisolone (12,20).

iv. Alterations in Intestinal Motility

Bacterial toxins, specifically E.coli heat labile toxin and cholera enterotoxin, produce a diarrheogenic myoelectric pattern, the migrating action potential complex (MAPC). The MAPC is produced by the B subunit of cholera toxin, requires binding at the membrane binding site and requires aggregation of B subunit components in a form more complex than the monomeric B subunit. E.coli heat labile toxin (LT), produces similar activity in similar concentration as cholera toxin. The antigenic similarities between CT and LT are insufficient to block MAPC activity by preincubation of toxin with heterologous antiserum. (30,31,33)

A primate model for chronic intestinal myoelectric activity recording has been developed. Our initial work has been in validation of the model by comparing fasting and fed changes in the interdigestive myoelectric complex (IDMEC) as monitored by computer analysis of spike burst activity (32). Two animals have developed clinical Shigella infection with diarrhea and dysentery (results not published).

In the monkey model previously described, we have tested common antibiotics and antidiarrheals by computer analysis to detect changes in myoelectric response. These studies will serve as control studies for tests of antibiotic/antidiarrheal medications during native infections described above.

v. Liver Injury and Repair

After any injury, the liver can recover its normal structure and functions, or alternatively, fibrosis can occur, with permanent severe impairment of function. Fibrosis can be defined as the deposition of an excessive amount of connective tissue, composed mainly of collagen, in an organ in such a way as to interfere with normal achitecture and circulation. Understanding the regulation of fibrosis is therefore central to promoting recovery from any liver injury.

The best model disease for studying liver fibrosis is schistosomiasis. This is so because in schistosomiasis, liver fibrosis results from a well-defined inflammatory and immunologic host response to parasite antigens. In all other forms of experimental liver injury, either the antigenic stimulus is unknown or the initial injury is so globally disruptive of all metabolic pathways that it is not possible to elicit clear

information on regulation of fibrosis. The findings from such studies with schistosomiasis have had general relevance for the entire problem of liver injury and fibrosis (34).

One control mechanism of fibrosis is the substrate supply of free proline, a major constitnent of collagen. Using mice with schistosomiasis, we showed that the metabolic handling of proliine is quite different from normal within the location granulomas where collagen is synthesized. Our data on the activites of enzymes that form and degrade proline suggest that this critical metabolite may be selectively trapped within granulomas and channeled into collagen petide synthesis (35). The net disposition of collagen-rich fibrous tissue must result from either increased synthesis or deficient degradation of collagen. Our studies in mice (36) and rabbits (37) with schistosomiasis show that both collagen synthesis and degradation proceed at higher than normal rates in injured liver, and that the imbalance between these two competing processes determines whether fibrosis will progress (36) or resolve (37).

During liver injury and repair there has been no quantitive way of monitoring the actual metabolic reserve of the liver in the same way that one can asses lung capacity, cardiac output, or kidney excretory capacity. We found that the synthesis of urea, a metabolic process unique to liver cells, can be reliably measured in rats after administration of a saturating substrate load. Maximal urea synthesis rates reflected functional liver cell mass in these animals when subjected to partial hepatectomy. Unlike other tests of liver function that fail to reflect liver reserve then liver structure or circulation are altered, the urea, synthesis measurement remained valid in rats with cirrhosis or with shunting of the liver's portal circulation (38).

Future Plans and Recommendation

i. Role of Mucosal Adherence in Bacterial Colonization

Over the next year we intened 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 substitued with hydrophobic ligands.

Based on the confirmation of pili as important determinants of adherence for pathogenic human isolates of E.Coli, and on 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 preparations 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 for testing of this vaccine.

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. Secretory IgA is the principle immunoglobulin class of antibodies expressed in the intestine. Augmentation of intestinal secretory immunity might be possible if the IgA stimulating factor found in human milk cells could be studied in an animal model.

iii. Alterations of Intestinal transport

Based on our results to date the normal mechanisms of salt and water transport in the intestine and the mechanism involved in hydrostatic pressure-induced secretion of water and electrolyes appear to be quite well understood. Future studies should be emphasized on obtaining answers to the following questions. How are the cAMP- and cGMP-protein kinase, protein kinase-electrolyte pumps, and calmodulin involved in modulating the intestinal secretion of water and electrolytes induced by bacterial toxins and other secretory stimuli? How do absorptive and secretory processes interact? Do they share any pathways? What are the mechanisms of action of berberine in reversing the cholera toxin- and ECST-induced secretion of water and electrolytes? What are the mehanisms whereby purified Shigella toxin influences small intestinal function? Finally, studies using a combination of drugs which inhibit stimulated secretion and drugs which enhence basal absorption, to maximize the antisecretory action should be carried out.

iv. Alterations in Intestinal Motility

Future investigations in the toxin area will move to an in vitro muscle system. Reproduction of MAPC activity in denervated loops of bowel has been reported. Extension of previous work to this system will allow more complete delineation of mechanisms of toxin action.

Future work in this area will be to test Shiga toxin in animals and compare responses to native infection. We are interested in quantitating the timing and deviation of changes in intestinal smooth muscle that occur with infection and clinical diarrhea.

v. Liver Injury and Repair

Future work on liver injury and repair should attempt to define control mechanisms of the critical steps in fibrosis. Experimental therapeutic interventions directed at the metabolism of proline for collagen synthesis, or at the activation of tissue collagenase for collagen degradation, may permit us to promote recovery and prevent fibrosis. Extension of the urea synthesis measurement to other animal test systems and ultimately to humans seems a promising way of assessing functional liver reserve.

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Patent

Reid R.H.: Method of Indentification of Surface Proteins of Cancer Cells, Clinical Test and Method of Immunization; United States Patent No. 4,174,385 issued 13 Nov 1979.

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	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORD'N MSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A875	875AA	161
b. [redacted]	62780A	3E162780A843	00	002
c. CONTRIBUTING	STOC 80 7 2 1			

11. TITLE (Precede 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: NA

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	0.3	16
FISCAL YEAR CURRENT 81	2.0	232

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

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TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

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Division of Experimental Therapeutics

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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

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) 79 10-80 09 Resynthesis of ten radioprotective compounds has been completed. Studies of acute toxicity in mice are in progress and 50-percent effective dose determinations for radiation-induced hematopoietic and gastrointestinal syndromes are being made. Additional quantitative radioprotection experiments will be initiated. Hydroxycobalamine successfully reversed acute cyanide poisoning in mice. At 300 to 500 mg per kg, it increased the dose of cyanide required for 50-percent lethality from 8.4 to 19.0 mg per kg. When hydroxycobalamine was given in conjunction with sodium nitrite and sodium thiosulfate, the 50-percent lethal dose of cyanide was further increased to 52 mg per kg. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79-30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3S162772A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT
* Project 3E162780A843 MEDICAL SYSTEMS IN CHEMICAL DEFENSE

Work Unit 161 Chemoprophylaxis of Chemical & Ionizing Radiation Injury
* Work Unit 002 Chemoprophylaxis of Ionizing Radiation Injury

Investigators:
Principal: COL David E. Davidson, Jr.

Associate: CPT Irving McConnell
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 chemicals 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 extend drug development efforts to develop orally effective radioprotective drugs which could be used to protect military personnel in a nuclear environment.

PROGRESS:

Radioprotective Drugs: Ten radioprotective compounds, selected from the previous program because of superior oral effectiveness and tolerance in animal models, have been resynthesized in 100-1000 gm quantity. Experiments have been initiated to quantitatively characterize the efficacy and tolerance of these compounds in mice. Acute toxicity studies in mice are in progress, and studies to determine radioprotective potency (DRF), minimum effective doses, optimal time of administration, optimal route of administration, and duration of protection are programmed. Experimental studies to calibrate and standardize radiation conditions for mice have been completed.

Chemical Antidotes: Hydroxycobalamine (Vitamin B_{12}) administered subcutaneously 30-60 seconds after cyanide administration substantially reduced the lethal effects of acute cyanide poisoning in mice. Hydroxycobalamine was effective alone, and it was even more effective when given in conjunction with the standard antidotes, sodium thiosulfate and sodium nitrite. Hydroxycobalamine alone increased the LD-50 of cyanide from 8.4 to 19.0 mg/kg (a 2.3-fold reduction in toxicity); the three drug combination raised the LD-50 of cyanide to 52.0 mg/kg (a 6.2-fold reduction in toxicity). Rodent experiments to further evaluate hydroxycobalamine as a cyanide antidote are in progress.

FUTURE PLANS:

The quantitative studies of 10 selected, orally effective radioprotective compounds in mice will be continued. These studies will be extended to larger animals if the results of rodent studies show promise. Analogs of non-nitrogen-containing radioprotectors and of amidine radioprotectors now being synthesized will be screened and evaluated in rodents. Prodrugs and novel formulations of the best protector, WR 2721, will be evaluated for oral efficacy as these become available. Neutron protection studies are also programmed.

Studies with hydroxycobalamine as an antidote for cyanide poisoning will be pursued. The characteristics and limitations of this approach will be evaluated, and chemical analogs will be studied. Investigations of mechanism of action will be initiated. Work with chemical antidotes will be extended to include antidotes for organophosphate agents.

PUBLICATIONS:

1. Davidson, D.E., Grenan, M.M., and Sweeney, T.R., 1980. Biological Characteristics of Some Improved Radioprotectors. (In Press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6478	80 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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A875	875AB	162
b. [redacted]	62780A	3E162780A843	00	001
c. CONTRIBUTING	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: 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 80	1	74
	CURRENT 81	2	156

19. RESPONSIBLE DOD ORGANIZATION
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20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Hick, Robert O., MAJ MSC
TELEPHONE: (301) 427-5422
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Klayman, D.L., Ph.D.
NAME:

21. GENERAL USE
Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Antiradiation Drugs; (U) Drug Development; (U) Aminoalkylthiols; 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) 79 10--80 09: Synthetic efforts directed towards latentiated WR 2721 and analogs as cyclophosphamides proved very difficult and are being phased out. Seven samples of the no-nitrogen type have been prepared and testing will commence in the next FY. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 65 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 3S162772A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

* Project 3E162780A843 MEDICAL SYSTEMS IN CHEMICAL DEFENSE
Work Unit 162 The Synthesis of Antiradiation Drugs
* Work Unit 001 Synthesis of Antiradiation Drugs

Investigators:

Principal: MAJ Robert O. Pick, Ph.D.
Associate: Thomas R. Sweeney, Ph.D; Daniel L. Klayman, Ph.D; William Y. Ellis, BS.

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 effort in latentiating WR 2721 has involved difficult problems in chemistry. The synthesis contract in which this is being attempted is being phased out.

The contractual effort in the synthesis of adamantyl amidinium disulfides and trithiocarbonate blocking of essential thiol has progressed through most of the intermediate steps and targets are expected shortly for screening. Seven samples of the "no nitrogen" type have been submitted, and testing is expected to commence early in calendar 1981.

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	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. [illegible]	9a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A878	878 3B	041
b. [illegible]	62771A	3E162771A805	00	041
c. CONTRIBUTING	STOG 80-7.1.4			

11. TITLE (Procede with Security Classification Code)

Biological Interactions with and Hazards of Microwave Radiation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

014100 Radiobiol 012900 Physiol 014000 Rad Chem 017000 Wave Prop 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: NA EXPIRATION

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE:

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING 80	6	1000
CURRENT 81	3	356

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
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RESPONSIBLE INDIVIDUAL
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TELEPHONE: 202-576-3551

20. GENERAL USE

Foreign intelligence not considered.

20. PERFORMING ORGANIZATION

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Larsen, L.E.
TELEPHONE: 202 576-3615
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Jacobi, J.H.
NAME: Hunt, E.L.

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Microwave Hazards; (U) Biophysics; (U) Dosimetry; (U) Bioeffects; (U) Military Medicine; (U) Psychology

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede 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) 79 10 - 80 09 Progress has included the demonstration of feasibility for non-invasive microwave dosimetry using scattering parameters and time delay spectrometry; development of network analysis methods for high speed, broad band measurement of permittivity in biological tissues; development of methods for improved spatial resolution in microwave images of isolated organs; preliminary studies to assess functional states of cells and tissue by in situ permittivity analysis; development of microwave transparent electrodes for temperature measurement and induced electric field strength in situ; and development of high power pulse exposure facility for radar simulation. Conducted studies of thermal cataracts of the ocular lens. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3E162777A878 HEALTH HAZARDS OF MILITARY MATERIEL

* Project 3E162771A805 MICROWAVE INJURY

Work Unit 041 Biological Interactions with and Hazards of Microwave Radiation

* Work Unit 041 Biological Interactions with and Hazards of Microwave Radiation

Investigators.
Principal: LTC Lawrence E. Larsen, M.D. MC
Associate: Edward L. Hunt, B.A.; John H. Jacobi, M.S.; CPT G. Rufus Sessions, Ph. D., MSC; Peter V.K. Brown, M.S.

ADMINISTRATIVE ACTIVITIES

The present report period encompasses several significant accomplishments of an administrative nature. Primary among these were completion of the Tri-Service Memorandum of Understanding and Tri-Service RFR Research Plan; the completion of the interagency research plan on the Biological Effects of Nonionizing Electromagnetic Radiation; the initiation and near completion of construction for new laboratory space for the in vitro and millimeter wave segments of the program; and the conclusion of a Memorandum of Understanding between USAMRDC and the Canadian Department of National Defense on microwave bioeffects research.

The major administrative actions still pending are completion of the construction at the Forest Glen microwave exposure facility and department reorganization to remedy the staff shortages which presently limit productivity.

A. Construction

This report marks the beginning of the fourth year of a construction project for renovation of the WRAIR microwave exposure facilities at Forest Glen. This project was originally estimated to take 6 to 9 months. The delays we have encountered relate chiefly to problems in environmental control for the animal modules and environmental control of the 4 exposure chambers. Beneficial occupancy did take place in December of 1979 with the expectation that progress would be accelerated on the heating, ventilation and air conditioning problems in the 4 exposure chambers (notably, correction of poor temperature/humidity control and high static air pressure). Whereas these expectations remained unrealized, beneficial occupancy did permit progress on several items of improvement in the exposure equipment that could not have taken place without beneficial occupancy. These will be detailed in a later section.

In contrast to the situation in Bldg 502/503, construction at Bldg 40 has not only commenced but also has made good progress such that the projected time for occupancy is 30 days in advance of the completion date required in the contract. The millimeter wave anechoic chamber has benefited from design refinements and an empirical test of a pilot room. It does appear that when we take occupancy of labs in Building 40, this anechoic chamber will be the first millimeter wave room in any biomedical research establishment within either the Tri-Service or the civilian communities involved with medical electromagnetics.

B. Tri-Service Electromagnetic Radiation Panel (TERP)

LTC Larsen served as Chairman of this group for an extended period term in order that the Tri-Service Memorandum of Understanding and Radiofrequency Research Plan could be completed under the same leadership under which they began. Both documents were completed by July of 1980.

In addition, the Army hosted the third TERP topical symposium. The topic for 1980 was Electromagnetic Dosimetric Imagery. This symposium was organized by LTC Larsen and Mr. Jacobi (both of the WRAIR Department of Microwave Research) to bring together for the first time those elements of radio-frequency and microwave technologies which bear upon electromagnetic propagation in biological dielectrics with spatial resolution sufficient or potentially sufficient to produce an image. In this way, the spatial distribution of microwave constitutive parameters and thence the spatial distribution of absorbed energy may be provided as a noninvasive method for dosimetry. The conference involved 12 invited speakers, ca.85 attendees and the production of a proceedings. The symposium was co-sponsored by the IEEE Professional Group on Microwave Theory & Techniques (PGMTT) and was held as a workshop in the 1980 International MTT Symposium.

C. Biological Effects of Nonionizing Electromagnetic Radiation (BENER)

Concurrently with the TERP proceedings, the Office of Science & Technology Policy (Executive Office of the President) appointed the Department of Commerce as the lead agency to call an interagency task force to prepare a national research plan on the subject of BENER. Significant contributions were made in the areas of dosimetry, ocular effects, thermoacoustic expansion and biomedical applications. The BENER plan was formally promulgated by the National Telecommunications & Information Administration (NTIA-SP-80.7) in June of 1980 and the working group's function was completed in November 30, 1979.

D. Canadian Department of National Defense (DND)

In the present report period, a Memorandum of Understanding (MOU) was signed between the USAMRDC and the Canadian DND on the subject of research on the biological effects of nonionizing electromagnetic radiation. This MOU served to formally link the research programs of the two research organizations by means of report exchanges and joint studies. The latter have been delayed, of course, by the construction problems at Bldg 502/503, but it is our intention to pursue joint studies of blood brain barrier alterations and share technologies for induced electric field measurements. The exchange of reports has already proven to be useful in that the DND has completed a study of emissions/dosimetry for the US Army manpack radio set (AN/PRC-77) that is common to both armed forces.

E. Exposure Facility Management

Contractor operation of microwave exposure facility at Forest Glen has been in place for nearly one year. The contractor for this period has been the Electromagnetic Compatibility Analysis Center (ECAC). On the basis of revised policy decisions at ECAC, it was agreed that ECAC would begin a phase-out period starting in FY 81 during which time a new request for quotation (RFQ) would be developed. Because of increasing costs and decreasing budget, the scope of the RFQ will be diminished somewhat from that described in the previous annual report. Specifically, the positions of facility manager and microwave engineer will be combined into a single position. The expectation is that in the second quarter of FY 81 a new contractor will take over operations. Hopefully, the remaining problems with environmental control in the exposure chambers will be solved by that time.

MICROWAVE ENGINEERING

This section enumerates a number of engineering steps taken to expand the range of exposure conditions available at the Forest Glen Facility. These steps represent two classes of engineering problems: the production of high peak power pulsed exposures for studies of thermoacoustic expansion; and the creation of free field exposure conditions which maintain relatively constant energy coupling into experimental animals without the need for restraint. These steps relate to the high peak powers as well as modulations used by Army radars and the need to provide free field validation of waveguide based in vivo exposure systems, respectively. Both were possible because of beneficial occupancy.

A. High Peak Power Pulsed Radiation

This category of engineering development includes two items: the development of a 1.5 megawatt (peak power) transmitter system and an elliptical reflector to focus the power into a small region of space with plane wave characteristics. The transmitter project consisted of modifications to the model 2007 transmitter which previously operated with an X band klystron to become the power supply, modulator and monitoring base for a 1.5 million watt (peak) pulsed klystron which operates in the radar L band. Because the 2007 was so conservatively designed, the 400 kw power supply (40 kilovolts at 10 amperes with 1% regulation) could be used with a pulse transformer to provide the 80 kilovolts at 40 amperes and 1% duty factor needed for the beam supply to the pulsed klystron. Further modifications included magnet supplies, modulator & various monitoring functions specific to the VA963 tube. The VA963 is a derated version of a 5 megawatt tube, thus good reliability should result. The average power rating of the tube is 11 kilowatts, but it is unlikely that in actual use average power will exceed 10 kilowatts. Again, good reliability should result. The transmitter project is presently estimated to be concluded by the end of CY 80. At the present time, mechanical installation is complete, electrical installation is complete while only system integration and testing remain to be accomplished.

The elliptical reflector is a "dish" 6 feet in diameter which has been preoviously used with WR 284 and WR 340 feed in the radar S band. It is

presently being modified for a WR 650 feed in order to be operated with the 1.5 megawatt L band transmitter. Design, mechanical installation and machine work has been completed. The steps that remain are to map the focal points and position the feed in order to achieve maximum power concentration at the specimen zone. The antenna is installed in a specialized exposure chamber (Chamber D) in the Forest Glen faciltiy. In the case of operation with the focused antenna, maximum average power will be limited to the low kilowatt range, but high crest factors will be available (ca. 10^3 to 10^5) due to the very high peak power.

B. Vertical Circularly Polarized (CP) Antenna

The high peak power transmitter will also be used in Chamber C in combination with a vertical feed that will supply circularly polarized fields of very high purity (axial ratio ca.0.2 dB) over a region of ca.15 cm in diameter with vertex presentation to quadrepedal animals. The antenna design is a corrigated conical horn with very low (-30 dB) sidelobes. The intention is to maintain relatively constant coupling to the experimetnal animal since the electric field will always have the same high value regardless of the orientation of the long axis of the animal within the plane of the specimen zone. Thus, restraint will not be necessary as is presently the case with linearly polarized radiation. This will permit free space validation of experimental findings in circular cross section transmission lines with respect to constant coupling and freedom from restraint, but without the wavelength dilation that takes place in dominant mode waveguide.

At the present time, design work has been completed for the antenna, feed through and mechanical support. The WR650 waveguide run has been completed to the top of Chamber C, and we are presently awaiting delivery of the antenna. Installation of the antenna will also involve new absorber material for Chamber C in order to convert the floor to the load wall and maintain good axial ratio.

RESEARCH ACTIVITIES

A. Dosimetry: Introduction

Dosimetric studies are among the most fundamental lines of inquiry with respect to radiofrequency radiation (RFR) bioeffects. The biological consequences of RFR exposure depend largely upon the spatial and temporal distribution of absorbed RFR energy. In this research topic, there presently exists two classes of projects: those based upon invasive methods of dosimetric analysis and those based upon noninvasive methods of dosimetric analysis. The former includes projects related to implantable, RFR transparent electrodes for biological transduction and RFR compatible telemetry. The second category contains projects related to microwave antenna developments, microwave scattering parameter measurement systems for spatially dependent insertion loss measurements, polarization transformation measurements, microwave differential propagation delay with high spatial and temporal resolution and electronic scanning systems to apply these microwave devices to biosystem characterization.

1. MIC Electrodes

Problem: Measure temperature and induced electric fields in biological dielectrics during microwave exposure.

Objective: Provide dosimetric estimates without field perturbation.

Progress: Both the temperature and electric field electode programs were discontinued in FY 80. This fact reflects several factors: a) a reordering of priorities to support the new millimeter wave program without additions to the staff; b) the inability to accomplish fabrication of the electrodes in Army facilities with both thick film and thin film capabilities; c) the prospect of successful technology transfer now that our U.S. Patent (#4,148,005) has been issued; d) the appearance of commerical temperature electrodes based upon MIC methods rather the liquid crystal methods; and e) the unsuitability of recommendations that we embark on a 5 year plan for development of manufacturing methods. Concurrently, the telemetry program has been discountinued. Since the budget projections for FY 81 are for a 35% decrement and our staffing problems remain unsolved, there is little grounds for optimisim that the project will be resuscitated in the near future. Indeed any consideration of such a step would be deferred until the commerical MIC electrode manufactured by Narda can be evaluated.

Plans: The project will remain terminated in FY81.

The previous years of this program have been reasonably productive in that one U.S. Patent and 3 papers have been produced (1,2,3,4). At this stage the R&D problems are virtually solved. The next phase in one of development of manufacturing methods for quantity production.

2. Non-invasive Dosimetric Analysis

Problem: Provide estimates of the spatial distribution of energy dissipation and energy storage within biosystems at the regional and target organ level for frequencies in the radar S band.

Objective: Describe microwave propagation in biosystems noninvasively at sufficient spatial resolution that the dosimetric information may be presented as image in order to assist pathologic and physiologic interpretation.

Progress:

a. Polarization Transformation

A new property of target organs with respect to the radar S band of frequencies was developed in the present report period. The new property is polarization transformation. In order to put this into perspective, it is necessary to recall that prior studies achieved high spatial resolution by means of a patented water coupled element (5,6), but that both the prior scattering parameter (7,8) and differential propagation delay (9,10) images were obtained with both transmitting and receiving antennas linearly polarized in the vertical direction. Insofar as microwave radiation interacts with all dielectrics as a vector quantity, the polarization properties of the target-

field interaction constitute an important aspect of dosimetry. This is well established at the level of the whole-body average power absorption as prior work in this laboratory has amply demonstrated (11,12,13). The importance of polarization is equally great for internal dosimetry at the target organ level. For complete description in the far field a four element, complex valued matrix can describe the polarization transformation properties of targets such as radar reflectors where no imagery is possible. When high spatial resolution permits target description as on image, then 4 complex valued spatial series result. Of the 4 cases, we have studies two. There are the co-polarized vertical and cross polarized vertical/horizontal cases for scattering parameter S_{21} The tentative interpretation from the findings thus far available is that the cross polarized foreward scattered image represents linear or piecewise linear boundaries within the organ. In the case of canine kidney, this enhances the juxtramedulary cortex interface with the outer medulary stripe and the inner medulary stripe interface with the pelvis. These result have appeared in the scientific literature during the present report period (14).

In the present report period, the two U.S. patents granted during the prior annual report (5,13) have been expanded in scope by a continuation in part for which all claims have been allowed and the new application (#41,374) has proceeded to issue.

b. Water Coupled Phased Array

Another aspect of this program is the development of a water coupled, phased array antenna. The computer aided design (CAD) phase of that project was completed in the last report period with the surprising result that the concave (quadratic) conformal array was exceeded in performance by a planar array with synthesis of a volumetric array by scanning in the third dimension. By CAD simulation, this proved to be the best solution to suppression of axially polarized components when the array was steered off bore sight. The performance parameter that was compromized in the process was, of course, speed; but it still appears possible to reduce data collection times by at least one order of magnitude. All elements were constructed, tuned and matched for both the transmit and receive arrays. This final configuration was a tight hexagonal lattice of 151 elements in the transmit array and 127 elements in the receive aray. The antennas have been assembled, the transmit and receive control units have been completed, and the software for focusing as well as propagation medium characterization has been completed. What remains at this point is calibration of the array and fitting it to the new scanner.

c. Isometric and Raster DART Display

In this reporting period, two systems have been under development for display of noninvasive microwave dosimetric measurements. One project that is nearing completion is the 3-D display system. It consists, physically, of two units: one oscilloscope-type vector display and one control console. The latter contains all the electronics controls to simulate 3-D images, test patterns and interface to a 16 bit parallel output from a computer. This is a highly complex system that uses analog techniques to magnify images, perform zoom fucntions and rotate perspective. This in-house development resulted in a considerable cost saving over commercially available

units. The application intended is for use with the noninvasive dosimetric projects. This system has now been interfaced to the Hewlett Packard computer which is a part of the phased array imaging system. Both the hardware and software portions of the interface have been completed and tested. Future developments are planned to increase the refresh rate and reduce flicker. The second system developed displays the spatial variation of absorbed microwave energy as a gray scale map or as a color map. These systems will aid in prescreening data prior to transfer to the U.S. Army Topographic Laboratories for final analysis.

d. Differential Propagation Delay

Microwave hardware improvements to the differential propagation delay system now allow spatial resolution limit of ca. 5 mm and a propagation delay resolution of ca. 40 picoseconds. The propagation delay system was shown to be less sensitive to multipath propagation by dielectric guiding at the organ/water bath interface. Interior details of the kidney were, of course, different for the propagation delay and scattering parameter systems, but the general comparability of results at the level of major regional specialization within the kidney was most encouraging. In this reporting period, work was performed to improve the performance of the system. This included construction of a microwave stimulus sytem that has greater linearity than the backward wave oscillator. Work is also progressing on modifying the fourier analyzer which will result in substantial improvement to the time delay spectroscopic images.

The recent developments cited above were presented in a series of 3 papers at the Dosimetric Imagery Workshop held in conjunction with the IEEE Microwave Theory and Techniques International Symposium (15,16,17).

Plans: The phased array calibration and preliminary imaging tests will take place in FY 81. We expect to have results from dielectric phantoms by the third quarter of FY 81. Simultaneously, a new scanner is under development that will allow multistatic scattering in addition to the foreward scatter system presently in use. The new scanner will also permit extracorporal maintenance of the target organ and, hopefully, data acquisition times in the order of minutes rather than hours.

3. Dosimetric Modeling

Problem: Provide computational predictions and empirical verification for whole body average energy absorption.

Objective: Determine the role of 180° and 90° reflectors in the vicinity of dielectrics on their specific absorption rates.

Progress: This project has been completed. The results are described in a paper published during the present report period (11). In summary, 180° reflector can introduce as much a 6 dB gain and 90° reflectors can introduce as much as 18 dB gain for dielectrics at the "optimum" distance from the reflector under conditions of maximum coupling via choice of frequency and polarization.

Plans: Empirical verification will continue based upon latency to seizure in experimental animals.

B. Analytical Studies of Army Radar

Problem: Predict power density contours for the IHAWK system under conditions of ground reflections and nearly conductors.

Objective: To examine the role of co-located communications and electric systems that could affect the normal definition of safe distances in an actual site deployments.

Progress: Some single condition have been studied. It appears that ground reflections do not significantly increase the zone of hazard, but reflectors certainly do. Even distant reflectors in the main beam can increase power density such that zones previously below 10 mW (peak) are increased by a factor of 3.

Plans: Work has been temporarily suspended due to a lack of funds. Hopefully, the project will continue in FY 81 and a larger variety of reflecting environments will be analyzed.

C. Theoretical Studies of Pulse Propagation

Problem: Energy dissipation in water dominated dielectrics is different under transient conditions (pulse) than steady state (CW) conditions. What are the relevant parameters of radiation and their relationship to the dielectric?

Objective: Define the conditions of maximum energy dissipation as a function of the dielectric medium, its relaxation frequency(s), the carrier frequency, and the pulse width.

Progress: Preliminary results are available for the Debye model of the frequency dependent properties of the dielectric; however, the project has been temporarily suspended due to lack of funds. To date, the results indicate that the absorption diminishes linearly with pulse width for carrier frequencies in the order of the relaxation frequency for the case of the damped harmonic oscilator model. In this case, if the carrier frequency in well removed from the relaxation frequency, transient absorption may exceed steady state absorption by an order of magnitude when the pulse width in short relative to the relaxation time.

Plans: This project has been suspended due to lack of funds. Hopefully, it will continue in FY 81. In the event that funds are available, the next step is to examine the limitations of the dielectric model employed and plan empirical verifications.

D. Dielectric Relaxation

1. In Situ Permittivity

Problem: Conventional permittivity measurements take place *in vitro* where physiological variables such as local blood flow are not included. The measurement of permittivity *in situ* does account for these very important

factors. The role of heating and vasodilation are important determinants of dosimetry with respect to local variations in absorbed energy.

Objective: Develop and test methods for in situ measurement of complex permittivity under conditions known to represent standard dielectrics as well as physiological and pathophysiological conditions.

Progress: The ANA based methods were applied to a modified electrical model in order to extend its data collection advantages to the in situ setting. Prior report periods describe the electrical model which will be briefly recapitulated here: A monopole antenna radiating into a lossy dielectric medium exhibits a driving point admittance/impedance which is dependent upon the complex permittivity of the medium. Thus, a measurement of the reflection coefficient at the driving point may be used to estimate the complex permittivity of the medium in which the antenna is "imbedded". The antennas have been open-ended coaxial lines of .085" and .142" OD. Frequencies of operation vary between 100 MHz and 10 GHz. These variously have small ground planes attached to the outer conductor and/or small probes attached to the inner conductor. The dielectric volume at the radiating element to which the method is sensitive is ca. 9 cubic mm. Prior work has established the accuracy and sensitivity of the method (18).

Research in the present report period confirmed and extended the pilot results from last year. Specifically, various brain regions were mapped and were found to be significantly different between dura, pia, gray matter and white matter. These differences were consistent with known circulatory patterns. For example, gray matter was characterized by higher regional cerberal blood flow than white matter with a consequently higher relative dielectric constant. Antemortem-postmortem studies were made under anoxia and cardiac depolarization. The result of depolarization was a prompt increase in blood pressure followed by a fall in perfusion. This was detected as a spike in relative dielectric constant and loss tangent co-incident with the blood pressure rise followed by a gradual deadline in dielectric constant over the next 20 minutes. Physiologic studies indicate that increased regional cerebral blood flow can be measure by means of in situ permittivity.

Plans: This is a very fruitful research over which we expect to continue in FY 81. The two areas of emphasis will be renal and CNS physiology/pathophysiology. Physiologic response to microwave exposure will be included later.

2. In Vitro Permittivity

Problem: Disperison mechanisms in biological dielectrics are a basic factor in frequency dependent absorption. To the extent that spectrum management and safety standards reflect frequency dependencies, it is important to characterize dielectric relaxation over wide frequency ranges. In the present context, this means HF band, UHF band and radar S band frequencies.

Objective: Characterize the role of cell the membrance in relaxation processes within the HF band, the role of bound water in UHF band relaxation, and the intra cellular environment in S band relaxation.

Progress: In prior report periods, a method based on automatic network analaysis applied to a lumped element model was described for the HF band (19). This method and its application to cell membrane analysis has been the subject of two successful U.S. patent applications (20,21) all claims of which have been approved and have passed to issue during the present report period.

The infinite loss method that was applied to the radar S band has not been further pursued. This is a result of insufficient staff time available due to the millimeter wave projects.

Plans: There projects are suspended until such a time that additional staff can be recruited. The methods are very promising and the decision to suspend the project reflects merely the reality of too many projects and too few staff given the priority of the millimeter wave projects.

E. Thermoacoustic Expansion

Problem: Given the proper combination of dielectric and thermal properties, carrier frequency and pulse parameters, the energy in the electromagnetic wave may be efficiently transduced into a pressure wave via thermal expansion. What are the hazards of this transduction and are they adequately addressed in a simple description of average power?

Objective: Compare thermoacoustic mechanisms with simple heating by the use of continous wave (CW) and modulated (pulse) fields of the same average power when the efficiency of thermoelastic transduction in maximized.

Progress:

1. Neural Membrane Studies

The change in resting birefringence of a crab nerve coincident with propagation of the action potential was used as a measure of peripheral nerve response to microwave radiation. This study was completed and an article has been published in the scientific literature (22). Statistical analysis of the data indicated that pulsed microwave energy degraded the birefringence amplitude a greater amount and more rapidly than did either continuous wave (CW) energy of the same average power or commensurate heating. CW energy and heating caused no changes from the control condition.

2. Erythrocyte Membrane Studies

Red blood cells from sheep were exposed to both CW and pulsed non-ionizing electromagnetic energy. The frequencies used were at the cell membrane relaxation frequency (1 MHz) and frequencies far removed (918, 2450 MHz) in a parallel plate waveguide apparatus. This study was completed after equipment assembly and calibration, and an article is in preparation for submission to the literature. Membrane permeability was assessed in the supernate of the cell suspension by analysis for LDH and CPK. No significant difference in enzyme levels occured between control and expired cells for any frequency or modulation used. No future work is planned on this experiment.

A future objective is to use a laser light source to increase sensitivity so that individual axons, as opposed to nerves, can be studied. The microwave effect will be sought, and then the mechanism of interaction will be explored.

3. Ocular Effects

The studies of cataractogenesis have also progressed significantly. It was previously established that temperature elevation can produce putative cataract changes in the murine lens. These results have been published as a short paper in Experimental Eye Research (23). Studies done in the present report period controlled temperature, power level and modulation in a 3 factor design. The results have been published in the IEEE Proceedings of the 1980 International Symposium (24). In brief, temperature effects appear to be separable from field effects and pulse modulation differs in its ultra-structual consequencies from CW radiation of the same average power.

This aspect seeks to measure the approximate amplitude of displacement of an eye lens when exposed to high power pulsed electromgnetic radiation. The resonant frequency of the eye lens is also sought. The method of laser interferometry is being applied to this problem. The required equipment, including an isolation table, temperature regulated premature legs has been ordered and acquired. An appropriate sample holder for the lens has been consructed. Necessary electronic equipment is being constructed. This includes a very high speed (10 MHz), high sensitivity photodetector-amplifier.

Preliminary studies have resulted in stable interference patterns being obtained from a simulated (glass bead) lens, and from a real lens surrounded by air. Future objectives will be to obtain stable interference patterns from a real lens surrounded by a physiologically compatible medium. Exposure to high power pulsed electromagnetic radiation will be coupled with computerized observation of the fringe pattern.

Plans: This is a very fruitful area of research. The program will be continued to in situ exposures with high peak powers and large crest factors followed by organ culture prior to ultrastructural and biochemical analysis.

F. Behavior

Problem: What are the behavioral consequencies of microwave exposure? Can behavioral end points be used to assess motivational states and central nervous system effects of microwave energy? Can behavioral findings in transmission lines be generalized to free space exposure?

Objective: Determine if microwave exposure can serve as the unconditioned stimulus in conditioned taste aversion. Determine interactions between microwave exposure and psychotropic drugs. Establish comparability between free field and transmission line exposure.

Progress: Behavioral Assessment of Microwave Induced Alterations in Drug Susceptibility in Rats. This protocol was based on the recent findings that low average doeses of 2450 MHz pulsed microwave radiation have been shown to enhance the effects of chlordiazepoxide HCI (CDP) on fixed-interval bar press responding in rats. The first study of the protocol explored the generality

of this finding by studying microwave/drug interactive effects on variable-interval (VI) responding. Due to equipment limitations, a direct replication of the Thomas et al. experiment could not be immediately attempted. Therefore studies of microwave-drug synergy were initiated using a waveguide exposure device (25).

Two follow-up experiments have been initiated which attempt to further examine these issues relating to microwave-drug synergistic interactions. In the first experiment the same animals involved in the initial 915 MHz experiment are being exposed to 2450 MHz pulsed radiation in waveguide prior to being testing on performance on a VI schedule of reinforcement. Testing occurs after chlordiazepoxide injections to determine if pulsed 2450 MHz radiation in waveguide will alter drug-induced increases in response rates on the VI schedule.

Another experiment, using a separate group of rats, applies the same experimental design as above in investigating the effects of 2450 MHz pulsed radiation on chlordiazepoxide-induced response rate increases on a fixed-interval (FI) schedule of reinforcement. This experiment, most closely follows the procedures of the experiment described by Thomas et al (26).

Baseline and drug response testing have been completed for both these experiments and a limited amount of data have been collected examining microwave-drug combinations. Preliminary analysis of those data reveal no evidence of microwave-drug synergy in rats responding on a VI schedule of reinforcement. Similar negative results have been obtained in five out of six animals trained on a FI schedule, although one animal does appear to respond at a higher rate during drug sessions preceded by microwave exposure than in sessions preceded by sham exposure.

Plans:

1. Drug Susceptibility

The work completed so far suggests that the reported synergistic interaction between pulsed microwave fields and CD2P is either not a robust phenomenon, or that the effect cannot be duplicated in a waveguide exposure system. Objectives for FY81 include: Complete waveguide experiments at 915 and 2450 MHz, using both VI and FI behavioral test paradigms; and initiate experiments designed to test the comparability of waveguide exposure systems with free field exposure conditions.

2. Comparison of Free Field and Transmission Line Exposures

Plans were developed for a comparison of transmission line (circular waveguide) and free field (anechoic chamber) exposure conditions. Since the wavelength of the incident radiation in the transmission line is ca. 3 times longer than that in free space at the same frequency, there are significant questions about the comparability of experimental results under the two circumstances and the generality of findings in transmission line exposures.

3. Energy Absorption and Distribution

With completion of the Bldg 502/503 research facility, research on measurement technologies for energy absorption and distribution in experimental animals and models simulating humans will be resumed. These include further development of whole-body absolute calorimetry for determining average whole-body dose and the in vivo testing of the radio transparent electrodes for temperature and tissue electric fields induced by RF irradiation.

4. Behavioral and Nervous System Effects

With completion of the Bldg 502/503 research facility, research on effects of plane wave fields in various field configurations to investigate low level radiation effects on behavior and nervous system function will be resumed. Such studies will include further research on changes in excitability with high peak power pulsed fields, phenomenology of field escape behavior and effects of stressors (such as high ambient humidity) in combination with RF exposure.

G. Inactivator

Problem: A recognized technique for determining the concentrations of brain neurotransmitters at a point in time uses microwave inactivation to heat and destroy heat labile enzymes, leaving the neurotransmitters (27). An older inactivator unit has low power and is very unreliable. It was decided that a new unit was needed to replace the old unit before it completely failed.

Objective: The objective was to specify and obtain a reliable inactivator unit that would provide faster inactivation in rodents.

Progress: Such a unit was specified and after considerable searching, a suitable vendor found. The contract was written and awarded to Georgia Institute of Technology. They have completed the unit and are ready for final acceptance testing at their facility. The unit appears to fulfill the design objectives. The completed unit will be installed in the Neurochemistry lab as soon as suitable power wiring is installed.

Plans: The inactivator will be installed in FY 81 after acceptance testing of the contractor's laboratory. No further design or construction steps are anticipated.

H. Millimeter Waves

Problem: Characterize the millimeter wave region of the spectrum with respect to its interactions with biological dielectrics.

Objective: Identify narrow band absorption in the range 40-60 GHz and study ocular (corneal) effects of millimeter wave radiation on experimental animals under free-field (plane wave) conditions.

Progress: The present report periods marks the beginning of the millimeter wave program. The only progress reportable is programmatic planning plus the

generation, evaluation, and award steps on four RFQs in addition to the laboratory construction (described in Administrative Activities) notably, the developement/construction of a millimeter wave anechoic chamber.

1. Millimeter Wave Synthesizer

The six-port network analyzer discussed in this section requires a stimulus signal for measurement of the scattering parameters. Reports in the literature concerning biological effects of millimeter wave radiation indicate that these effects are very narrow band in nature. This means that the frequency of the stimulus signal must be accurately known and it must be possible to vary the frequency in very fine steps to study these effects. For this purpose, specifications were developed for a 40 to 60 GHz synthesizer which has extremely high frequency accuracy, tuning steps of 100 kHz, and can be computer controlled. A competitive procurement was conducted resulting in a contract being awarded to Georgia Institute of Technology.

2. Six-Port Network Analyzer

In order to measure the complex permittivity of biological materials at millimeter wave frequencies, it is necessary to measure the scattering parameters of a sample holder containing the material. Two basic methods of measuring scattering parameters at millimeter wave frequencies were investigated. These were the reflectometer method using a phase/amplitude receiver and the method employing a six-port network and an amplitude detector. It was found that at millimeter wave frequencies, the six-port method was the only acceptable method. This was verified by consultation with the National Bureau of Standards who had experience in this subject area. A set of specifications was developed for the six-port analyzer covering 40 to 60 GHz and a competitive procurement was conducted. Proposals were received and evaluated resulting in a contract being awarded to Georgia Institute of Technology.

3. Millimeter Wave Exposure System

The millimeter exposure system consists of a millimeter wave anechoic chamber, a millimeter wave CW transmitter and a millimeter wave focusing antenna. The chamber is a custom designed and fabricated shielded enclosure of modular construction with a minimum attenuation of 100 dB at frequencies between 35 GHz and 100 GHz. The enclosure is lined with a custom fabricated absorber material with better than -40 dB reflectivity over the range 35 to 100 GHz. Heating ventilation and air conditionings for the chamber is provided by waveguide below cut-off windows lined with aquadag to increase loss followed by absorber lined ducts. This total configuration was tested in a pilot room and the performance specifications were verified prior to building the chamber on site.

The transmitter provides 1 kW CW output power at 35 GHz. The output tube is a VA 928A 5 cavity klystron. Modulation is provided by cascaded PIN diodes to achieve an on-to-off ratio of ca. 60 dB. Spectral purity is acheived by a phase locked Gunn oscilator and harmonic rejection filters.

The antenna consists of one meter elliptical reflector with a polarization twist feed from WR28. This design offers minimal blockage and very compact

dimensions. The elliptical shape permits plane wave exposures at the second focal point with a very narrow beam width (ca. 1/2°) thanks to the freedom from blockage.

Plans: The millimeter wave synthesizer and six-port network analyzer will be combined in a computer controlled instrument for spectral scanning in FY 81. After system integration and softwave development, the spectral scanning will take place in cell suspensions and macromolecules in aqueous solution. The transmitter and antenna will be used for ocular studies.

I. Electronic Development Projects

Problem: Often off-the-shelf electronic systems cannot meet the needs of our research program as a result design and custom fabrication of electronic equipment must be provided on site.

Objective: Provide electronic support for all phases of department research activities.

Progress:

1. Assembly of a Temperature-Controlled Hot and Cold Water System.

The device is used in an experiment involved microwave exposure of eye lenses of rats. The device contains a mixing bath in plexiglass, heating rods, an electric coller, a temperature controller, a heater-strirrer, a controlled and metered flow pump, an exposure chamber in quartz mounted inside a wave guide and connecting tubes.

2. Assembly of a BASIC-Language Converter with 2K Bytes of Memory for use with a Microprocessor-Trainer.

The device permits the use of the English-like BASIC language as opposed to Machine language. Programming becomes much easier this way, and permits the use of a standard terminal.

3. Design and Construction of Eight Temperature-Controlled Exposure Waveguides.

The waveguides are used to maintain blood at a constant temperature while it is being exposed to microwaves.

4. Refurbishing of Six Portable Emergency Lanterns.

New rechargable batteries have been installed in all of them. Some required new projection bulbs, others new power cords.

5. Renovation of Eight Cumulative Recorders.

The instruments were thoroughly cleaned and oiled and new parts were installed where needed. Each device was given a complete check out and calibration.

6. Design and Assembly of a Special Photo-Amplifier to be used in a Laser study.

Design and assembly of a high-voltage regulated power supply for the amplifier. This work is still in progress and will take another month to be completed.

Plans: Design antenna test range transducers for the elliptical reflector and vertical feed. Design system interfaces for processor controller of multiple exposure tubes. Provide regulation for transmitter voltages and design safety interlock.

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21. Jacobi, J.H., Larsen, L.E.: U.S. Patent Application S/N 938,570, Calibration Method for Lumped Capacitance Measurement of Complex Permittivity at HF, VHF and UHF Frequencies.

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3. Jacobi, J.H., Larsen, L.E.: Linear FM pulse compression radar techniques applied to biological imagery. Proc. Symp. Electromagnetic Dosimetric Imagery, in press, 1981.

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3. Jacobi, J.H., Larsen, L.E.: Ridged waveguide antenna submerged in dielectric liquid. No. 4,162,500, S/N 891,256, Filing Date 29 Mar 78, Issued Date 24 Jul 79.

Claims Allowed

4. Jacobi, J.H., Larsen, L.E.: Calibration method for lumped capacitance measurement of complex permittivity at HF, VHF and UHF frequencies. S/N 938,570, Filing Date 31 Aug 78.

5. Larsen, L.E., Jacobi, J.H.: An electromagnetic method for the non-invasive analysis of cell membrane physiology and pharmacology. S/N 938,625, Filing Date 31 Aug 78.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 372	80 10 01	DD-DRA&E(AR)636

3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. OTHER INSTR'S	9a. SPECIFIC DATA: CONTRACTOR ACCESS	9b. LEVEL OF SUM
79 10 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A878	878AB	042
b. CONTRIBUTING	61102A	3M161102B501	00	148
c. CONTRIBUTING	STOG 80-7.2:3			

11. TITLE (Include with Security Classification Code)

(U) Non-auditory effects of blast overpressure

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

017100 Weapons Effects 013300 Protective Equipment 016700 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: 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 80	7	600
	CURRENT 81	2	358

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

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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., CPT(P), MC

TELEPHONE: 202 576-3014

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: JAEGER, James J., CPT(P), MSC

NAME: HESS, JEFFREY, MAJ, VC

21. GENERAL USE

Foreign Intelligency Not Considered

22. KEYWORDS (Provide each with Security Classification Code)

(U) Impulse noise; (U) Blast overpressure; (U) Human Volunteer; (U) Pulmonary Physiology; (U) Chest wall impact; (U) M198 155mm Howitzer

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief statement paragraphs identified by number. Provide last item with Security Classification Code.)

23. (U) To define the physiologic effects of blast overpressure exposure upon the human. To develop a laboratory model of blast injury. To assist in a special study of the M198 Howitzer firing the M203 charge as directed by headquarters.

24. (U) Full hemithoracic chest wall impact will be used to simulate pulmonary blast injury. Appropriate cardiopulmonary measurements and biochemical assays will be used to define the pathophysiology of impact/blast injury. Requirements for blast-thorax modelling will be defined. Surgical techniques for chronic tracheostomies, carotid loops, lymphatic drainage, and chronic instrument, implantation will be developed. Tests of ovine pulmonary function will be refined.

25. (U) 79-10-8009 In July 1980 a field study was conducted at APG during which 98 sheep were exposed to the blast field of the M198/M203 Howitzer. Lung, laryngeal, and ruminal injury were noted. Final histopathologic data is pending. 3000 serum samples were collected and are stored at -70°C pending biochemical analysis. Lovelace Foundation has completed a study of laryngeal injury in sheep for multiple blast exposure and have obtained blood and tissue samples from severely blast damaged animals and appropriate controls. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

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

Project 3E162777A878 HEALTH HAZARDS OF MILITARY MATERIEL

* Project 3M161102BS01 BLAST OVERPRESSURE

Work Unit 042 Non-Auditory Effects of Blast Overpressure

* Work Unit 148 Medical Effects of Blast Overpressure: Applied Studies

Investigators

Principal: Yancy Y. Phillips, CPT(P), MC

Associate: James J. Jaeger, CPT(P), MSC; Jeffrey L. Hess, MAJ, VC; Marvin Stein, GS-11; Earl Massey, GS-9; G. D. Ross, SSG; John Callahan, SP4; Dr. D. R. Richmond; Mr. H. Evans

The WRAIR is tasked with establishing "a research program in the pathophysiology of blast overpressure" (BOP). The project is charged with evaluating the potential for non-auditory injury of exposure to impulse noise (BOP) generated by the firing of Army weapons systems. Of immediate concern is the M198 155mm Howitzer which, when firing the M203 extended range charge, creates overpressures in the crew positions that exceed the levels suggested as safe by MIL-STD 1474. It is the long term goal of the Department of Clinical Physiology to develop generally applicable non-auditory damage risk criteria (DRC) for human exposure to BOP. This work is being coordinated with a similar program on the auditory effects of BOP being conducted at USAARL.

Exposure of sheep to the muzzle blast field of the M198/M203 Howitzer was first conducted at Aberdeen Proving Grounds (APG) in Nov 79. This pilot study involved 20 animals. In July 1980 98 sheep were exposed in predetermined positions such that subgroups were subject to approximately 15, 7.5, 3.5, and 0.5 PSI peak overpressure for 50 repeated firings. Three thousand blood samples were collected with various preservatives prior to, during and after blast exposure. All animals were necropsied and preliminary gross pathologic results show an apparent increase in the incidence of lung, larynx, and rumen abnormalities with increasing severity of BOP exposure. A final report on the histologic examination of tissue is expected during the first quarter of FY 81.

Much of the work of the BOP project has been done on contract. JAYCOR Corporation has assisted us in acquisition and analysis of blast data from the M198 and the shock tube at Albuquerque, NM. They have developed a simple fluid dynamic model of blast wave far field propagation which accounts for ground reflection and is in good agreement with field data. JAYCOR has begun a survey of biomechanical modelling of blast and impact effects on the thorax.

The Biodynamics Group of the Lovelace Institute of Inhalation Toxicology Research has carried out protocols under contract at their shock tube. They have demonstrated that laryngeal petechiae in sheep are a function of both blast number and intensity and can be produced in a majority of animals at overpressures as low as 2 PSI with 100 blasts. They have also collected blood and tissue from severely blast damaged animals for histologic and biochemical analysis at WRAIR.

In FY 81 full hemithoracic chest wall impact will be used to simulate pulmonary blast injury. Appropriate cardiopulmonary measurements and biochemical assays will be used to define the pathophysiology of impact/blast injury. Requirements for blast-thorax modelling will be defined. Surgical techniques for chronic tracheostomies, carotid loops, lymphatic drainage, and chronic instrument implantation will be developed. Tests of ovine pulmonary function will be refined for use in evaluating chronic blast exposure. WRAIR is prepared to assist in a special study of the M198 Howitzer firing the M203 charge as directed by USAMRDC headquarters.

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PUBLICATIONS:

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PROJECT 3E162777A879

FACTORS LIMITING SOLDIER EFFECTIVENESS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOC 6453	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCT	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	879AA	041
b. CONTRIBUTING	62771A	3E162771A804	00	022
c. CONTRIBUTING	STOG 80-7.2:4			

11. TITLE (Precede with Security Classification Code)

(U) Military Preventive Psychiatry

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 013400 Psychology 021900 Physiology

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: 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	7.5	446
	CURRENT 81	7.5	535

19. RESPONSIBLE DOD ORGANIZATION

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

ADDRESS:

RESPONSIBLE INDIVIDUAL

NAME: Russell, COL P.

TELEPH (202) 576-3551

21. GP... E

Foreign Intelligence Not Considered

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: Harris, LTC, J.

NAME: Knudson, CPT, K.

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Psychiatric Illness; (U) Military Adjustment; (U) Environmental Factors; (U) Social and Psychological Factors; (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 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 minim' ion of psychiatric casualties.

24. (U) The ' hods 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.

25. (U) 79 10-8' 09 Pilot field studies of women in the Army and the factors affecting their mental and ysical health have been completed. Follow-on studies are under development as ... studies of the military families' impact on the health and deployability of active duty members. A study of the impact of a mass tragedy on the helping institutions of a military post has been carried out and completed. An historical review of factors involved in generation and prevention of combat psychiatric casualties has been completed as has review of the relationship of systems of combat psychiatry to battle intensity. A field study was initiated with the 82nd ABN Div on the relationship of health factors and health perceptions to troop deployability and transition states with special emphasis on the relationship of group norms to stress responses. Studies designed to delineate patterns of drill sergeant stress and stress coping in BT/AIT have been developed and will commence in the 1st Qtr of FY81. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79-30 Sep 80.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

SA 1 NOV 65

Project 3E162777A879 FACTORS LIMITING SOLDIER EFFECTIVENESS
* Project 3E162771A804 MILITARY PSYCHIATRY

Work Unit 041 Military Preventive Psychiatry
* Work Unit 042 Military Preventive Psychiatry

Investigators.
Principal: David H. Marlowe, Ph.D.
Associate: LTC Jesse J. Harris, MSC; LTC Jacob M. Romo, MSC; LTC Norman M. Camp, MC; MAJ Robert J. Schneider, MSC; CPT Robert H. Stretch, MSC; CPT Linda K. Jellen, MSC; CPT Kathryn H. Knudson, MSC; CPT Darlene M. Vernon, 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; SP5 Calvin Cummings; SP5 James Hall; SP5 Richard Lynk; SP5 William Rigney; SP5 Richard Pickle

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 dysfunctiontional behaviors, and decrements in military performance.

Progress

Pilot field studies of women in the Army and the factors affecting their physical and mental health have been completed. Pilot studies indicate that there may be significant differences in perceived health status, perceived well being, and rates of utilization of medical facilities based upon whether or not female personnel are assigned to units and MOSs considered to be traditional for women; e.g., clerks, medical personnel, etc., or non-traditional for women; e.g., engineers, signal, etc. These studies have also highlighted the psychological and emotional problems that seem to mark a number of women soldiers in attempts to achieve status equality in predominantly male units and in dealing with parenting and marital responsibilities.

A study of the impact of a mass tragedy on the helping institutions of a military post was carried out and completed during the past year. The tragedy involved the death of a number of dependent teenagers in an automobile accident. This study has indicated that at the post in question, helping systems seem to be inadequately integrated and did not provide optimum support, information, and organization to the members of the community. It was observed, as well, that the local military community did not coalesce in support of the families of the victims of the tragedy. This study indicates that the problem of provision of integrated supports to dependents in the event of mobilization and war, particularly with the possibility of high initial casualty levels in the RDF for example, may directly affect morale and performance as well as the health of the military community.

A field study has been initiated with the 82nd Airborne Div, North Carolina, on the perceptions of health and illness and the organization of behavior in respect to such perceptions in relation to troop deployability and performance. This study will be carried out and completed during the course of FY 81.

An historical review has been completed of the relationship between unit cohesion and structure and the generation of psychiatric casualties in combat. This work utilizing data from WW II, Korea and Vietnam conflicts, demonstrates that the social support system provided by military unit cohesiveness acts as the primary mitigating factor of the breakdown of men in battle. The more militarily cohesive the unit, the smaller percentage of men who break down in battle of a given intensity. The same review also demonstrates that the primary forces conducing to break down in battle are battle intensity over time. The more intense the battle, the more rapidly personnel are at risk for behavioral breakdown. Other work also tends to indicate that there may be a need for revising treatment doctrine for psychiatric casualties based upon intensity of conflict.

In addition to the above, studies designed to delineate the patterns of stress and stress coping and their relationship to performance among drill sergeants in BT/AIT, have been developed and will commence in the early part of FY 81.

Future Recommendations and Objectives

Future research will include 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.

Papers Presented at Scientific Meetings

1. Harris, J.J., LTC. Report of President's Commission on Mental Health - Sub Panel on Women. National Association of Social Work, San Antonio, TX, Nov 1979.

2. Jellen, L.K., CPT. Social Workers' Role on the Rape Crisis Team. Biannual AMEDD Social Work Conference, Mar 1980.

3. Knudson, K.H., CPT & Kagan, Spencer. Relationships Among Affective Role-Taking and Prosocial Behavior in a Sample of Anglo American and Mexican American Children. Piagetian Conference - University Affiliated Program, University of Southern California, Los Angeles, 1980.

4. Romo, J.M., LTC & Harris, J.J., LTC. The Military Family of Occupation. AMEDD Social Work Symposium, unpublished paper presented at U.S. Army Academy of Health Sciences, San Antonio, Tx, 17-21 Mar 1980.

5. Romo, J.M., LTC. Triple Jeopardy: Ethics and the Military Social Worker. AMEDD Social Work Symposium, unpublished paper presented at U.S. Army Academy of Health Sciences, San Antonio, TX, 17-21 Mar 1980.

6. Romo, J.M., LTC. The Discovery of the U.S. Military Family: Where It's Been, Where It's At, and Where It's Going, Canadian Forces, Regional Social Work Meeting, Ottawa, Ontario, Canada, 31 Mar-2 Apr 1980.

7. Romo, J.M., LTC. Psychiatric Casualties in the Vietnam Combat Zone: Types, Triage and Treatment. Canadian Forces Combat Psychiatry Meeting, Halifax, Nova Scotia, Canada, 17-18 Apr 1980.

8. Romo, J.M., LTC & Schneider, R.J., MAJ. Disaster, Mass Casualties, and Psychiatric Reactions: Applications to the Modern Battlefield, US Army Medical Department Division and Combat Psychiatry Short Course, Monterey, California, 29 Apr-2 May 1980.

9. Vernon, D.M., CPT. Factors Affecting the Health of Women in the Army. Women on the Military Panel, First National Conference on Women in Crisis, Washington, DC, Jun 1980.

Publications

1. Knudson, Kathryn H.M. & Kagan, Spencer. Relationships among affective role-taking and prosocial behavior in a sample of Anglo American and Mexican American Children. In Piaget and the Helping Professions (Ninth Annual Proceedings from 1980 Conference). University of Southern California Bookstore, in press.

2. Knudson, Kathryn H.M. & Kagan, Spencer. Differential development of affective role-taking abilities and prosocial behavior. Journal of Genetic Psychology, in press.

3. Stretch, R. & Figley, C. Beauty and the Boast: Predicators of Interpersonal Attraction. Psychology, 17(1), 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOC 6454	80 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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	879AA	042
b. CONTRIBUTING	62771A	3E162771A824	00	047
c. CONTRIBUTING	SCOO 80-C-[illegible]			

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: 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	5.5	245
FISCAL YEAR CURRENT 81	5.5	329

19. RESPONSIBLE DOD ORGANIZATION

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ADDRESS: Washington, DC 20012

RESPONSIBLE INDIVIDUAL

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TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

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Division of Neuropsychiatry

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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.

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) 79 10-80 09 A field station has been established at Ft Bragg, NC, and data is being gathered on the relationship of outpatient medical contact to troop deployment. Data gathering is presently under way to determine patterns of use of psychotropic medication in the Vietnam conflict. Materials have been gathered on two Divisions. Analysis of the past and present patterns of psychiatric disease, psychosomatic illnesses and behavioral dysfunctions is under way utilizing IPDS and other DA reporting systems, as are studies analyzing the psychological and health problems of women in the Army, and the epidemiology of neurological syndromes following penetrating wounds and studies of factors involved in suicide of military personnel. Cohort analysis with selected accessions of FY72 personnel to determine precursors of dysfunctional behavior continue. Studies are under development: to determine patterns of usage of Army medical facilities; and of Vietnam veterans still serving in the Army to determine differential impacts of support systems on health outcomes. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79-30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3E162777A879 FACTORS LIMITING SOLDIER EFFECTIVENESS
* Project 3E162771A804 MILITARY PSYCHIATRY

Work Unit 042 Military Psychiatric Epidemiology
* Work Unit 047 Military Psychiatric Epidemiology

Investigators:
Principal: David H. Marlowe, Ph.D.
Associate: LTC Jesse J. Harris, MSC; LTC Jacob M. Romo, MSC; LTC Norman M. Camp, MC; MAJ Robert J. Schneider, MSC, MAJ Robert E. Blaik, MC; CPT Robert H. Stretch, MSC; CPT Linda K. Jellen, MSC; CPT Kathryn H. Knudson, MSC; CPT Darlene M. Vernon, 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; SP5 Calvin Cummings; SP5 James Hall; SP5 Richard Lynk; SP5 William Rigney; SP5 Richard Pickle

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 fiscal year, a field station was established at Ft Bragg, North Carolina. Extensive data gathering on the relationship of presenting symptoms and patterns of outpatient medical contact to patterns of deployment of line units was begun. Data is being gathered on one Infantry Brigade and selected units in divisional support elements. Posting and organization of data for ADP is presently under way. It is anticipated that this data collection will continue throughout the coming fiscal year. Data gathering is presently under way to determine patterns of use of

psychotropic medication during the Vietnam conflict and the relationship of such patterns to combat intensity and the distribution of non-combat casualties generated by injury and disease. Materials on two divisions have thus far been gathered at the National Records Center.

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.

Studies of suicide among Army active duty members continue. Suicide remains a major cause of death among active duty Army personnel in the past and accounting for approximately 10% of all soldier deaths. The most significant change thus far found has been an increase in the annual rate of suicides among female personnel. Studies analyzing the psychological and health problems of women in the Army, based upon selective cohorts accessed in 1977-78, continue more slowly than had been hoped because of ADP difficulties. Studies of the epidemiology of neurological syndromes following penetrating wounds in combat melding Army and VA data continue as well. Cohort analyses of accessions of FY 72 personnel designed to determine the outcomes of military careers and the precursors of dysfunctional behavior and performance disruption continue as well. Studies are presently under development designed to determine patterns of usage of Army medical facilities and the impact of "high usage" populations on such facilities and the medical system as a whole.

The development of an instrument for the examination of perceived social supports on the part of military personnel was completed during the past fiscal year.

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 battle field of the present and the future.

Developments in the course of the next fiscal year will focus particularly on the relationship between unit cohesion, patterns of well being, and health outcome and the utility of medical data for describing the state of cohesiveness of 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.

Papers Presented at Scientific Meetings

1. Datel, W.E. Fort Ord's Merit-Reward System: a contingency management program in basic combat training. (Paper presented at symposium entitled "Applied Behavior Analysis in the Military," American Psychological Association meetings, Montreal, Quebec, Canada, 3 September 1980.) Alexandria, Virginia: Defense Documentation Center, 1980. Document AD No. A 088 475.

2. Blaik, R., MAJ & Genser, S., LTC. Perceived Social Support & Risk of Depression, American Psychiatric Association Meetings (New Research) San Francisco, CA. 4-9 May 1980.

3. Blaik, R., MAJ & Genser, S., LTC. Perception of Social Support as a Risk Factor in Depression, Society for Epidemiological Research Meetings, Minneapolis, MN, June 18-20, 1980.

4. Blaik, R., MAJ & Genser, S., LTC. Social Support Satisfaction: Scale Development, American Psychological Association Meetings, Montreal, Canada, 1-5 September 1980.

5. Blaik, R., MAJ & Genser, S., LTC. Social Support, Life-Events and Depression, American Psychological Association Meetings, Montreal, Canada, 1-5 September 1980.

6. Blaik, R., MAJ & Genser, S., LTC. Stress (Support-Stressors) and Depression, American Psychiatric Association Meetings, New Orleans, LA, 9-15 May 1981.

7. Mann, M.R. & Rothberg, J.M. Reliability Methodology to Think About Human Behavior, Joint National Meeting The Institute of Management Sciences/Operations Research Society of America, May 4-7, 1980.

Publications

Datel, W.E., Jones, F.G., & Esposito, M.E. Suicide in United States Army personnel, 1977-1978. Military Medicine, in press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6457	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	879AC	043
b. CONTRIBUTING	62771A	3E162771A804	00	048
c. CONTRIBUTING	STOG 80-7,2	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: NA
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 80	5.0	347
CURRENT FISCAL YEAR 81	5.0	507

19. RESPONSIBLE DOD ORGANIZATION

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

RESPONSIBLE INDIVIDUAL
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TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Hegge, F.W. Ph.D.
TELEPHONE: 202-427-5521
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Graeber, MAJ R.C.
NAME: Thorne, D. Ph.D.

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) Performance; (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) 79 10 - 80 09 Long term laboratory studies have been initiated to simulate rapid deployment across time zones and to improve the effectiveness of countermeasures shown to be beneficial in field studies for reducing the effects of fatigue. Completion of the first experiment has resulted in development of a microcomputer assisted test battery which is sensitive to the influence of circadian rhythms and sleep loss on cognitive performance. Use of this test battery plus continuous physiological monitoring of circadian rhythms is currently underway in a second experiment involving a simulated 6-hr. eastward time zone shift. Data collection has been completed on civilian firefighters and fire/rescue dispatchers in relation to the stress of occupational life threat and shiftwork. Heart variability has been found to interact in a circadian fashion with the impact of stressful events. Further analyses are underway to determine the usefulness of the measure as an index of stress in military populations. Studies of rhythmic aspects of affect, activation, and verbal and auditory perception are underway in an attempt to clarify the functional rhythms of cerebral hemispheric laterality. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 OCT 79 - 30 SEP 80.

(Available to contractors upon additional approval)

Project 3E162777A879 FACTORS LIMITING SOLDIER EFFECTIVENESS
* Project 3E 62771A804 MILITARY PSYCHIATRY
Work Unit 043 Military Stress: Circadian and Ultradian Factors
* Work Unit 048 Military Stress: Circadian and Ultradian Factors

Investigators.
Principal: Frederick W. Hegge, Ph.D.
Associate: 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); John Sapp, M.A.; Helen Sing, M.S.

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

Laboratory simulation studies of rapid deployment across time zones have been initiated to confirm and improve the effectiveness of countermeasures shown to be beneficial in two previous field studies of troop deployments to Europe. Emphasis has been placed on the assessment of cognitive performance shifts through use of a microcomputer automated test battery. Initial experiments required subjects living on local time to complete a variety of tests every 3 hrs. during the waking day for 5 days. Those tests most sensitive to time of day were combined into a final test battery to examine disruptions of the circadian system including sleep loss. These tests involve a variety of cognitive abilities which span the performance requirements demanded of soldiers and commanders in operational settings. A second series of experiments has begun in which use of this battery is combined with the continuous monitoring of physiologic circadian rhythms and rest-activity patterns. Groups of four subjects live for two weeks in an isolation facility in which all time cues are controlled by the investigator. Following four days of baseline U.S. measurements the subjects undergo a 6-hr. advance of their daily routine to simulate the time zone shift typically experienced during deployments to Europe. Adjustment to the circadian phase shift is monitored for the remaining ten days to determine the course of "post-flight" adaptation.

Two other laboratories have been assembled to determine the contribution of cerebral hemispheric activation patterns to time related variations in military performance. Complex verbal or patterned stimuli may be presented either visually or auditorally in a manner which assures initial reception by a chosen cerebral hemisphere. Perceptual tasks have been

selected on the basis of known differences in performance by the dominant and non-dominant hemispheres. Related data regarding circadian variations in mood, subjective activation, and bilateral motor activity suggest that the two hemispheres may function out of phase with each other.

Data collection has been completed on civilian firefighters and fire/rescue dispatchers in relation to the stress of occupational life threat and shiftwork. Heartrate variability has been found to vary in a circadian fashion with greater variability appearing during the usual span of daily rest. The impact of stressful events on cardiac function depends on the phase of the circadian cycle at time of impact.

Future Objectives. Immediate goals are directly related to ongoing laboratory research and data analyses. The deployment simulation facility will enable us to determine the effectiveness of different chronobiologic countermeasures in reducing the deleterious effects of rapid deployment by air over multiple time zones, thus leading to the development of recommendations for use by the RDF in future tactical operations. Future efforts will focus on ameliorating the combined effects of deployment and quasi-continuous operations. Analyses of firefighter heart-rate variability data will proceed to determine the usefulness of this measure as an index of stress in military population. Initial studies of cerebral hemispheric activation will investigate whether the two halves of the brain differ in their level of functioning at different times of the day. Implications regarding performance variations on related military tasks will then be pursued.

Presentations and Publications

1. Graeber, R.C., Cuthbert, B.N., Sing, H.C., Schneider, R.J., and Sessions, G.R. Rapid transmeridian deployment: Cognitive performance and chronobiologic prophylaxis for circadian dyschronism. Army Science Conference, USMA, West Point, N.Y. 17-20 June 1980.

2. Graeber, R.C. Implications of chronobiologic countermeasures for pilot fatigue and circadian dyschronism. NASA Workshop on Pilot Fatigue and Circadian Desynchronosis, San Francisco, CA. 26-28 August 1980.

3. Redmond, D., Sing, H., and Hegge, F. Multiple complex demodulation and the analysis of biologic time series. In F. M. Brown and R.C. Graeber (Eds.), Behavioral Aspects of Biological Rhythms. Erlbaum Assoc.: Hilldale, N.J., in press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

AGENCY ACCESSION: DA OC 6452

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 EFFORT
79 10 01	D. Change	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	879AC	044
b. CONTRIBUTING	62771A	3E162771A804	00	044
c. CONTRIBUTING	CARDS 114E			

11. TITLE (Precede with Security Classification Code)

(U) Neuroendocrine Response to Military Stress

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 012600 Pharmacology 002300 Biochemistry
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: N/A EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PRECEDING 80	3	318
CURRENT 81	3	523

19. RESPONSIBLE DOD ORGANIZATION
NAME: Walter Reed Army Institute of Research
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TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION
NAME: Walter Reed Army Institute of Research
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NAME: Meyerhoff, J.L., M.D.
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Holaday, J.W.
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) Neurotransmitters; (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) 79 10 - 80 09

Studies with hypophysectomized or adrenalectomized rats suggest that pituitary endorphins are involved in mediating the depression of cardiovascular systems in shock states. These studies also indicate a central nervous system site of action for naloxone's therapeutic effects in both hemorrhagic, endotoxic and spinal shock. In spinal shock, the decrease in cardiac performance appears to be vagally mediated. Preliminary studies indicate that cholinergic stimulation produces rapid and marked increases in plasma levels of beta-endorphin. We find that insertion of a new human subject into a competitive working group produces marked changes in that subject's testosterone levels, which correlate with degree of success in competition for a preferred work/rest schedule. For technical report, see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Project 3E162777A879 FACTORS LIMITING SOLDIER EFFECTIVENESS
* Project 3E162771A804 MILITARY PSYCHIATRY
Work Unit 044 Neuroendocrine Response to Military Stress
* 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
Bates, V.E., M.D., MAJ, MC
Mougey, E.H., M.S.
Pennington, L.L., B.S.
Faden, A.I., M.D., MAJ, MC

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 shock, extremes of heat and cold, other physical stressors, continuous performance requirements, desynchronization of circadian rhythms, as well as psychological stressors such as competitive interaction in groups. Studies are carried out in laboratory, clinical or military operational settings as appropriate. Initial goals must be the identification of endocrine systems which are activated by the various stressors. Subsequent goals include identification of those endocrine systems which when activated, increase, or decrease the probability of physical and/or psychiatric breakdown. Ultimate goals include identification of physiological or environmental manipulations which may decrease the potential for breakdown.

Progress:

Therapeutic role of naloxone in shock studies. Studies with adrenalectomized and hypophysectomized rats were performed to ascertain if the endorphins responsible for the pathophysiological effects in shock states were of pituitary or adrenal origin. Adrenalectomy potentiated shock sensitivity, elevated circulating beta endorphin concentrations, and adrenalectomized rats subjected to shock responded to the therapeutic effects of parenterally administered naloxone. By contrast, hypophysectomized rats subjected to shock failed to respond to naloxone injections. Thus, data are consonant with the hypothesis that pituitary endorphins are involved in the depression of cardiovascular systems in shock states. These studies further indicated a CNS site of action for naloxone's therapeutic effects in both hemorrhagic and endotoxic shock. In the rat and cat spinal shock model, the effects of naloxone were also shown to be centrally mediated. Moreover, since vagotomy and atropine-like drugs blocked the improvement in blood pressure produced by naloxone, we believe that endorphins are affecting the decreased cardiac performance in spinal shock via vagal cholinergic efferents to the heart. In additional studies, naloxone was shown to diminish the extent of the paralysis resulting from spinal cord injuries by improving spinal cord blood flow and thereby preventing ischemic death of critical spinal cord nerve cells. To ascertain the types of opiate receptors involved in mediating the pathophysiological effects of shock, we conducted studies with naloxazone, a long lasting opiate antagonist that permanently blocks high-, as opposed to low-affinity opiate receptor

sites. Since naloxazone blocked the pharmacological effects of morphine but did not alter shock susceptibility or reverse shock hypotension, we concluded that endorphins primarily affect the cardiovascular system via low-affinity opiate receptors.

Role of beta endorphin in stress response and physiology regulation. In collaboration with Dr. Bruce Cuthbert of the Physiology and Behavior Branch, we have studied the effects of beta endorphin in the awake, behaving primate. The results are similar in the two animals studied to date. Beta endorphin infusion produced a transient increase in blood pressure, followed by a more pronounced and sustained decrease. The magnitude of this effect was dose-dependent, and was larger for systolic than for diastolic pressure. Dose-related increases in heart rate were observed. Respiration rate was only slightly and transiently affected in either monkey. Behavioral responding was slowed to a small extent for a maximum of five to seven minutes.

Cholinergic interactions with endocrine systems. Cholinomimetic agents have been shown to be potent releasers of pituitary corticotrophins (1). Inasmuch as corticotrophins and endorphins may share a common biochemical precursor (3-5), it is reasonable to expect that cholinomimetics or cholinesterase inhibitors might release endorphins as well as corticotrophin. Moreover, endorphins released in very large amounts might reach brain regions which influence respiration such as the ventral surface of the medulla or the floor of the fourth ventricle. In preliminary studies we have demonstrated that administration of oxotremorine (a muscarinic agonist), nicotine bitartrate (a nicotinic agonist), or physostigmine (a cholinesterase inhibitor) will produce marked and rapid elevations of plasma levels of beta endorphin.

Psychoendocrine studies of stress in small group interactions. In a collaborative study at Johns Hopkins University, we have found that when an uninitiated volunteer is introduced into an established working group, his urinary testosterone increased or decreased over baseline values in relationship to his success or failure to gain access to a work station according to a schedule that was least disruptive to his previous wake-sleep routine. The results clearly show that testosterone levels are not fixed, but rather are sensitive to changes in environmental conditions. The relationships are similar to those observed in studies of lower primate social behavior, and they suggest a continuity of behavioral biological processes across species, including man.

Future objectives:

Future research will be directed toward the elucidation of other pharmacological and physiological agents in the therapeutics of circulatory shock states. Related studies will examine the endogenous opiate circuitry involved in the maintenance of cardiovascular and thermoregulatory homeostasis. Experiments are planned to measure the pituitary beta endorphin response to environmental stress and to examine the mechanisms of endorphin-induced hypotension. In addition, we will compare the respiratory and cardiovascular effects of equianalgesic doses of morphine and beta endorphin; beta endorphin might provide pain relief without respiratory depression. The studies on hormonal effects of social interaction will be extended to include the effects of replacement of members of a group while the group is engaged in cooperative or competitive tasks.

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Presentations

Emurian, J., Brady, J.V., Mougey, E.M., and Meyerhoff, J.L. "Testosterone Responses to a Change in Group Composition and Size", Annual Research Staff Conference, Department of Psychiatry and Behavioral Science, Johns Hopkins University, Baltimore, Maryland. May 1980.

Holaday, J.W. "Endorphins in the Pathophysiology of Shock", Grand Rounds, Department of Surgery, University of Iowa Hospital and Clinics, Iowa City, Iowa. Oct. 1979.

Holaday, J.W. Departmental Seminar, Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA. Mar. 1980.

Holaday, J.W. "Naloxone Reverses the Pathophysiology of Shock Through an Antagonism of Endorphin Systems", given at the "Neurosecretion and Brain Peptides" Symposium, Sea Island, GA. Mar. 1980.

Holaday, J.W. "Implications for Endorphins in Psychiatry and Neurology", Military Psychiatry Symposium, William Beaumont Army Medical Center, El Paso, TX. Mar. 1980.

Holaday, J.W. Grand Rounds, Department of Endocrinology, Walter Reed Army Medical Center, Washington, DC. April 1980.

Holaday, J.W. "Endorphin Involvement in the Pathophysiology of Shock", Departmental Invitational Lectureship, University of Arizona Health Sciences Center, Tucson, AZ. May 1980.

Holaday, John W. "Endorphins and Shock", presented at the Gordon Conference, "The Mode of Action of Opiates", Plymouth State College, Plymouth, NH. June 1980.

Holaday, J.W. "Endorphin Involvement in Electroconvulsive and Circulatory Shock", Lectureship in biological psychiatry, Biological Psychiatry Branch, National Institutes of Mental Health, Bethesda, MD. July 1980.

Holaday, J.W. "Endorphin Involvement in the Pathophysiology of Shock and Trauma: Therapeutic Effects of Naloxone", Satellite Symposium of the International Congress of Physiology - "Homeostasis in Shock and Injury", Budapest, Hungary. July 1980.

Holaday, J.W. "Endorphins in Shock and Trauma", Grand Rounds, Department of Anesthesiology, Technischen Universität München, Munich, Germany. July 1980.

Holaday, J.W. "Pathophysiological Role of Endorphins in Shock and Trauma", institute seminar, Max-Planck Institute of Psychiatry, Munich, Germany. July 1980.

Holaday, J.W. "Endorphins in Shock - Therapeutic Effects of Opiate Antagonists", institute seminar, Langley Porter Neuropsychiatric Institute, University of California, San Francisco, San Francisco, CA. April 1980.

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1. Holaday, J.W., Belenky, G.L., Faden, A.I. and Loh, H.H. Possible function of beta endorphin, in: Neuro-Psychopharmacology, ed. B. Saletu et al, Pergamon Press, Oxford, p. 503-514. (1979).

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3. Belenky, G.L. and Holaday, J.W. Electroconvulsive shock (ECS) in rats: Naloxone modification of post-ECS behaviors provides evidence for functional endorphin release. In: Endogenous and Exogenous Opiate Agonists and Antagonists, edited by E.L. Way, Pergamon Press, New York, p. 479-482. (1979).

4. Holaday, J.W. and Faden, A.I. Naloxone improvement of shock pathophysiology: Evidence for opiate receptor involvement. In: Endogenous and Exogenous Opiate Agonists and Antagonists, edited by E.L. Way, Pergamon Press, New York, p. 479-482. (1979).

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14. Belenky, G.L. and Holaday, J.W. Electroconvulsive shock (ECS) in rats: naloxone modification of post-ECS behaviors provides evidence for functional endorphin release. Int. Narcotics Research Conf. (Falmouth, Mass., June 1979).

15. Holaday, J.W. and Faden, A.I. Naloxone reverses the pathophysiology of shock through an antagonism of endorphin systems. Meeting on Neurosecretion and Brain Peptides. (Sea Island, GA, March 1980).

16. Holaday, J.W. Implications of endorphin research in neurology and psychiatry. AMEDD Military Psychiatry Symposium. (El Paso, TX, April 1980).

17. Holaday, J.W. and Faden, A.I. Endorphins act through vagus nerves to depress cardiovascular function in spinal shock. Abstracts, Fed. Proc. 39(3). 1980.

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19. Holaday, J.W. and Faden, A.I. The role of endorphins in the pathophysiology of shock and the therapeutic benefit of opiate antagonists. Army Science Conference. (West Point, NY, June 1980).

20. Holaday, J.W. and Faden, A.I. Endorphin involvement in the pathophysiology of shock and trauma: Therapeutic effects of naloxone. Homeostasis in Injury and Shock. (Budapest, Hungary, July 1980).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6450	80 10 01	DD-DR&E-AR(...)

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
79 10 01	D. Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. MO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	879AC	045
b. CONTRIBUTING	62771A	3E162771A804	00	041
c. CONTRIBUTING	STROG 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 Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: 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	3	124
	CURRENT 81	2	202

19. RESPONSIBLE DOD ORGANIZATION

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

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TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

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Division of Neuropsychiatry

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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 (Furnish 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) 79 10 - 80 09 Major findings: Behavioral studies have revealed that moment to moment heart rates changes can be correlated with the level of attention directed to the task. Studies of the stomach indicate that gastric acidity is phasic in nature and that these oscillations may correlate with the rest-activity cycle. Studies of the behavioral and physiologic effects of endogenous opiates indicates minor changes in behavior accompanied by longer lasting heart rate increases and blood pressure decreases. Respiration and body temperature were unaffected. In contrast, morphine also reduced respiration and had a much more marked effect on behavior. Systems to measure analgesia in monkeys were established. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 79-31 Sep ...

* Available in contractors work unit summary approved

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

Project 3E162777A879 FACTORS LIMITING SOLDIER EFFECTIVENESS
* Project 3E162771A804 MILITARY PSYCHIATRY

Work Unit 045 Behavioral Variables in Autonomic Function and Disease in Military Personnel

*Work Unit 041 Behavioral Variables in Autonomic Function and Disease in Military Personnel

Investigators
Principal: Hursh, MAJ S.R.
Associate: Faden, MAJ A.I., Cuthbert, CPT B.N., Hamilton, CPT B.E.

1. Problem and objectives: Military personnel are subjected to unique forms of stress that alter behavior and the nervous system and that can foster disease. The objective of this work unit is to develop and use laboratory models to define and describe the organ system responses and disease states caused by stressors in the military environment.

2. Progress: Stress and cardiovascular system. The cardiovascular stress laboratory has completed transition to a totally computer based system. A reliable and non-invasive method for measuring heart rate was devised. Behavioral studies have revealed that moment to moment heart rate changes can be correlated with the level of attention directed to the task. For example, in a task requiring a subject to estimate a 60-sec time interval, the level of heart rate preceding the estimate can indicate whether the response will be correct; the higher the level, the more accurate the response. The high heart rate suggests heightened attention or arousal. This finding helps us link successful behavior to somatic indications and may be a useful tool for the study of continuous performance and sleep disturbance, two military specific stressors.

Stress and the gastro-intestinal system. A computerized laboratory for the study of the relationships between environmental events and the gastro-intestinal system was established. Methods were developed to implant and protect recording transducers, to allow for twice weekly endoscopic examinations, and to promote the healthy restraint of the subjects. Basal determinations of gastric dynamics measured remotely are now in progress. In collaboration with the Division of Medicine, studies of migrating myoelectric discharge along the intestinal tract are underway using an automated system.

Endogenous opiate effects on physiology and behavior. Additional animals have been prepared to study the effects of beta-endorphin, an endogenous opiate, on physiology and behavior. Blood pressure decreased and heart rate increased with little or no effects on behavior, respiration or body temperature. Morphine, on the other hand, caused dosed related decreases in blood pressure and respiration, as well as disrupting behavior much more than beta-endorphine. Phentolamine mimics the physiological effects of beta-endorphin but had no effect on behavior. As an extension of this work a method has been developed to assess the analgesic effect of beta-endorphin using nonhuman primates and a self-report system.

Long distance deployment and "jet-lag". In collaboration with the Department of Military Medical Psychophysiology, a human test facility has been completed that is capable of comfortably housing subjects with sufficient isolation that simulations of day-night phase shifts (jet-lag) can be performed. The

effects of these shifts on performance can be automatically and continuously monitored using a newly implemented computer test system. In addition to performance measures, procedures have been devised for the analysis of urinary hormones and electrolytes partly under outside contract and partly in collaboration with the Department of Nephrology.

3. Future objectives. Studies of cardiovascular changes during performance will be extended to observations of changes around the clock, during sleep deprivation, and during long periods of vigilance in visual monitoring tasks. Studies of gastrointestinal changes during stress and performance will reach the point of producing informative data. These initial tests wll be preliminary and exploratory in nature, setting the extent and limitations of the controlling relation between environmental stress and gastric activity. Studies of the effects of beta-endorphin on behavior will be completed during the second quarter. Studies of "jet-lag" will be concentrated in the first two quarters with data analysis in the third quarter and re-evaluation of future directions in the fourth quarter. Another field study of "jet-lag" may be required but is doubtful.

Paper Submitted

Cuthbert, B.N., Kristeller, J., Simons, R., Hodes, R., and Lang, P.J. Strategies of Arousal Control: Biofeedback, Meditation, and Motivation. Submitted to Journal of Experimental Psychology.

Presentation

Cuthbert, Bruce N., and Sodetz, Frank J. Interactions of operant and classical conditioning paradigms in rhesus monkeys. Poster paper presented at the convention of the Society for Psychophysiological Research, Cincinnatti, October 1979.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOC 6470	80 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
79 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	879AA	046
b. CONTRIBUTING	62771A	3E162771A804	00	046
c. XXXXXXXXX	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 Physiology 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
7710	Cont'	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	2	40
FISCAL YEAR CURRENT 81	3	60

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Walter Reed Army Institute of Research
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TELEPHONE: (Avn 435) 740/626
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Manning, MAJ F.
NAME: Schneider, MAJ R.

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) 79 09-80 09 During this period efforts centered on three areas identified by commanders as important concerns within the European Theater. These were (a) "psychological autopsies" of drug overdose victims, (b) unit cohesion and peacetime performance of combat arms battalions, and (c) community consultation studies. Investigations of drug overdose incidents indicate neither personal nor unit characteristics distinguished victims from the general population. No pattern of organizational climates was evident; both task and people oriented commanders with either prominent or low key drug suppression programs experienced overdose incidents. While there were no clues on preventing drug overdoses, per se, measures to prevent death by overdose are available and can be easily implemented. Studies of unit cohesion resulted in a reliable measurement instrument that can be used successfully by minimally trained personnel serving on the inspector general site visits. Scale scores also correlate well with various measures of unit performance in garrison. Community consultation studies represent exploratory work defining the nature and function of military communities and their relation to individual health and well being.

*Available to contractors upon originator's approval

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

Project 3E162777A879 FACTORS LIMITING SOLDIER EFFECTIVENESS
* Project 3E162771A804 MILITARY PSYCHIATRY
Work Unit 046 Medical Factors Limiting Soldier Effectiveness
*Work Unit 046 Medical Factors Limiting Soldier Effectiveness

Investigators.

Principal: Ingraham, MAJ L.H.
Associate: Manning, MAJ F.J.

Description

This field unit, stationed in West Germany with the U.S. Army Europe and Seventh Army, identifies and investigates physical, psychological, social, and organizational factors bearing on individual and unit performance and combat readiness. During this period efforts centered on four areas identified by commanders as important concerns within the European theater.

DRUG OVERDOSES AMONG SOLDIERS OF U.S. ARMY EUROPE

Problem. This exploratory project investigated three fundamental questions: (1) Are there personalities or social environments that make death by overdose a higher risk for some people than for others? (2) What is the significance of an overdose casualty for either assessing current drug use within an Army unit or predicting future use? (3) Can death by overdose be prevented?

Progress to Date. Part I of the study consisted solely of an intensive records screen involving the files of the Casualty Branch of First Personnel Command. Available materials on all active duty deaths for 1978 and 1979 were reviewed, and a total of 91 cases identified as "overdoses," that is to say, these were cases in which death was the direct result of injection or ingestion of an intoxicant, legal or illegal. Three facts emerged from this phase of the study: first, USAREUR drug deaths are quite different from U.S. civilian drug deaths in terms of victim characteristics and circumstances; second, the victims did not differ markedly from USAREUR's junior enlisted population on the variables available for comparison; last, far more information than routinely recorded would be necessary for suggestions on prevention. This information was collected in Part II, which consisted of on-site investigations involving interviews with spouses, friends, associates, and leaders of the casualty as well as looking at medical and administrative records. The object was to reconstruct, insofar as possible, the environment and personality of the casualty and the events surrounding the overdose.

Subjects in Part II consisted of all active duty soldiers put on the seriously ill or very seriously ill list at any USAREUR medical facility with an initial diagnosis of including suspected overdose of a drug abuse (N=21) during the period 15 June 1978 to 1 June 1980. An additional 16 cases in which USAREUR soldiers died before reaching a medical facility were also investigated, these cases being accepted only when an investigator was immediately available. The total was thus 37. Results showed the following:

The typical victim was male, SP4, 20-24 years old, single, a high school graduate, in excellent health, serving his first tour beyond AIT. Equally often black as white, in Germany 18 months, liked his job, and was seen as a better-than-average worker by his supervisors and was inclined toward reenlistment, despite having received at least one Article 15. Although almost half the casualties were or had been enrolled in CDAAC's, better than 60% were suspected of hard drug use by only their closest friends. Less than one third could be called barracks rats, better than half played on one or more unit sports teams, over 60% had a steady German girlfriend, and one third were seen as leaders among their peers. Only 10% of the victims did not use drugs before entering the Army, though only one in seven admitted to heroin use before reaching Germany. Only 4 or 5 out of 37 could be called addicts, but only one was not an experienced heroin user. Use itself was typically off duty with a small group of friends.

Excepting suicides or suicide gestures, the drug involved was invariably heroin, although the typical case followed partying of some sort, with alcohol the primary intoxicant, followed by a "night cap" of heroin in a barracks room or a girlfriend's apartment. Most often the heroin was injected, but in 5 cases it was inhaled. We have no evidence, including eye witnesses in half the cases, that the dose was larger, stronger, or more adulterated than the victim's usual dose. Sometime in the next two hours, the victim goes to bed or is put to bed, vomits during the night, and chokes on his stomach contents. If discovered before dying, he is subjected to a round of dubious buddy aid that may last 2 or 3 hours before competent medical aid is summoned. The latter may or may not be given helpful information about the victim's condition and how he got that way.

Unit climate and discipline ranged from a model of paternal concern to something resembling the stereotype of the French foreign legion. Possibly of greater significance was the fact that only 13 of 31 units had not seen a change in battalion commander, company commander, or first sergeant in the six months prior to the overdose, and in only one third of the cases was membership in the victim's section, platoon or company a source of pride for its junior enlisted.

In conclusion, the data unfortunately indicated that there is no unique overdose personality, nor is there a type of unit environment closely associated with drug overdoses. In fact, the evidence more clearly suggests we abandon 3 myths which dominate thinking in this area: that heroin use invariably degrades job performance; that an overdose death is a clear indication that drug use in a unit is out of control and/or leadership is inadequate; and that what we call "overdoses" are the result of extra potent, or perhaps extra-tainted heroin.

Future Recommendations. Data collection is complete, and future efforts in this area will focus on dissemination of results.

UNIT COHESION AND PEACETIME PERFORMANCE OF COMBAT ARMS BATTALIONS

Problem. Although it is now widely conceded that unit cohesion is the primary deterrent to combat psychiatric casualties, its importance in peacetime has been a topic of far less agreement. The present study, undertaken at the request of the VII Corps deputy commander, aims (a) to develop a measure of unit cohesion which could serve as a useful tool in the Corps IG's assessment of tactical units, and (b) to investigate the relationship, if any, between such measures of unit cohesion and more traditional measures of unit performance.

Progress-to-Date. A total of 16 battalions have been surveyed to date, in conjunction with unannounced IG inspections. Members of the inspection team have interviewed cross-sections of each battalion, asking a standard series of questions on unit life and relations with superiors, subordinates, and peers. Interinterviewer reliability has consistently been high (0.90) and face validity has been more than acceptable (correlations of unit scores with subjective ratings of the IG teams have been high, and such renown hign-cohesion units as the armored

cavalry have shown far and away the highest scores). Item analysis is currently being conducted, to eliminate non-descriminating items and/or rank or position subgroups.

Correlations with traditional performance measures have ranged from non-existent (in the cases of administrative discharges and disciplinary actions) to highly significant (results of an operational readiness test (alert), physical fitness testing, crime rate, reenlistments and performance on a standardized field exercise). The poor intercorrelation of the performance measures themselves preclude strong relations between the cohesion measures and all the performance measures, but the correlation between cohesion and the average standing of the units in 9 major areas of performance was 0.80.

The 3 high cohesion units were reliably differentiated from the 3 lowest by only 5 questions. All five were from those directed to the junior enlisted ranks, and four of the five pointed to beneficial effects of increased informal interaction across ranks.

Future Recommendations. Data collection continues, and future plans include analysis of individual scores for the influence of demographic variables (sex, race, age, rank, type of unit, time on the job, etc). Further in the future is the possibility of organized intervention to build cohesion in low scoring units, and/or the extention of the methodology to the level of military communities.

EMPLOYED WIVES OF U.S. ARMY MEMBERS IN GERMANY FARE BETTER THAN UNEMPLOYED

Problem. A considerable body of literature indicates that rates of mental illness, particularly depression, tend to be higher for women than men. However, this apparently holds true only among the married; and some have suggested that this implies some "protection" by employment outside the home. Research findings thus far have been equivocal, but resolution of this question is of no small importance for an increasingly married Army, amid the frequent moves required of an Army family make employment outside the home particularly difficult.

Progress to Date. Questionaires were distributed to 111 soldiers and 111 wives from a single field artillery battalion stationed in a small city in central West Germany.

Basic demographic information was solicited, although no names were required and participants were advised that cooperation was purely voluntary (in fact, only 49% of questionaires were completed). Other questions inquired about aspects of daily living, marriage and family living, and problem-solving in their military community.

Of 18 measures on which employed wives' scores differed from those of wives not working outside the home, 15 pointed to better adjustment or more satisfaction among the wives with paying jobs ($p=0.008$). The soldiers themselves shared a similar pattern of scores, i.e., those with wives working outside the home were more satisfied. These differences were not explicable in terms of differences in age, education, months in Germany, type of residence (Army or German), or proximity to Army shopping and medical facilities. Interview data instead pointed to the importance of outside employment in the development of social support networks.

Future Recommendations. Data collection is complete, and future efforts in this area will focus on dissemination of results.

COMMUNITY CONSULTATION STUDIES

Problem. Community consultation research began at the invitation of two community commanders in Europe who asked the research team to visit their facilities and make recommendations for improvements. Aside from performing a service to the Army, the research objectives were two. First, to explore the organization and function of military communities in Europe as they impact on general health and well being. There is considerable literature showing the relation of social factors on health, but none specifically concerned with those features unique to militar communities. Therefore this research represents an initial attempt to describe military communities. The second research objective was to continue developing a consultative model of research which simultaneously provides a service to the Army in a timely manner while at the same time permitting the collection of scientifically useful data.

Progress to Date. Four observers spent one month living in one military community and two observers spent two weeks working in a second community. The mode of data collection was passive observation supplemented by interviews and examinations of archival record sources such as newspaper files. Community commanders received reports summarizing the observations and organized around topics the research team felt deserved further command attention.

These data were the basis for four lectures on the organization and function of military communities that were presented at a Corps level community planning conference. The data suggest military communities differ from their civilian counter-parts in their political structures, their social structures, and the interaction of the political and social structures. These factors encourage a lack of involvement with consequent feelings of isolation, depression, anger, and frustration that most typically must be resolved within the military family. The data also suggest that the principle function of military communities is to reinforce tactical readiness. This is accomplished by: (a) providing for the civil defense, (b) fostering and maintaining viable social support networks, (c) reinforcing professional identity, and (4) insuring a minimal level of community service.

Future Recommendations. The consultative research model appears viable, and a start has been made on describing some of the basic parameters of military communities. The relationship with health and the incidence of disease or dysfunction remains undocumented. Future research should focus on health and well being as related to individual social networks which are in turn influenced by community organization and functioning. One possibility is an examination of housing patterns as they encourage or discourage informal social network development and in turn affect the incidence of health related problems.

FORMAL PRESENTATIONS

INGRAHAM, L.H. Psychological Sense of Community as Requisite to Drug and Alcohol Abuse. Presented at V Corps Community Life Council on 23 January 1980, Frankfurt, West Germany.

INGRAHAM, L.H. Anatomy of an Elephant: The Shoeleather Epidemiology of Drug Use in the U.S. Army. Presented at U.S Air Force Europe COUNTERPUSH Training Workshop on 21 March 1980, Ramstein Air Force Base, West Germany.

MANNING, F.J. Cohesion and Peacetime Performance by Selected Combat Units. Presented at VII Corps Battalion Commanders' Conference on 23 April 1980, Nuernberg, West Germany.

INGRAHAM, L.H. Neuropsychiatric Battle Casualties. Presented at 7th Medical Command Medical/Surgical Conference May 1980, Garmisch, West Germany.

MANNING, F.J. Drug Overdoses in USAREUR: Two Years of Psychological Autopsies. Presented at Annual European Medical Service Corps Conference June 1980, Garmisch, West Germany.

INGRAHAM, L.H. (1) Why Bother About Military Communities?
(2) Who Needs You?: Functions of Military Communities
(3) Dimensions of Social Support in Military Communities
(4) How Do We Get There from Here? Criteria Toward Utopia
(5) Sense and Nonsense in the Army Drug Abuse Prevention Program

All presented at V Corps Community Planning Conference August 1980, Viernheim, West Germany.

INGRAHAM, L.H. Sense and Nonsense in the Army Drug Abuse Prevention Program. Presented at U.S. Army Europe Clinical Consultants/Directors Conference 28 September 1980, Viernheim, West Germany.

PUBLICATIONS

MANNING, F.J. Continuous Operations in Europe: Feasibility and Effects of Leadership and Training. Parameters, 9, 8-17 (1979).

INGRAHAM, L.H. and F.J. MANNING Psychiatric Battle Casualties: The Missing Column in a War Without Replacements. Military Review, 60, 18-29 (1980).

MANNING, F.J. and L.H. INGRAHAM The Face of Waste: Personnel Attrition in the U.S. Army. Armed Forces and Society (in press).

MANNING, F.J. and E.M. DeROUIN Employed Wives of U.S. Army Members in Germany Fare Better than Unemployed. Military Medicine (in press).

MANNING, F.J. Cohesion and Readiness. Air University Review (in press).

MANNING, F.J. Assessment of Unit Cohesion and Its Relation To Unit Performance. Proceedings of 15th Annual Anglo-American Psychiatry Symposium (in press).

INGRAHAM, L.H. and F.J. MANNING Cohesion in the U.S. Army: Who Needs It, What Is It Anyway, and How Do We Get It To Them? Proceedings of the 1980 Conference of the Inter-University Seminar on Armed Forces and Society (in press).

MANNING, F.J. and L.H. INGRAHAM Continuous Operations: Who Melts, When and Why? Proceedings of the 1980 Conference of the Inter-University Seminar on Armed Forces and Society (in press).

INGRAHAM, L.H. Sense and Nonsense in the Army Drug Abuse Prevention Effort. Parameters (in press).

FY 80 PROJECT AND WORK UNIT NUMBER

TERMINATIONS

PROJECT 3M261102BS01

BASIC RESEARCH ON MILITARY INJURY AND DISEASE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6431	80 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
79 10 01	H. Termination	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS01	00	121
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Ecology and Control of Disease Vectors and Reservoirs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 005900 Environmental Biology 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
54 09	30 June 1980	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 79	6	318
FISCAL YEAR CURRENT 80	6	300

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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20. PERFORMING ORGANIZATION

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

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TELEPHONE: 202-576-3719

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Hoch, CPT, A.L.

NAME: Gargan, CPT, T.P.

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Arboviruses; (U) Ecology; (U) Mosquitoes; (U) Disease Vectors; (U) Control; (U) Trombiculid mites

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

23. (U) Studies emphasize control of vectors of arbovirus, rickettsial and parasitic diseases of military significance. Objectives are incrimination of vectors and understanding of host-parasite relationships initially, understanding of vector biology and disease transmission mechanisms ultimately in order to develop more effective control procedures.

24. (U) Invertebrate vectors and vertebrate reservoirs and hosts are collected in areas of known disease activity. Seasonal changes in size of an infection rates in vector populations are determined; biological processes of vector species, such as pathogen transmission, diapause and reproductive physiology, are studied in the laboratory.

25. (U) 79 10-80 06 The seasonal transmission of Eastern equine encephalitis (EEE) and Highlands J (HJ) viruses was monitored in the Pocomoke Cypress Swamp (PCS) by sentinel quail, from 30 April - 11 December 1979, EEE virus transmission to sentinel quail was first detected the last of May and peaked in late June when 100 percent of the birds developed neutralizing antibodies, EEE virus transmission then gradually declined through the summer and fall and was last detected during the period from 27 November - 11 December. Over 35,000 mosquitoes, representing 23 species, were processed for virus isolation from the PCS. Four viral isolates were recovered. EEE virus was isolated from a pool of Culiseta melanura for an overall infection rate of 0.06 percent (1 per 1788). JC virus was isolated from 2 pools of Aedes canadensis for an infection rate of 0.007 percent (2 per 27644). Another, as yet unidentified, isolate was found in Aedes canadensis. Due to the transfer of function of the arbovirology portion of the Department of Entomology at the Walter Reed Army Institute of Research to the U.S. Army Medical Research Institute of Infectious Diseases, this work unit was terminated. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

* Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM A D 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE OV 69

Project 3M161102BS01 BASIC RESEARCH ON MILITARY INJURY AND DISEASE

Work Unit 121 Ecology and Control of Disease Vectors and Reservoirs

Investigators
Principal: Charles L. Bailey, MAJ, MSC
Associate: CPT Thomas P. Gargan, MSC; CPT Michael W. Hastriter, MSC; CPT Alfred L. Hoch, MSC; 1LT Brooke T. Elias, MSC; David E. Hayes, Ralph F. Tammariello and SP5 Ardith J. Regdon; SP4 Vicki Paraschos

Objectives

Conduct laboratory and field investigations on arthropods of medical importance. Field studies also provides for training of medical entomologists in epidemiological and arboviral surveillance techniques. Also, examination of the physiological basis of the overwintering mechanism of a mosquito vector.

Progress

Studies on the overwintering ecology of Culex pipiens were directed towards determining the environmental factors (i.e. temperature and/or photoperiod) that stimulate diapausing Cx. pipiens to blood feed. Overwintering mosquitoes were collected on 3-4 March 1980 from bunkers at Ft. Mott in New Jersey. The mosquitoes were held in the laboratory under 4 temperature/photoperiod regimes and were offered a blood meal daily. For the first week of the experiment it appeared that temperature was more important than photoperiod for stimulation of the blood feeding response. Eventually, however, all the mosquitoes maintained under the various experimental conditions took a blood meal and exhibited ovarian development which indicated that the mosquitoes were not in diapause when originally collected.

The seasonal transmission of Eastern equine encephalitis (EEE) and Highlands J (HJ) viruses were monitored in the Pocomoke Cypress Swamp (PCS) by sentinel quail from 30 April - 11 December 1979. EEE virus transmission to sentinel quail was first detected the last of May. A peak of EEE virus transmission was observed during late June when 100% of the birds exposed in the swamp were infected with virus. EEE virus transmission then gradually declined through July, August, and September. There were no EEE virus seroconversions in quail from the middle of September through the last of November. EEE virus transmission was last detected in quail exposed during the period 27 November - 11 December.

There was very little transmission of HJ virus in the PCS during the summer of 1979. A total of 8 quail developed neutralizing antibody to HJ virus. The first and last detection of HJ virus transmission occurred during early July and early November, respectively.

Over 35,000 mosquitoes, representing 23 species, were processed for virus isolation from the PCS. Four viral isolates were recovered from these mosquitoes. EEE virus was isolated from a pool of 18 Culiseta melanura. The infection rate for EEE virus in Cu. melanura was 0.06% (1 per 1788). Jamestown Canyon (JC) virus was isolated from 2 pools, each containing 50 female Aedes canadensis for an infection rate of 0.007% (2 per 27644). Another isolate was obtained from a pool of 40 Ae. canadensis, however, the virus has not been identified.

Studies on the ecology of California group encephalitis viruses in the Delmarva peninsula were directed towards determining the vector(s) of Keystone (KEY) and JC viruses in the Chincoteague National Wildlife Refuge (CNWR) and determining the incidence of KEY virus infection in the cottontail rabbit population in the area.

The seasonal transmission of KEY and JC viruses was monitored in the CNWR by sentinel rabbits and also by trapping sylvan cottontail rabbits from 28 April to 29 September 1980. None of the sentinel rabbits developed antibodies to KEY or JC viruses. Neutralizing antibody to KEY virus was found in 47% (8/17) of the sylvan cottontail rabbits. However, these rabbits showed high KEY virus antibody titers when they were initially collected, suggesting that they were infected with this virus in previous years.

A new program directed at collecting Ae. triseriatus was initiated in the PCS and the CNWR. Transovarial transmission of JC virus in this species has been reported by Berry et al. (1977). Ovitraps, gallon cans, and tires, all filled with water, were deployed in the two study areas to collect Ae. triseriatus eggs and larvae. These collection devices proved to be an efficient means of collecting large numbers of Ae. triseriatus, yielding between 50-300 adults reared from each collection. The adults have been frozen for future virus isolation attempts.

Current Fiscal Year Plans: This work unit has been transferred to the United States Army Medical Research Institute of Infectious Diseases.

Literature Cited

Reference:

Barry, R. L., B. J. Lalonde, C. H. Calisher, M. A. Parsons, and G. T. Bear. Evidence for transovarial transmission of Jamestown Canyon virus in Ohio. Mosquito News 37: 494-496, 1977.

Presentations:

1. American Society of Tropical Medicine and Hygiene, Tucson, AZ, 16 November 1979.

Bailey, C. L., T. P. Gargan., M. E. Faran, and D. E. Hayes. Winter survival and ovarian development in Culex pipiens.

Gargan, T. P., C. L. Bailey, and M. E. Faran. Isolation of Keystone virus from Aedes infirmatus D. & K. in the Chincoteague National Wildlife, VA

2. Medical Entomology Workshop A00425, Academy of Health Sciences, Ft. Sam Houston, TX, 7 February 1980.

Bailey, C. L. Overview of the entomological program at the WRAIR and the overseas laboratories.

Gargan, T. P. Entomological and arboviral investigations in the Pocomoke Cypress Swamp and Chincoteague National Wildlife Refuge.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DA OC 6445	80 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
79 10 01	H. Termination	U	U		NI.	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS01	00	123
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Biochemical Research on Cellular Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002300 Biochemistry 003000 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	Terminated	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	6.0	379
FISCAL YEAR CURRENT 81	0.0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Walter Reed Army Institute of Research

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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

Foreign Intelligence not considered

ASSOCIATE INVESTIGATORS

NAME: Wolfe, Alan D., Ph.D.

NAME: Hansen, Brian D., Ph.D. POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Cell Membrane; (U) Parasists; (U) Metabolism; (U) Transport

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 (a) definition of the biochemical processes and transport systems in parasites, (b) the elucidation of process which are amenable to chemotherapeutic and immunological modulation, (c) the determination of biochemical differences between parasite host and vector stages, (d) biochemical relationships between host and parasite and elucidation of mechanism of action of chemotheraputic drugs.

24. (U) The approaches employ animal and cell culture model systems. Isotopically labelled metabolic precursors and drugs are employed to determine metabolic pathways, transport processes, drug and metabolite distribution, binding sites, and process inhibition. Cellular sub-fractions are isolated, identified, and quantitated by chromatography, electrophoresis and gradient centrifugation. The function of significant enzymes and membrane preparationsis analyzed to determine drug uptake and resistance mechanisms.

25. (U) Both P. berghei and Leishmania braziliensis panamensis absorb purine nucleosides by mediation and diffusion. Rat erythrocytes possess two transport loci, one for adenine and hypoxanthine, and a second one for adenosine and inosine, while parasitized erythrocytes possess one for adenosine and inosine. In Leishmania, three transport loci may exist, and substrates appear competitive. Mefloquine and WR 122,455, uniformly inhibit cellular macromolecular synthesis, while an acetylpyridine thiosemicarbazone preferentially inhibits RNA acid synthesis. Lysis of spheroplasts and inhibition of membrane bound NADH oxidase suggests mefloquine and WR 122,455 are deleterious to cell membranes. Cellular drug adaptation is accomplanied by (a) a reduction in the amount of labeled mefloquine bound to the outer membrane, (b) altered membrane gradient profiles, and (c) altered gel electrophoretic patterns of membrane proteins. For Final Technical report see WRAIR Annual Report 1 Oct 79 to 30 Sep 80.

*Available to contractors upon originator's approval

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498A 1 NOV 65 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE.

Project: 3M161102BS01 BASIC RESEARCH ON MILITARY DISEASES

Work Unit: 123 Biochemical Research on Cellular Injury

Investigators:
Principle: Bhupendra P. Doctor, Ph.D.
Associate: Brain D. Hansen, Ph.D., Ruth Brown, Clarence Emery, Sp-4 Jose Perez-Arbelo, Sp-5 Frank Stancato, A. David Wolfe, Ph.D., Sp-4 John Walkony

The objective of this work unit is the elucidation of parasitic biochemical processes which significantly influence cell viability, and are, or may be, modulated by chemotherapeutic or immunological agents.

The following studies were conducted:

1. Determination of purine base nucleoside uptake in Plasmodium berghei, Leishmania braziliensis panamensis, and parasitized and nonparasitized rat erythrocytes.

2. Analysis of short interval metabolism of amino acids in Leishmania braziliensis panamensis.

3. Determination of the mechanism of action of mefloquine, the phenanthrenemethanol WR 122,455, and an acetylpyridine thiosemicarbazone.

4. Elucidation of molecular changes which occur upon cellular adaptation to mefloquine and WR 122,455.

1. (A) Purine Base and Nucleoside Uptake in Plasmodium Berghei and Host Erythrocytes.

The absorption of ^{3}H-labeled adenine, adenosine, hypoxanthine and ^{14}C-labeled inosine by normal rat erythrocytes, Plasmodium berghei-infected erythrocytes and saponin released "free parasites" was measured. The uptake of these labeled substrates by the normal rat erythrocytes occurs both by diffusion and mediated transport systems. Similar absorptive mechanisms for these substrates were also observed for both Plasmodium berghei-infected erythrocytes and "free parasites". Data from inhibition studies using purine base nucleoside analogues indicate the presence of three distinct transport loci in the normal erythrocyte for adenosine-inosine, hypoxanthine, and adenine and two loci in the infected erythrocyte and "free parasite" for adenosine-inosine-hypoxanthine and adenine.

The initial metabolism of ^{3}H-adenosine by the "free parasite" was also examined. A double isotope technique was used to follow the separate

metabolic fates of the purine base and ribose moieties of adenosine. The date suggest a possible conversion of adenosine to the purine base and ribose moiety and subsequent uptake of the purine base by the parasite. In addition, a powerful adenosine deaminase inhibitor (2-deoxycoformycin) significantly reduced the uptake of ^{3}H-adenosine by the "free parasites". Chromatographs of aliquots from post incubation media show the tritium label to be associated predominately with adenosine in the presence of 2-deoxycoformycin and with inosine and hypoxanthine in the absence of the inhibitor.

B. The Specificity of Purine Base Nucleoside Transport in Leishmania braziliensis panamensis:

Promastigotes of L.b. panamensis absorbed the purine adenine, hypoxanthine, adenosine and inosine by a combination of diffusion and mediated components. When the uptake rates for these substrates are corrected for diffusion and compared, the purine bases adenine and hypoxanthine were transported at a greater rate than the purine nucleosides adenosine and inosine. Competitive interactions among these four purines confirmed the presence of mediated and diffusion components and suggested three transport loci may be operating. The first transport locus, designated as Locus 1, transported inosine, Locus 2, hypoxanthine and adenine and Locus 3 adenosine, with adenine, hypoxanthine and inosine binding nonproductively. In addition, adenine inhibited the uptake of labeled hypoxanthine in a fully competitive manner.

2. Analysis of Short Interval Metabolism of amino acids in Leishmania braziliensis panamensis (WR008).

The percent distribution of ^{14}C from the added labeled amino acids among free pool amino acids of amastigotes and promastigotes of Leishmania braziliensis panamensis was determined. In addition, the total number of CPM's found in the free pool and the protein hydrolysate were calculated. The summarized observation for the short interval metabolism of those amino acids studied are as follows:

A. The percent distribution of the radiolabel from added ^{14}C-amino acids among free pool amino acids were approximately the same for both promastigotes and amastigotes. Glutamate, aspartate and glutamine were readily metabolized to alanine while alanine was converted to glutamine. These data suggest the presence of glutamate pyruvate transaminase activity in both amastigotes and promastigotes of Leishmania SP. These data were confirmed directly when transaminase activity (GOT and GPT) was found in promastigotes and amastigotes of Leishmania braziliensis panamensis although significantly less activity was found in the amastigote.

B. After 10 min, ^{14}-glycine and ^{14}C-leucine in the free pool remained largely unmetabolized in both promastigotes and amastigotes. Moreover, nearly 50% of the total ^{14}C-label from added leucine was found in the protein hydrolysate of the promastigote. Significantly less ^{14}C-leucine was found in the amastigote hydrolysate.

C. Although the percent distribution of the ^{14}C-label from the added proline is approximately the same for both amastigotes and promastigotes, the total amount of activity found in the proline free pool of the amastigote is negligible compared to that found in the promastigotes. This suggests that while proline may be readily utilized as an energy source by promastigotes (as suggested by Krassner and Flory, 1972) such is not the case for the amastigotes. However, nearly as much label from ^{14}C-arginine was absorbed and metabolized in the amastigote as in the promastigote suggesting that arginine may replace proline as an energy source once conversion to the amastigote has occurred.

3. Determination of the Mechanism of Action of Acetylpyridine Thiosemicarbazone, Mefloquine and the Phenanthrenemethal, WR122,455.

Thiosemicarbazone H induces bacteriostasis in E. coli AT-9. Bacterial cultures incubated with graded concentrations of H exhibit reduced, linear growth when measured by turbidimetry, while colony count reveals viability that remains constant. Drug H influence on growth was also analyzed by comparison of isotopically labelled macromolecular precursor incorporation rates in drug-free and drug-containing cultures. Uracil incorporation was more severly inhibited than incorporation of either deoxyadenosine or phenylalanine. Probit analysis of data obtained from turbidimetric measurement of growth inhibition and isotope incorporation showed that a causal relation exists between suppression of RNA synthesis and growth inhibition.

Inhibition rates were also determined after forty-five minutes preincubation of bacteria with drug. Double-labelling experiments again revealed RNA synthesis to be the process most sensitive to H. However, a more severe, time dependent inhibition of DNA synthesis was now observed; preincubation of achieve more complete expression of drug action resulted in the following distinction between the sensitivity of these processes to H: RNA>DNA>protein.

Plasmodia and bacteria are sensitive to the 2-acetylpyridine thiosemicarbazone. In addition, we have found both pro and amastigotes of Leishmania braziliensis and mexicana, grown in vitro, are killed by H concentrations between 10^{-8} and 10^{-9}M.

The antimalarial drug mefloquine, a quinoline-4-methanol, has been found to be a potent bactericidal agent. A concentration of 5×10^{-5}

positive bacteria. Mefloquine at a concentration of 1 x 10^{-4}M reduced the viability of a culture of E. coli AT-9 by 34% upon mixture of drug and bacteria; a concentration of 3 x 10^{-4}M mefloqine immediately reduced viability of more than 99%. In each instance, further decreases in viability, accompanied by decreases in turbidity, occurred upon subsequent incubation. Growth inhibitory concentrations of mefloquine inhibited the uptake or incorporation of isotopically labelled precursors of DNA, RNA, and protein within one minute of drug addition, while prelabelled cells released 50% of their DNA, 40% of their RNA, and 25% of their protein during a four hour incubation with drug. The results suggest that mefloquine may be a membrane active drug. This hypothesis was tested by analysis of the influence of mefloquine on intact bacteria, on spheroplasts, and on isolated membrane fractions. The uptake or association of (^{3}H-CH_3)-2methylalanine (aminoisobutyric acid) with E. coli was suppressed within one minute by concentration of 10^{-4}M mefloquine. Spheroplasts derived from mefloquine sensitive bacteria were lysed in direct relation to the concentration of drug; 21% of the spheroplasts were lysed in 2.5 minutes by 10^{-4} M mefloquine, while this drug concentration lysed only 9% of spheroplasts derived from mefloquine adapted bacteria. Enzymatic oxidation of NADH by isolated E. coli membranes was inhibited by 50% at a concentration of 10^{-4}M drug. The results suggest that mefloquine affects the function or integrity of E. coli membranes and by analogy, offers a viable hypothesis for the antimalarial action of mefloquine.

The exposure of exponently growing Escherichia coli to there antimalarial drug WR 122,455, at concentrations above 1 x 10^{-5} resulted in rapid suppression of cell growth. A concentration of 5 x 10^{-5} M drug exponentially reduced uptake of the isotopically labelled macromolecular procursors uracil, deoxyadenosine, phenylalanine, and glycerol. Despite tne bactericidal properties of WR 122,455, growth inhibition at all tested drug concentrations was transient, with the duration of inhibition a direct function of the drug concentration. Cell 'adaptation' required 3 hours at a drug concentration of 2 x 10^{-5}M, and 70 hours at 10^{-4} drug. The growth of adapted cells was characterized by biosynthetic rates similar those of drug-free organisms although cells were elongated when grown in medium containing fresh drug at concentrations above 6 x 10^{-5} M. Electron micrographs indicated incomplete septa formation. The influence of drug upon selected reactions catalyzed by membrane preparation from E. coli and P. berghei was tested, and inhibition of NADH oxidation was observed. The results suggest that WR 122,455 may affect a number of reactions and structures related to cell growth, morphology and viability.

4. Elucidation of Molecular Changes Which Occur Upon Cellular Adaptation to Mefloquine and WR122,455.

Membranes from bacteria adapted to WR 122,455, or mefloquine, have been isolated, and analyzed by sucrose gradient centrifugation, and SDS-

gel electrophoresis. Sucrose gradients of membranes from bacteria adapted to WR 122,455 possess A_{280} profiles which contain an additional peak, in contrast to membranes from either mefloquine adapted, or normal bacteria. Membranes from normal, and drug-adapted cells were extracted with trition X-100, and thermally reduced in the presence of sodium dodecyl sulfate and B-mercaptoethanol. Gel electrophoresis of such samples revealed the membranes of drug adapted cells to contain abnormal bands of approximately 35,000 daltons after staining with Coomassie brilliant blue R-250. Additional, minor alterations were also observed. Membranes were separated into inner and outer portions of the cell envelope, either by sucrose gradient, or by incubation of source bacteria with sarkosyl, and the abnormal proteins were found to be contituents of the outer envelope, the initial cell permeability barrier.

Mefloquine uptake and binding by P. berghei infected rodent reticulocytes and erythrocytes has been under investigation. Parasitized cell take up more than twice as much mefloquine as do uninfected cells, althought the kinetics of uptake appear similar. Considerable binding occurs upon mixture of cells and (^{14}C)-mefloquine, and slowly continues during a one hour incubation at 37C. Approximately 75% of drug cell association occurred upon mixture, and the remainder during incubation. Bound (^{14}C)-mefloquine remains attached to membranes and high molecular weight cell components through saponin lysis of red cells, isolation of plasmodia, and their destruction in a French pressure cell. Membranes were identified by the presence of (^{3}H)-glycerol administered to parasitized rats, and analyzed by discontinued sucrose gradient. The distribution of (^{14}C)-mefloquine in such gradients is identical to that obtained by prior isolation of membranes, and subsequent incubation with (^{14}C)-mefloquine. The peaks observed in these gradients have been separated, and their constituent proteins extracted for electrophoretic study.

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2. Hansen, B.D. 1979. Trypanosoma gambiense: Membrane Transport of amino acids. Exp. Parasitol. 48, 296-304.

3. Hansen, B.D. 1980. Purine Base and nucleoside uptake in Plasmodium berghei and host erythrocytes. J. Parasitol. 66, 205-212.

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1. Brown, R.E., Stancato, F.A., and A.D. Wolfe. 1980. Mefloquine: evidence for a mode of action. Current chemotherapy and infectious diseases, Pgs. 1101-1103. Eds. Nelson, J.D., and Grassi, C. The American Society for Microbiology, Washington, DC.

2. Brown, R.E., Stancato, F.A., and A.D. Wolfe. 1980. Studies on the mode of action of the phenanthrenemethanol, WR 122,455. 80th Annua. Meet., American Society for Microbiology.

3. Hansen, B.D., N.D. Brown and L.D. Hendricks. 1979 Short-Internal Metabolism of Radiolabeled amino acids in Leishmania braziliensis panamensis (WR-008). Abstract. American Society of Tropical Medicine and Hygiene Nov, 1979.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6450	80 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
79 10 01	H. Term	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY				
b. CONTRIBUTING	61102A	3M161102BS01	00	140
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)
(U) Military Hematology

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008800 Life Support 02600 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: NA EXPIRATION:
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c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING: 79	10	417
FISCAL YEAR CURRENT: 80	10	542

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
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22. KEYWORDS (Procede EACH with Security Classification Code) (U) Coagulation; (U) Hematopoiesis; (U) Blood; (U) Marrow Failure; (U) Erythrocytes; (U) Leukocytes

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

23.(U) To define the hematologic pathophysiology of trauma, burns, shock, marrow toxic drugs or radiation, and infectious diseases of military importance; to identify modalities to restore hemostasis, to protect against hematopoietic stem cell injury, and to augment host defense systems; to study hematologic aspects of chemical defense.

24.(U) 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) 79 10 - 80 09 Studies of platelet function have identified the importance of membrane associated scialotransferase in platelet mediated clotting. Studies of red blood cell acetylcholinesterase--ACH receptors have led to model systems for the study of nerve agents. Studies of human neutrophil storage granules have led to an understanding of mechanisms by which inflammatory neutrophils may influence specific immune responses and wound healing. Studies of bone marrow hematopoietic stem cells and their behavior in tissue culture have identified effects of chemotactic factors and mediators from activated lymphocytes upon the regulation of stem cell proliferation and differentiation. Hematopoietic stem cells that have undergone malignant transformation have been established in tissue culture for study of basic mechanisms of hematopoietic regulation and the effects of radiation and drug toxins. Studies of vitamin B_{12} analogues have identified B_{12a} as a potent antidote for acute cyanide poisoning. Studies of purine metabolism in blood borne parasitic diseases (malaria and leishmania) in in vivo animal models and in in vitro cultures of blood cells have identified basic metabolic pertubations that accompany these infections as well as purine metabolic pathways unique to the parasite that are potential targets for development of new antibiotic chemotherapy. For Technical Report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

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Project 3M161102BS01 BASIC RESEARCH ON MILITARY INJURY AND DISEASE

Work Unit 140 Military Hematology

Investigators

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Description

Five distinct research tasks have been carried out under this work unit. 1) Studies of hematopoiesis and bone marrow failure: 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 (viral in particular) which interfere with cell division or differentiation. Our objectives are to study basic mechanisms involved in the regulation of hematopoiesis using tissue culture of stem cells and committed hematopoietic precursor cells from human, mouse, and rabbit marrow, using leukemic cell lines that can be induced to differentiate in vitro, and using allogenic transplantation of bone marrow tissue in mice. The effects of mediators derived from inflammatory processes (e.g. chemotactic factors), from bone marrow adventitial cells, and from mature leukocytes upon the proliferation and differentiation of blood precursor cells are studied using these experimental systems. These systems are also used to study the toxic effects of drugs, certain families of chemicals (e.g. toluene and benzene) and radiation.

2) Studies of coagulation and plasma proteins: Blood coagulation factors, blood platelets, and plasma protein systems (e.g. kinin-kallikrein, fibrinolytic and complement systems) are critical for the development and outcome of acute responses to traumatic, thermal and infectious injury. Our studies are directed at changes in clotting and plasma proteins and in blood platelets during stress, exercise, trauma, burns, and infection which lead to clinically significant abnormalities of hemostasis. Also underway are studies of the therapeutic uses of intravenous immunoglobulins that have military relevance (e.g. in thermal injury and in certain viral infections) and studies of the toxicities of IV immunoglobulins.

3) Studies of blood phagocytes: Phagocytic blood leukocytes are critical to host defense against bacterial and fungal infections and to the development and outcome of inflammatory responses. Studies of human neutrophil and monocyte function have been done with a special emphasis upon understanding the secretion of soluble mediators by these cells and the mechanisms by which pathogenic microorganisms circumvent the normal microbicidal functions of these cells. Studies of soluble mediators released by phagocytic cells are concerned with tissue healing and the reorganization of connective tissue cells and substrates (e.g. collagen and fibronectin) and with the activation of immunoresponsive functions in macrophages and lymphocytes. Studies also involve the use of neutrophils harvested from normal donors for transfusion into profoundly neutropenic subjects.

4) Blood cell membranes and membrane associated enzymes (e.g. acetylcholinesterase): Studies of the physiology of blood cell membranes have concentrated upon the ectoenzyme, acetylcholinesterase, and the acetylcholine receptor complex which are 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 with the membrane lipoprotein structure as this relationship affects enzyme activity. This work also involves the quantification and characterization of acetylcholine receptors on mature blood cells and on blood precursor cells separated from normal human marrow.

5) Vitamin B_{12} and serum B_{12} binding proteins: 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_{12a} might be a useful cyanide antidote, our work represents the first vigorous pharmacologic studies of this question. Our object is to study the feasibility of using B_{12}, both as a therapeutic and a prophylactic measure against cyanide poisoning as may be encountered by military personnel during chemical warfare.

Progress

1) Studies of hematopoiesis and bone marrow failure: The toxic effects of toluene and di-nitrotoluene derivitives on hematopoiesis as well as factors that regulate the proliferation and differentiation of hematopoietic precursor cells have been studied. The variable effects of different toluene and di-nitrotoluene derivatives upon two enzyme systems critical for heme synthesis have been studied in particular: aminolevulinic acid (ALA) synthetase and ferrochelatase. Variable inhibition of ALA synthetase by these compounds has permitted a delineation of structure-activity relationships for the inhibitory phenomena. These studies have now been extended to intact cellular systems using erythroleukemia cell lines which are capable of the differentiated function of heme synthesis.

Studies of murine and rabbit hematopoietic stem cells have investigated techniques of purifying spleen-colony forming cells from marrow aspirate specimens. Techniques have been developed to partially purify early erythroid precursor cells using a sequence of discontinuous and continuous density gradient separations. In studies of spleen lymphocyte conditioned media, a soluble mediator has been identified which appears to facilitate erythroid differentiation by hematopoietic precursor cells in marrow (murine).

Studies of murine committed granulocyte/macrophage precursor cells isolated from marrow have demonstrated that small N-formylated peptides which exhibit chemotactic activity can inhibit the proliferation and differentiation of these cells. The structure-activity relationships for this inhibitory phenomenon is similar to those that have been demonstrated for other biologic effects of these small molecules. These studies have laid the methodologic foundation for the identification processes interfere with normal hematopoiesis to produce marrow failure. These studies are relevant to the understanding and prevention of marrow failure from chemical, physical, and infectious toxins that are of special interest to the military.

2) Studies of coagulation and plasma proteins: Studies with normal volunteers who were subjected to controlled, strenuous exercise demonstrated significant increases in fibrinolytic activity following exercise, as well as quantitative increases in the plasma levels of factors VIII and V. Exercise was also found to be associated with decreases in platelet ADP levels which were reflected in altered responsiveness of platelets to aggregating stimuli.

As part of these studies, a sensitive radioimmunoassay was developed for detection of the earliest degradation products of fibrinolysis. In related studies, hypercoagulable states that occur with certain neoplastic conditions were investigated with respect to platelet sialyltransferase activity, factor VIII levels and fibrinolysis. Fibrinolysis and the sequential changes of hemostasis associated with disseminated intravascular coagulation were studied in detail in an animal model of systemic Trypanosome infection.

A detailed understanding of coagulation changes that accompany stress, acute exercise, and infection is of particular relevance to the treatment of traumatic injury in military personnel.

3) Studies of blood phagocytes: Various studies of human blood neutrophils and monocytes and their function in inflammatory responses have been carried out. Studies of purified neutrophil secondary granule protein have identified a neutrophil derived mediator which stimulates the transformation of blood monocytes to macrophages in vitro. It is anticipated that these studies will clarify the mechanisms by which inflammatory macrophages acquire functional capabilities important for immune recognition phenomena and microbicidal activity. Studies of the membrane lipid content of purified primary and secondary cytoplasmic granules from human neutrophils have identified differences in these membranes which may explain functional differences in the mobilization and use of these organelles by the neutrophil. Studies of neutrophils recovered from the oral mucosa in humans in mouthwash specimens have led to the development of a new technique for quantifying the ability of an individual to supply his tissues with neutrophils. This technique will be particularly useful in evaluating host defense deficits in patients with profound neutropenia or marrow failure.

These studies are relevant to other research on-going in military labs concerned with microbial infection and vaccine development. They are also relevant to the investigation and understanding of marrow failure syndromes encountered in both military and civilian settings.

4) Blood cell membranes and membrane associated enzymes: This laboratory was established during the current fiscal year to study acetylcholinesterase and acetylcholine receptors on blood cell membranes. This enzyme system, which is the target of nerve agents, is richly represented on blood cell membranes. Study of acetylcholine/acetylcholinesterase on blood cells is directed towards understanding how the structural considerations of enzyme membrane interactions influence enzyme activity and susceptibility to inhibitors. To date this laboratory has established the methodology for studying acetylcholinesterase and acetylcholine receptors in whole cell and isolated membrane preparations.

Acetylcholine receptors on red blood cells and neutrophils have been quantified and characterized. Moreover, the ACh receptor on red blood cells has, for the first time, been shown to be functional with receptor-ligand interactions resulting in stimulated calcium influx into the cells and in increased intracellular cyclic GMP levels. The studies have provided the basis for detailed evaluation of nerve agents (acetylcholinesterase-inhibitors) in readily accessible human cellular systems.

5) Vitamin B_{12} and serum B_{12} binding proteins: This laboratory has explored the use of hydroxycobalamin ($B_{12}a$) as an antidote to acute cyanide poisoning using an animal model. The capacity of $B_{12}a$ to increase the LD_{50} of sodium cyanide (given IV and sq. to mice and rats) up to four fold has been demonstrated. Furthermore, the effectiveness of $B_{12}a$ in detoxifying cyanide was found to be additive to the antidotal effects of nitrates and sodium thiosulfate.

These studies have led to the organization of a collaborative effort between this laboratory and the Division of Experimental Therapeutics (WRAIR) to develop B_{12} compounds as cyanide antidotes which is of considerable interest to the military at this time. This work is of special interest to military chemical defense because of the possibility that B_{12} compounds can be used in cyanide prophylaxis.

Future Plans

The future plans for each component of this work unit are presented in outline form.

1) Hematopoiesis

a) Initiation of studies concerned with the hematologic toxicity of toluene and di-nitrotoluene compounds in whole cell systems. These studies will use erythroid cell lines that are capable of induced differentiation and heme synthesis. These studies will extend our current studies of these toxic compounds upon heme synthetic enzyme systems.

b) Continued studies of the effects of inflammatory mediators (such as chemotactic stimuli) upon the proliferation and differentiation of human myeloid precursor cells.

c) Use of partially purified myeloid and erythroid precursor cells prepared from normal human marrow and myeloid and erythroid cell lines (see above) for studies of radiation sensitivity and drug sensitivity (e.g. chloramphenicol) relative to the maturity and proliferative state of the precursor cells.

d) Biochemical characterization of mediators recovered from mature lymphoid (splenic) or phagocytic cells that can alter the proliferative state of hematopoietic precursor cells.

2) Blood coagulation and plasma proteins

a) Continuation of studies concerned with hemostatic abnormalities, and their pathophysiology, that accompany acute protozoal infections, using the normal human volunteer program of malaria as a major focus.

b) Initiation of studies of hemostatic abnormalities associated with acute thermal injury using animal models and burn patients treated in Army hospitals.

c) Initiation of studies concerned with the toxicity of intravenous gamma globulin preparations as mediated by acute phase plasma protein systems.

d) Initiation of studies concerned with the direct effects of proteases recovered from phagocytic cells and experimental inflammatory lesions upon the coagulation and thrombolytic system.

3) Blood Phagocytes

a) Continuation of studies concerned with the effects of partially purified, human secondary granule protein upon the differentiation of human blood monocytes into macrophages in vitro. Biochemical characterization of neutrophil derived mediators involved in monocyte stimulatory effects.

b) Continuation of studies concerned with the effects of partially purified, human secondary granule protein upon the proliferation and differentiation of human granulocytic precursor cells using fractionated, normal human marrow and the HL-60, promyelocytic leukemia cell line.

c) Continuation of studies of neutrophil granule membrane lipid content; generation of artificial membranes (liposomes) from extracted phospholipid of neutrophil granules and use of these artificial membranes for studies of membrane fusion potential.

d) Use of the HL-60 cell line to study the potential influence of activated complement and secondary granule proteins recovered from mature neutrophils upon myeloid precursor cell proliferation and differentiation.

e) Initiation of studies concerned with the effects of extracellular lipid environments upon phagocytic cell function: chemotaxis, phagocytosis, and microbial killing. Of particular concern in these studies will be the effects of variations in extracellular free fatty acids and cholesterol.

4) Blood cell membranes and membrane associated enzymes

a) Continuation of studies in which purified acetylcholinesterase (from RBC) is reconstituted with artificial membranes and with ghosts of normal and abnormal RBC (e.g. RBC from patients with PNH) to explore relationship of enzyme activity to structural placement of enzyme in membrane. Initiation of studies in which the effects of acetylcholinesterase inhibitors are evaluated when acetylcholinesterase is associated with artificial membranes in different ways.

b) Continuation of studies concerned with the quantitation of ACH receptors on RBC, RBC precursor cells, and leukocytes. Initiation of studies on the effects of ACh agonists upon RBC function (e.g. deformability) and leukocyte function; initiation of studies concerned with the effects of ACh agonists and antagonists upon hematopoietic precursor cell proliferation and differentiation.

5) Vitamin B_{12} and serum B_{12} binding proteins

a) Comparison of in vitro binding affinities of different B_{12} analogues for cyanide ion (CN); studies of in vivo CN detoxification in mouse model by B_{12} analogues to determine if in vitro binding affinity correlates with antidote activity.

b) Development of simple assays for the detection of B_{12} in urine in which B_{12}-CN complex is distinguished from free B_{12}.

c) Studies of the relative avidity of B_{12}a for CN when the B_{12}a is free and when it is associated with various B_{12} binding proteins.

Abstracts and Presentations at National and International Meetings

1) Hematopoiesis

1. Williams, H.L., Johnson, D.J., Slater, S., and Wright, D.G.: The in vitro effect of selected organic compounds on heme synthesis. Presented at 18th Congress of the International Society of Hematology, Montreal, Canada, August, 1980.

2. Mora, P.A., Valle, J., Jozsa, N., and Wright, D.G.: Inhibition of bone marrow myeloid precursor cell proliferation by chemotactic peptides. Presented at 18th Congress of the International Society of Hematology, Montreal, Canada, August, 1980.

3. Butler, W.M., Jozsa, N., and A.J. Salvado: Effect of rabbit anti-mouse brain sera (RAMBS) on CFU-E and BFU-E in a plasma clot system. Blood, 54 (Suppl.1):134a, 1979.

4. Salvado, A.J. and Sytkowski, A.J.: An improved assay for erythropoietin using cryopreserved rabbit bone marrow cells. Clin. Res., 1981 (in press).

5. Salvado, A.J. and Sytkowski, A.J.: Identification and characterization of multiple erythroid progenitors in the rabbit: a basis for their purification. Presented at the 2nd conference on Hemoglobin Switching, Airlie House, VA, June, 1980.

2) Coagulation and Plasma Proteins

1. Saito, H. and Scialla, S.J.: Isolation and properties of a non-functional Hageman factor (HF, Factor XII) from a CRM^+ Hageman trait plasma. Blood, 54 (Suppl. 1):301a, 1979 (Presented at the annual meeting, American Society of Hematology, Dec., 1979).

2. Barr, C., Taylor, G., Collins, G.J., Shaker, S., Conyers, W. and Scialla, S.: Platelet sialytransferase activity in hemostatic disorders. Circulation, 62:III-105, 1980 (Presented at the national meeting, American Heart Association, Oct. 1980).

3. 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., 1981 (in press).

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3) Blood phagocytes

1. Wright, D.G. and Greenwald, D.: Increased motility and maturation of human blood monocytes stimulated by products released from neutrophil secondary granules. Blood, 54 (Suppl. 1):95a, 1979 (Presented at the annual meeting, Amer. Soc. Hematology, Dec., 1979).

2. 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. Blood, 54 (Suppl. 1): 130a, 1979 (Presented at the annual meeting of the Amer. Soc. of Hematology, Dec., 1979).

3. Fontana, J.A., Wright, D.G., Schiffmann, E. and Deisseroth, A.B.: The development of chemotaxis in differentiating myeloid precursor cells: studies with a human leukemia cell line. Blood, 54 (Suppl. 1):74a, 1979 (Presented at the annual meeting of the Amer. Soc. of Hematology, Dec., 1979).

4. Thompson, A.R., Takaki, A., Enfield, D.L. and Wright, D.G.: Calcium-dependent activation cleavages of ^{125}I-human Factor IX by a human granulocyte constituent. Clin. Res., 28:326A, 1980 (Presented at the annual meeting, AFCR, May, 1980).

5. Wright, D.G.: Leukocyte Transfusions. Presented at the International Symposium on Infections in the Immunocompromised Host, Eindhoven, The Netherlands, June, 1980.

6. Wright, D.G., Karsh, J., Fauci, A.S., Klippel, J.H., Deisseroth, A.B. and Decker, J.L.: Lymphocytapheresis. Presented at the American Red Cross Symposium on The Lymphocyte, May, 1980.

7. Wright, D.G. and Meierovics, A.I.: Assessing the numbers of neutrophils supplied to tissues: Studies with neutrophils recovered from mouthwash specimens. Clin. Res., 1981 (in press).

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4) Blood cell membranes and membrane associated enzymes

1. Schoomaker, E., Butler, W.M., and Diehl, L.F.: Increased heat sensitivity of red blood cells in hereditary elliptocytosis with acquired cobalamin (B_{12}) deficiency. Clin. Res., 1981 (in press).

2. Tang, L., Schoomaker, E. and Weissmann: Cholinergic stimulated calcium uptake and cGMP formation in human red blood cells. Clin. Res., 1981 (in press).

3. Tang, L.C. and Schoomaker, E.B.: Evidence for muscarinic receptor stimulated cGMP and Ca uptake in human red blood cells. Trans. Amer. Soc. Neurochemistry, 12:244, 1981.

5) Vitamin B_{12} and B_{12}-binding proteins

1. Kale, M.P., Grenan, M.H. and Altstatt, L.B.: Studies of hydroxycobalamin in the treatment of acute cyanide poisoning. Clin. Res. 28:238A, 1980 (Presented at the annual meeting of AFCR, May, 1980.)

2. 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. Clin. Res. 28:(in press), 1980. (Presented at Midwest Section, AFCR, Nov. 1980).

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1) Hematopoiesis

1. Sytkowski, A.J., Salvado, A.J., Smith, G.M., McIntyre, C.J. and deBoth, N.J. Erythroid differentiation of clonal Rauscher erythroleukemia cells in response to erythropoietin or dimethylsulfoxide. Science. (in press). 1980.

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6. Crosby, W.H.: The Spleen, Chapter 5. In Blood Pure and Eloquent, A Story of Discovery, of People, and of Ideas. M.M. Wintrobe, ed. McGraw-Hill, New York, pp. 96-138, 1980.

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8. Crosby, W.H.: Iron Deficiency: Seven Rules for Corrective Therapy. Consultant 20:49-63, Jan. 1980.

9. Crosby, W.H.: Red Cell Mass: Its Precursors and its Perturbations. Hospital Practice 71-81, Feb. 1980.

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11. Crosby, W.H.: The hypereosinophilic syndrome. JAMA 244:78-79, 1980 (Questions and Answers).

2) Coagulation and Plasma Proteins

1. Ferguson, E.W., Barr, C.F., and Bernier, L.L.: Fibrinogenesis and fibrinolysis with strenuous exercise. J. Appl. Physiol. 47:1157-1161, 1979.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6532	80 09 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. SUMM MATG	9. T/SCIFIC DATA-BANK/FACTOR ACCESS	10. LEVEL OF SUM
79 10 01	Termination	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

11. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS01	00	142
b. CONTRIBUTING				
c. CONTRIBUTING				

12. TITLE (Precede with Security Classification Code)

(U) Pathophysiology of Systemic Responses to Shock and Trauma

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Stress Physiology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
73 07		DA	C. In-House

18. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

19. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 80	2	113
FISCAL YEAR CURRENT 81	0	0

20. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME:

22. GENERAL USE

Foreign intelligence not considered

23. KEYWORDS (Precede EACH with Security Classification Code)

(U) CPK, (U) LDH, (U) Isoenzymes, (U) Mesenteric, (U) Infarction

24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with security classification code.)

23 (U) The technical objective was to evaluate the isoenzyme systems of creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) as possible markers of ischemic injury to the gut, such as that seen in various pathologic states (i.e., shock) which cause hypoperfusion of the mesentery. The hypothesis was that ischemic injury would cause release of the isoenzymes into the blood. This release would allow their early detection by analyzing peripheral serum.

24 (U) The specific injuries which were studied included laparotomy, acute peritonitis, acute infarction of the superior mesenteric artery, acute arterial colonic infarction, simple small bowel obstruction, simple large bowel obstruction, and strangulatory closed loop infarction such as seen in hernias. The serum obtained in each study was analyzed for total activity of each enzyme system by automated spectrophotometric analysis. Isoenzyme fractions were determined by agarose gel electrophoresis.

25 (U) 79 10 - 80 09 The dog was established as an appropriate model in which to study ischemic necrosis and subsequent changes in CPK and LDH. Results from the study of acute superior mesenteric artery infarction (MAI) in dogs showed that this condition can be differentiated from the effects of laparotomy or peritonitis by serum isoenzyme analysis of CPK. In MAI, serum CPK and each of its isoenzyme fractions become elevated in the first 24 hours after injury. CPK-BB, the isoenzyme most prevalent in smooth muscle, peaks in the serum at six hours after injury, CPK-MM, the dominant isoenzyme in the elevation, peaks at nine hours after injury, and CPK-MB peaks between 12 and 24 hours. This project is being terminated because of a change in the mission of the Department of Experimental Surgery. The new mission is to study the physiology of blast injury. For technical report see Walter Reed Army Institute Research Annual Progress Report 1 Oct 79 - 30 Sept 80.

*Available to contractors upon originating's approval.

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

Project: 3M161102BS01 BASIC RESEARCH ON MILITARY INJURY AND DISEASE

Work Unit: 142 Pathophysiology of Systemic Responses to Shock and Trauma

Investigator:
Principal: MAJ Geoffrey M. Graeber, MC

I. Determining an appropriate model to study severe ischemic gut injury.

Background and Objectives:

At the current time there are no specific laboratory aids in determining the presence of severe ischemic gut injury. The enzymes creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) are potential markers of this injury because they are easily measured and have particular isoenzymes which are present in the bowel in large quantities. The first objective was to determine an appropriate laboratory animal in which to look at this entity. The canine emerged as the animal of choice.[1,2]

Progress:

Fresh postmortem specimens were obtained from both human and canine subjects. One gram samples of large and small bowel were taken from each of the major segments of each part of the bowel. Each of these samples was homogenized individually in 10 cc;s of Ringer's lactate, centrifuged and the supernatant was taken off for analysis. Total enzyme activity was determined by automated spectrophotometry and the isoenzymes were determined by agarose gel electrophoresis. Isoenzyme concentrations were analyzed and calculated from the electrophoretic patterns by a computerized densitometer. CPK and LDH were found to have all of their respective isoenzymes present in both the large and small bowel. LDH 2, 3 and 4 were present in greater quantity than LDH 1 or 5. CPK isoenzymes were present in equal concentrations. In man and dog both CPK and LDH were found to be analogous. Thus, the canine is an appropriate model for the study of ischemic gut injury using these isoenzymes as markers of this injury. (See publications)

II. Acute mesenteric artery occlusion.

Background and Objectives:

Currently there is no specific diagnostic test for evaluating acute mesenteric artery occlusion in its early stages. This is one of the reasons that the mortality rate of this entity is reported at 85%.[1] Observations have shown that intestinal necrosis may be associated with the appearance of CPK-BB isoenzyme in the serum.[2,3,4] Our objective is to determine if CPK and/or LDH isoenzymes are valid markers of severe ischemic injury to the gut.

Progress

Using a randomized block design, canines were assigned to one of three groups: laparotomy control, laparotomy with peritonitis, and laparotomy with superior mesenteric artery ligation. Serum specimens were drawn for 30 hours postoperatively after each procedure. Specimens were analyzed using spectrophotometry for total enzyme activity and agrose gel electrophoresis for isoenzyme distribution. Results were as follows: (1) LDH total enzyme activity remained near the preoperative levels, however, there was a shift in isoenzyme pattern showing a predominance of LDH 2, 3, and 4 in mesenteric necrosis. (2) Total CPK became elevated in all procedures. In mesenteric necrosis it was elevated to 10 times the upper limits of normal whereas the laparotomy and peritonitis groups showed elevations of only twice the upper limits of normal (normal for our laboratory being 100 IU/L). The isoenzymes CPK-MB and CPK-BB came elevated in mesenteric necrosis as well. CPK-BB elevated within the first 12 hours and CPK-MB elevated in the second 12 hours after onset of the mesenteric occlusion. It is concluded that the CPK and LDH isoenzymes appear to be valid markers of severe ischemic injury to the gut. Changes in these systems can be measured in the peripheral serum. The isoenzyme pattern changes of serum CPK displayed with mesenteric necrosis may be of particular diagnostic significance.[2,3]

Recommendations for the Future

The mission for FY 81 has been changed to evaluation of injuries caused by blast. This is particularly applicable to our previous work since recent studies by the Blast Overpressure Project indicate that blast can cause hemorrhagic injury of the stomach and the colon at the overpressures studied. In order to detect these injuries early enough to effect treatment and prevent death, a serum marker will be a very necessary component of any clinical screening program. The work conducted in FY 80 on the isoenzymes of CPK and LDH found in the serum after acute mesenteric infarction has great promise for a potential avenue to detect the gastrointestinal injuries associated with blast. In FY 81, our work will be conducted in three areas:

1) The normal values for CPK and LDH will be established for sheep both in serum and in the organs of the GI tract showing the greatest affect from blast injury.

2) The frozen sera from sheep subjected to blast injury during the last round of experiments conducted by the Blast Overpressure Project will be assayed for total CPK and LDH. Isoenzyme fractions will be determined by agarose gel electrophoresis. The results will be tabulated and assessed for trends.

3) Acute blast experiments using sheep will be conducted throughout FY 81. The sera from the animals will be assayed for changes in these two serum enzyme systems. All results will be correlated with the autopsy findings.

Project: 3M161102BS01 BASIC RESEARCH ON MILITARY INJURY AND DISEASE

Work Unit: 142 Pathophysiology of Systemic Responses to Shock and Trauma

Literature Cited:

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Publications:

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2. Graeber, G.M., Cafferty, P.J., Reardon, M.J., Curley, C.P., Ackerman, N.B., Harmon, J.W.: Changes in serum total creatine phosphokinase (CPK) and its isoenzymes caused by ligation of the superior mesenteric artery in canines. In Press - Annals of Surgery.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6527	80 09 30	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GRP'N INSTR'N	9A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. Termination	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS01	00	144
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Control Mechanisms of Regional Circulation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 07		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	1	133
	CURRENT 81	0	0

19. RESPONSIBLE DOD ORGANIZATION

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NAME:

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Hemorrhagic shock; (U) Oxygen delivery; (U) Oxygen consumption; (U) Hemodilution

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

23 (U) To obtain data on the physiological, biochemical and circulatory effects of hemorrhaging. Excessive blood loss contributed to the cause of death in more than 23% of patients dying once they reached hospitals in RVN during 1969. These studies will provide insite to the effects of blood loss on oxygen delivery and consumption and the effects of intentional hemodilution during resuscitation.

24 (U) Blood was removed by phlebotomy in both an acute and a chronic canine model. During chronic blood loss, 100% to 150% of the estimated blood volume was removed over a period of 17 to 34 days. Blood loss was replaced by an electrolyte solution, a high protein diet, and supplemental iron. Physiological responses and biochemical alterations were carefully monitored. During acute blood loss 40 to 60% of the EBV was removed. Cardiac output, oxygen delivery and consumption, systemic vascular resistance, and colloidal onconic pressures were monitored.

25 (U) 79 10 - 80 09 The chronic loss of blood up to 125% of the estimated blood volume over a period of 3 to 6 weeks is physiologically and biochemically well tolerated if supplemental iron and a high protein diet is administered. The route of administration of supplemental iron was not important. The acute loss of 40 to 60% of the estimated blood volume led to a marked increase in systemic vascular resistance, a decrease in oxygen delivery and consumption, and a marked acidosis. Despite the replacement of shed blood, systemic vascular resistance remained high and oxygen delivery did not return to normal. Hemodilution decreased systemic vascular resistance and resulted in a net increase in oxygen delivery and consumption. The optimum hematocrit during acute resuscitation may be between 30 to 34%. This project is being terminated because of a change in the mission of the Department of Cardiovascular Physiology. The new mission is to study military blast injury. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 - 30 Sept 80.

* Available to contractors upon originator's approval.

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

Project: 3M161102BS01 BASIC RESEARCH ON MILITARY INJURY AND DISEASE

Work Unit: 144 Control Mechanisms of Regional Circulation

Investigator:
Principal: COL Arthur W. Fleming, MC

Background and Objectives

Blood loss due to penetrating injuries is one of the most common causes of morbidity and mortality in combat casualties. Our understanding of the relative importance of the loss of the two major components of blood, the red cells and the plasma, has been significantly influenced by practices developed for non-combat surgical procedures. Previous work by us (Fleming, 1977) demonstrated that even patients with severe cardiovascular disease tolerated the loss of 10 to 15% of their estimated blood volume repeatedly without red blood cell replacement. Furthermore, we demonstrated that complex operations can be performed with maximum hemodilution and minimal red cell transfusions, and recommended guidelines for blood component therapy during such procedures (Fleming and Garcia, 1980). The experience that has been gained over the past several years has led us to question the necessity or desirability of massive red cell transfusions during resuscitation in combat casualties. The objectives of this research project were: to determine the biochemical and physiological tolerance to graded blood loss and intentional hemodilution; to document the maximum rate of blood production; to evaluate methods of accelerating erythropoiesis; and to determine if a graded blood loss increases or decreases tolerance to massive blood loss. These studies may provide insite to the effects of blood loss on oxygen delivery and consumption and help define the limits of the beneficial effects of intentional hemodilution during resuscitation.

Progress

A canine model was chosen so that a constant diet could be used, phlebotomies could be easily performed, and efforts to increase erythropoiesis could be quantitated.

Baseline blood volume studies were performed in 32 dogs using sodium chromate (^{51}Cr) for the red cell mass and radioiodinated (^{125}I) serum albumin for the plasma volume. The values are shown in Table 1.

TABLE 1

Blood Volume Measurements

Component	Volume in ml	ml/kg
Red cell mass (RCM)	848.9±29.6	34.8±1.5
Plasma volume (PV)	1246.8±35.0	51.1±1.5
Total blood volume (TBV)	2095.7±59.1	85.9±2.4

Blood was removed by percutaneous jugular vein punctures on eight occasions over a period of 17 days. On each occasion 12.5% of the estimated blood volume was removed. A volume of blood equivalent to the total blood volume was thus removed over the 17 days. Blood volume measurements were made prior to each phlebotomy and 3 days following the last phlebotomy in the first 8 experiments. In the remaining 24 experiments, blood volume measurements were made at 4 specific intervals.

Physiological measurements (blood pressure, EKG, cardiac output, blood gases, and oncotic pressure); biochemical measurements (electrolytes, 2,3 DPG, total proteins, albumin, globulin, serum iron, TIBC, lactates, pyruvates and glucose); and tolerance to a massive bleed were then studied 4 days following the last phlebotomy (the 21st day following the first phlebotomy).

Iron was administered in one of several ways; intravenously as one bolus dose; intravenously following each phlebotomy; intramuscularly following each phlebotomy and orally. Intravenous amino acids, were administered along with 500 ml of 1/3 N sodium chloride following each phlebotomy. A high protein diet and water were given ad libitum.

When 12.5% of the estimated blood volume was removed on each occasion, 3 times per week for 17 days, the RCM dropped following each phlebotomy to its lowest point after the 4th phlebotomy. At this point approximately 50% of the estimated TBV had been removed. The TBV, however, was 95.6% of normal, compensated for by an increase in the PV. The rate of decrease in the RCM between the 4th and 8th phlebotomy was attenuated by a marked increase in erythropoiesis which had increased 4.6 fold following the 5th phlebotomy. The overall rate of erythropoiesis was accelerated 3.3 fold over the period of 20 days. After the 8th phlebotomy, the mean RCM was 76.6% of the prephlebotomy values and the hematocrit (HCT) was 30.7%. The PV was increased by 8.2 to 14.9% and the TBV averaged 95.9% of predicted. The decrease in the HCT ranged from 22.4 to 30.9% and the decrease in the Hgb ranged from 25.3% to 30.1% between the period before the 1st phlebotomy and three days following the last phlebotomy (see Table 2).

TABLE 2

Change in the Hematocrit and Hemoglobin
Following Multiple Phlebotomies

Group	Decrease in Hgb	% Decrease in Hgb	Decrease in Hct	%Decrease in Hct
Oral Iron	4.01 gm%	25.3%	10.16 %	22.4%
Bolus Iron	3.98 gm%	30.1%	9.58 %	25.2%
Multiple Dose Iron	3.82 gm%	29.3%	12.34 %	30.9%

The route of administration of supplemental iron did not statistically influence the rate of erythropoiesis. Apparently, the quantity of iron given, rather than the route is the most important factor.

Biochemical Data

Baseline values for the biochemical determinations are shown in Table 3.

TABLE 3

Laboratory Values

	Baseline
Platelets	275,833 ± 8,950/cu mm
Fibrinogen	461.05 ± 15.85 mg%
Sodium	154.4 ± 1.22 mEq/L
Potassium	4.25 ± .07 mEq/L
Chloride	100.5 ± 1.3 mEq/L
Glucose	100 ± 2.6 mg%
Total Protein	6.77 ± 0.09 gm/100 ml
Albumin	3.35 ± 0.05 gm/100 ml
Globulin	3.42 ± 0.12 gm/100 ml
2,3-diphosphoglycerate	18.4 ± 1.1 μMoles/gm Hgb
Lactate	21.2 ± 1.1 mg/100 ml
Pyruvate	1.01 ± 0.06 mg/100 ml
Serum Iron	154.2 ± 5.8 mcg/dl
Total Iron Binding Capacity	326.2 ± 8.8 mcg/dl
Hemoglobin	14.5 ± 0.27 gm%
Hematocrit	42.3 ± 0.63 %

Following 8 phlebotomies of 12.5% of the EBV, the total proteins decreased from 6.94 to 5.55, the albumin from 3.25 to 2.71 and the globulins from 3.52 to 2.77. These changes, although small, were significant ($p<.05$). The level of the serum iron is the only other value that decreased significantly (from 140-176 to 108-98). These values were, however, higher than those seen in iron deficiency anemia.

The only value that increased significantly was the platelet count which increased by 64.5 and 62.0% in the groups receiving bolus iron and multiple dose injectable iron respectively. The platelet count increased by 37.5% in the group receiving only oral iron.

The levels of electrolytes, 2,3-DPG, lactate, pyruvate, glucose and fibrinogen did not change significantly during the course of the phlebotomies.

Physiological measurements. Four days following the last phlebotomy, each dog was anethetized with sodium pentobarbital (30 mg/kg), intubated and ventilated by a positive pressure respirator with a 47.5% oxygen, 2.5% carbon dioxide, 50% nitrous oxide mixture. Cardiac output measurements were made using the thermodilution technique. Blood pressure and blood gas measurements were made at 30 minute intervals.

Physiological measurements were made (see Table 4) in 21 dogs which had been bled 100% of their estimated blood volume and in 9 dogs who were not previously bled (controls).

TABLE 4

Physiologic Variables

Variable	Post-Bleeding Values (N=21)	Non-Bled Values (N=9)
Mean Systemic Arterial Pressure ($\overline{SAP}$) mm Hg	112.5	122.1
Mean Right Arterial Pressure ($\overline{RAP}$) mm Hg	6.3	2.8
Cardiac Output (C.O.) L/min	3.70	3.30
PaO_2	282.3	308.2
$PaCO_2$	33.9	37.5
pH	7.36	7.38
Percent Saturation (% SAT)	99.37	99.98
Systemic Vascular Resistance dsc	2474	2896
Oxygen Delivery ml O_2/min	566.7	638.5
Oxygen Consumed ml O_2/min	138.8	125.3
Percent Oxygen Utilized	25.9	19.6

The mean arterial pressure was 9.6 mmHg lower in the group that had been bled, yet the right arterial pressure was higher (6.3 vs 2.8). The cardiac output was minimally increased in the animals which had been bled as compared to the non-bled controls. The slight decrease in the systemic vascular resistance in the bled animals accounted for the decrease in the mean arterial pressure since the cardiac output was higher than normal. The percent saturation was normal in both groups of animals. Oxygen delivery was minimally decreased in the animals which had been bled while oxygen consumption remained normal. This was accomplished by a higher percentage of utilization in the bled animals in comparison to the controls (25.9% and 19.6% respectively).

Tolerance to massive hemorrhaging. Animals were bled to 50 mm Hg and held there for 90 minutes; and then bled to 30 mm Hg and held there for an additional 2 hours. The mean survival time in 11 dogs who had been bled 100% of their estimated blood volume was 6.4 hours following resuscitation. A 12th animal survived for greater than 24 hours. The mean survival time in five unbled animals was 2.2 hours. Although the animals that had been previously bled on multiple occasions survived almost 3 times as long, the degree of shock proved to be fatal in less than 12 hours in 91.6% of the cases.

Recommendations for the Future

The mission of the Department of Cardiovascular Physiology in the Division of Surgery has been changed for FY 81. Certain facets of this research will be continued under accession number DA OG 2221 and OMA supported activities. Future objectives will be to correlate physiologic function with changes in the number and ratio of mature cells to immature cells, and to determine if the increase in the number of platelets facilitates obtaining hemostasis following bleeding. A long range objective is to determine how much blood (red cells and/or plasma) can be lost before there is a change in exercise tolerance. The ultimate goal of this objective is to determine whether or not soldiers in a combat zone can donate blood for stockpiling and still maintain combat readiness; and if so, how often can they donate blood.

The new mission of the Department of Cardiovascular Physiology is "The Management of Military Blast Injury." Our technical objectives will be 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 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 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.

Project: 3M161102BS01 BASIC RESEARCH ON MILITARY INJURY AND DISEASE

Work Unit: 144 Control Mechanisms of Regional Circulation

Literature Cited:

References:

1. Fleming, A.W., Green, D.C. Radcliffe, J.H., St. James, D.M., and Fleming, E.W.: Development of a practical autologous blood transfusion program. Amer. Surg. 43:794, 1977.

2. Fleming, A.W. and Garcia, C.S.: Use of blood components in cardiac surgery, in Blood, Blood Components and Derivatives in Transfusion Therapy. Washington, DC, American Association of Blood Banks, 1980, Chapter 9, pp 173-209.

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PROJECT 3M162770A802

MILITARY PREVENTIVE MEDICINE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6465	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY TYPE	5. SUMMARY SECURITY	7. REGRADING	8. SMRY INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
78 10 01	M, Term	U	U	N/A	N/L	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A802	00	008
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Tropical and Subtropical Diseases in Military Medicine

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 08	CONT	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 79	9	580
FISCAL YEAR CURRENT 80	9	573

19. RESPONSIBLE DOD ORGANIZATION

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ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: ECHEVERRIA, P.E., MAJ; USSERY, M.A., CPT

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Infectious Diseases; (U) Hepatitis; (U) Gonorrhea; (U) Fectors; (U) Diarrhea

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 ecological and biological factors that predispose U.S. military personnel to tropical infectious diseases. Characterization of disease organisms and response to infections is done in support of vaccine development.

24. (U) The etiology, epidemiology, and ecology of disease organisms are studied in the field and in hospital. In vitro cultivation, serological procedures, microbiological assays, mosquito inoculation, vector colonization, and other techniques are used to characterize disease organisms.

25. (U) 79 10 - 80 09 Autopsies revealed viral antigen predominantly in the reticuloendothelial cells. Dengue virus was recovered from 5 percent of A. aegypti mosquitoes collected. Virus was isolated from patients by intrathoracic inoculation of mosquitoes. Virtually 100 percent of Thai adults had antibody to HAV. HAV accounted for 75 percent of cases of acute hepatitis in young children, 25 percent in young adults, and less than 10 percent in adults over 30 years. HNANB has been found to be most common in adults. Diarrheal diseases in Americans new to the Asian environment were studied. Nine chigger species were found to harbor the scrub typhus rickettsia. The infection rate was eight percent. An ELISA test was developed for diagnoses of scrub typhus. Among penicillin-resistant N. gonorrhea, 18.5 percent produced penicillinase. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval.

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

Project 3M162770A802 MILITARY PREVENTIVE MEDICINE

Task 00 Work Unit 008 Tropical and Subtropical Diseases in Military Medicine

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1. Ecology and Epidemiology of Dengue Viruses in Din Daeng, Bangkok

PROBLEM: The overall objectives of this study 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 and utilization of artificial containers by this species for oviposition.

PROGRESS: Analysis of present data reveals definite seasonal changes in the densities of Ae. aegypti in Din Daeng, corresponding to both temperature and precipitation. These changes, however, are not of a very large magnitude. During the first two years of the study data indicated the aegypti density in the study area was gradually increasing. During the last year, however, this increasing trend stopped and population densities did not increase as expected. The reason(s) for these changes are not currently understood.

These surveys continue to demonstrate that of the three housing types sampled, i.e. slums, shops and high-rise flats, the slums have the most infestations of Ae. aegypti, while the high-rise flats have the fewest infestations. These data are influenced by the slums having at least two times as many water jars as the shops and high-rise flats. In addition to fewer water jars the high-rise flats often have screens. Data from the container surveillance aspects of this study definitely indicate a correlation of high aegypti densities with lower standard housing.

FUTURE OBJECTIVES: This project is scheduled for termination at the end of this calendar year. Sufficient data have been accrued during the past three years to evaluate population densities and container utilization aspects of this study. The analysis of data will continue into next year.

REFERENCES:

1. Watts, D.M., Harrison, B.A., Johnson, D.E., Klein, T.A. Ecology and Epidemiology of Dengue Viruses in Din Daeng, Bangkok. AFRIMS Annual Progress Report, October 1978 to September 1979.

2. Weather and Dengue Hemorrhagic Fever Case Rates

PROBLEM: Reported case rates of DHF in Bangkok follow an annual cycle with peak case rates in the mid-rainy season (June to October), falling case rates in the late rainy season and cool season (November to March) and rising case rates in the hot season (April to May). Although the cycle pattern is relatively constant the amplitude may vary by a factor of ten-fold between mild and severe years This analysis was undertaken to determine if meteorologic variables played a role in determing the severity of DHF seasons in Bangkok.

PROGRESS: Linear regression analysis of monthly case rates versus total yearly case rates for the years 1962 through 1978 showed that case rates in May of a given year, at the onset of the rainy seasons, are strongly correlated with the total number of cases in that year ($N=17$), $r=.858$ $p< .001$). Case rates in March of a given year (typically the nadir of DHF activity) are also significantly correlated with the total number of cases in that year ($N=16$, $r=.696$ ($p< .01$).

These observations suggested that factor(s) which govern DHF case rates are operative well before the onset of the rainy season. Meteorologic data from the Bangkok weather bureau were obtained from the Ministry of Communications and analyzed in relationship to DHF rates as recorded by the Ministry of Public Health by linear regression analysis. A strong positive correlation ($p < .001$) was observed between the mean monthly temperatures of December and January and the DHF case rates in December, January and February; in years with "relatively warm" cool seasons DHF case rates tended to remain higher than years with lower temperatures.

In this analysis hot and rainy season temperatures were found not to be closely correlated with DHF cases rates. Other meteorologic variables evaluated included total rainfall per month, number of days with rain per month, mean relative humidity per month, days with minimum relative humidity less than 70% per month, mean wind velocity per month, and days with mean wind velocity per month greater than 10km/hr. None of these variables were found to closely correlate with DHF case rates at anytime throughout the year.

Although high DHF case rates can be correlated with high cool season environmental temperatures, the mechanism is unknown; one good explanation is that temperature may exert an effect on the extrinsic incubation period of dengue virus in Aedes aegypti.

FUTURE OBJECTIVES: Although there is an association between ambient temperature and DHF case rates in Bangkok, the role of weather in DHF epidemiology in other countries requires study. A relationship between minor weather changes covering broad geographic areas and dengue activity in these areas seems likely.

3. Effects of Temperature on the Extrinsic Incubation Period of Dengue Virus in Aedes aegypti

PROBLEM: Analysis of weather data and DHF case rates in Bangkok has shown an association of relatively "warm" cool seasons with subsequent increased DHF activity. Laboratory experiments were therefore undertaken to evaluate the effect of temperature on the rapidity with which an Aedes aegypti mosquito can become infected and transmit dengue virus from one primate to another (the extrinsic incubation period).

PROGRESS: Experimental studies were conducted to determine the ability of a local strain of Aedes aegypti to transmit a local strain of dengue virus type 2 to Rhesus monkeys at temperatures comparable to those of the different seasons in Bangkok, Thailand. After feeding on a dengue 2 infected monkey with an undetectable viremia, mosquitoes maintained at 20°C, 24°C, 26°C, 28°C, and 30°C, became infected but only the mosquitoes maintained for 25 days at 30°C transmitted virus to monkeys. Infection rates ranged from 25% for mosquitoes at 20°C to 58% for mosquitoes at 30°C. In a second experiment, Ae. aegypti were fed on a viremic monkey with $2 \times 10^{3.0}$ plaque forming units per 0.3 ml of plasma. Virus transmission was not demonstrated using mosquitoes maintained for 3, 7, 12, 18, and 25 days at 26°C while mosquitoes maintained at higher temperatures transmitted virus to monkeys. Mosquitoes maintained at 32°C and 35°C were capable of transmitting virus 4 to 7 days after feeding on infected monkeys, while 8 to 12 days were required for mosquitoes maintained at 30°C to transmit virus. These experimental findings indicate that the efficiency of Ae. aegypti as a vector of dengue virus type 2 was influenced markedly by the amount of virus ingested and by minor changes in temperature. The significance of the findings under field conditions is unknown; however, these data may account for the marked decrease in human virus infections during the cool season (mean daily temperature of 24-25°C) and the rapid and sustained rise in the incidence of human infections during the hot and rainy seasons (a mean daily temperature of 31°C and 27°C, respectively) in Bangkok, Thailand.

FUTURE OBJECTIVES: The universality of the demonstrated effect of temperature on the extrinsic incubation period should be confirmed with other virus types and strains and other mosquitoes species and strains. The nature of the block of transmission at temperatures below 27°C should be defined.

4. Dengue Viruses in Mild Febrile Illness and Hemorrhagic Fever in Bangkok

PROBLEM: Previous studies from the SEATO Lab have estimated that only one of every 50 to 100 dengue virus infections in Bangkok results in hemorrhagic fever; most produce little or no clinical disease. This study was undertaken to determine the role of the infecting virus type and the patient's past flavivirus exposure history in the development of dengue hemorrhagic fever.

PROGRESS: During the calendar year 1979, 229 children with clinical hemorrhagic fever and 189 children with undifferentiated febrile illnesses (pyrexia of uncertain origin, PUO) were studied. Among 219 cases of serologically confirmed DHF in children older than one year, 215 demonstrated a secondary flavivirus type HAI antibody response; only four (2%) showed a primary antibody response. Fifty-five virus isolates were obtained from these children; one was dengue type 1, 53 were dengue type 2, and one was dengue type 4. Among 11 confirmed cases of DHF younger than one year, all 11 had primary type antibody responses; seven virus isolates, all dengue type 2, were obtained from these children. Although good paired sera were obtained from 161 of the 189 PUO patients studied, only 10 (6%) were shown to be caused by flaviviruses. The infecting virus type was successfully identified in all 10 flavivirus PUO cases: in all eight with a secondary type antibody response dengue type 2 was isolated, while among the two primary cases dengue type 1 was isolated from one patient and the other had a monospecific antibody rise to Japanese encephalitis virus.

Sixty-nine of 174 (39%) PUO patients who did not show evidence of a current flavivirus infection 69 had detectable HAI antibodies in their acute serum (32 of 122, or 26% of children six years old or less and 37 of 52, or 71% of children seven through 13 years old).

These studies show that (1) dengue type 2 virus was by far the most important cause of both mild and severe illness due to flaviviruses in Bangkok in 1979 and (2) a higher proportion of children with either mild or severe illness in

1979 were experiencing secondary flavivirus infections than would be expected based on the flavivirus age specific antibody prevalence in non-dengue PUO patients.

FUTURE OBJECTIVES: Although an association between prior flavivirus exposure, current infection with dengue type 2, and clinical illness was clearly shown during the 1979 season in Bangkok, role of prior flavivirus exposure in the development of clinical illness in infections with other dengue virus types remains unestablished. This study should be repeated in a year in which multiple dengue types are circulating in the Bangkok area.

5. Elevated Serum Acid Phosphatase Activity in Dengue Hemorrhagic Fever

PROBLEM: The mechanism whereby increased vascular permeability, intravascular coagulatin, 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. We therefore studied serum levels of a known readily measured lysosomal enzyme, acid phosphatase, to determine if lysosomal enzyme release might be associated with the pathophysiology of hemorrhagic fever and shock.

PROGRESS: Fresh serum specimens from 66 healthy children at a Bangkok school, 68 children older than one year with pyrexia of undetermined origin but proven serologically to be not due to dengue (ND-PUO), and 74 children older than one year with serologically confirmed DHF were assayed for acid phosphatase activity. Among the healthy children (ages 4-12) there were no significant age related differences in AcPtase activity. Overall mean (+1S.D.) values for healthy children with non-dengue PUO's, and children with DHF were $1.31 \pm .28$, $1.11 \pm .27$, and $2.05 \pm .72$ units/ml respectively. ($p < .001$ for healthy children vs DHF cases). Children under one year old with DHF had much higher serum AcPtase value than age matched PUO controls, 3.54 ± 1.05 ($n = 10$) vs $1.33 \pm .30$ ($n = 11$). The highest single value observed (5.55 units) was in a seven month old female child who died with DHF; dengue type 2 was isolated from her acute blood.

The mean serum activity of nine children with uncomplicated serologically documented dengue fever was $1.17 \pm .31$; in seven of these cases dengue virus was isolated from the acute blood.

In measles virus infections, (another virus known to preferentially infect monocytes and other reticuloendothelia cells) the mean serum AcPtase activity was 1.41 $\pm$ .34 ($n = 20$; $p < .01$ compared to DHF mean).

FUTURE OBJECTIVES:

1. The isozyme pattern of the elevated serum AcPtase should be characterized to identify the cell type of origin of the enzyme.

2. The temporal association of elevated AcPtase activity and shock needs to be defined.

3. Other markers of lysosomal enzyme release (B-glucuronidase, lysozymes, lactoferrin) should be sought in urine and serum of DHF patients.

6. A Prospective Study of Acute Viral Hepatitis in a Military Hospital in Bangkok

PROBLEM: Hepatitis viruses type A (HAV) and type B (HBV) are endemic in Thailand; most healthy adult Thais have serum antibodies to both viruses. The proportion of acute hepatitis cases caused by HAV, HBV and other (NANB) viruses in Thailand was determined to assess the relative impact and priority for control of each of these viruses in Thailand.

PROGRESS: During the one year period 1 July 1979 through 30 June 1980, 122 consecutive adult Thai patients who presented to the Royal Thai Army Hospital with a clinical diagnosis of symptomatic acute viral hepatitis were prospectively studied. Criteria for acceptance as a confirmed case of viral hepatitis were (1) elevated serum SGOT or SGPT ($\geq$80 units/ml) and serum total bilirubin ($\leq$2.0 mg%) (2) lack of history of hepatotoxic drug exposure and (3) no alternative diagnosis ultimately disclosed. On each clinic visit a history and physical examination questionnaire form was completed and blood was obtained for SGOT, SGPT, bilirubin, anti-HAV IgM, HBsAg, anti-HBs, anti-HBc, anti-cytomegalovirus, and anti-Epstein-Barr virus tests. The average number of hospital visits and blood samples per patient was 3.4 $\pm$ 1.6 (mean $\pm$ 1S.D.). Of the 91 cases that met study criteria for confirmed viral hepatitis, a follow-up period of one week or longer, one month or longer, or three months, or longer was obtained in 83%, 71%, and 52% respectively. Seventy-five cases were male and 16 female; 40 were military active duty and 51 were civilian.

Overall 21 cases could be attributed to HAV, 59 to HBV, four to simultaneous HAV and HBV, and none to either CMV or EBV; in seven cases no known viral etiology could be assigned (NANB). One patient with HBV died of fulminant hepatic failure. Among 62 patients with follow-up laboratory studies obtained 12 weeks or more after presentation, 1/19 HAV cases, 1/38 HBV cases, and 1/4 NANB cases had persistent abnormalities of serum SGOT, SGPT, or bilirubin levels.

FUTURE OBJECTIVES:

1. Studies to define the mechanism(s) of spread of hepatitis viruses in Thailand, especially HBV should be undertaken.

2. The importance of HAV, HBV, and NANB viruses in acute hepatitis in age groups other than young adults remains to be established.

7. Development of Solid Phase Radioimmunoassays to Detect IgM and IgG anti-Hepatitis B Core Antibodies

PROBLEM: Hepatitis B virus (HBV) is endemic in the population of most SEA countries; five to 10% of all healthy persons in Thailand are chronic carriers of this virus. Although viral surface antigen (HBsAg) can be detected in the blood of chronic carriers and of patients with acute hepatitis due to HBV, at present there is no reliable method to rapidly distinguish between these two types of patients. Clinical evaluation of anti-viral compounds and vaccines against HBV will be critically dependent on the ability to accurately differentiate between acute and chronic infections with HBV. This project was undertaken in an effort to develope such an assay.

PROGRESS: A SPRIA for IgM anti-HBc was developed which utilizes the following procedure: (1) sensitize a polyvinyl 96 well microtiter plate with goat anti-human μ chain anti-sera at a 1:100 dilution in carbonate buffer (2) add 100 microliters of the test serum at a 1:100 dilution in PBS for screening (3) add 25 microliters of a crude two percent suspension of a liver from a chronic carrier gibbon diluted in 20% normal human serum (4) add 25 microliters of purified high titered anti-HBc IgG from the serum of an HBs antigenemic human blood donor labelled with I^{125}, approximately 150,000 CPM/well (5) count CPM in a gamma counter.

In order to evaluate assays for specific IgM and IgG anti-HBc activity a test panel of 40 sera were selected as follows: (A) Acute sera from 10 patients with a diagnosis of

acute HBV with HBsAg in acute sera with subsequent disappearance in convalescent sera (B) 90 day convalescent sera from the 10 patients in group A; (C) sera from 10 healthy adult blood donors in whom HBsAg was detected by CIEOP at the time of blood donation; (D) acute sera from 10 patients with a diagnosis of acute hepatitis A virus infection (HAV) based on the demonstration of anti-HAV in acute blood which was not absorbable with staph aureus (presumably IgM - anti-HAV) and with no detectable HBsAg in the acute blood specimens. The 10 patients were specifically selected to have anti-HBs in the acute specimen.

All 40 sera in the test panel were subjected to sucrose density gradient rate-zonal fractionation and the total anti-HBc activity of fraction three (the peak of IgM activity as detected by immunodiffusion) and fraction eight (the peak of IgG activity as detected by immunodiffusion) of each gradient were measured by CORAB (R).

In all four groups the P/N results obtained with the IgM HBc assay correlated well with the blocking anti-HBc activities in the respective "19S" serum fractions, and showed no correlation with the activity of the "7S" fraction.

Acute sera from patients initially diagnosed as having acute viral hepatitis were tested by the IgM HBc SPRIA. All 65 of the HBsAg positive serum specimens tested were positive for IgM anti-HBc. One specimen initially diagnosed as HBsAg positive but IgM anti-HBc negative was subsequently found to be HBsAg negative on retesting. Of the nine HBV patients with low levels of IgM anti-HBc (1-2.0 units) six had follow-up sera available for testing 80 days or later after presentation; all six (100%) were persistently positive for HBsAg. Of the 59 HBV patients with higher titers (>2 units), 33 had sera available for testing 80 days or later after presentation; two (6%) of these were persistently positive for HBsAg. Eight of 25 HAV patients, all negative for HBsAg, had low levels of anti-HBc IgM, and three of 24 patients without other evidence of either HAV or HBV had detectable IgM anti-HBc.

FUTURE OBJECTIVES:

1. The technique should be applied to detect IgA and IgG anti-HBc as well as IgM anti-HBc.

2. The temporal course of IgM, IgA, and IgG anti-HBc in HBV infections should be defined; this will almost certainly lead to the ability to differentiate between acute and chronic HBV antigenemias.

8. Hepatitis A Virus (HAV) Infections in Primates

PROBLEM: Two primate species are generally accepted as models for acute HAV infection, chimpanzees and marmosets, both are endangered species and their use in biological experimentation is severely restricted. Recently we have found that a high percentage of cynomologous monkeys (Macaca fascicularis) have serum antibodies to HAV, suggesting previous infection. As this species is plentiful in SEA, we conducted studies to determine if HAV transmission was occurring between cynomologous monkeys in the wild, and if HAV could be transmitted to cynomologous monkeys in the laboratory.

PROGRESS: Blood specimens were obtained from 98 freshly trapped cynomologous monkeys in central Malaysia in October-December 1979. Overall 20% had serum antibodies to HAV (HAVAB (R)). Antibody prevalence was related to monkey weight: 1 kg, 5%; 1-1.9 kg, 14%; 2 kg, 76%. Twenty-eight monkeys with serum specimens taken in late 1979 were again bled in June 1980 after 5-8 months in captivity in Kuala Lumpur, 2/2 (100%) of those previously positive remained positive, but in addition 25/26 (96%) of these previously sero-negative had developed serum HAV antibodies, suggesting that virus transmission was occurring in captivity.

In a challenge study, 10 primates (2 HAV (+) cynos, 2 HAV (-) cynos, 2 HAV (+) Rhesus, 2 HAV (-) Rhesus, 1 HAV (+) gibbon, and 1 HAV (-) gibbon) housed in the AFRIMS Vet Med facility in Bangkok were inoculated intravenously with 0.5 ml of a 0.2% suspension of a known HAV infectious stool filtrate (strain FJ-1-2-19, supplied by MAJ Stanley Lemon, WRAIR). Animals were examined clinically daily and blood specimens were obtained twice weekly for determination of complete blood count, total bilirubin, SGOT, SGPT, alkaline phosphatase, and anti-HAV. Stool specimens were collected daily for HAV antigen detection. Open liver biopsies were planned if the animals developed abnormal liver function tests.

No animals became clinically ill during the 90 day study period. None developed abnormal liver function tests, therefore no liver biopsies were done. One previously healthy cynomologous monkey which had seroconverted to HAV died suddenly 92 days post inoculation; no significant gross or microscopic pathology was found. No change was seen in the anti-HAV percent blocking in sera from HAV immune animals. All five HAV non-immune challenged primates developed HAV antibodies after inoculation; greater than 50% blocking occurring at four to five weeks for the two cynos, four weeks

for the gibbon, and at 10 to 11 weeks for the two Rhesus monkeys.

Attempts to detect HAV antigen in the stools of the sero-converting cynomologous monkeys have thus far been unsuccessful.

FUTURE OBJECTIVES:

1. Field studies should be conducted to determine the role of a possible natural monkey HAV reservoir in the epidemiology of human disease in countries with large primate populations.

2. The cynomologous monkey model of human HAV infection should be developed further to determine the sites of virus replication, the suitability of the model for vaccine testing, and the effects of adaptation of local human strains to monkeys by serial passage in monkeys with the ultimate goal of cultivation of local strains in vitro in cynomologous monkey derived cell cultures.

9. Rapid Diagnosis of Japanese Encephalitis Virus Infection by Solid Phase Immunoassays

PROBLEM: Infections with JEV are difficult to diagnose in the laboratory with existing techniques, and rapid diagnosis of this disease is currently impossible. JEV is an important cause of morbidity and mortality throughout South East Asia; an improved method of diagnosis would be an important step toward improved control.

PROGRESS: A solid phase "reverse" radioimmunoassay was developed to detect IgM class specific antibodies to JEV. Steps in the assay are (1) sensitize a polystyrene or polyvinyl solid phase surface with goat anti-serum to human U chains (2) add the test serum or CSF sample (3) add sucrose acetone extracted mouse brain JEV antigen at a 1:100 dilution (4) add labelled (I^{125} for RIA or alkaline phosphatase for ELISA) IgG anti-flavivirus. The test is highly sensitive, detecting IgM anti-JEV in acute blood serum specimens at dilutions of 10^{-4} or greater. The proportion of patient sera with detectable activity ($P/N \geq 2.1$ at a 1:100 dilution) are as follows: acute JEV sera 9/10, convalescent JEV sera 10/10, acute and convalescent sera from patients with encephalitis not due to JEV 0/20, normal flavivirus antibody positive adults 1/20, and normal flavivirus antibody children 0/10. False positive reactions were found with sera from patients with recent dengue infections; with these sera results obtained using JEV

antigen were invariably lower (P/N <10) than when the homologous antigen (dengue) was used in the (P/N> 20-150). Low level false positive were also occasionally encountered when high titered rheumatoid factor sera were tested; these were characterized by persistent positive reactions when normal mouse brain antigen was used in the test.

Insufficient late convalescent serum specimens were available to determine the persistence of serum IgM anti-JEV in humans after clinical encephalitis. In two experimentally infected Rhesus monkeys the P/N value was maximum 10 days after inoculation, then progressively fell to below the cut-off by 60 days.

Twelve acute CSF specimens from patients with encephalitis serologically proven by HAI to be due to JEV were tested; all were positive with P/N ratios higher than found in simultaneously obtained serum samples. Zero of seven CSF specimens from patients with encephalitis not due to JEV were positive.

The SPRIA was modified to use the enzyme-linked immunoassay method. In preliminary tests negative and positive reactions could easily be differentiated by naked eye.

FUTURE OBJECTIVES:

(1) Define the duration of persistence of IgM anti-JEV in humans after mild and severe infections.

(2) Evaluate the magnitude of the IgM-anti-JEV response as a measure of disease severity.

(3) Determine if CSF IgM anti-JEV is a reliable marker of active viral replication in the central nervous system, and can therefore be used as a measure of efficacy of anti-viral drugs.

(4) Evaluate the usefulness of the reverse ELISA IgM anti-JEV to replace the hemagglutination inhibition technique in existing sero-epidemiological studies which utilize dried blood specimens sent by mail.

10. Immunization of Americans with the Biken Japanese Encephalitis Virus Vaccine

PROBLEM: Encephalitis caused by infection with the Japanese Encephalitis virus is a major disease problem throughout SEA.

Previous trials of the only currently available vaccine (Biken Co., Osaka, Japan) in Americans produced conflicting results, but in the main suggested that the vaccine was poorly immunogenic in flavivirus non-immune Caucasians. In 1979 the U.S. State Department elected to inoculated U.S. Peace Corps and U.S. AID personnel stationed in Nepal with the Biken vaccine. A prospective blinded cooperative study was designed to critically evaluate the immunogeneicity of the Biken JEV vaccine in Americans.

PROGRESS: The study group consisted of 55 subjects. Twenty were associated with the American diplomatic mission or with U.S. AID. Two of these subjects were dependents nine years of age; the remainder were aged 29 through 62 years. Three adults subjects were of non-American extraction (one each from Columbia, Pakistan, and Philippine origin). In addition 35 Peace Corps Volunteers participated in the immunization series. Completion of the full immunization program with three serum titers, particularly a post-immunization titer was hampered by remoteness of the work/residence sites for many of the volunteers.

Lyophilized vaccine was obtained from Biken in Osaka and shipped to Kathmandu refrigerated on ice by commercial air freight. Upon receipt the vaccine was stored at $4^{o}C$ until being reconstituted with diluent per the manufacturers instructions. Reconstituted vaccine was refrigerated at $4^{o}C$ then discarded after one week if not used.

All subjects received a series of three intramuscular infections of 1.0 c.c. Biken JBE vaccine. Immunizations were administered on days 0, 7-14, and day 28. Serum was drawn on day 0 (pre-immunization), on day 28 (mid-immunization) and from one to seven weeks after the last immunization (post-immunization). Sera were split with aliquots being submitted to each of the three participating labs: AFRIMS, NIH, and Biken. Specimens were coded with alphabetical tags so that none of the labs was aware of whether a sample was a pre, mid, or post immunization titer. All sera were shipped on ice and hand carried by courier aboard commerical airlines from the American Embassy Clinic in Kathmandu to the respective labs. Serum titer reports were submitted by each of the labs and thereafter decoded by the medical staff of the embassy clinic.

The percent of specimens with detectable neutralizing activity before and after the immunization sequence as determined by each laboratory on the coded specimens were: AFRIMS 5%→60%, NIH 40%→92%; Biken 6%→83%.

FUTURE OBJECTIVES: The Biken JEV vaccine does appear to produce low level neutralizing antibodies in most vaccinees, and therefore its use should be considered to protect at risk field and laboratory workers. The persistence of neutralizing antibody activity should be determined in order to formulate appropriate booster immunization schedules.

11. A Longitudinal Serologic Study of A Bangkok School Population

PROBLEM: To determine the incidence of clinical and sub-clinical togoviral infections during the "disease season" and during the period of the year without a large amount of clinical illness. Also to establish reasonable definitions for primary and secondary infections based on HAI survey data.

PROGRESS: The results of serologic samples obtained between June 1977 and June 1979 have been presented in a previous report (1). In that report definitions of primary and secondary infection were taken as generally accepted in the literature (2-4), namely:

Primary Dengue Infection: The acquisition of one or more type specific dengue HI antibodies in a person previously exhibiting no such antibodies (a titer of 1:20 is considered evidence of such antibody).

Secondary Dengue Infection: Evidence of a four-fold or greater titer increase in one or more type specific HI antibodies in a person exhibiting a 1:$\geq$20 titer to one or more dengue antigens.

Because the definition of a secondary dengue infection gave results inconsistant with clinical hospital admission data, the effect of more rigorous definitions was investigated in those sera collected between June 1977 and January 1978 during the "dengue season" of an "epidemic year". Stricter definitions (i.e. eight fold titer rise) dropped cases by 25-50%% depending on the definition. However, if the definitions were applied to the January 1978-January 1979 data where fewer cases would be expected, the case drop was 60-95%. Meaning that during the clinical quiesent period. A much higher proportion of seroconversions have only the minimum titer elevation required for consideration as a case. If, in fact, an eight fold titer elevation is applied to Table 2 in reference 1. The resulting incidence for the three periods is 12.0%, 3.8%, and 4.9% (vs 18.4%, 9.2% and 21.0% as given)

and are more in line with the incidence of primary infections and the incidence of reported hospitalizations.

FUTURE OBJECTIVES: In January 1980, this survey was continued with 998 samples collected from Phibunprachasan Schools. These specimens are currently having HI tests performed upon them.

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12. Investigation of the Epidemiology and Microbiology of Wounds and Wound Infections in the Royal Thai Army (RTA)

PROBLEM:

1. To describe the extent and distribution (by agent and anatomical site) of wounds, incurred as a result of combat, appearing at 1^{o} and 2^{o} surgical centers.

2. To establish the types and quantities of microbial flora coexisting with these injuries.

3. To relate microbial occurrence to risk of overt infection, severity and type of injury, subsequent treatment and residual morbidity.

4. To ascertain the value of the injury/microbiology/infection approach for use in predicting the infection potential of combat injuries.

PROGRESS: This report represents the conclusion of this investigation as reported in the two previous annual reports. Case acquisition and specimen collection were completed prior to FY 80, therefore follow-up and summary conclusions are presented in this report.

Follow-up: Follow-up was completed on all of the 275 patients enrolled in the study from Phramongkutklao Hospital and all 78 patients acquired through the two hospitals at Phitsanulok on 15 February 1980. Fourteen patients (5%) from Phramongkutklao remained hospitalized at that time and all were in excess of the 300 day limit for length of hospitalization calculation. Mean length of hospitalization from the Phitsanulok hospitals was less than 40 days with over 75% of patients requiring less than 30 days hospitalization. The corresponding figures for Phramongkutklao Hospital were a mean stay of slightly in excess of 105 days and 28% of patients requiring less than one month in the hospital.

Summary conclusions: Significant variance occurs between the patient populations of the primary and secondary care facilities, especially in regard to the agent of injury, anatomical location of injury and the prevalence of complicated injuries.

By antibiotic sensitivity patterns, there is no difference in the bacteria that are contaminating combat injuries in Bangkok and Phitsanulok. The relative frequency of isolation of the various types of bacteria between the two levels of care is, however, significantly different. Wound contamination at the primary level of care is probably autogenic contamination from skin (Staph. epidermitis), urine (Mima sp) or feces (Enterobacter and Klebsiella sp). Isolates at the secondary level of care are most likely of nosocomial origin (Staph. aureus and Pseudomonas sp). Universal use of multiple antibiotics in irregular dosages make assessment of the impact of antimicrobials on this situation impractical. Generalized bacterological complications (septicemia, UTI, pneumonia) are, in fact, rare and hospital mortality is very low.

Bacteriological contamination of injuries is not totally benign, however, but is associated with a prolonged hospitalization (1).

From the point of view of either individual resultant disability or societal cost of hospitalization, the use of the anti-personnel land mine by insurgent forces in Thailand has had the largest impact on the patients followed during this investigation.

FUTURE OBJECTIVES: This study is complete.

REFERENCES:

1. Annual Progress Report, AFRIMS, 1978-1979.

13. Studies on Venereal Disease

PROBLEM: Venereal disease is a major problem for military personnel (1). In the Far East multiple antibiotic resistance is common among N. gonorrhoeae (2) however resistance to penicillin (due to a plasmid encoding for β lactamase) has been restricted to the Philippines, Indonesia, Thailand and Malaysia but has not spread to Taiwan, Korea or Japan. Plasmids encoding for β lactamase in N. gonorrhoea isolated from different regions of the Far East will be characterized and compared with plasmids encoding for β lactamase in other bacterial pathogens for example Haemophilus influenzae type B, enterobacteriae etc. and compared with each other to determine if there are regional variations in plasmids coding for lactamase.

Chancroid is the second most common venereal disease among US Army troops in South Korea (personal communication). Within the last year doctors at the CDC and at the University of Mannitoba in Canada have successfully isolated Haemophilus ducreyi in the United States, Canada, and Africa (3,4). There appears to be considerable differences in these organisms depending on geographical source. Antimicrobial resistance appears to be common. At present this organism has not been isolated in Korea and physicians do not know how to treat soldiers infected with this organism.

PROGRESS: N. gonorrhoeae, lactamase + and -, strains have been collected in the Philippines (with the USAF), Indonesia (with the US Navy), Hong Kong and Malaysia (with Dr. Keith Arnold) and by ourselves in Thailand. Plasmid DNA have been isolated and plasmids coding for lactamase have been compared with plasmids encoding for β lactamase in H. flu type B(6). Geographic differences in the plasmids is being examined.

FUTURE OBJECTIVES: To perfect our techniques of isolating H. ducreyi in Thailand. To subsequently determine the point prevalence of infections among US Army troops in Korea, determine the antibiotic susceptibilities of these isolates and then perform treatment trials.

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14. The Etiology and Epidemiology of Acute Diarrhea in Southeast Asia

PROBLEM: Acute diarrhea is responsible for considerable morbidity among newly arrived troops in poorly sanitated tropical and subtropical areas (1-3). Some of the agents that cause diarrhea are known but a great many remain to be determined. The epidemiology, risk factors, and pathogenesis of some of the recently discovered enteropathogens, (enterotoxigenic E. coli, Rotavirus, Norwalk agent, Campylobacter etc.) are being examined. In addition other potential enteric bacterial pathogens, Aeromonas hydrophila, Plesiomonas shigelloides, and non agglutinable vibrios are being investigated. Attempts to protect travelers who have recently arrived from acquiring diarrhea, and an evaluation of the environmental sources of enteric pathogens is being pursued. The epidemiology and characterization of plasmids coding for enterotoxin production in Escherichia coli is being investigated.

PROGRESS: The etiology of diarrheal disease has been determined in five separate Thai populations:

1. Mothers and newborns. This study was performed to determine the role of medical transmission of enteric pathogens and to gain some insight into the protective value of IgG acquired by the infants from their mothers in protecting against enteric pathogens.

2. The prevalence of enteric pathogens in 100 children with diarrhea and 100 children without diarrhea in Bangkok.

3. The prevalence of enteric pathogens in adults admitted to Bamrasdura Hospital in Bangkok over a three month period.

4. The etiology of diarrhea in patients seen at a rural hospital over a two month period.

5. The etiology of diarrhea in three rural Thai villages over a two month period.

The age related acquisition of antibodies to Norwalk agent has been determined in populations in the United States, Taiwan, Philippines, and three populations, one urban and two rural, in Thailand. The age related acquisition of antibody to rotavirus has been determined in a rural Thai population.

The effect of antibiotics on the point prevalence of enterotoxigenic E. coli has been examined in two populations in the Philippines and a population of children in Thailand.

An investigation of the epidemiology of V. cholerae infections at Rangsit refugee camp has been performed.

Travelers' diarrhea acquired by one hundred and twenty-one American Peace Corps volunteers during their first five weeks in Thailand has been investigated. Initially 35 PCV were studied to determine the etiology of their diarrhea, and the extent of antibiotic resistance among enteric pathogens. Subsequently 87 have or are being studied to determine the value of doxycycline in preventing travelers' diarrhea in rural Thailand.

The pathogenesis of Aeromonas hydrophila and Plesiomonas shigelloides has been investigated by comparing the prevalence of these organisms in American and Thai patients with diarrhea and matched controls without diarrhea. Furthermore these organisms have been fed or injected into more than 20 rhesus monkeys, adult and infant mice, and rabbits to determine the enteropathogenicity of these organisms.

Klongs, animals, flies, food etc. in rural villages in Thailand have been investigated to determine the potential sources of enteric pathogens in rural villages in Thailand.

Plasmids coding for heat-stable toxin (ST) have been sent to Dr. Maggie So at the University of California who has found considerable heterogeniety in the genes coding for ST (unpublished observation). Multiply resistant tox+ E. coli have been found to usually contain separate plasmids coding for other LT, ST or antibiotic resistance. Methods to identify CFA I and CFA II in tox+ E. coli have been completed.

FUTURE OBJECTIVES:

1. To further characterize plasmids coding of LT, ST, antibiotic resistance and colonization factors in over 1000 tox+ E. coli collected from previous studies.

2. To expand the rural village study to gain further insights into the epidemiology of enteric pathogens in rural Thailand.

3. To continue to maintain surveillance at Bamrasdura Hospital to gain further insights into the seasonal prevalence of enteric pathogens, their pathogenesis, and hopefully their treatment.

4. To characterize the antibody response to CFAs in E. coli, Campylobacter, and rotavirus by ELISA techniques or radiolysis.

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15. Effects of Plasmodium Infections on the Longevity of the Mosquito Host, Anopheles dirus

PROBLEM: To determine if Plasmodium infections have detrimental effects on the viability of Anopheles dirus, a primary vector of human malaria parasites in Thailand.

PROGRESS: Studies of the effects of P. cynomolgi (Strain B) infections on Anopheles dirus have been completed and manuscripts are in preparation. These data show that: (a) the longevity of An. dirus was significantly effected by P. cynomolgi infections; and (b) a reduction in the daily survival rate was directly correlated to the infection load as measured by oocyst numbers in the midgut, and was especially significant during days 9-20, post infection. Current WHO malaria epidemiological assessment models such as the "entomological inoculation rate" and the "vectorial capacity" are based on the assumption that malaria infections do not affect the daily survival rate (viability)of the mosquito vector. Data from the present studies suggest that this basic assumption may be false and needs re-evaluation. Efforts have been initiated to study the effects of P. falciparum and vivax infections on the viability of An. dirus.

FUTURE OBJECTIVES: Future studies will concentrate on the effects of human malarial infections on the mosquito host, Anopheles dirus. Data from these studies will provide a much better understanding of the epidemiology of malaria transmission.

16. Comparative Susceptibility of Suspected and Known Vectors of Human Malaria to Plasmodium Species

PROBLEM: This study is designed to compare the susceptibility of known and suspected vectors of human malaria in Southeast Asia to Plasmodium cynomolgi (Strain B), P. falciparum and P. vivax.

PROGRESS: Considerable progress was made during this period and sufficient data have been accrued to evaluate the susceptibility of three anopheline species, An. philippinensis (Rayong strain), An. maculatus (Huai Kuum, Thai strain) and An. balabacensis (Perlis form) to P. cynomolgi (Strain B). Additional feeding replicates are needed before at least four other anopheline species or strains can be evaluated. Preliminary evaluations indicate that An. maculatus (Huai Kuum) and An. philippinensis (Rayong) are not very susceptible to P. cynomolgi (Strain B) infections. On the other hand, An. balabacensis (Perlis form) was found highly susceptible to infections of this parasite. Comparisons were based on the control species, An. dirus, an extremely susceptible and efficient laboratory vector of this simian parasite (1).

FUTURE OBJECTIVES: Additional replicates will be run to complete the comparisons to P. cynomolgi (Strain B), and studies have been initiated to compare the susceptibility of the seven available anopheline taxa to the human malarias, P. falciparum and P. vivax.

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17. Mosquito Survey and Taxonomic Studies

PROBLEM: This is a continuing study to identify and determine the distribution of the mosquito fauna of Thailand and Southeast Asia, with primary emphasis on the identification of diagnostic characters for the separation of vector species, groups containing vector species and potential vectors of human pathogens.

PROGRESS: During this period major efforts on the Leucosphyrus Group resulted in: (a) the description and elevation of An. takasagoensis Morishita 1946, from Taiwan, to species status (1); (b) the discovery of several good pupal characters to distinguish balabacensis (Perlis form), from dirus and takasagoensis; and (c) the discovery of An. leucosphyrus, a known vector of human filarial and malarial parasite in Sawawak, in the southern peninsular area of Thailand. Other progress includes: (a) the completion and sending to press of a 195+ page comprehensive revision (2) of the Minimus Group. culicifacies and jeyporiensis in Thailand, with extensive notes on the five additional species in the Myzomyia Series of Anopheles (Cellia) not found in Thailand; (b) the location of a good population of the suspected malaria vector, Anopheles philippinensis in Rayong Province, for comparison with the more common species, nivipes. The pupal paddle characters described by Reid (3) are apparently the only reliable characters for separating these two species; (c) the first collections of An. minimus, a primary malaria vector, from Phangnga Province in a number of years; (d) the discovery and preparation for publication of a new species of Aedes (Finlaya) from Chiang Mai, that appears most closely related to formosensis; (e) the discovery of additional larval, pupal and adult characters to identify An. indefinitus, subpictus and vagus in Thailand; and (f) the near completion of a 72 page manuscript called "A guide to the genera of mosquitoes of Thailand, with illustrated keys, biological notes and preservation and mounting techniques."

FUTURE OBJECTIVES: Morphological studies will continue on the Leucosphyrus Group in Thailand and Southeast Asia, the philippinensis-nivipes problem in Thailand, the surveillance of the vector species densities and distributions in Thailand, and the completion of manuscripts. These objectives are all of major importance to the ellucidation of the mosquito vector species of Thailand.

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3. Reid, J.A. Two Forms of Anopheles philippinensis in Malaya. J. Me. Entomol. 4:175-9, 1967.

18. Mosquito Cytogenetics, Cross Mating and Electrophoresis Studies

PROBLEM: To use the latest cytogenetic, cross mating and electrophoresis 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 populations of vector species and correlate this variation with the susceptibility of the vector(s) to infection with human pathogens.

PROGRESS: Colonies of two Thai strains of An. maculatus and a Thai strain of An. philippinensis were established, while colonization of An. nivipes (Thai strain) is underway. Studies comparing the Thai maculatus with the Malaysian vector strain have begun and similar studies will start between philippinensis (a suspected secondary malaria vector in Thailand) and nivipes as soon as the nivipes colony is firmly established. An upgrading of cytological techniques is in progress, with a switch over to the use of ovarian nurse cell chromosome preparations whenever possible. Considerable progress was made in discriminating the sibling species in the Balabacensis Complex. A map of the salivary polytene chromosomes of An. dirus was completed and published (1). This map has been used to

compare the chromosomes of dirus with those of balabacensis (Perlis form) and balabacensis (Taiwan form). These cytological studies, combined with morphological studies, cross mating studies (in preparation) and karyological studies (2) have revealed that these three taxa are distinct biological entities, deserving full species status. Discriminating features were: (a) length and banding differences in the sex chromosomes; (b) centromere and heterochromatin differences in the metaphase chromosomes; (c) sterility in F_1 hybrid males with distinct morphological abnormalities in the reproductive organs; (d) considerable asynapsis in the autosomes and sex chromosomes of F_1 hybrid larvae; and (e) external morphological differentiating characters on the larval, pupal and/or adult stages. Subsequently, the balabacensis (Taiwan form) has been elevated to full species status as, takasagoensis Morishita 1946 (3). The recognition of these sibling species make it imperative that their roles or potential roles in the transmission of human malaria parasites be re-examined.

FUTURE OBJECTIVES: Continue studies stressing objectives (a) and (b), particularly on the malaria vector species or species complexes in Thailand, e.g., the Balabacensis Complex, maculatus, nivipes and philippinensis. Once objectives (a) and (b) have been attained, objective (c) can be initiated.

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19. Epidemiological and Ecological Studies of Scrub Typhus in Royal Thai Army Field Training Facilities

PROBLEM: Overall objectives for this study were reported previously (1). Objectives for this study period were: (a) to determine the level of Rickettsia tsutsugamushi antibody in sera from small mammals collected in the study area; and (b) the identification of engorged chiggers collected on the small mammals, and their association with unengorged chigger species

collected by the black plate method.

PROGRESS: Tests for rickettsia antibodies were run on the sera of 634 small mammals of 15 species, using commercial conjugate and the micro-indirect fluorescent antibody technique. Antibody seroconversions occurred in 343 of 580 specimens of six mammal species: Rattus bukit bukit (35%), R. koratensis (44%), R. rattus (56%), R. sabanus (68%), R. surifer (88%), and Tupaid glis (13%). Positive animals were collected in all three of the basic habitats in the study area, i.e., grass, early regenerating evergreen forest and secondary evergreen forest. Over 24,000 engorged chiggers were collected on a total of 718 small mammals. These specimens have been mounted on slides and nearly all have been identified. This task should be completed in the near future.

FUTURE OBJECTIVES: These studies are nearing termination, with limited identifications still required. The analysis of the black plate chigger collections, the rodent collections and ecological data, rodent serology data and the engorged chiggers are currently in progress for the preparation of a manuscript. This manuscript will also include the human serology studies reported previously (1).

REFERENCES:

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20. Ectoparasite and Rickettsia tsutsugamushi Studies in Thailand

PROBLEM: To identify 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: A checklist of the ticks of Thailand is now in 4th draft stage and is nearly ready for outside review. A major effort during this year has been the analysis of habitat, weather and seasonal data for the chigger collections and rickettsia isolations discussed in the last annual report (1). In addition, ecological data are being analyzed for the small mammals collected in association with the chigger species

detected with rickettsia in the last annual report. A manuscript (2) "Rickettsia tsutsugamushi strains found in chiggers collected in Thailand" has been prepared and is nearly ready for clearance.

FUTURE OBJECTIVES: Additional collection trips are planned to help define the ecology and identify the potential and known chigger hosts of Rickettsia tsutsugamushi in Thailand. Also, the analysis of the currently accrued ecological data is continuing so that a companion manuscript to that above (2) can be prepared.

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21. Studies on Canine Viral Enteritis (CVE)

PROBLEM:

1. To identify and describe the etiologic agent producing a severe, bloody, often fatal diarrheal disease of canines in the military working dogs at the Royal Thai Army National War Dog Center in Pak Chong and the Royal Thai Navy Military dog units at Sattahip.

2. To evaluate the immune response of military working dogs to Feline Panleukopenia Vaccine (FPLV) a killed parvovirus vaccine, and the protection produced against CVE in the field at the Military Working Dog Center (MWDC), Pak Chong, Nakornrajsima Thailand.

3. To produce Canine Viral Enteritis in susceptible, weanling dogs.

PROGRESS: This is a continuation of a study on Canine Viral Enteritis begun last year at the request of the Royal Thai Army following a severe outbreak among the dogs at the Military Working Dog Center at Pak Chong, Thailand.

Paired sera and stool specimens from military dogs were submitted to Dr. Leonard Binn, Department of Veterinary Medicine, WRAIR for serologic and/or virologic analysis. Eighty -four percent (84%) of the paired sera were positive for Canine parvolike virus HI antibody. Of the antibody positive sera, 48.5% were from dogs with a history of enteritis while 35.2% were from dogs with no history of illness. (Probably explained by the fact that the epidemic had been in progress for over six months before the first specimens were collected).

Antigens which agglutinated rhesus monkey RBC were isolated from feces from four of 25 dogs submitted. The HA titers ranged from 1:5,120 to 1:20,480. Fecal suspensions from these four dogs produced CPE when inoculated onto canine A-72 cells. Intranuclear inclusion bodies were observed in H & E stained cell cultures which were inoculated from all dogs examined. Each of the viruses recovered from cell cultures agglutinated rhesus monkey RBCs and was inhibited by Feline Panleukopenia Virus antiserum.

Since canine parvovirus and feline panleukopenia virus are antigenically similar, Feline Panleukopenia Vaccine (FPLV) has been demonstrated to protect dogs in the face of an outbreak of canine viral enteritis. In an effort to reduce the death loss from CVE, 300 doses of FPLV were purchased locally and inoculated into 120 dogs at MWDC (2 inoculations at two week interval). From May 1979 until May 1980, 235 puppies died out of 355 puppies born at the MWDC. Many of these deaths were attributed to CVE. In order to evaluate the effectiveness of FPLV in the field and also to determine the longivity of protection afforded from this vaccine, serum specimens were collected before vaccination and will be collected every 90 days post immunization and titers determined. This serology will be done within the Department of Veterinary Medicine by our staff which has just undergone training in HA & HI procedures. (Taught by members of the Virology Department, AFRIMS).

Additionally, fecal and serum specimens from dogs experimentally infected with canine parvovirus will be analyzed.

A paper is being prepared for publication that describes the Canine Viral Enteritis outbreak in Thailand.

FUTURE OBJECTIVES: To evaluate the efficacy of the FPLV under field conditions. To evaluate the efficacy of the new Canine parvovirus vaccine when it becomes available for use by the Royal Thai Army.

22. Studies on Canine Brucellosis at the Military Dog Center In Pak Chong

PROBLEM: Canine brucellosis is caused by Brucella canis which has been found to be the cause of many abortions and drops in conception rates in canine colonies in the United States. Over the past few years the Military Dog Center at Pak Chong has been having many reproductive problems in their breeding colony which are suggestive of brucellosis. The objectives of this study are as follows:

1. To determine if canine brucellosis is present in the colony.

2. To do semen evaluation on the male breeders to determine testicular damage from the brucella organism.

3. To evaluate various therapeutic regimens to decrease the loss of reproductive performance due to the organism.

PROGRESS: One hundred and seventy eight canines have been bled at Pak Chong. Of these, thirty animals were from the breeding colony. The serum from the breeding animals showed that Brucella canis is present in the breeding colony. The breeding males will be evaluated this fall to determine the degree of semen abnormalities and testicular damage. Various antibiotic regimens are being discussed and the final choices will depend on cost and availability.

FUTURE OBJECTIVES: Since canine brucellosis is almost impossible to eliminate from breeding colonies, the study will be shifted to determining if antibiotic therapy will be effective in reducing losses in production to an acceptable level.

23. Studies on Filariasis in Small Mammals in Northeast Thailand

PROBLEM: To establish a consistently reproducible, readily available, genetically controlled laboratory animal model for the study of infection with nematodes of the Superfamily Filarioidea.

PROGRESS: Efforts to concentrate on Dunnifilaria ramachandrani and the new species Dunniflaria dilli were abandoned due to too few specimens to work with. A decision was made to concentrate on studying Brugia tupaia in the tree shrew Tupaia glis. Since a high percentage of Tupaia glis are found to be naturally

infected with Brugia tupaia, efforts were directed toward establishment of a breeding colony of Tupaia glis within our laboratory.

Sixteen (11♂, 5♀) Tupaia glis were live-trapped in the area of Sakaerat; Thailand and transported to our laboratory. Four females and four males were paired and placed in breeding cages that had been equipped with a specially designed nesting box. As of the end of this fiscal year, no young had been born in the laboratory.

FUTURE OBJECTIVES: Life cycle, periodicity, and mosquitoe-transmission studies will be pursued when adequate numbers of Tupaia glis are available for study.

24. Epidemiological Studies on Leptospirosis in Thailand

PROBLEM: Leptospirosis has become one of the world's most wide-spread contemporary zoonoses. Symptoms of this disease are similar to many of the diseases seen in military personnel in tropical regions and serves to further confuse an accurate diagnosis by the clinician. There has been a need to determine the sero-varieties that are prevalent in various countries, the role of various mammal vectors, and the pathogenicity of these sero-varieties in humans. Leptospirosis is a wide-spread problem in Malaysia (1,3) and was seen in Vietnam (2). There has been limited studies in Thailand which suggests a high incidence of leptospirosis and has led to the following objectives:

1. To determine sero-varieties of leptospirosis seen in various locations in Thailand; (2) to determine what sero-varieties of leptospirosis infect and are shed by water buffalo, cattle, swine and sylvatic mammals in these various locations; (3) to determine what sero-varieties are prevalent in human populations in these various locations; and (4) to isolate new sero-varieties from domestic and sylvatic animals.

PROGRESS:

Field Studies - This project was started on 15 May 1980. From 19 March to 10 April, 1,500 serum samples were collected from water buffalo in various sites around Khon Kaen and Yasothorn in Northeast Thailand. These areas are primarily rice growing regions and there had been several cases of leptospirosis in humans reported in the provincial hospitals. Serology is pending on arrival of necessary supplies. We also

are in the process of doing a mammal trapping study in Bangpu near Nakornpathom. Animals are caught, bled for serology, and the kidneys are cultured in EMJH media. Several isolations have been made so far and final identification awaits serologic capability. We have done limited rodent trapping in the Bangkok area and three isolations have been made. These will also be serologically identified when possible.

Laboratory studies - Twenty-one sero-varieties of leptospirosis representing sixteen sero groups of leptospirosis that are pathogenic to man were obtained from the FAO/WHO Leptospirosis Reference Laboratory in Brisbane, Australia. These cultures were received 15 July 1980 and have been stored until the culture media was obtained from the United States. All serological studies and identification will be done with microagglutination test using live antigens. We currently are now in the progress of growing the cultures to begin the microagglutination testing.

FUTURE OBJECTIVES: With the limited success of the project so far it appears that leptospirosis is prevalent within Thailand. We are also interested in studying what sero-varieties are prevalent in canine populations with emphasis being placed on military working dog populations. Leptospirosis has not been investigated in these populations and could pose a serious problem to those animals in a field situation. We plan to serologically investigate the populations of working dogs at Pak Chong, Sattaheep and those animals working in the eastern parts of Thailand. Cultures will also be made of canine populations in Bangkok and various geographical regions in Thailand.

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Presentations:

1. Etiology of Acute Hepatitis in Thailand, Donald S. Burke, (presented by Herbert Segal) at the Medical Session, US Army Medical Command - Korea Seminar, Yongsan Military Reservation, Korea, 10 April 1980

2. Progress on Dengue Vaccines, Phillip K. Russell, (presented by Donald S. Burke) at the Meeting on Research in Viral Hemorrhagic Fevers of the Eastern Mediterranean, South East Asian, and Western Pacific Regions of the World Health Organization, New Delhi, India, 10 March 1980.

3. Hepatitis A, B, and Non A-Non B in Thailand: An Overview. Presented by Donald S. Burke at a Symposium on Viral Hepatitis, Third Asian Congress of Pediatrics, Bangkok, Thailand, 22 November 1979.

4. Age Specific Prevalence of Anti-Hepatitis A Virus Antibody in Thailand. Presented by Donald S. Burke at the Seminar entitled "Virology: Education, Service, and Research for National Development," Mahidol University, Bangkok, Thailand, 8 November 1979.

5. Evaluation of Toxorhynchites splendens as a Bioassay Host for Dengue Viruses. Presented by Douglas M. Watts. Seminar entitled "Virology: Education, Service, and Research for National Development," Mahidol University, Bangkok, Thailand, 8 November 1979.

6. Isolation of Dengue Viruses from Plasma and Cellular Components of the Blood of DHF Patients at Children's Hospital. Presented by Dr. Ananda Nisalak at the Seminar entitled Virology: Education, Service, and Research for National Development, " Mahidol University, Bangkok, Thailand, 8 November 1979.

7. Recent Progress in Arthropod Vector Studies in Thailand and Southeast Asia. B.A. Harrison. Asian Meeting on Parasitic Infections, The Parasitol. Trop. Med. Assoc. Thailand, 26-28 February 1980, Bangkok.

8. Longitudinal Dengue Virus Serological Surveillance Study in a Lower Socio-Economic Sector of Bangkok, Thailand. Johnson, D.E., Watts, D.M., Harrison, B.A., Klein, T.A. 16th Annual Scientific Seminar, Malaysian Soc. Parasitol. Trop. Med., 1-2 March 1980, Kuala Lumpur.

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10. Recent Morphological and Biological Studies on the Anopheles minimus Species Group in Thailand. B.A. Harrison. Meeting for Thailand Malaria Group, 25 March 1980, Bangkok.

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12 Evidence of Sibling Speciation in the Balabacensis Complex of South East Asia (Diptera: Culicidae). V. Baimai, B.A. Harrison. Abstract submitted to 10th Intern. Congr. Trop. Med. and Mal, 9-15 November 1980, Manila.

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Publications:

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3. Blacklow, N.R., Cukor, G., Bedigian, M.K., Echeverria, P., Greenberg, H.B., Schreiber, D.S., Trier, J.S. Immune Response and Prevalence of Antibody to Norwalk Enteritis Virus as Determined by Radioimmunoassay. J. Clin. Micro. 10:903-909, 1979.

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5. Burke, D.S., Snitbhan, R., Johnson, D.E., Scott, R.M. Age Specific Prevalence of Hepatitis A Virus Antibody in Thailand. Accepted for publication by the American Journal of Epidemiology.

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7. Burke, D.S., Snibhan, R. Evaluation of the Staphylococcus Protein A Absorption Method for Detecting Acute Phase Hepatitis A Virus Antibodies in Clinical Specimens. Submitted to the Journal of Clinical Microbiology.

8. Cukor, G., Blacklow, N.R., Echeverria, P., Bedigian, M.K. Basaca-Sevila V, Cross JH. Comparative Study of the Acquisition of Antibody to Norwalk Virus in Pediatric Populations. Infect. Immun. (In press).

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10. Echeverria, P., Mejia, P.A., Duangmani, C. Effect of Antibiotics on the Prevalence of Enterotoxigenic Escherichia coli in Two Populations in the Philippines. Anti. Microb. Agent Chemo. (Submitted for publication).

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6447	80 09 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. CHRON MONTH	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. Term	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M 162770A802	00	011
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Health Care and Management of Laboratory Animals

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: 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 79	3.5	557
	CURRENT 80	4.0	300

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: 202 576-3019
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Binn, Leonard, M., Ph.D
NAME:

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Disease surveillance; (U) Klebsiella pneumoniae; (U) Aotus; (U) Guinea pig salmonellosis; (U) Laboratory dog viruses; (U) Cat viruses

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 diseases and conditions affecting laboratory and military owned animals used as military working and research animals. To enhance production quality and health management and to provide animals free of known or potential pathogens. The ability to provide veterinary disease diagnosis to military facilities is critical to the operation and maintenance of programs which depend on working and research animals. The establishment of a disease data storage and retrieval system will provide unique epizootiological information not available from any other laboratory source.

24. (U) Conventional epidemiologic, pathologic and microbiologic methods are employed; unconventional procedures are developed as needed,

25. (U) 79 10 - 80 09, Bacteriological data is still being compiled for entry into a computer for future use. The resistance transfer plasmids of Klebsiella pneumoniae were isolated from parent strains and recipients and characterized on agarose gels. Recipient modification of plasmids was observed. Selection and enrichment of low levels of K. Pneumoniae in samples was continued. Oral administration of antibiotics to guinea pigs was not found to be practical for treatment. An antigen from Salmonella typhimurium was used as an in vivo indicator of past or present infection.

Canine corona and parvoviruses were associated with outbreaks of canine diarrhea at government-owned facilities. Inactivated and attenuated vaccines were tested for protection of dogs to parvovirus diarrheal diseases.

A cytopathic corona virus was recovered from a cat with feline infections peritonitis. Coronavirus, Reovirus 2 and Calici viruses were recovered from sick cats at the WRAIR Farm cat colony. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 to 30 Sep 80.

*Available to contractors upon originator's approval

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

Project 3M 162770A802 MILITARY PREVENTIVE MEDICINE

Work Unit 011 Health Care and Management of Laboratory Animals

Investigators:

Principal: M. Rogul, Ph.D and L. N. Binn, Ph.D.

Associates: MAJ C.R. Bartz, VC; CPT B. Boedeker, VC; CPT S.R. Lamb III, VC; CPT D.G. Martin, VC; R.L. Marchwicki, BS; J. Brendle, BS; K.F. Noon, MSc; R.E. Sims, BS; SGC F. Eckertt; SP5 G. Lathrop, SP5 R.E. Thomas, BS; SP5 E. Wolff, SP4 W.T. Gietz, SP4 C. Misky, SP4 L.D. Palmer, SP4 M.P. Riley; SP4 R. Soltero; SP4 R.A. Von Der Porten; CPT L.A. McKinney, VC; CPT C. Keenan, VC; P. Gemski, Ph.D; and MAJ R.M. Bunte, VC

Problems and Objectives

Objectives are to investigate diseases and conditions affecting government owned animals, in particular military working and research animals. To aid in the provision, production and health management of these animals. The problems undergoing study are (1) an emphasis on the diagnosis of bacterial and viral diseases, of laboratory animals in the WRAIR colonies, (2) characterization of Klebsiella pneumoniae antibiotic resistance, (3) isolation techniques for low levels of K. pneumoniae, (4) chemical methods for gastric cytoprotection, (5) determine etiology and epizootiology of viral diarrhea in dogs, (6) Evaluate the optimal immunization regimen to prevent parvoviral diarrhea in military dogs.

Progress

(U) Laboratory diagnosis during routine and special surveys have found potential pathogenic problems of K. pneumoniae in the Aotus monkeys; Pasteurella pneumotropic and Corynebacterium kutscheri, pneumonia virus of mice, Sendai virus, mouse hepatitis virus, Kilham rat virus and sometimes rat coronavirus in the rodent breeding colonies. Bordetella bronchiseptica appears to be the major bacterial pathogen in the WRAIR Cat Colony and P. multocida the major pathogen in purchased rabbits. A broth selection and enrichment method for isolation of K. pneumoniae is still being evaluated. Data is accumulated and entered into a computerized system. The antibiotic resistance of K. pneumoniae isolates from Aotus monkeys was found to be transmissible and due to plasmids.

(U) Salmonella typhimurium is a constant threat to the survival of the guinea pig breeding colony. Antibiotic therapy via drinking water does not appear to be feasible as the guinea pigs will not drink the mixtures used. A palpebral antigen hypersensitivity test shows promise of using this detection method for culling S. typhimurium carriers.

(U) Caliciviruses, reovirus type 2 and a cytopathic coronavirus have been recovered from sick cats at the WRAIR Farm. The feline coronavirus is neutralized by canine coronavirus and transmissible gastroenteritis virus of swine antiserum. The relevance of these isolates to the diseases are being investigated.

(U) Cases of suspected viral caused diarrhea have occured in government facilities at Lackland Air Force Base, TX; Ft Jackson, SC and Ft Benning, GA and the Canal Zone. A canine coronavirus was correlated with the disease at Lackland AFB. Canine parvovirus was associated with the disease in the canal zone. Killed and attenuated vaccines to prevent parvovirus disease are being evaluated.

(U) Models for human stress ulcers and pill produced esophagitis have been produced in rabbits and rats. A class of chemical compounds have been found which are very promising for the prevention of the stress ulcer.

Recommendations

1. Continue surveillance for disease and provide diagnostic microbial and veterinary support for government-owned animals.

2. Correlate biotype isolations of K. pneumoniae through enrichment broths with conventional isolation methods.

3. Develop a palpebral test for guinea pigs and other animals which will indicate recent or past cryptic Salmonella typhimurium infections and eliminate the carriers.

4. Study the etiology and epizootiology of viral diarrhea in dogs at government-owned facilities.

5. Determine optimal immunization practices for prevention of canine parvovirus infections in military dogs.

6. Determine pathogenicity of feline coronavirus isolates for cats, dogs, and pigs.

7. Evaluate the presence of adenvirus and cytomegaloviruses in Aotus monkeys in the disease.

8. Pursue the chemical prevention and treatment of stress ulcers.

Presentations

1. Holmes, K.V., Binn, L.N., Behnke, J.N., and Marchwicki, R.H. Purification of Canine Coronovirus and Characterization of Vision Polypeptides. American Society for Microbiology, Annual Meeting, Miami, Florida, 1980 Abstract p. 251.

2. Rogul, M., Brendle, J.J. and Sims, R.E. Effects of Glyoxylate on Cultures of Ureaplasma, Mycoplasma and Acholiplasma Species. American Soc Microbiol. 1980 Abstract.

Publications

1. Noon, K.F., Rogul, H., Binn, L.N., Keefe, T.J., Marchwicki, R.H. and Appel, M.J. An Enzyme-linked Immunosorbent Assay for the Evaluation of Antibody to Canine Distemper Virus. Am J Vet Res 41:605-609, 1980.

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5. Jackson, N.N., Wall, H.G., Miller, C.A. and Rogul, M. Naturally Acquired Infections of Klebsiella pneumoniae in Wistar Rats. Laboratory Animals 140:1-7, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	80 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
79 10 01	H, Term,	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A802	00	015
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Chemotherapy and Chemoprophylaxis of Schistosomiasis

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: 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: 80	1.0	129
FISCAL YEAR CURRENT:		

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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

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TELEPHONE: (301) 427-5029

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: GREENE, Lyford K., CPT

NAME:

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Animal Models; (U) Schistosomiasis; (U) Drug Development; (U) Antiparasitic

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 chemoprophylactic or chemotherapeutic activity against Schistosoma mansoni, which can be used by military personnel to prevent or treat the disease in endemic areas.

24. (U) Compounds previously shown to be active in screening tests will be re-examined to determine their optimum prophylactic and/or therapeutic treatment regimens in the mouse and/or subhuman primate models. Prophylactic agents for either topical application or systemic administration are being developed. Available chemical analogues will be tested and efforts will be made to determine the relationships between chemical structures, modes of antiparasitic action and modes of toxicity. Analogues with increased therapeutic efficacy and decreased host toxicity will be identified, and where justified, submitted for preclinical studies.

25. (U) 79 10-80 09. During this fiscal year, ninety-seven compounds were evaluated in the secondary, topical prophylactic, antischistosomal drug screen. Thirty-seven compounds were active. Twenty-four compounds retained activity even after a water wash of one-half hour duration. The most active classes were: 4-aminoquinolines (12/24), diamidines (1/1), heavy metals (1/2), hexachlorophene analogues (5/25), quinazolines (1/2), triazines (1/3), and miscellaneous (3/22). The avian schistosome, Giganto-bilharzia huronensis, was collected in Michigan. Parakeets and canaries were infected, a laboratory breeding colony of Physa gyrina snails established, and quantitative mouse studies initiated to develop a new schistosome model suitable for phase II topical, prophylactic, antischistosomal studies. This Work Unit is being terminated by consolidation with Work Unit 086 into Work Unit "Experimental Drug Development." For technical report, see Walter Reed Army Institute of Research Annual Report, 1 Oct 79 - 30 Sep 80.

*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 88 (FOR ARMY USE) ARE OBSOLETE.

3M162770A802 MILITARY PREVENTIVE MEDICINE
WORK UNIT 015 - Chemotherapy and Chemoprophylaxis of Schistosomiasis

Investigators:

Principle: CPT Lyford K. Greene

Associate: COL David E. Davidson, Jr.
Marie M. Grenan

PROBLEM AND OBJECTIVES:

Species of human schistosomes occur in fresh water environments in the Caribbean, Northern South America, Africa, the Middle East and the Far East. To date, there is no drug which has been approved for prophylactic use against any human schistosome. New drugs must be developed which can be used by military personnel in endemic areas to prevent or treat the disease.

PROGRESS:

In addition to coordination and support of screening operations conducted at the U.S. Army Medical Research Unit at the University of Brasilia, Brazil, where candidate drugs are tested for systemic prophylactic activity and for curative activity, studies have been conducted in-house for screening and evaluation of topically applied chemicals for antipenetration activity against the cercariae of the Puerto Rico strain of Schistosoma mansoni. During this past year, ninety-seven compounds were evaluated in the secondary topical prophylactic antischistosomal drug screen. Thirty-seven compounds were active when the prophylactic topical treatment was not followed by a water wash prior to cercarial exposure. Twenty-four of these compounds retained activity after a water wash of one-half hour duration. The most active compounds in terms of wash-resistant activity were found among the 4-aminoquinoline and the hexachlorophene classes. The diamidine, heavy metal, quinazoline and triazine classes also had active members.

Additional studies were conducted on hexachlorophene's topical prophylactic activity. Effective hexachlorophene residual remains on skin, even after a five-hour running-water wash, to enable cercaricidal amounts to leach into small volumes of water surrounding treated skin. Because isopropyl alcohol would be a more acceptable solvent than methanol for human topical application, the efficacy of hexachlorophene in this vehicle was examined. It was observed that hexachlorophene in isopropyl alcohol was effective against S. mansoni infection when applied to skin either by immersion or by wipe applicators.

Further studies were conducted on WR 234927, which had been observed not to prevent cercarial penetration, but rather to kill schistosomula after penetration. It was found that when cercariae gain access to mice at

sites other than treated skin, no protection was afforded by topical treatment with the compound. It appears that the topical protection afforded by WR 234927 is due to a delayed effect on schistosomula penetrating through treated skin rather than due to systemic action afforded by percutaneous absorption of the compound.

The avian schistosome, Gigantobilharzia huronensis, was collected in Michigan. Parakeets and canaries were infected, a laboratory breeding colony of Physa gysina snails was established. Quantitative mouse studies were initiated to develop a new schistosome model system to be used for preclinical evaluation of antipenetrants.

FUTURE OBJECTIVES:

Coordination and support of screening operations at the University of Brasilia will continue. Compounds which are shown to be active in screening tests will be studied to determine optimum prophylactic and/or therapeutic treatment regimens in the mouse and/or subhuman primate models. Available chemical analogues will be tested and efforts will be made to determine relationships between chemical structure, mode of antiparasitic action and mode of toxicity. Analogues with increased therapeutic activity and decreased toxicity will be submitted for preclinical studies. This work unit is being terminated by consolidation with Work Unit 086, A803, Acession No. DAOB 6535, page 239, into Work Unit: "Experimental Drug Development."

PROJECT 3M162770A803

DRUG DEVELOPMENT

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6471	80 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
79 10 01	H. Term	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A803	00	089
b. CONTRIBUTING				
c. CONTRIBUTING	CARDS 114F			

11. TITLE (Precede with Security Classification Code)

(U) Field Studies on Drug Resistant Malaria

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	CONT	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 79	7.5	580
	CURRENT 80	7.5	375

19a. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

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

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21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Malaria, (U) Drug Resistance; (U) Chemotherapy; (U) Immunology; (U) Monkey; (U) Vectors

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 strains of human plasmodia in continuous in-vitro culture. To evaluate candidate antimalarial drugs against simian malaria. These studies are in support of the Army Drug Development Program.

24. (U) Chemotherapeutic drugs are studied in rhesus monkeys with P. cynomolgi. In-vitro procedures are evaluated for growing erythrocyte-free intact parasites.

25. (U) 79 10 - 80 09 Candidate dengue vaccine virus strains were isolated from mosquitoes and humans. Purification procedures of virus strains provided reliable materials for potential vaccine development. The response of lymphocytes in malaria infected patients were being characterized. Malarial antigen isolated by production of intact parasites freed from erythrocytes. Cell mediated immunity was altered in humans naturally infected with malaria and worked to determine the interactions of lymphocytes, serum factors and parasite antigen during malaria infection. The rhesus monkey - P. cynomolgi system was used to test 32 drugs for radical curative effect. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 79 - 30 Sept 80

*Available to contractors upon originator's approval.

DD, FORM 1498 MAR 88 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE.

3M162770A803 DRUG DEVELOPMENT

Project 089 Field Studies on Drug Resistant Malaria

Investigators.

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1. 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 lymphycytotoxic 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 response to malaria infection.

PROGRESS: A comparison of Con A generated suppressor cell activity in various patient and control individuals show that the mean level of suppressor cell activity is substantially reduced in the malarious patients' mononuclear cell population. Suppressor cell activity was lower in cultures stimulated with phytohemmaglutinin (PHA), Concanavalin A (Con A), Pokeweed mitogen (PWM) and in the mixed lymphocyte cultures.

FUTURE OBJECTIVES: 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.

REFERENCES:

1. Wells, R.A., Pavanand, K., Zolyomi, S., Permpanich B., 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., MacDermott, R.P. Lymphocytotoxic Antibody in Sera of Thai Adults Infected with Plasmodium falciparum or Plasmodium vivax Malaria. Clin. Exp. Immunol. 1980 (In press).

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. 1980 (In press).

2. Nature of Malaria Cold-Reactive Lymphocytotoxic Antibody

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.

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 severally 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 mononuclear cells incubated with high ALA sera or normal sera.

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.

FUTURE OBJECTIVES: It is important to consider the significance of ALA activity at both 4° and 15°C in respect to immunoglobulin classes with activity directed at specific target cells. Also, it would be worthwhile to investigate the role of blocking immunoglobulin, possibly IgA in regulating lymphocytotoxicity during various stages of malaria infection.

REFERENCES:

1. Wells, R.J., Pavanand, K., Zolyomi, S., Permpanich, B., MacDermott, R.P. Lymphocytotoxic Activity in Sera of Thai Adults Infected with Plasmodium falciparum or Plasmodium vivax Malaria. Clin. Exp. Immunol. 1980. (In press).

3. Measurement of Antibody Dependent Cellular Cytotoxicity (ADCC) by Peripheral Blood Mononuclear Cells from Patients with Naturally Acquired P. falciparum or P. vivax Malaria

PROBLEM: It has been reported that cells responsible for antibody dependent cellular cytotoxicity (ADCC) increase in both human and mice during malarial infection (1,2). However, Wells, et al. (3) recently showed that although T cell subpopulations in humans infected with malaria are reduced, they found no alteration in the K cell (Null) or B cell subpopulations. In our search for a correlation between assays of cellular immunity and protection against, or immune regulation by malaria parasites in humans, we investigated ADCC response of lymphocytes from Thais with naturally acquired malaria. We have investigated the ADCC response using both the Chang target cell and the Chicken Red Blood Cell Systems. In addition, we have examined the effect that coating of patient and/or control effector cells with sera from malarious individuals has on the ADCC response.

PROGRESS: Small differences exist between ADCC activity of patient and control cells, however, additional data are needed to determine the level of significance.

To date 14 malaria serum samples have been tested in the Chick RBC System to determine if malaria sera can alter ADCC activity of macrophage depleted mononuclear cells obtained from healthy individuals. No significant difference has been found at any of the effector/target cell ratios ranging from 1:1 to 50:1.

FUTURE OBJECTIVES: It would be important in future studies to determine an individual patients' ADCC response in relation to the cellular responses of their lymphocytes in assays measuring several different cellular immune responses (i.e. lectin induced cellular cytotoxicity, spontaneous cellular cytotoxicity) to determine an individual's broader immune responsiveness to malaria infection and the variability of responses among individuals.

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1. Greenwood, B.M., Oduloju, A.J., Stratton, D. Lymphocyte Changes in Acute Malaria. Trans. Roy. Soc. Trop. Med. Hyg. 71:408, 1977.

2. MacDonald, V. and Phillips, R. Increase in Non-Specific Antibody Mediated Cytotoxicity in Malarious Mice. Clin. Exp. Immunol. 34:159-163, 1978.

3. Wells, R.A., Pavanand, K., Zolyomi, S., Permpanich, B, 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.

4. 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

PROBLEM: Delineation of the host immune response to infection with malaria should include examination of infected humans. Studies in this area have only recently begun to be carried out utilizing peripheral blood mononuclear cells (MNC) from patients. We have previously observed that Thai adults naturally infected with either P. falciparum or P. vivax have a decrease in the percentage and concentration of T lymphocytes, an increase in the percentages but no change in the concentrations of B and "Null" lymphocytes, and no change in either the percentage or concentration of Fc receptor bearing lymphocytes (1). Thus, peripheral blood MNC from patients who have malaria exhibit a true loss of T cells without any real change in B cells, Fc receptor bearing cells, or Null cells. In addition, we have recently demonstrated that sera of Thai adults naturally infected with both P. falciparum and P. vivax contain cold reactive lymphocytotoxic antibodies with marked reactivity at 15°C. A number of individuals also had lymphocytotoxic antibodies which were effective at 37°C. Although the subpopulations against which these antibodies are directed have not yet been elucidated, the ability of sera components to interact with peripheral blood MNC in functional assays clearly needs to be ascertained. Therefore, in order to examine the functional capabilities of peripheral blood cells from patients with malaria and to also examine the effects of their sera on cellular immune function, we have begun to examine the peripheral blood MNC and sera of Thai adults naturally infected

with P. falciparum and P. vivax in a number of in vitro cellular immune assays. The present work describes our results using mitogen induced lymphocyte transformation and mixed leukocyte culture systems.

PROGRESS: We have previously observed that Thai adults who are infected with malaria, have a loss of peripheral blood T cells and that patient sera contain lymphocytotoxic antibodies. In the present study, we have examined peripheral blood mononuclear cells (MNC) from Thai adults naturally infected with P. falciparum and P. vivax for the capacity to undergo blastogenesis in response to phytohemagglutinin (PHA), concanavalin A (Con A), pokeweed mitogen (PWM) and allogenic cell surface antigens in a one way mixed leukocyte reaction (MLR). In addition, sera from actively infected patients were examined with regard to suppressive capabilities toward normal lymphocyte blastogenesis using the same assays. We found that patient MNC exhibited normal reactivity to PHA, Con A, and PWM when compared to controls. However, peripheral blood MNC from patients had a decreased stimulatory capacity in the allogeneic MLR and P. vivax but not P. falciparum lymphocytes exhibited decreased responsiveness in the MLR. Furthermore, sera from patients with active malaria induced decreased responsiveness by normal MNC to PHA and Con A but not PWM and pooled P. falciparum sera caused decreased responsiveness to allogeneic cell surface antigens in the MLR. These studies indicate, that despite the loss of circulating T cells during the course of infection with malaria, blastogenic responsiveness remains intact and that sera from patients with malaria are capable of exerting negative immunoregulatory effects.

FUTURE OBJECTIVES: Further studies are necessary to determine if serum regulatory factors or lymphocyte response to specific and non-specific antigens vary with the course of malaria infection. In order to totally understand the immunological significance of malaria infection in humans one needs to determine whether or not the abnormalities in the immune function result in alteration of immunoregulation or of immune effector function.

REFERENCES:

1. Well, R.A., Pavanand, K., Zolyomi, S., Permpanich, B., MacDermott, R. Loss of Circulating T Lymphocytes with Normal Levels of B and "Null" Lymphocytes in Thai Adults with Malaria. Clin. Exp. Immunol. 35-202-209, 1979.

5. Lectin-Induce Cellular Cytotoxicity (LICC) in the Pheripheral Blood Mononuclear Cells from Malarious Thai Adults

PROBLEM: An in vitro model of cellular cytotoxicity involving non-immune effector cells, lectin induced cellular cytotoxicity (LICC), has been studied in animals and man in hopes of further classifying cytolytic mechanism that may be important in specific immune cytotoxicity in vivo (1). Some studies indicate that most LICC effector cells primarily belong to Fc receptor positive lymphocyte populations (2) whereas others have shown that Fc receptor-negative cells also can be cytotoxic in the presence of lectins (3).

We are investigating the LICC response in humans with naturally acquired malaria since this non-specific response may be of importance in the early stages of infection. In addition we are collecting basic information that may be of importance in future vaccine studies if non-specific immune responses are needed to enhance immune protection.

PROGRESS: Using chicken erythrocyte (CRBC) target cells in a 51 chromium release assay, LICC mediated by macrophage depleted peripheral mononuclear cells from malarious patients (8 P. falciparum and 2 P. vivax) is significantly reduced when compared with effector mononuclear cells from healthy controls. Significant reduction is seen at effector/target cell ratios of 10/1 and 25/1 while no significant reduction is seen at the lower 5/1 ratio.

We are presently assessing LICC of malarious patient lymphocytes using the nucleated Chang target cell system and unfractionated patient mononuclear cells. Additional studies are comparing the response of individual patients mononuclear cells in both assay systems to determine if different effector cells are involved in host's LICC response to malaria parasites.

FUTURE OBJECTIVES:

1. Measure LICC in patients peripheral mononuclear cells during acute, early convalescent and convalescent stages of illness to determine if the LICC response can be correlated with the patients response to therapy.

2. Compare individuals' LICC response with antibody dependent cellular cytotoxicity (ADCC), spontaneous cell

mediated cytotoxicity (SCMC) and presence on absence of serum regulatory factors to access the broader human immune response during the malarial disease process.

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1. Holm, G., Pearlman, P., Werner, B. Phytohemagglutinin Induced Cytotoxic Action of Normal Lymphoid Cells on Cells in Tissue Culture. Nature. 203:841, 1964.

2. Bonavida, B., Robins, A., Saxon, A. Lectin-Dependent Cellular Cytotoxicity in Man. Transplantation. 23:261, 1977.

3. Waller, C.A., Campbell, A.C., MacLenan, I.C.M. Two Populations of Lymphocytes Involved in Phytohemagglutinin-Induced Cytotoxicity of a Dividing Target Cell. Scand. J. Immunol. 5:931, 1976.

6. Suppression of Monkey Lymphocyte Mitogenic and Allogeneic Responsiveness by Serum Obtained from Malarious Monkeys

PROBLEM: Serum inhibition of lymphocyte responsiveness has been reported in several infectious diseases, including human malaria (1,2). Although serum obtained from malarious patients during the acute stage of illness inhibits the response of lymphocytes from healthy control individuals to mitogens and xenogeneic cell surface antigens it is not known when the suppressive activity first appears, its relationship to disease state and its relationship to peripheral blood parasite levels.

Likewise, a study in which plasma obtained from Aotus owl monkeys, during the acute phase of Plasmodium falciparum infection, was shown to suppress the responsiveness of normal monkey peripheral blood lymphocytes, did not determine if suppression and parasitemia were correlated throughout the course of the illness. Suppression of PWM response of lymphocytes was shown, however, to depend on high (50%) levels of parasitemia.

In our study we obtained sera from rhesus monkeys, prior to, and during their course of infection with Plasmodium cynomolgi malaria. We investigated the relationship of suppressive activity, severity of disease, presence of blood parasites, and fluctuations in blood parasite levels. The information obtained from this study may help to understand the role of suppressive factor in regulating the host's response to parasite antigens.

PROGRESS: In preliminary analysis the data indicate that suppression appears 5-7 days post infection, but 6-8 days prior to the appearance to detectable levels of peripheral blood parasites. Phytohemmagglutinin (PHA), Concanavalin A (Con A), Pakeweed mitogen (PWM) and xenogeneic cell surface antigen stimulation are all significantly suppressed up to 3 months after infection. Suppressive activity appears to decrease at 3 months regardless of whether chemotheraphy was initiated or not. The decrease in suppressive activity in the untreated monkeys may indicate that the natural immune protective mechanism against malarial parasites may be regulated by serum factors.

FUTURE OBJECTIVES: Further studies should chemically characterize suppressive factor and determine if specificity exists for peripheral blood target cells. Long term goals may be directed at investigating if a relationship exist between suppressive activity and individual parasite antigen(s).

REFERENCES:

1. Levene, G.M., Turk, J.L., Wright, D.J., Grimble, A. Reduced Lymphocyte Transformation Due to a Plasma Factor in Patients with Acute Syphilis. Lancet ii, 246-247, 1969.

2. 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. 1980. (In press).

3. Taylor, D.W., Siddigui, W.A. Suppression of Lymphocyte Transformation by Plasma from Owl Monkeys Acutely Infected with Plasmodium falciparum. Infect. Imm. 21(1):147-150, 1978.

7. Fansidar and Human Lymphocyte Response to Plant Lectins

PROBLEM: Fansidar is presently used widely in Thailand as an anti-malarial drug by the National Malaria Eradication Project, the Ministry, and for self-treatment. However, questions have arisen concerning the possible adverse effect on the hematopoietic system that long-term prophylactic use of the drug may have (1,2). Both components of Fansidar, pyrimethamine and sulfadoxine, have been shown to adversely effect hematopoiesis in humans when the drugs are taken daily for extended periods (1,2,3). Thus, it is possible that Fansidar, taken routinely for prolong prophylactic purposes may likewise adversely effect an individual's immune competence.

We are presently using mitogenesis inhibition assays and one way mixed lymphocyte cultures to investigate the effect that either pyrimethamine, sulfadoxine, or sera from individuals on long-term (24 weeks) Fansidar chemoprophylaxis has on lymphocytes from healthy, non-treated, individuals.

PROGRESS: Results of the in vitro pyrimethamine or sulfadozine drug assays show that a 10^{-4} molar concentration of pyrimethamine inhibits ^{3}H-thymidine uptake by 61.3%, 53.5% and 51.7% in cultures stimulated with phytohemmagutinin (PHA), Concanavalin A (Con A) and Pokeweed mitogen (PWM) respectively. Inhibition in MLC assays was 34.7%. No significant inhibition was found in mitogenesis or MLC assays in which sulfadoxine was added. The marked suppression of lymphocite blastogenesis was found not to be due to drug toxicity.

Preliminary analysis shows that serum obtained from Thais during the 6th week of Fansidar chemoprophylaxis was suppressive when mixed with lymphocytes from healthy individuals and assayed in the mitogenesis and MLC assays.

We are presently analysing the raw data to determine if any relationship exists between the level of blastogenic stimulation in the various cultures and the level of sulfa detected in the sera.

FUTURE OBJECTIVES: This study is near completion. However, the techniques may be used to study the effect that long term Fansidar chemoprophylaxis has directly on the lymphocytes. 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.

REFERENCES:

1. Castles, T.R., et.al. The Effect of Folic or Folinic Acid on the Toxicity of Pyrimethamine in Dogs. Toxicol. Appl. Pharmacol. 20:447-459, 1971.

2. Hamilton, L., et. al. Haematological Effect of Certain 2, 4-diaminopyrimidine Antagonists of Folic Acid. Bolld, 9:1062-1081, 1952.

3. TenPas, A., Abraham, J.P. Hematological Side-Effects of Pyrimethamine in the Treatment of Ocular Toxoplasmosis. Amer. J. Med. Sci. 249:448-453, 1965.

8. 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 schizontocidal 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 lymphocytes 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.

FUTURE OBJECTIVES: Further chemical isolation of the different constituents will be performed on the plant extract exhibiting the activity that inhibits parasite growth.

REFERENCES:

1. Ketusinh, O. Report on Experimental Anti-Malarial Therapy of Thai Medicinal Plants. Proceeding of the Siriraj 60th Anniversary Meeting, 271-281, 1950.

9. Drug Tolerance Study of Primaquine in Rhesus Monkeys and Drug Tolerance Study of WR225448 in Rhesus Monkeys

PROBLEM:

1. To determine the maximum tolerated dose of primaquine in rhesus monkeys.

2. To determine the maximum tolerated dose of WR225448 in rhesus monkeys.

3. To determine the nature of the toxic effects, including a determination of the organ system(s) affected by primaquine and by WR225448.

PROGRESS: While certain chemical compounds are known to have excellent schizonticidal activity, they are, at the same time, toxic to the host. The purpose of this study was to determine the toxic dose of primaquine and the toxic dose of WR225448 in rhesus monkeys and also to determine what organ system(s) were affected by each of drugs.

The study was conducted using the primary test phase format of fixed dosages (the dosage levels remain fixed throughout the test). Primaquine was tested at two dosages levels: 10 mg/kg body weight/day for 7 days and 3.16 mg/kg body weight/day for 7 days. WR225448 was tested at only one dosage level: 3.16 mg/kg body weight/day for 7 days.

The monkeys were divided into three groups of five monkeys each. (Four test monkeys and one control monkey). All drugs were suspended in 3 ml. of 0.3% methyl cellulose and were administered orally via nasogastrotube followed by a 3 ml. water flush. Control monkeys received only the vehicle, 3 ml of 0.3% methyl cellulose. Group I test monkeys received primaquine at 10mg/kg body weight/day; Group II test monkeys received primaquine at 3.16 mg/kg body weight/day; Group III test monkeys received WR225448 at 3.16 mg/kg body weight/day. All drugs were given seven consecutive days.

Blood was collected from all monkeys once each week for two weeks prior to the administration of the test drugs and the following laboratory tests performed: WBC, RBC, differential, hematocrit, methomoglobin, glucose, total protein, creatinine, BUN, SGPT, SGOT. Additional blood specimens were collected and the above listed tests performed on three occasions during the testing of the drug; day 1 (during treatment), post treatment day 1, and post treatment day 8.

Complete necropsies were performed on two test monkeys from each group plus one control monkey. Based on gross necropsy observations and preliminary laboratory results the liver appears to be the target organ of the toxic effects of both compounds tested.

FUTURE OBJECTIVES: Final results of this study are awaiting complete analysis of the laboratory data and histopathological examination of the tissues.

10. Evaluation of Experimental Antimalarial Drugs for Radical Curative Activity in the Rhesus Monkey

PROBLEM: To evaluate the radical curative effectiveness of selected experimental drugs in rhesus monkeys (Macaca mulatta) infected with Plasmodium cynomolgi malaria.

PROGRESS: This is a continuation of studies initiated by this Laboratory in 1974. A chronological report of the methodology and results are available in previous SEATO/AFRIMS Annual Reports. These studies are conducted in association with the Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research.

Rhesus Monkeys were inoculated intravenously with sporozoites produced in Anopheles dirus mosquitoes. A. dirus mosquitoes were fed on P. cynomolgi infected Rhesus monkeys. This feeding was conducted on the second or third rise or peak in parasitemia when both male and female gametocytes were present as confirmed by examination of blood smears. The sporozoites were harvested on post-feeding day 14 from salivary glands of infected mosquitoes and diluted in a saline-normal monkey serum solution (1:1) to a concentration of 5-20 X 10^5 sporozoites per ml. Pre-selected, malaria-negative rhesus monkeys were immediately inoculated intravenously with one ml of the sporozoites suspension.

Each monkey was monitored daily, beginning on day 6 post-inoculation for development of a parasitemia. When parasitemia reached 5-25 X 10^3 parasites per cmm., test drugs were administered for 7 days at a predetermined dosage level based on a mg/kg/body weight/day. To permit evaluation of drug activity against tissue parasitic forms independently of blood schizonticidal activity, chloroquine phosphate was administered simultaneously with each test drug at a level of 6.2 mg/kg body weight/day for 7 days. Each monkey is followed by obtaining blood smears every other day for 80 days. Monkeys negative at this time are considered "cured". Monkeys that "break" with parasitemia in under 20 days post-treatment are reused for a second drug test using a different drug.

A total of 37 experimental drugs were evaluated using 109 rhesus monkeys during the year.

FUTURE OBJECTIVES: Due to the nonavailability of Indian Rhesus monkeys; the result of a moritorium on the export of these monkeys imposed by the Indian Government, the future

testing of experimental drugs in his model at AFRIMS will be terminated. There are 25 rhesus monkeys remaining in our animal colony which can be used for antimalarial drug testing. Attempts to develop a new animal model system using laboratory reared cynomolgus monkeys (Macaca fasicularis) are under investigation.

11. Entomological Evaluations of Human Malaria Transmission in a Village-Rice Field Scenerio on the Korat Plateau of Thailand

PROBLEM: To obtain malaria epidemiological and vector information of the study area. This includes (a) the presence of known primary, secondary and/or suspected vectors; (b) identify the vector(s) of malaria parasites by dissection; (c) determine the human malaria prevalence and ratio of endemic:migrant infections; (d) determine the parity rates, nocturnal biting cycle and host propensity of the vector(s); and (e) determine all aspects of An. philippinensis and/or nivipes involvement in this study area.

PROGRESS: This study was recently initiated and a study site in Nakhon Ratchasima Province was located with the assistance of the Thailand Malaria Division. The Thailand Malaria Division records consider An. philippinensis to be the probable vector in the study area. However, preliminary collections by AFRIMS personnel resulted in large numbers of An. nivipes, but no philippinensis from the study area. In addition, other potential vectors such as aconitus, annularis and campestris were also collected in the village, but in very low numbers. To date none of the primary vectors, An. dirus, maculatus or minimus, have been collected in the village. Sufficient numbers of An. nivipes were collected to initiate colonization attempts.

FUTURE OBJECTIVES: The project will proceed as planned, with major efforts beginning at the start of the calendar year.

12. Lymphocytotoxic Antibody in Serum from Patients with Dengue Hemorrhagic Fever (DHF)

PROBLEM: Our laboratory previously reported that Thai children with dengue hemorrhagic fever (DHF) showed a major shift within the component cell populations of the immune system (1). The major changes noted in the lymphocyte subpopulations were a decreased percentage and number of T lymphocytes with an

increased percentage and number of non-T, non-B and non-Fc receptor bearing cells during the acute stage of illness. Various causes for the alteration in lymphocyte subpopulations during viral infections have been suggested by several authors (2,3). Recently, Boonpucknavig (4) used indirect immunofluorescence to detect anti-T lymphocyte antibodies in sera from patients with DHF. However, the study did not determine the incidence of Anti-T lymphocyte antibodies in the sera from patients with DHF or whether these antibodies were cytotoxic for autologous lymphocytes. The present study was undertaken to determine if sera from Thai children with DHF is cytotoxic for autologous peripheral blood mononuclear cells and if such activity could be responsible for the alteration of T lymphocyte numbers during DHF illness.

PROGRESS: 59/90 sera from patients with confirmed DHF showed optimal cytotoxic activity against peripheral blood mononuclear cells at temperatures below 37°C. No significant cytotoxic activity was found in assays done at 37°C. Cytotoxic activity was not found in plasma from DHF patients, 18/25 sera obtained during the convalescent period (30 days post-admission) of illness from patients whose sera demonstrated cytotoxic activity during the acute stage of illness had a lower level of cytotoxicity than the sera obtained on the day of presentation to the hospital.

When 13 DHF sera that were known to have high lymphocytotoxic activity were tested against enriched T-cell, B-cell or Null cell populations 23% demonstrated no activity, 31% showed activity against both T-cells and B-cells at 4°C, 8% showed activity against only T-cells at 4°C, 15% showed activity against B-cells at 4°C, 23% showed activity against B-cells at both 4°C and 37°C. No activity was found when enriched null cell populations were used as target indicator cells.

Sucrose gradient serum fractionation studies show that lymphocytotoxic activity is found in the fractions containing IgM when assayed at 4°C. Additional experiments are underway to determine if IgG fractions contain blocking factors that interfere with IgM lymphocytotoxicity at 37°C.

FUTURE OBJECTIVES: Future considerations should focus on determining the target cell specificity of specific (IgG, IgM, IgA) sucrose gradient fractions at both 4° and 37°C and to determine if any relationship is present between lymphocytotoxic activity and age, sex, day to illness, grade of illness, HI titer, and blood cell picture.

REFERENCES:

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Presentations:

1. Lymphocytotoxins in Dengue Hemorrhagic Fever. Presented by Gilbreath, M.J. at the 16th Annual Scientific Seminar of the Malaysian Soceity of Parasitology and Trop Med Meeting, in Kuala Lumpur.

Publications:

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	80 10 01	[illegible]

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. [illegible]	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. Term.	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A803	00	92
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procode with Security Classification Code)

(U) Chemotherapy and Chemoprophylaxis of Leishmaniasis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 10	CONT	DA	C. In-House

17. CONTRACT/GRANT: NA
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	2.0	147
FISCAL YEAR CURRENT:		

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NAME: CHILDS, George E., MAJ

21. GENERAL USE

22. KEYWORDS (Procode EACH with Security Classification Code)

(U) Leishmaniasis; (U) Drug Development; (U) Biology; (U) Chemistry; (U) Toxicology

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

23. (U) To find new drugs with chemoprophylactic or chemotherapeutic activity against the cutaneous and visceral forms of leishmaniasis, which pose a serious hazard to military personnel operating in most of the tropical and subtropical regions of the world. The only drugs currently available are antimonials, which are hazardous and frequently ineffective.
24. (U) Selected chemical compounds will be tested for antileishmanial activity (1) in vitro, (2) in laboratory rodents, (3) in subhuman primates or canines in a series of leishmania models which have been developed or modified by this laboratory.
25. (U) 79 09 - 80 10 In cultures of axenic amastigotes of Leishmania braziliensis panamensis which have been used for drug screening, it has been found that there are two distinct parasite populations on the basis of susceptibility to antimony. In collaboration with WHO radiorespirometric method for taxonomic characterization of leishmania species and strains has been established. Currently the identification data base is being expanded, and is being subjected to a computerized analysis. Radiorespirometric methodology is being adapted to a drug screening application. A new cutaneous model using L. b. panamensis in the golden hamster is being evaluated. Pilot experiments for development of models for study of visceral leishmaniasis, L. donovani, have been completed in Aotus monkeys and opossums. Both models show promise for use in evaluating drugs. This Work Unit is being terminated by consolidation with Work Unit 086 into Work Unit "Experimental Drug Development." For technical report, see Walter Reed Army Institute of Research Annual Report, 1 Oct 79 - 30 Sep 80.

*Available to contractors upon originator's approval

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

Program 3M162770A803 DRUG DEVELOPMENT

WORK UNIT 092 - Chemotherapy and Chemoprophylaxis of Leishmaniasis

Investigators:

Principle: LTC Larry D. Hendricks
MAJ Jonathan Berman
MAJ George E. Childs
CPT Lawrence K. Lightner

PROBLEM AND OBJECTIVES:

Visceral and cutaneous leishmaniases have a world-wide distribution and can pose a serious health hazard to military personnel. The antimonial drugs, currently the drugs of choice for treatment of this disease, are toxic and often ineffective. New drugs which are more effective and less toxic are needed for the treatment of leishmaniasis.

PROGRESS:

Improved *in vitro* methods of drug screening continue to be evaluated in research efforts in-house. A procedure to identify and differentiate species and strains of leishmania as well as their susceptibility to various antileishmanial drugs is being evaluated (1). Candidate drugs at levels which are expected to be attainable in serum are being evaluated *in vitro*; this should provide insight as to their potential effectiveness *in vivo* (2). Parasite metabolism studies have provided data that will assist in selection or formulation of compounds which may be useful in blocking metabolic pathways specific to the parasite (3). Basic research studies of *in vitro* cultivation of leishmania organisms have led to the development of simple media for the rapid production of large quantities of both the promastigote and amastigote stages of the parasite which may be employed in a wide variety of studies (4).

Because of the need for laboratory models, the evaluation of the susceptibility of various mammals to both cutaneous and visceral leishmaniasis has continued. Within this reporting period a program of such experiments has yielded data indicating that a series of strains of in-bred mice provide models with a wide range of susceptibility and histopathology when infected with various cutaneous leishmania species of man (5). A collaborative study conducted in-house indicates that young German shepherd dogs are much more susceptible to visceral leishmaniasis than are other types of dogs which have been investigated (6). In studies with other animals, it has been discovered that the owl monkey, *Aotus trivirgatus* (7) and the common opossum, *Didelphus marsupialis* (8) are quite susceptible to visceral leishmaniasis. These new models will be used for the advanced evaluation of the lepidine, WR 6026, as well as the liposome-encapsulated delivery system for drugs.

WORK UNIT 092 - Chemotherapy and Chemoprophylaxis of Leishmaniasis

FUTURE OBJECTIVES:

Clinical formulations of the lepidine and the liposomal preparations will be evaluated to ensure efficacy during the development of these two novel approaches to therapy of leishmaniasis. Continued evaluation and improvement of *in vitro* techniques of parasite identification, and determination of susceptibility to clinical and experimental drugs is planned. Efforts to identify potential prophylactic antileishmanial drugs will be continued in screening systems both *in vitro* and *in vivo*. This work unit is being terminated by consolidation with Work Unit 086 A803. **Accession No.** DAOB 6535, page 239, into Work Unit: "Experimental Drug Development."

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3. Specificity of Purine Transport in Promastigotes of Leishmania braziliensis panamensis (WR 008). B. Hanson, L.D. Hendricks. Submitted to J. of Parasitology, 1980.

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WORK UNIT 092 - Chemotherapy and Chemoprophylaxis of Leishmaniasis

PRESENTATIONS:

1. Anti-Leishmanial Activity of Ketoconazole and an 8-Aminoquinoline *in vitro*. J. Berman, E. Peterson, L. D. Hendricks. Abstract and presentation at the Annual Meeting of the Infectious Disease Society of America: Internal Science Conference on Antimicrobial Agents and Chemotherapy. September 1980, New Orleans, LA.

WORK UNIT 092 - Chemotherapy and Chemoprophylaxis of Leishmaniasis

PUBLICATIONS:

1. Specificity of Purine Transport in Promastigotes of Leishmania braziliensis panamensis (WR 008). B. Hanson, L.D. Hendricks. Submitted to J. of Parasitology, 1980.

2. Present Knowledge of the in vitro Cultivation of Leishmani. L.D. Hendricks, and G. Childs (1980). WHO Bulletin. Tropical Diseases Research Series: 3, Chapter 32:251-272. Schwabe & Co., AG, Basel.

3. Leishmania donovani in the Owl Monkey (Aotus trivirgatus). W. Hanson, W. Chapman and L. Hendricks. Submitted to Trans. Roy. Soc. Trop. Med. & Hyg., 1980.

4. Leishmania donovani in the Opossum (Didelphis marsupialis). W. Chapman, W. Hanson and L. Hendricks, 1980. J. Parasitol.66:700-701.

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PROJECT 3S162772A814

BASIC RESEARCH ON MILITARY INJURY AND DISEASE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2409	80 09 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. Termination	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	006
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Gastrointestinal Sequelae of Combat Casualties

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 01		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 79	0	0
FISCAL YEAR CURRENT 80	1	100

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ASSOCIATE INVESTIGATORS

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PII Redacted

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Gastric mucosal barrier; (U) Abdominal visceral blood flow; (U) Septic shock; (U) Portacaval shunt; (U) Stress ulcer; (U) Shock

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

23 (U) Combat casualties are subject to gastric stress ulceration if they sustain multisystem injury. Studying the pathophysiology of gastric stress ulceration in experimental animals is an objection of this unit. Major abdominal trauma can result in massive loss of small bowel and the short bowel syndrome. Another goal of this unit is to develop methods for expanding the small bowel absorptive mucosa. Finally blood flow to the liver is being studied as it relates to liver function.

24 (U) The methods used include study of mucosal injury in a dog and a rabbit model which are compared. The esophagus of the rabbit is also studied. Injury is produced by bile acids and acid loss, ion fluxes, potential difference as well as gross and microscopic examination are used to assess injury. The control of acid secretion in the gut is being studied in a collaborative effort at Georgetown University. The growth of small bowel mucosa is being studied in a rabbit model.

25 (U) 79 10 - 80 09 We have studied the effects of indomethacin, prostaglandins and bile acids on the gastrointestinal mucosa. We have shown that prostaglandin E_2 does not prevent hydrogen ion back diffusion in the rabbit esophagus. We have studied the permeability of the rabbit esophagus to water as measured by tritiated water and shown that bilateral water fluxes are increased by bile and acid injury. Our attempts to expand the surface area of the small bowel mucosa have lead us to the use of vascularized pedicle grafts of abdominal wall muscle as a template for the growth of "neogut." The mucosa we have grown looks essentially normal by light and electron microscopy and absorbs glucose in a Ussing chamber in a manner similar to normal mucosa. This project is being terminated because of a change in the mission of the Department of Surgical Gastroenterology. The new mission is to study the physiology of blast injury. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 79 - 30 Sept 80.(NOTE: Work continued from DAOA 6467,Proj: S01, Work Unit 143)

*Available to contractors upon originator's approval.

Project: 3S162772A814 BASIC RESEARCH ON MILITARY INJURY AND DISEASE

Work Unit: 006 Gastrointestinal Sequelae of Combat Casualties

Investigators:
Principal: John W. Harmon, LTC, MC
Co-investigators: Keith Lillemoe, CPT, MC
William Berry, CPT, MC

I. Pathophysiology of Gastric Stress Ulcers

Background and Objectives

Bile, with ischemia and acid, have been implicated in the etiology of gastric stress ulcers. Gastric stress ulcer bleeding is a major cause of morbidity and mortality among combat casualties. Our objective has been to study the effects of various bile acids and drugs on the gastric mucosa of dogs to more precisely understand their injurious effects. A paper from our laboratory entitled "Gastric Stress Ulceration: Current Concepts of Pathophysiology and Therapy" which appeared in Military Medicine, 144:291-6, 1979, provides extensive background on this subject.

Progress

Dogs were prepared with chronic Heidenhain pouches with 2 cannulas each for through and through perfusion. Acid loss was measured with a pH stat and transmucosal electrical potential difference was measured with a voltmeter, KCL agar bridges, and Calomel electrodes. Reduction in potential difference and increased permeability to hydrogen ion were the indices of injury measured.

Completed during this time period was a study assessing the injurious effects of Capmul (mono-octaoin - MO). MO (glyceryl-1-mono-octanoate) rapidly dissolves cholesterol gallstones in vitro, faster than cholic acid or heparin. Clinical experience with the agent has been promising when it has been perfused into the common bile duct via a T-tube to dissolve retained stones. Because MO perfused in this manner can be refluxed into the stomach, and because of an anecdotal report of gastric bleeding during its use, it is important to know its effect on the gastric mucosa. We performed perfusion experiments using 4 dogs with chronic Heidenhain pouches of fundic gastric mucosa with cannulas at either end for through-and-through perfusion. Isotonic acid test solutions with 10 mM HCl plus NaCl were used. MO was added to create solutions of 1, 5, and 25%. Net acid back diffusion (NABD) in μEq/10 min was measured with pH stat technique and transmucosal electrical potential difference (PD) in millivolts (mV) was measured using agar KCl bridges and standard electrodes and voltmeter. Experiments without the gastric mucosa in the system showed that MO did not alter baseline NABD or PD measurements. Thus, changes observed could be attributed to the effects of MO on the gastric mucosa. A fall in PD or a rise in NABD are considered to be evidence of damage to the gastric mucosa.

Both of these stigmata of injury were present in experiments with 1, 5 and 25% MO solutions as shown below. Mean differences (Δ) between the values before and after exposure are shown.

Percent MO	1%	5%	25%
# Dogs	2	4	3
Δ PD (mV)	-14	-25	-32
Δ NABD (μEq/10 min)	+21	+82	+63

While these experiments demonstrated damage to the gastric mucosa, it should be noted that we see similar effects from exposure to bile salts. In addition, gross bleeding was never observed in the above experiments nor in 2 other experiments with pH 1, 25% MO solutions perfused for three hours continuously.

As an additional approach to understanding the pathophysiology of gastric stress ulceration, a collaborative project has been undertaken with Dr. Dick Gillis of the Department of Pharmacology, Georgetown University. We are investigating the role of gamma-aminobutyric acid (GABA) sensitive centers in the central nervous system in controlling gastric acid secretion and motility.

Previous results from Dr. Gillis' laboratory indicate that GABA inhibits central parasympathetic outflow to the heart by interacting with brainstem neurons located in the nucleus ambiguus (Science 204:1106, 1979). The purpose of the present study was to determine whether GABA receptor control of central parasympathetic activity also extends to the gastrcintestinal tract. Experiments were performed in chloralose-anesthetized cats subjected to bilateral adrenal ligation and splanchnic nerve section. Strain gauge force transducers were sutured to the antrum and pylorus to record circular muscle activity. Gastric motility was monitored during microinjections of drugs into the nucleus ambiguus. Drugs used were the GABA receptor antagonist, bicuculline methiodide, and the GABA receptor agonist muscimol. Microinjection of bicuculline (100 ng) into either the left or right nucleus ambiguus resulted in pronounced increases in gastric motility. Both frequency and force of antral and pyloric contractions were increased. The subsequent micro-injection of muscimol (50-100 ng) completely abolished the bicuculline-induced increases in motility. In addition, intravenous administration of picrotoxin (2 mg/kg), another agent that antagonizes GABA receptor mechanisms, produced increases in gastric motility similar to that seen with bicuculline. The increases were abolished by bilateral cervical vagotomy. These results suggest that the neurotransmitter GABA may exert central inhibitory effect on parasympathetic outflow to the stomach by interaction with neurons located in the neurons located in the nucleu ambiguus.

II. Pathophysiology of Reflux Esophagitis

Background and Objectives

The human esophagus may be exposed to acid and/or alkaline gastric juice during periods of gastroesophageal reflux. The acid pH of refluxed gastric juice results from gastric secretion of HCl, while the alkaline pH persumably results from the pyloric reflux of alkaline duodenal contents into the stomach. This exposure of the esophageal mucosa to gastric contents is thought to result in esophagitis. One argument in support of this contention is the observation that reducing gastro-esophageal junction, leads to disappearance of esophagitis. This incriminates the refluxed gastric contents as the cause of the esophagitis. Experimentally is has been shown that esophagitis can be produced in dog, monkey, and rabbit esophagus by exposing the esophageal mucosa to various components of gastric juice. It has also been shown in man, dog, and rabbit that increased hydrogen permeability of the esophageal mucosa is seen in the early phases of esophageal injury.

Progress

In the present experiments we measured hydrogen ion loss from the rabbit esophagus with a pH stat autoburette system. We used increased hydrogen permeability as an index of esophageal mucosal injury. The rabbit esophagus normally neither absorbs nor secretes acid. When the mucosa is injured a flux of hydrogen ion down the concentration gradient from the lumen to the blood is seen, with disappearance of hydrogen ion from the esophageal lumen. We performed most of our experiment at pH 2. At pH 2 minimal loss of hydrogen ion from the lumen normally occurs for up to four hours of observation.

In this time period we have looked at the flux of tritiated water (THO) as a marker of permeability of the rabbit esophageal mucosa. We have compared the fluxes of THO and acid to assess the kinds of permeability changes that bile creates in the mucosa.

Back diffusion of hydrogen into the esophageal mucosa has been proposed as a major pathogenic mechanism in peptic esophagitis. The observation that disruption of the esophageal mucosal barrier with bile salts is accompanied by rapid disappearance of hydrogen ion from the esophageal lumen has been viewed as supporting evidence for this hypothesis. The possibility that this phenomenon is attributable to hydrogen ion back diffusion, was explored by examining the effect of bile salt on the permeability of the esophageal mucosa. The esophageal lumens of anesthetized rabbits were cannulated and perfused for one hour with and without 5 mM taurodeoxycholate (TDC). Net hydrogen ion disappearance was measured with a pH stat, whereas unidirectional fluxes were determined by measuring the disappearance of tritiated water (H_2O) and C14 urea out of the lumen. Perfusion of the rabbit esophageal lumen with 5 mM TDC for 1 hour caused a 126 fold increase in the net hydrogen ion disappearance. In contrast perfusion with TDC increased

unidirectional fluxes of H_2O and C14 urea by only 5.3 and 6.7 fold respectively. (Mean of 4 experiments each) These results are inconsistent with the hypothesis that the disappearance of hydrogen ion can be attributed to an increase in the cross sectional diameter of pores in the esophageal mucosa which should result in proportional increases in diffusion for all small molecules. Furthermore, these results indicate that although bile salts increase the permeability of the esophageal mucosa, this mechanism by itself cannot account for the net disappearance of hydrogen ion. Other mechanisms such as neutralization of H^+ by hydroxyl ion or bicarbonate must also be considered.

III. Developing Strategies for Expanding the Small Bowel Mucosal Area

Background and Objectives

Injury to the intestine occurs frequently in combat injury. Massive loss of small intestine is a debilitating problem. We have been investigating strategies for expanding the absorptive area of the small bowel. We are searching for ways to grow sheets of small bowel mucosa from small remnants of mucosa. We are also assessing the function of the mucosa we grow by studying its electrophysiological properties as well as its absorptive capacity.

Progress

New Zealand white rabbits weighing 4 to 7 pounds were prepared surgically. The anti-mesenteric surface of the distal ileum was opened for a length of 7 cm. A patch was then sewn in place in the defect with running 4-0 Tevdek suture. As the diameter of the rabbit ileum is about 1.5 cm this represented a 1/3 enlargement of the bowel circumference. Rabbits returned to regular diet after 5 days of fasting. The following observations were made: 1) Mortality, 2) Gross examination of the patched ileum at 3 weeks (1 rabbit), 2 months (1 rabbit), or 3 months (5 rabbits), 3) Light microscopic evaluation of the neogut mucosa with hematoxylin and eosin staining in 3 rabbits, 4) Evaluation of the electrophysiologic properties of the neogut which grew over the Dacron patches in comparison to normal ileum. Two to three pieces of the neogut from each of 4 rabbits were studied. Mucosa in Ussing chambers separates two small reservoirs contining test solutions and agar bridges. Using electrodes and a voltmeter the potential difference and short circuit current were measured and the conductance was calculated.

In this time frame we completed our studied in which we used Dacron patches in the rabbit ileum. The rabbits survived this operation, but we finally concluded that only a minimal expansion of the small bowel mucosal area was possible with this technique. We subsequently tried two other materials: de-epithelialized rabbit skin and Dexon Polymer obtained from USAIDR. Neither was as successful as Dacron as a patching material. We are currently working on a project using vascularized abdominal wall pedicle muscle graft. We are very encouraged by our initial work here.

IV. Evaluation of the Effects of Porta Caval Shunts

Background and Objectives

Porta-caval shunts have been extensively utilized in the treatment of bleeding esophageal varices secondary to hepatic cirrhosis. This procedure also has a potential role in the treatment of intestinal schistosomiasis and in the reconstruction of the traumatized portal system. Although porta-caval shunts are effective in controlling variceal hemorrhage, hepatic failure following shunting can occur acutely or chronically and appears in a significant percentage of shunted patients. Hepatic decompensation has been attributed to the loss of portal inflow to the liver by the shunt. Because of this possibility the interposition (H-graft) mesocaval shunt was developed, and its proponents claimed that it was hemodynamically superior to the standard porta-caval shunts.

This laboratory previously studied the portal hemodynamics of three porta-systemic shunts using gamma-labeled microspheres to evaluate portal blood flow. A comparison was made between the hepatopetal portal blood flow following end-to-side and side-to-side porta-caval shunts and mesocaval interposition shunts. The microsphere technique showed that about 60% of the portal flow to the liver was preserved by the mesocaval shunt and virtually none by the side-to-side porta-caval shunts. A project was undertaken to determine the effect of end-to-side porta-caval shunt on the maximal rate of urea synthesis by the rat liver. Urea synthesis is an exclusively hepatic function. In theory, the rate of urea synthesis after administration of an excess of substrate should reflect functional liver mass even in the presence of shunting or structural distortion. Three such measurements of urea synthesis rates in humans (JCI 52:2241, 1973; JCI 56:1170, 1975; Gastro 78:1419, 1980) have given conflicting results. The validity of these measurements as indicators of functional hepatic mass is unknown because this relationship has never been tested directly in experimental animals.

Progress

To assess the relationship between urea synthesis rates and functional hepatic mass, we studied urea synthesis in rats after partial hepatectomy (50%, 70% or sham), end-to-side porta-caval shunt, or induction of cirrhosis with CCl_4. A liquid diet was administered that contained neomycin to minimize intestinal ureolysis. The substrate load, 20 g casin/kg body wt, was administered in 8 gavage feedings over four hours.

Urinary urea excretion increased promptly, reached a peak at four hours and persisted at peak levels for at least three 2 hour periods. Higher protein loads gave no higher peak urea excretion but prolonged its duration. Recovery of ^{14}C urea given i.p. was 80-100%. Urea synthesis was calculated as the sum of urinary urea excretion over the six hour peak period, and the net increment in total body urea derived

from estimated body water and increase in BUN. Rats were killed and liver weight, protein and DNA content were determined. After partial hepatectomy, urea synthesis rates correlated positively with liver weight (r=0.63), protein (r=0.86), and DNA (r=0.76) (21 rats, $p<0.001$). Urea synthesis, 153 $\pm$ 28 (1 SD) umoles/gm liver/hr, agreed with rates attained under optimal conditions in isolated perfused rat liver (J. Biochem 77:659, 1975). Urea synthesis after shunts (147 $\pm$ 17 umoles/gm liver/hr, n=7) and in cirrhotic rats (129 $\pm$ 30 umoles/gm liver/hr, n=11) was comparable with that of normal liver on a weight basis. We conclude that urea synthesis accurately reflects hepatic mass under these conditions in rats. The biological meaning and accuracy of various methods to measure urea synthesis in humans require similar validation in experimental animal models.

Recommendations for the Future

The Division of Surgery has a new mission to study the physiology of blast injury. This will give a new focus to the work in the Department of Surgical Gastroenterology. Blast injury is known to cause gastrointestinal hemorrhage and necrosis in experimental animals. Thus much of the department's past research on basic mechanisms of gastrointestinal mucosal injury and cytoprotection will remain relevant to this new mission. Our plan will be to continue our projects investigating basic mechanisms of injury and cytoprotection while developing protocols to specifically investigate the type of gastrointestinal injury which is seen in the blast overpressure setting. Our current plans in this regard include monitoring GI injury in the blast setting with serum enzymes and stool hematest techniques. We would also be interested in assessing the importance of the size and composition of air bubbles within the alimentary tract on the severity of injury due to blast overpressure.

Project: 3S162772A814 BASIC RESEARCH ON MILITARY INJURY AND DISEASE

Work Unit: 006 Gastrointestinal Sequelae of Combat Casualties

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1. Harmon, J.W., Johnson, L.F., Maydonovitch, C.L.: Do bile salts cause hydrogen ion back diffusion from the rabbit esophagus? Clin Research 28:277 A, 1980.

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