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FISCAL YEAR 1982

(1 October 1981 – 30 September 1982)

1 October 1982

Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington, D.C. 20307

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

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

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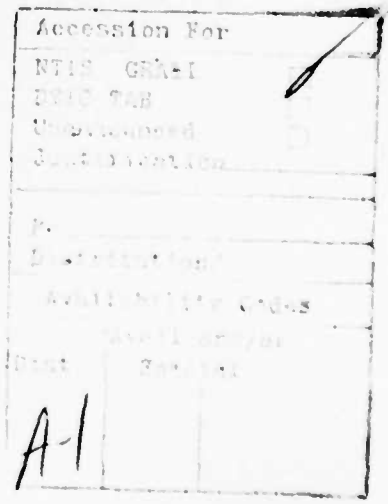

FOREWORD

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

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PROJECT 3A161101A91C

IN-HOUSE LABORATORY INDEPENDENT RESEARCH

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300022	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWN GRADING	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	104 WWH8
b. CONTRIBUTING				
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11. TITLE (Procede with Security Classification Code)

(U) Development of Monoclonal Antibody Producing Hybridomas

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
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19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

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NAME: Solow, R. POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Monoclonal; (U) Antibody; (U) Protein; (U) Enzyme; (U) Toxin; (U) LPS

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

23. (U) The technical objective of this work unit is to develop monoclonal antibody producing hybrid cell lines by using cell-cell fusion procedures and to investigate host antibody responses to microbial antigens of military interest. Antigens to be explored include select toxins; enzymes; viruses; and LPS. Both mouse and human myelomal cell systems will be employed in fusions with lymphocytes from diverse origins (spleens, peripheral blood, lymph nodes, Peyers patches).

24. (U) Antigens or chemical-immunogen hapten complexes will be injected into mice to elicit immune reaction. The purified spleen cells from these animals will be fused with appropriate murine myeloma cells. The fused cells will be subjected to specific selection by growth in selective media. The survivors from actively growing cell cultures will be screened for production of specific monoclonal antibody. Human monoclonal antibodies will be developed by fusing peripheral blood lymphocytes with a human lympho blastoid cell line.

25. (U) 81 10 - 82 09. Hybridoma-produced monoclonal antibodies to dengue-2 virus were characterized, and three distinct antigenic determinants on the virus have been defined. Intestinal IgA and serum IgM anti-shigella LPS antibodies were induced in Lipid A non-responder C3H/Hej mice as well as in normal C3HeB/Fej mice by intragastric immunization with LPS preparations. A potentiated murine systemic antibody response to meningococcal Group B polysaccharide was developed by immunization with an outer membrane meningococcal protein Group B polysaccharide complex. Human hybridoma cell lines secreting anti-tetanus and diptheria toxoid antibodies were developed. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 30 Sep 82.

*Available to contractors upon Originator's approval

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 104 Development of Monoclonal Antibody Producing Hybridomas

INVESTIGATORS:
Principal: Peter Gemski, Ph.D.
Associates: Mary K. Gentry, M.S.; SP4 Karla K. Kopec, B.S.; Mary Ann Sodd, M.S.; and CPT Rikki Solow, M.S., MSC

IN COLLABORATION WITH: W.E. Brandt, Ph.D. (DCD&I); H. Collins (DCD&I); CPT D.E. Griffin, M.S., MSC; CPT E.A. Henchal, Ph.D., MSC (DCD&I); LTC G. Lowell, M.D. (DCD&I); W.D. Zollinger, Ph.D. (DCD&I)

DESCRIPTION:

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

A. Monoclonal Antibodies to Dengue Virus

1. Development of Monoclonal Antibodies for Identification of Dengue Virus Serotypes.

Extensive serological cross-reactions occurring among dengue virus serotypes frequently interfere with their identification. We developed hybridoma-derived reagents directed against the prototype dengue virus strains. DEN-1 (Hawaiian), DEN-2 (New Guinea C), DEN-3 (Philippines H-87), and DEN-4 (Philippines H-241). Lymphocyte hybridomas were prepared by fusing P3x63Ag8 mouse myeloma cells with spleen cells from mice immunized with dengue virus antigens. Antibodies secreted by hybridomas were detected by solid phase radioimmunoassay (SPRIA) using antigen extracts from dengue-infected C6/36 (Aedes albopictus) cells. Hybrid cells from selected lines were cloned and injected into Pristane-primed mice for preparation of ascitic fluids containing higher concentrations of anti-dengue antibody. Each ascitic fluid was evaluated by plaque reduction neutralization (PRNT), hemagglutination inhibition (HAI) and indirect immunofluorescence (IFA) for type-specificity.

Several anti-DEN-1 monoclonal antibody preparations have been selected for use as serotyping reagents. Two cell lines produced high-titered (1:320; 1:1280) type-specific antibody by IFA on virus-infected cells. However, these monoclonal antibodies were not reactive by PRNT and HAI. A third cell line produced an antibody that titered 1:1100 to DEN-1 and 1:10 to DEN-3 by PRNT but was cross-reactive to higher titers by IFA and HAI. Those cell lines type-specific by IFA have recently been used successfully to type isolates from a recent dengue

outbreak in Jamaica.

Monoclonal antibodies to DEN-2 virus, reported at the previous annual meeting, include 5 preparations that are type-specific by IFA, one of which reacts specifically to high titer by PRNT, but not by HAI, and four of which react specifically to high titer by HAI but not PRNT. These monoclones also reacted by IFA with other DEN-2 strains from Asia and Caribbean geographical regions.

Monoclonal antibody preparations to DEN-3 included one that was serotype specific to low titer by IFA and HAI, and another that was type-specific to high titer (1:2560) by IFA only. Three monoclonal antibody preparations to DEN-4 were serotype specific by HAI. One of these was type-specific by IFA (1:640) and two were cross-reactive by IFA. There was little, if any, PRNT activity by these DFN-3 or DEN-4 monoclonal antibodies.

Characterization of monoclonal antibodies to the dengue viruses has shown that there are distinct antigenic determinants that react in the PRNT and HAI tests, both of which involve reactions with the major glycoprotein of the virus. Thus, it is unlikely that a single monoclonal antibody preparation would be available for all serological tests that depend on the virion as the test antigen. It is clear, however, that the IFA test can be used with these monoclonal antibody preparations at the present time in field laboratories to identify each serotype of dengue virus, and that it can continue to be used if the antigenic determinants on the intracellular viral specific polypeptides remain stable.

2. Rapid Identificaiton of Dengue Virus Serotypes Using Monoclonal Antibodies in an Indirect Immunofluorescence Test.

Dengue and dengue hemorrhagic fever occur in epidemic and endemic form throughout tropical areas of the world and pose a potential threat to military operations in those areas. Monoclonal antibodies were produced against the four dengue virus serotypes. These antibodies demonstrated four categories of reactions by immunofluorescence: flavivirus group reactive, dengue complex specific, dengue subcomplex specific (DEN-1, DEN-3), and dengue serotype specific. This is the first time that monospecific antibodies have been available for these unique antigenic determinants. Type-specific monoclonal antibodies prepared against the four dengue virus serotypes were evaluated for their ability to rapidly identify low-passage human and mosquito isolates from Jamaica and West Africa by an indirect immunofluorescence assay. Serotyped human isolates from Jamaican dengue fever patients included 12 DEN-1, two DEN-2, and five DEN-4 viruses. Viruses form West Africa included 84 DEN-2 mosquito strains as well as two DEN-1 and one DEN-2 from humans. Results obtained using the immunofluorescence assay were consistent with virus identifications obtained using the more classical but costly and time-consuming plaque-reduction neutralization test. More viral isolates and higher virus yields were obtained using the C6/36 clone of Aedes albopictus cells rather than LLC-MK2 (monkey kidney) cells. The DEN-2 type-specific monoclonal antibody detected prototype viral antigens 12 to 24 hours postinfection in C6/C36 and LCC-MK2 cells. The use of monoclonal antibodies in the manner described should make dengue virus isolation and identification a rapid and routine procedure.

3. Production and Characterization of Monoclonal Antibodies to Shiga Toxin.

Hybridoma cell lines which produce monoclonal antibodies to Shiga toxin from Shigella dysenteriae 1 were prepared by fusion of spleen cells derived from BALB/cJ mice immunized against glutaraldehyde-inactivated Shiga toxin with the P3x63Ag8 mouse myeloma cell line. Preliminary screening for hybrids which secreted antitoxin was based on the neutralization of Shiga toxin cytotoxicity to HeLa cells and on detection of antibodies to Shiga toxin by a solid phase radioimmunoassay. Six hybrid lines producing monoclonal antibodies to Shiga toxin were identified and cloned twice to soft agarose. The antibodies were found to be of the IgG class. Monoclonal antibodies from the six hybrid lines, amplified in mouse ascitic fluids, differed about 500-fold in their ability to neutralize cytotoxicity; the difference among these lines in antibody bound in the radioimmunoassay was about 12-fold. Autoradiographic detection of the binding of monoclonal antibodies to preparations of pure and crude toxin that were electrophoretically fractionated on polyacrylamide gels and transferred to nitrocellulose indicate that the monoclonal antibodies are monospecific for Shiga toxin.

B. Host Antibody Response and Immunopotentiation

1. Studies on Mitogenicity and Adjuventicity of Meningococcal Outer Membrane Proteins in Mice.

It has previously been shown that meningococcal (Mgc) outer membrane proteins (MP) are B and T cell mitogens and confer immunogenicity upon Mgc group B polysaccharide (Bps) in man. A mouse model was developed to investigate these two immunologic properties of MP. MP was found to be mitogenic for splenic lymphocytes of two normal mouse strains, BALB/cJ and C3HeB/FeJ, as well as for C3H/HeJ mice which respond poorly to Mgc lipopolysaccharide. A solid phase RIA was used to detect anti-Bps IgG and IgM in sera and in splenic lymphocyte culture supernatants. As in man, MP complexed to Bps (MP-Bps) induced anti-Bps IgM in mice whereas Bps alone did not. Immunization with MP alone or MP mixed with but not complexed to Bps did not induce anti-Bps IgM. MP therefore is unlike adjuvants which do not require complexing in order to be effective. Bps complexed to the protein carrier, bovine serum albumin, was also ineffective. Furthermore, priming with MP ten days prior to immunization with MP-Bps did not enhance the immunogenicity of MP-Bps. These data suggest that the function of MP in MP-Bps is not similar to that of a typical protein carrier. Accordingly, we suggest that the immunopotentiating nature of MP may be related to its ability to activate lymphocytes mitogenically while presenting antigens to the immune system in an immunogenic configuration.

2. Induction of Anti-Shigella Lipopolysaccharide Antibodies in C3H/HeJ Mice Following Oral Immunization.

Oral immunization is thought to be necessary for the development of protective immunity against enteric diseases such as shigellosis. Previous reports indicate that C3H/HeJ mice which are non-responsive to lipid A of lipopolysaccharide (LPS) have elevated IgA responses to intragastically administered protein antigens. We have investigated the ability of C3H/HeJ mice to respond to intragastric immunization with: 1) acetone-killed-dried (AKD) Shigella flexneri X16, 2) protein-containing Boivin X16 LPS (BLPS), 3) protein-free Westphal LPS

(WLPS) and 4) alkaline-hydrolized X16 LPS (ALPS). A solid phase RIA was used to analyze anti-LPS IgA, IgM and IgG in sera and splenic lymphocyte culture supernatants. AKD shigellae, LPS and WLPS induced serum anti-WLPS IgA and IgM but not anti-WLPS IgG. The dose responses to BLPS and WLPS were similar. ALPS, in contrast, was not immunogenic even when coated to sheep red blood cells. Co-administration of Concanavalin A with BLPS or WLPS altered the anti-WLPS IgA response but not the anti-WLPS IgG and IgM responses. In addition, anti-WLPS antibodies were detected in splenic lymphocyte culture supernatants following immunization with AKD shigellae, BLPS or WLPS. Our data indicate that specific anti-shigella LPS IgA and IgM can b e induced in lipid A non-responder C3H/HeJ mice by intragastric immunization with LPS.

3. Stimulation of Secretion of Anti-Shigella Lipopolysaccharide (LPS) Secretory IgA by Mucosal Immunization with Meningococcal Outer Membrane Protein-LPS Complexes.

Induction of anti-LPS secretory (S-) IgA is considered important for protection against shigellosis. The protein-free hot-phenol-water (Westphal) preparation of LPS (LPSw) isolated from Shigella flexneri, however, is ineffective as a mucosal immunogen. Meningococcal outer membrane proteins (MP) have previously conferred immunogenicity upon meningococcal group B polysaccharide. We now report that MP can also confer immunogenicity upon LPSw when co-precipitated with LPSw to form a non-covalent MP-LPSw complex. Rabbits were immunized once im or weekly X 3 intra-lumenally (il) in chronic Thiry-Vella intestinal loops. Specific anti-LPSw S-IgA and IgG in loop fluids and in sera were measured by an ELISA. MP-LPSw induced specific intestinal fluid S-IgA or serum IgG following il or im immunization respectively. Immunogenicity of LPSw was enhanced by complexing it with either 41K or ca. 30K MW MP fractions. Boivin LPS containing shigella membrane proteins was also effective without MP. In contrast, little anti-LPSw S-IgA or IgG was induced by immunizing il or im with LPSw alone or complexed to bovine serum albumin. These data suggest that oral vaccination with LPS complexed to membrane protein "helper" antigens may be effective against enteric diseases such as shigellosis.

PUBLICATIONS:

1. Gentry, M.K., Henchal, E.A., McCown, J.M., Brandt, W.E. and Dalrymple, J.M. 1982. Identification of Distinct Antigenic Determinants on Dengue 2 Virus Using Monoclonal Antibodies. Am. J. Trop. Med. Hyg. 31: 548-555.

2. Henchal, E.A., Gentry, M.K., McCown, J.M. and Brandt, W.E. 1982. Dengue Virus-specific and Flavivirus Group Determinants Identified with Monoclonal Antibodies by Indirect Immunofluorescence. Am. J. Trop. Med. Hyg. 31: 830-836.

3. Brandt, W.E., McCown, J.M., Gentry, M.K. and Russell, P.K. Infection Enhancement of Dengue type 2 Virus in the U-937 Human Monocyte Cell Line by Antibodies to Flavivirus Cross-reactive Determinants. 1982. Infect. Immun. 36: 1036-1041.

4. Henchal, E.A., McCown, J.M., Seguin, M.C., Gentry, M.K. and Brandt, W.E. Rapid Identificaton of Dengue Virus Isolates by Using Monoclonal Antibodies in an Indirect Immunofluorescence Assay. Am. J. Trop. Med. Hyg. (in press)

ABSTRACTS:

1. Brandt, W.E., McCown, J.M., Gentgry, M.K. and Russell, P.K. 1981. Immune Enhancement of Dengue-2 Virus Replication in the U-937 Human Monocyte Cell Line by Cross-reactive Monoclonal Antibodies. FASEB, Annual Meeting, Atlanta, GA.

2. Gentry, M.K., McCown, J.M., Harrison, S.A., Henchal, E.A., Brandt, W.E. and Dalrymple, J.M. 1980. Characterization of Monoclonal Antibodies Directed Against Dengue-2 Virus. Am. Soc. for Trop. Med. Hyg. Annual Meeting, Atlanta, GA.

3. Griffin, D.E., Gentry, M.K., Limrotlert, C.T., Alvarado, M.I. and Fishbein, J.D. 1982. Production and Characterization of Monoclonal Antibodies to Shiga Toxin. ASM E39.

4. Henshal, E.A., McCown, J.M. Gentry, M.K., Dalrymple, J.M. and Brandt, W.E. 1981. Evaluation of the Serological Characteristics of Monoclonal Antibodies Produced Against Dengue Virus Antigens. FASEB, Annual Meeting, Atlanta, GA.

5. Solow, R., Sodd, M.A., Zollinger, W.D. and Lowell, G.H. 1982. Studies on the Mitogenicity and Adjuventicity of Meningococcal Outer Membrane Proteins in Mice. Federation Proceedings 41(3): 830.

6. Sodd, M.A., Solow, R., Gemski, P. and Lowell, G.H. 1982. Induction of Anti-Shigella Lipopolysaccharide Antibodies in C3H/HeJ Mice Following Oral Immunization. Federation Proceedings 41(3): 830.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

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

11. TITLE (Procede with Security Classification Code)

(U) Neuropharmacology of Performance and Fatigue

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012900 Physiology 016200 Stress physiology 010340 Psychology 000000 Pharmacology

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

17. CONTRACT/GRANT

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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	1.5	69
FISCAL YEAR CURRENT 83	2.0	80

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS
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NAME: Mobley, W.C., MAJ, MC

21. GENERAL USE

Foreign Intelligence Considered

POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

23. (U) To continue ongoing studies initiated to evaluate the role of endogenous neuromodulators in performance, fatigue, and sleep in order to derive potential insights into the mechanisms by which these behaviors are mediated. Presently available stimulant drugs, such as amphetamine and related substances, have the adverse consequence of severely impairing judgement while providing their stimulant effects, thus limiting military applications. If insights into the body's own systems for producing arousal, sleep, and other behaviors relating to vigilance and performance are elucidated, it may be possibile to develop novel substances to enhance endogenous stimulants and depress endogenous sedatives and thus improve performance.

24. (U) Experimental animals will be entrained on an eight arm maze, and performance will be assessed with and without prior pharmacological treatment. This general paradigm will also be employed with descriminative operant tasks specifically designed to evaluate stereotypic responses relative to general stimulant effects. The effects of transauricular electroconvulsive shock will also be evaluated to determine endogenous factors which may play a role in physiological and behavioral compensation to applied stress. Immunohistochemical procedures will be utilized to correlate changes in endogenous substances with changes in their receptors.

25. (U) 81 10 - 82 09 Thyrotropin-releasing hormone (TRH), a neuropeptide found in the brain, was shown to have stimulant properties when injected into rats. Doses of this substance which increased locomotor activity failed to alter maze running time or accuracy. By contrast, amphetamine doses which increased locomotor activity increased maze running time and decreased correct responses. Electroconvulsive shock (ECS) was further shown to activate endogenous opiates and increase their receptor numbers. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

(Attach list of contractors upon originator's approval)

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

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 105: Neuropharmacology of Performance and Fatigue

Investigators:
Principal: John W. Holaday, GM-14
Associates: Gregory L. Belenky, LTC, MC
W. C. Mobley, MAJ, MC

Problem:
To evaluate the role of endogenous neuromodulators in performance, fatigue, and sleep in order to derive a potential understanding into the mechanisms by which these behaviors are mediated. Such information will enable novel approaches to the development of neuropharmacological agents which will rely on manipulations of such endogenous biochemicals to enhance performance, diminish fatigue and prevent sleep.

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

Approaches:
Initial experiments have involved training animals to perform on an eight-arm maze; responses were monitored for speed and accuracy of traversal of all eight arms. Secondary studies have involved evaluation of alterations in conditioned responses using food and other rewards to maintain operant performance. Both of these studies have been performed prior to and following neuropharmacological manipulation of endogenous substances which may be linked to states of arousal. Further studies are planned to evaluate brain and plasma concentrations of neuropeptide substances which are likely candidates for a role in endogenous behavioral regulation. Such substances will be evaluated in conjunction with measurements of locomotor activity, post-electroconvulsive shock depression, sleep, sleep deprivation, and performance of entrained operant tasks.

Results:
Initial results have demonstrated that thyrotropin-releasing hormone (TRH), a tripeptide material with an established endocrine

role, also has stimulant properties as demonstrated by its effects in increasing locomotor activity. At the same doses required to improve locomotor activity, TRH failed to alter maze running time or accuracy. By contrast, amphetamine doses which increased locomotor activity were associated with an increased maze-running time and a decrease in number of correct responses. Additional studies with electroconvulsive shock in rats demonstrated that the function of endogenous narcotics (the endorphins) was significantly activated in temporal association with the decreased behavioral responses associated with cessation of the convulsive state. These studies provide potentially novel stimulant drugs, namely TRH and opiate antagonists, which will be further evaluated along with other potentially useful substances in paradigms as outlined above.

References:

Belenky, G.L., Cardenales, L., Robles, L.E., Arday, D., and Holaday, J.W. Amphetamine but not TRH Disrupts Performance on the Radial Arm Maze. Soc. Neurosci. Abstr., Vol. 8, p. 104, 1982

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOC 6468	82 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		NL	☒ YES ☐ NO	A. WORK UNIT

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

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology 012900 Physiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2	90
FISCAL YEAR CURRENT 83	2	

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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

ADDRESS:* Washington, D.C. 20012

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: (202) 576-3593
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ASSOCIATE INVESTIGATORS
NAME: MAJ H. Kyle Webster (AFRIMS)
NAME:

21. GENERAL USE

Foreign intelligence considered

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

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

25.(U) 81 10 - 81 09 The metabolic pathways for purine salvage and interconversion have been determined for P. falciparum infected RBC in vitro and P. knowlese infected RBC in vivo. PRPP (phosphoribosyl-pyrophosphate) has been identified as a key cofactor for purine metabolism for both parasite and red cell host. High levels of purine salvage synthesis enzymes have been found in infected RBC. The role of polyamines in intraerythrocytic parasite growth have been studied with the use of inhibitors of ornithine decarboxylase and S-adenosyl-methionine decarboxylase. Inhibition of polyamine synthesis inhibits parasite growth. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

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

Project 3A161101A91C: IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 106 Intermediary Metabolism of the Malaria-Infected Erythrocyte

Investigators LTC June Whaun, MC; Dr. Nesbitt Brown, GS-13 (Div. of Biochemistry); MAJ H. Kyle Webster, MSC (AFRIMS); MAJ William Wiesmann (Dept. of Nephrology)

Description

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

Progress

Studies of purine metabolism in malarious RBC in vitro and in vivo have proceeded in collaboration with MAJ H. Kyle Webster, Chief, Dept. of Immunology, AFRIMS, Bangkok, Thailand and are described in the progress report from that department.

In separate studies of polyamine metabolism in RBC infected in vitro by P.falciparum, inhibitors of ornithine decarboxylase (ODC) and s-adenosylmethionine decarboxylase have been used to evaluate the relationships between polyamine metabolism and intraerythrocytic malaria growth and development. Interference with polyamine biosynthesis has been shown to inhibit intracellular parasite growth. In addition, it has been

found that the anti-cancer drug, methylglyoxalbisguanylhydrazone, is effective at micromolar concentrations at inhibiting parasite growth when combined with polyamine depletion induced by the drug DFMO, an inhibitor of ODC.

Future Plans

We plan to continue the study of purine and polyamine 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, and to continue to study the biochemical consequences of malaria infection for immunocompetent cells and host defense *in vivo*.

Abstracts

1. Whaun, J.M., Ip, S.H.C. and Hansen, W.P. Rapid identifi- cation and detection of parasitized human red cells by automated flow cytometry. Blood 58:38a, 1981.

2. Whaun, J.M. The effects of aspirin-containing serum in the continuous culture of P.falciparum-infected human erythrocytes. Clinical Research 30:381A, 1982.

3. Whaun, J.M. and Brown, N.D. Polyamine inhibition and the malarial-infected red cell: A model for studying polyamine metabolism and cell growth. Fifth International Congress of Parasitology. Toronto, 1982.

Publications

1. Webster, H.K. and Whaun, J.M. Anti-malarial properties of bredinin: prediction based on identification of differences in human host-parasite purine metabolism. Journal of Clinical Investigation 70:461-469, 1982.

2. Brown, N.D., Whaun, J.M. and Strickler, M.P. A femtomolar ion-pair high performance liquid chromatographic method for determining dazsylated polyamine derivatives of biological fluids utilizing an automated polyamine analyzer. Journal of Chromatography 245:101-108, 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2531	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

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

11. TITLE (Precede with Security Classification Code)

(U) Genetic Basis of Virulence of Bacterial Pathogens

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

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

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Lazere, Janet

NAME:

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

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

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

25. (U) 81 10 - 82 09. Studies of the genetic control and characterization of virulence properties of invasive enterics have continued. In addition to further study of Vwa plasmids (42-47 Mdal range) which relate to virulence, calcium dependency and V-W antigen production, a new plasmid (82 Mdal) has been discovered. This plasmid species was found to be present (in addition to the Vwa plasmid) only in serotypes of Y. enterocolitica associated with disseminating septic infections. In our studies related to E. coli K1, we prepared chromosomal hybrids which express both K1 and K27 antigens and examined the relative contributions of these capsules to serum resistance. Our findings indicate that K1 antigen can protect rough E. coli from serum kill whereas K27 fails to provide such a protective effect. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

WORK UNIT: 110 Genetic Basis of Virulence of Bacterial Pathogens

INVESTIGATORS:
Principal: Peter Gemski, Ph.D.
Associates: Janet Lazere, B.S.; SP4 Kathryn Kraus, B.S.; and PFC Dana H. Wells, B.S.

IN COLLABORATION WITH: L.S. Baron, Ph.D. (DCD&I); T. Cook, Ph.D. (University of Maryland); A.D Cross, MD (DCD&I); S. Opal, MD (DCD&I); K. Wachsmuth, Ph.D. (CDC); J.A. Wohlhieter, Ph.D. (DCD&I); N. Yamamoto, Ph.D. (Hannemann Medical College, Phila. PA)

DESCRIPTION:

Studies on the pathogenesis of enteric infections have established that some organisms evoke diarrheal disease by an invasive mechanism in which the pathogen penetrates and replicates within gastrointestinal tissue. Without doubt, several bacterial attributes must function in concert to allow expression of invasive events. The polygenic control of invasive virulence remains unelucidated at the present time. Our objective is to study that genetic control of invasive properties of enteric pathogens. An understanding of chromosomal and plasmid genes associated with invasive properties of enterics will provide basic formation needed to facilitate the development of (1) live attenuated vaccines and (2) improved methods for prevention and treatment of intestinal infections in military personnel operating in areas of poor sanitation. Although such diseases are temporary, they are sufficiently devastating to interfere seriously with military activities.

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

A. Studies of the Virulence of Yersinia

1. Vwa Plasmids of Yersinia enterocolitica Serotype 0:3.

We have previously shown that Y. enterocolitica serotype 0:8 and Y. pseudotuberculosis type III can have plasmids, designated Vwa, which are associated with production of V and W antigens and virulence of these organisms. Seven strains of Y. enterocolitica serotype 0:3, originally isolated from feces of children with diarrhea, have been examined for plasmids by means of agarose gel electrophoresis of their DNA and found to harbor a Vwa plasmid. As with 0:8 strains, 0:3 isolates with Vwa produced small, convex colonies when grown at 37C on Trypticase soy agar. Such colonies produced the V and W antigens as evidenced by calcium dependence (growth inhibition on magnesium

oxalate agar at 37C). Isogenic derivatives that had lost the Vwa plasmid were no longer calcium dependent. Unlike 0:8 strains, these 0:3 isolates failed to provoke conjunctivitis in guinea pigs, irrespective of the presence of a Vwa plasmid. We examined the similarity of Vwa plasmids from 0:3 and 0:8 strains of Y. enterocolitica by comparing DNA fragmentation patterns after digestion by restriction endonucleases. After treatment with several different restriction enzymes, Vwa plasmids from each serotype yielded different banding patterns, indicating a divergence in the microevolution of Vwa. However, some common restriction fragments of Vwa were observed, irrespective of serotype.

2. New Virulence-Associated Plasmid in Yersinia enterocolitica.

We found that 7 of the 100 study strains and the 3 positive controls were lethal for adult mice and that each contained one 42- and one 82-Mdal plasmid. Of the 93 strains not lethal for mice, only 1 contained 45- and 82-Mdal plasmids, and 2 contained only a 42-Mdal plasmid. Although the 82-Mdal plasmid has not been reported in previous studies of Y. enterocolitica, the data from those studies reflected the use of a sodium dodecyl sulfate-salt precipitation method for DNA extraction. In our hands, this procedure failed to detect the presence of the 82-Mdal plasmid, which was visible at low intensity in both routine and gradient preparations. One explanation for this is the sensitivity of large plasmid molecules to phenol and chloroform, which are used to extract protein in this method. Another explanation is the possibility that this large plasmid is present in low copy numbers or in a relatively small percentage of the cell population.

We believe that this 82-Mdal plasmid may be associated with virulence for the following reasons. (i) It was present in all study and control strains which were lethal for mice. (ii) The association of this plasmid with lethality was statistically significant ($P << 0.001$ [Fisher exact test]). (iii) The two strains containing only the 42-Mdal plasmid were not lethal for mice. (iv) A derivative, strain 1223-75-2, contained only the 82-Mdal plasmid and was as lethal for mice as was the parent strain, 1223-75-1. (v) Previous reports have shown the presence of a 42-Mdal plasmid in an avirulent Y. enterocolitica strain. (vi) These previous studies have not shown complete homology among 40- to 48-Mdal plasmids isolated from virulent Y. enterocolitica strains. (vii) The correlation between this plasmid and lethality in Y. enterocolitica serotypes other than 0.3, 0:8, and 0:9 was not expected but is consistent with the potential mobility of plasmid DNA.

Further work must be done to compare the 42- and the 82-Mdal plasmids in all of these strains and especially to determine the degrees of homology among the 82-Mdal molecules. Both plasmid species are statistically associated with lethality for mice, and one or both may be necessary to determine the virulence of Y. enterocolitica. However, the initial evidence suggests that the newly observed 82-Mdal plasmid is an excellent candidate for both plasmid profile screening and genetic probe construction. Either technique offers a protential diagnostic and epidemiologic tool not available at present.

3. Studies of Plasmids in Yersinia ruckeri.

Seventeen strains of Yersinia ruckeri, representing virulent and non-

virulent serotypes, were examined for plasmid DNA by means of agarose gel electrophoresis of partially cleared lysates. Of 6 avirulent strains examined, only one (strain 11.59) had a plasmid of about 2-3 Mdal. In contrast, all 11 virulent isolates that were studied had a 70-Mdal plasmid. Virulent Y. ruckeri strain 11.34 containing the 70-Mdal plasmid (pTC4) was examined further because it also has an additional 35-Mdal plasmid (pTC1). pTC1 was found to carry the genes for resistance to tetracycline (Tc) and is transmissible to E. coli by conjugation. Of 30 Tc^r E. coli transconjugants analyzed, 29 contained pTC1. The remaining Tc^r transconjugant, however, contained a 16-Mdal plasmid (pTC2) presumably derived as a result of a large deletion in pTC1. None of these transconjugants had inherited pTC4. In addition to producing Tc^s derivatives cured of pTC1, ethidium bromide treatment of strain 11.34 also yielded a Tc^s derivative which contains a 25-Mdal plasmid (pTC3) presumably derived from pTC1. Studies are in progress to assess the role of the 70-Mdal plasmids in virulence.

B. Studies of K Antigens

1. Effect of K Antigen Phenotype on Serum Sensitivity of Escherichia coli.

We have previously shown that the K_1 capsular antigen confers serum resistance to bacteremic E. coli isolates of rough phenotype. Yet, some evidence suggests that other K capsules provide little if any protection against serum. Thus, we prepared hybrids which express both K_1 and K_{27} antigens and examined the relative contributions of these capsules to serum resistance. E. coli Hfr strain F639 (rough, K_{27}^+, serum sensitive) was conjugated with E. coli recipient strain E_{412} (rough, K_1^+, his^-, trp^-, str^R, serum resistant). Transconjugants which inherited both the his and trp linked genes for K_{27} antigen synthesis were analyzed. These hybrids retained and expressed the K_1 antigen since the K_1 locus is non-allelic with K_{27} gene loci. Hybrid strains which express both K_1 and K_{27} antigens exhibit serum resistance, but not at the level of the K_1+ parental strain. An isogenic K_1- derivative of a hybrid which expressed only K_{27} antigen was serum sensitive, (>99% kill, 60 min.). These findings indicate that the presence of the K_1 capsular antigen can protect some rough strains of E. coli from serum bactericidal activity, whereas K_{27}, and perhaps other K antigens, fail to provide such a protective effect.

2. The Uniqueness of the K1 Antigen in the Epidemiology and Phagocytic Resistance of E. coli.

We examined 498 clinical isolates of E. coli (EC) from blood (248), urine (193) and wounds (57) for the synthesis of K1 antigen ($K1^+$) and lipopolysaccharide (LPS) smooth (S) or rough (R) phenotype using rough-specific and K1-specific phages. 50%(97/193) of urinary EC were R compared to 28%(70/248) of blood and 30%(17/57) of wound isolates. Urinary isolates were less likely to be $K1^+$: 11%(21/193) versus 22%(55/248) for blood EC and 21%(12/57) for wound EC. 47%(33/70) of R bacteremic EC were $K1^+$ while only 12%(22/178) of S bacteremic EC were $K1^+$ ($p<0.0001$). This strong association between $K1^+$ and R phenotypes was not seen with urinary EC where only 12%(12/97) were R-$K1^+$ and 9% (9/96) were S-$K1^+$. This epidemiologic data suggests that the EC phenotype is related to the ability to cause bacteremia. The sensitivity to killing (>80% of inoculum) in a phagocytic system with a neutrophil: EC ratio of 1:1 differed with the phenotype. Among bacteremic $K1^+$ isolates, only 5%(1/20) S-$K1^+$ and 21%

(6/28) R-K1$^+$ were killed. In contrast, 73%(32/44) of S-K1$^-$ and 91%(30/33) R-K1$^-$ were killed. The combination of S LPS and K1$^+$ confers resistance to phagocytic kill. Capsular types other than K1 in combination with S LPS may confer phagocytic resistance. Only the K1$^+$ phenotype, however, confers resistance to EC of the R LPS phenotype. This resistance may explain the high (47%) prevalence of K1$^+$ among the R bacteremic EC.

3. Ability of Murine Monoclonal Antibody Prepared Against Group B Meningococcal Polysaccharide to Kill K1-Positive E. coli.

In a phagocytic killing assay (neutrophil to bacterium ration 1:1) 78% (60/77) of bacteremic E. coli (EC) lacking K1 antigen were killed in excess of 80% by normal human serum. In contrast, only 15%(7/48) of K1-positive EC were killed under similar conditions ($p<0.0001$). The K1-polysaccharide (PS) is immunochemically identical to the Group B meningococcal (GBM) PS. An IgM monoclonal antibody (MA) was prepared against GBM PS by fusion of spleen cells of mice vaccinated with viable GBM with a non-producer mouse myeloma cell line. MA killed 90% of GBM with human complement (C) in the absence of neutrophils (N) to a dilution of 1:40,000. In addition, MA was able to kill 7 different clinical bacteremic strains of EC that had K1$^+$ phenotypes, regardless of whether the strain had a smooth or rough O-phenotype. Both human C and N were required for kill. There was greater than 99% phagocytic kill by MA at a dilution of 1:150,000 and 78% kill at a 1:500,000 dilution. There was no kill with rabbit C at any dilution. A prozone of at least 1:1,000 was seen in the 7 K1-positive strains killed. MA did not kill 2 EC strains lacking K1 antigen, thereby showing specificity. This MA has potential for the treatment of disease caused by K1-producing EC.

C. Studies of Interspecific Hybrid Phages

Hybrids between Salmonella phage P22 and coliphage ϕ80 were isolated after superinfection with P22 of a smooth Escherichia coli-S. typhimurium hybrid lysogenic for ϕ80. These hybrid phages, designated as ϕ80immP22 and ϕ80immP22dis, possess the ϕ80 protein coat and tail genes. The ϕ80immP22 hybrid group has acquired the immunity (immC) region of P22 and some adjacent P22 genes, but E. coli-S. typhimurium strains lysogenic for ϕ80immP22 hybrids remain sensitive to P22. The ϕ80immP22dis hybrids, found ten times more frequently than the ϕ80immP22 hybrids, confer on their hosts immunity to P22 infection. The ϕ80imm P22dis hybrids therefore contain a more extensive portion of the P22 genome which encompasses the immI as well as the immC region of P22. These ϕ80immP22dis hybrids also carry the P22 attachment region and either P22 tail gene 9 or antigen conversion gene a1, but not both of these genes.

PUBLICATIONS:

1. Opal, S.M., Cross, A. and Gemski, P. 1982. K Antigen and Serum Sensitivity of Rough Escherccchia coli. Inf. Immun. 37: 956-960.

2. Kay, B., Wachsmuth, K. and Gemski, P. 1982. New Virulence-Associated Plasmid in Yersinia enterocolitia. J. Clin Microbiol. 15: 1161-1163.

3. Yamamoto, N., Gemski, P. and Baron, L.S. 1982. Genetic Studies of New Hybrid Phage Species Between Coliphaze $\phi 80$ and Salmonella p. 22. J. Gen. Virology. (in press)

ABSTRACTS:

1. Lazere, J.R., Wohlhieter, J.A. and Gemski, P. 1982. Vwa Plasmids of Yersinia enterocolitica Serotype 0:3. Am. Soc. Microbiol., B42.

2. Cook, T. and Gemski, P. 1982. Studies of Plasmids in the Fish Pathogen, Yersinia ruckri. Abst. XIIIth Int. Cong. of Microbiol. P26: 5.

3. Opal, S., Cross, A. and Gemski, P. 1982. Effect of K Antigen Phenotype on Serum Sensitivity of E. coli. Am. Soc. Microbiol. Abst B163.

4. Cross, A.D., Sadoff, J.C. and Gemski, P. 1981. Uniqueness of K1 Antigen in the Epidemiology and phagocytic Resistance of E. coli. Am. Soc. Microbiol., 21st ICAAC Meeting. Abst 340.

5. Cross, A.C., Sadoff, J., Zollinger, W., Mandrell, R. and Gemski, P. 1981. Ability of Murine Monoclonal Antibody Prepared Against Group B Meningococcal Polysaccharide to kill K1-positive E. coli. ICAAC. Abst 682.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	3. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DAOG 2525	82 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. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	H. Term.	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	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	81 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	2.0	150
	CURRENT 82	2.0	92

19. RESPONSIBLE DOD ORGANIZATION

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NAME:

21. GENERAL USE

Foreign Intelligence Considered

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 antileishmanial drugs to infection sites and the effect on the disease will be studied in laboratory animals. Tolerance of the animals to liposomal preparations will be evaluated.

25.(U) 81 10-82 09. Retrospective data analysis of death rates and parasite suppression in hamsters experimentally infected with Leishmania donovani revealed infections with different degrees of virulence. Studies on liposome-encapsulated antimonial (both pentavalent and trivalent) and 8-aminoquinolines continued in L. donovani-infected hamsters. Experiments were initiated to test liposome-encapsulateo compounds in hamsters infected with Leishmania braziliensis oanamensis. Efforts were continued on the studies of the stability, temperature dependence, entrapment characteristics and shelf-life properties of liposome-encapsulated drugs. Also investigated were new methods for the measurement of liposome-encapsulated compounds and the lipid solubility of test compounds, most notably the 8-aminoquinolines. This Work Unit is being terminated by reason of expiration of its three-year period. For Technical Report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

PROJECT 3A161101A91C In-House Laboratory Independent Research

WORK UNIT 114 **Liposomes for Treatment of Leishmaniasis**

INVESTIGATORS:

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

PROBLEM AND OBJECTIVES:

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

RESULTS:

Retrospective data analysis of death rates and parasite suppression in hamsters experimentally infected with Leishmania donovani revealed infections with different degrees of virulence.

Studies on liposome-encapsulated antimonials (both pentavalent and trivalent) and 8-aminoquinolines continued in L. donovani infected hamsters. Experiments were initiated to test liposome-encapsulated compounds in hamsters infected with Leishmania braziliensis panamensis.

Efforts were continued on the studies of the stability, temperature dependence, entrapment characteristics and shelf-life properties of liposome encapsulated drugs. Also investigated were new methods for the measurement of liposome encapsulated compounds and the lipid solubility of test compounds most notably, the 8-aminoquinolines.

FUTURE OBJECTIVES:

This Work Unit has been terminated by expiration of its three-year period as a 91C project. Promising studies in progress will be continued as appropriate in support of drug development efforts under other work units.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2221	82 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
81 10 01	H. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

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

11. TITLE (Precede with Security Classification Code)

(U) 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	19. 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)
PRECEDING FISCAL YEAR 81	2.0	50
CURRENT FISCAL YEAR 82	2.0	101

16. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Substrates; (U) Energy; (U) Prostaglandins; (U) Stress; (U) Shock; (U) Reperfusion

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

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

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

25. (U) 81 10 - 82 09 The use of maximal conventional therapy (returning all of the shed blood and administering a volume of Ringer's Lactate equal to three times the volume of shed blood) led to a 100 percent survival in rats subjected to a standard protocol of hemorrhagic shock. During the fiscal year over 100 experiments were carried out with a variety of protocols of hemorrhagic shock to try to develop an LD 50 model. No protocol provided this result, demonstrating the importance of animal to animal variability in this type of study. This variability must be taken into consideration in evaluating the results of treatment required for hemorrhagic shock. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

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

Project 3A16110191C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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

Investigator:
Principal: COL Arthur W. Fleming, MD

Problem and Objectives:

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

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

Progress:

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

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

takes to remove the blood from the body as well as how long therapy is delayed is critical in standardizing a reproducible model. By removing the blood at a constant rate of 0.1 ml per 100 gm of body weight per minute, we have produced, preliminarily, a reasonably predictable model. The model, which needs refinement, still suffers from the rather steep slope of the line at the LD_{50}. Further work on this model has been carried out in this fiscal year with experiments on over 100 rats. We have not yet devised a method for consistently achieving 50% mortality. Attempts during this year to measure prostaglandins did not yield consistent results using the New England Nuclear assay kits.

The variability of the results demonstrate the importance of animal to animal variation in this kind of study, and must be taken into consideration in assessing the results of treatment studies.

Future Objectives:

This work unit is being terminated by expiration of its funding status.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1284	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. HEADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

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

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
002600 Biology 012900 Physiology

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

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	3.0	207
	CURRENT 83	2.0	200

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: MAJ Eric B. Schoomaker
NAME:

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with security Classification Code) (U) Erythrocyte; (U) Acetylcholinesterase; (U) Acetylcholine receptor; (U) Structural membrane proteins; (U) Leukocytes

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

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

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

25.(U) 81 10-82 09 AChR Studies: By using non-hydrolyzable, radiolabelled cholinergic agonists and various neurotransmitter antagonists, a specific, saturable muscarinic AChR has been identified, characterized, and enumerated on human RBC and WBC (neutrophils) as well as on blood precursor cells harvested from normal bone marrow aspirates. These studies were extensions of previous studies done with animals. AChR studies have been carried out both with whole blood cells and plasma membrane preparations. Methods have been established to study the effects of ACh, ACh analogues, and ACh-AChE blockade on RBC rigidity, deformability and membrane integrity using blood viscometry and RBC osmotic fragility measurements. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81-30 Sep 82.

*Available to contractors upon originator's approval

Project 3A161101A91C: IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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

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

Description

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

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

Work in this area is aimed at three problems:

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

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

3. To investigate the mechanism by which abnormal RBC structural membrane proteins result in premature RBC destruction. Techniques developed to study the above two issues have led to methods by which dysfunctional structural protein mutations may be examined. Abnormal interactions among these proteins appear to underlie RBC shape changes and cell lysis in disorders such as hemolytic hereditary elliptocytosis. We propose to extend our preliminary studies aimed at the recognition of major changes in the conduct and character of abnormal structural proteins to those of more subtle, qualitative changes in phosphorylation and protein-protein interaction.

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

Progress

1. ACHE Studies - By using non-hydrolyzable, radiolabelled cholinergic agonists and a variety of neurotransmitter antagonists, a specific, saturable muscarinic AChR has been found on human and rabbit RBC, neutrophils, and hematopoietic precursor cells from the bone marrow.

The AChR's have been identified both on whole cell and isolated membrane preparations. Studies of the ontogeny of human leukocytes have shown that these receptors appear early in the development of the cells during their intramedullary proliferation and maturation, indicating that a cholinergic response apparatus on blood cells may be important for the regulation of their production in the marrow.

Preliminary studies in mice have indicated that a muscarinc agonist (carbachol) promotes the fragility of circulating RBC resulting in transient hemolytic episodes.

2. Hemolytic anemia studies - Techniques have been established to measure RBC deformability and fragility using a rotating disk shear force apparatus adapted to allow for continuous monitoring of cell shape changes by direct microscopic visualization. Studies of exercise induced hemolysis in unconditioned and conditioned (marathon runners) individuals have defined relationships between cardiovascular and muscular conditioning and the occurrence of RBC hemolysis during vigorous and prolonged hemolysis. Preliminary definition of changes in RBC membrane structural proteins related to prolonged exercise has also been done.

Future Plans

Future plans are essentially those that have been outlined above in the description of this work unit, particularly as it relates to blood cell acetylcholinesterase function.

Abstracts

1. Schoomaker, E.B., W.M. Butler, and L.F. Diehl. Studies of erythrocyte (RBC) structural proteins in hereditary elliptocytosis (HE) with cobalamin (B_{12}) deficiency. Blood 58:48a, 1981.

2. Wright, D.G., A.I. Meierovics, E.B. Schoomaker, L. Tang, and D.L. Lucas. Muscarinic cholinergic receptors on human neutrophils during their development and function. Clin. Res. 30:382A, 1982.

3. Diehl, L.F., W.M. Butler, E.W. Ferguson, and E.B. Schoomaker. Intravascular hemolysis in marathon runners. Clin. Res. 30:314A, 1982.

Publications

1. Schoomaker, E.B., W.M. Butler, L.F. Diehl. Increased heat sensitivity of red blood cells in hereditary elliptocytosis with acquired cobalamin (Vitamin B_{12}) deficiency. Blood 59:1213-1219, 1982.

2. Butler, W.B., Sprattling, L., Kark, J.A. and Schoomaker, E.B. Hemoglobin Osler (B-145 tyr - Asp): Report of a new family with exercise studies before and after phlebotomy. Am. J. Hematol. (in press), 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OG 1296	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

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

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

010100 Microbiology 002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

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

19. RESPONSIBLE DOD ORGANIZATION

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NAME: BRANDT, WALTER E.

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

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

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

25 (U) 81 10-82 09 Mouse monoclonal antibodies prepared against all four dengue virus serotypes have been characterized with respect to the dengue antigen(s) with which they react. Flavivirus group reactive monoclonal antibodies which immunologically enhance dengue virus infection of human monocytes, radioimmune precipitate 8-10 virus-induced polypeptides in a manner similar to acute sera from DHF patients. On the other hand, type, specific mouse monoclonal antibodies react predominantly with the major envelope glycoprotein and possibly related non-structural proteins. Purification of individual dengue antigens is in progress. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*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 99 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE

Project 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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

Investigators:

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

Associates: SP5 Matthew Seguin
SP5 Lee Sosebee

Problems and Objectives

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

Progress

Dengue virus polypeptides intrinsically labelled with 35S and 14C were prepared from infected LLC-MK2 (monkey kidney) or C6/36 (*Aedes albopictus*) cells for use in radioimmune precipitation tests as described last year. The polypeptides were precipitated with anti-dengue antibodies (either polyclonal or monoclonal). The precipitated polypeptides were separated by polyacrylamide gel electrophoresis and identified using molecular weight standards and polypeptides from purified dengue virus.

An important difference was found between the reactivities of group-reactive and type-specific monoclonal antibodies with the polypeptides. Dengue complex-specific and flavivirus group-reactive monoclonal antibodies, which enhance dengue virus infection in vitro (1), radioimmune precipitate 8-10 virus-induced

polypeptides. This result was similar to that obtained by using acute sera from DHF patients. On the other hand, type-specific monoclonal antibodies react predominantly with the major envelope glycoprotein and related non-structural proteins. The evidence suggests that the dengue antigens which participate in the formation of immune complexes in DHF are shared in common between dengue serotypes and other members of the flavivirus group.

Recommendations

Polypeptides that combine with cross-reactive monoclonal antibodies should be studied by solid phase competitive radioimmune assay and peptic enzyme mapping to determine the precise epitopes involved in the DHF immune complexes. This work complements current extramural contracts on dengue genome mapping and the synthesis of viral antigens. Molecular studies of dengue viruses should lead to a more precise understanding of the pathogenesis of DHF.

Reference

1. Brandt, W.E., McCown, J.M., Gentry, M.K. and Russell, P.K. Infection Enhancement of Dengue Type 2 Virus in the U-937 Human Monocyte Line by Antibodies to Flavivirus Cross-Reactive Determinants. Infect. Immun. 36:1036-1041, 1982.

Presentation

1. Henchal, E.A., Scott, R.McN. and Brandt, W.E. Radioimmune Precipitation of Dengue Virus Proteins by Dengue Hemorrhagic Fever Patient Sera. 13th Annual Meeting American Society Tropical Medicine and Hygiene. San Juan P.R. 18 Nov 81

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DAOG 1295	2. DATE OF SUMMARY* 82 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 81 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8a. DISP'N INSTR'N NL	8b. SPECIFIC DATA: CONTRACTOR ACCESS ☒ YES ☐ NO

9. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	121
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Identification of Trypanosoma rhodesiense Protective Antigens

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 10 01	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	1.0	249
	CURRENT 83	1.0	250

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: Esser, Klaus

NAME:

21. GENERAL USE

Foreign intelligence considered.

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

23. (U) African sleeping sickness, a potential threat to military operations in Africa, has reached epidemic proportions in some areas. Currently no prophylaxis is available and the chemotherapeutic agents are toxic. The objective of the current work is to identify protective antigens of Trypanosoma rhodesiense. This work will be the basis for vaccine development.

24. (U) These studies employ an animal model to investigate immunity to both the infective insect form and the blood form of the parasite. To identify the antigen types involved in this immunity, monoclonal antibodies are prepared as markers for specific antigens. These reagents are used for the antigen type analysis of parasites obtained from the field and will also provide the means to isolate specific antigens.

25. (U) 81 10-82 09 Monoclonal antibodies were generated which neutralize T. rhodesiense metacyclic (insect form) infectivity and which identify discrete regions (epitopes) of metacyclic antigens relevant to protective immunity. Cloning of genes, coding for these antigens, is underway. Monoclonal antibody mapping of a single trypanosome antigen has identified eighteen epitopes, nine of which are relevant to protective immunity. Immunization of mice against trypanosome infection without the use of parasite material was accomplished by administration of anti-idotypic (anti-antibody) antibodies directed against trypanosome antigen-specific monoclonal antibodies. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

DD, FORM 1498 MAR 66 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 69 (FOR ARMY USE) ARE OBSOLETE

* U.S. GPO: 1974-540-943/8691

PROJECT 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 121 Identification of Trypanosoma rhodesiense protective antigens

Investigators:

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

Associates: Dr. Bruce T. Hall
CPT Donald E. Burgess, MSC
Mr. Maurice J. Schoenbechler
Mr. William L. Bowie
Sp4 Margaret Meadows

Problem and Objective:

African trypanosomiasis poses significant health hazards to troops operating in endemic areas. This is a progressive, generally fatal disease transmitted by the bite of infected tsetse flies. The fly vector and the causative protozoan parasite are prevalent throughout 30% of Africa. The current level of reported human disease is not high due primarily to restricted land use patterns and vector control measures in highly populated areas. However, the potential of this disease is evident from previous epidemics in which 20 - 30% of the population in some areas died. Although data on the risk of infection for military troops deployed in endemic areas is not available, a high incidence of infection would be expected. Currently no prophylaxis is available and therapeutic drugs are toxic. Vaccination against African trypanosomiasis is theoretically possible. Protection against infection with a single trypanosome antigen type can easily be achieved by immunization with attenuated parasites or purified antigens. However, multiple antigen types of the parasite are present in the fly vector and a large number arise by antigenic variation in the host. The objective of this work unit is the identification and isolation of the antigens which can elicit a protective immune response against the infective insect form of the parasite.

Progress:

Monoclonal antibodies have been generated which neutralize metacylic (insect form) infectivity. These antibodies identify discrete regions of metacylic antigens which are relevant to protective immunity. Expression of these relevant metacyclic antigens on blood form trypanosomes which occur early in the course of infection following metacylic inoculation of mice was demonstrated by monoclonal antibody analysis. Also, mice could be protected against metacyclic infection by immunization with these early blood form trypanosomes. These blood forms, in contrast to the metacylic forms, can be obtained in quantities sufficient for gene cloning. Gene clones which code for metacyclic antigens are currently being generated in collaboration with Dr. John Donelson at the University of Iowa. These cloned genes will provide a means to synthesize antigens for vaccine development.

In an effort to define the discrete regions (epitopes) of trypanosome antigens which are targets for an immune response, forty-five monoclonal antibodies reactive with a single trypanosome surface protein were generated and used to "map" this protein. Eighteen epitopes, at least seven of which are relevant to protective immunity were resolved. Defined synthetic antigens containing one or more relevant epitopes are likely to be the basis of future vaccines.

An alternative approach to traditional vaccination with antigens was tested using the trypanosome model. Mice were successfully immunized against trypanosome infection without the use of any parasite material by the administration of anti-idiotypic (anti-antibody) antibodies directed against trypanosome-specific monoclonal antibodies. These anti-idiotypic antibodies may stimulate a parasite specific antibody by interaction with antibody producing cells or by interaction with regulatory cells. This demonstration that manipulation of the immune system may provide an alternative to the use of specific target antigens for induction of antimicrobial immunity.

Studies have been done to determine if trypanosomes in a given endemic area are antigenically stable over time. Trypanosomes isolated from naturally infected humans in the Lambwe Valley, Kenya, were analyzed by use of a series of monoclonal antibodies and rabbit antisera defining 21 distinct

trypanosome antigen types. To date, no dramatic "antigenic drift" has been detected in trypanosome isolates obtained from 1972 to 1981. Antigenic analysis of metacyclic trypanosomes in tsetse flies infected with individual human trypanosome isolates is underway. This analysis will allow determination of the degree of metacylic antigen heterogeneity and stability. If metacylcic heterogeneity is restricted, as our current data suggests, then polyvalent vaccine development may be possible.

Recommendations:

In view of the findings that metacyclic heterogeneity appears to be restricted and that experimental immunization is possible, further work is indicated for the identification of antigens involved in eliciting a broad-spectrum immunity. Also, continued analysis of metacyclics from a range of different trypanosome isolates is necessary to determine the degree of metacyclic hetergeneity in a particular endemic area. Direct analysis of metacyclics present in tsetse flies in endemic areas will allow confirmation of laboratory findings. Monoclonal antibodies will continue to be the major tool for these studies. Refinement of serodiagnostic techniques is also needed to provide a clinically useful level of sensitivity and specificity. Studies on identification of discrete epitopes relevant to protective immunity should continue on an expanded scale. This will be relevant for trypanosomiasis vaccine development and also for establishing critical groundwork for production of synthetic vaccines is general.

Presentations:

1. Expression of metacyclic surface antigens on blood forms of Trypanosoma rhodesiense demonstrated by monoclonal antibodies. K. M. Esser, M. J. Schoenbechler and J. B. Gingrich. American Society of Tropical Medicine and Hygiene, 13th Annual Meeting, November, 1981.

2. Metacyclic-specific immunity produced by immunization of mice with Trypanosoma rhodesiense blood form trypanosomes. K.M. Esser, M. J. Schoenbechler and J. B. Gingrich. 5th International Congress of Parasitology, August, 1982.

3. Monoclonal antibody analysis of metacyclic populations of T. b. rhodesiense. K. M. Esser, M. J. Schoenbechler and J. E. Gingrich. Third WHO workshop on Immunology of African Trypanosomiasis, September, 1982.

4. Discrepancy between monoclonal antibody and conventional antiserum typing of trypanosome field isolates, and a possible molecular basis for the discrepancy. K. M. Esser, D. B. Burgess and B. T. Hall. Third WHO Workshop on Immunology of African Trypanosomiasis, September, 1982.

Publications:

1. Blood forms of Trypanosoma rhodesiense express all antigen specificities relevant to protection against metacyclic (insect form) challenge. K. Esser, M. Schoenbechler and J. Gingrich. J. Immunol. 129 (4): 1715-1718. 1982.

2. Properties of monoclonal antibodies capable of neutralizing Trypanosoma rhodesiense. K. Esser. Fed. Proceed. 41(3):583. 1982.

3. Immunization of mice against African Trypanosomiasis using anti-idiotypic antibodies D. Sacks, K. Esser and A. Sher. J. Exp. Med. 155:1108-1119. 1982.

4. Parasite (Antigen) specific stimulation of B and T cells in African Trypanosomiasis. G. Campbell, K. Esser and S. Phillips, J. Immunol. 129(3):1272-1274. 1982.

Patents:

Antibodies to connective tissue and basement membranes in Chagas' disease and African trypanosomiasis react with laminin. A. Szarfman, V. Terranova, K. Esser, and G. Martin. Application made May, 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6750	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. NIHD OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. HEDHADING	8a. DISB'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

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

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support 002600 Biology 012900 Physiology 003500 Clinical Medicine

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

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

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

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

21. GENERAL USE

Foreign intelligence considered

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

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

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

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

25.(U) 81 10-82 09 To develop an animal model for studying the protective effects of varying blood levels of B12a against CN^-, pharmacokinetic studies were carried out with female foxhounds. The distribution and excretion kinetics of CN-B12 and B12a were defined with computer modeling in these animals using single bolus, IV doses: 5, 10, and 25 mg/kg. These data were then applied to develop formulae for loading and maintenance B12a doses that would maintain B12a plasma levels in the animals constant at different discrete levels. The model is now being adapted to study of CN^- effects with and without B12a. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81-30 Sep 82.

*Available to contractors upon originator's approval.

Project 3A161101A91C: IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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

Investigators Mr. Harold Williams, MS (GS-13); MAJ Daniel G. Wright, MC; LTC Charles Pamplin, MC (Div. Exp. Therapeutics, WRAIR)

Description

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

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

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

Progress

We have completed research on the pharmacokinetics of blood clearance and kidney excretion of Vitamin $B_{12}a$ (Cbl-OH) and Vitamin B_{12} (Cbl-CN). Our studies of the pharmacokinetics of Cbl-OH and Cbl-CN in foxhounds indicate that these animals handle both forms of the vitamin in a fairly uniform manner from animal to animal. After bolus loading of the animals with 5, 10 and 25 mg/Kg of Cbl-OH and Cbl-CN, plasma levels attain a maximum content within one minute and thereafter decrease. Computer modeling indicates that the pharmacokinetics of both compounds after bolus infusion are best represented

by biphasic disappearance curves, with an initial distribution phase followed by an elimination phase. The distribution phase for both compounds is short with a $t_{1/2}$ of 0.038 hours for Cbl-OH and 0.047 hours for Cbl-CN. The $t_{1/2}$ of the elimination phase was 1.36 hours for Cbl-OH and 1.05 hours for Cbl-CN. The calculated volume of body distribution was slightly but not significantly greater for Cbl-OH (2.66L) than for Cbl-CN (2.37L), and the clearance of Cbl-OH is slightly less efficient (4.20 L/hr) than was that of Cbl-CN (4.92 L/hr).

We have found that clearance of Cbl-CN was almost entirely renal with essentially all the injected Cbl-CN being recovered in the urine by 4 hours. Cbl-OH excretion in urine begins almost immediately after IV injection; however, only about $1/2$ is recovered in urine after 4 hours. These data suggest that an alternate route of excretion occurs with Cbl-OH.

This pharmacokinetic information permits us to design loading and maintenance infusion doses of Cbl-OH in foxhounds that will result in steady-state plasma levels of this drug within the range of 1.0-20.0 µg/ml.

Sublethal doses of NaCN have been given to dogs in order to measure selected physiological responses to this drug. We have measured the effect that cyanide administration had on the dog's heartrate, blood pressure, EKG, and respiration. Low levels of IV cyanide administration cause a rise in blood pressure, tachycardia and hyperpnea. Continued administration of cyanide causes a drop in the blood pressure, acidosis, dyspnea and hypoxia. On the administration of near-lethal doses of cyanide, bradycardia occurs. Apnea is the final result when lethal doses are administered.

Blood cyanide levels follow a linear recovery pattern. Urinary recovery is not linear. This is perhaps due to the fact some of the cyanide has been converted to other metabolic forms.

Future Plans

1. To test schemes of loading and maintenance doses of $B_{12}a$ to confer stable plasma concentrations at different levels within a range of 1.0-20.0 µg/ml in foxhounds. To then evaluate the protective value of $B_{12}a$ loading against discrete measures of cyanide toxicity as described above.

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

Abstracts and Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 7012	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	123
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Test Systems for Specific Biological Effects of Chemicals

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 012600 Pharmacology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

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

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

Foreign Intelligence Considered

ASSOCIATE INVESTIGATORS

NAME: JACKSON, J.E.

NAME:

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

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

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

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

25. (U) 8110-8209 Testing in detail for hemolytic characteristics which might preclude consideration for further studies leading to clinical candidacy have been conducted on four compounds of interest. Results for test compounds are given relative to primaquine (WR 2975) in a radiorespirometric assay for determination of the μM/ml of test compound which results in a 50% hemolysis of normal human red blood cells (RBC). WR 225,448 is >120X more hemolytic than primaquine due to one or more nonoxidative lytic mechanisms. WR 242,511 is >14X more hemolytic than primaquine although drug-induced elevation of the hexose monophosphate shunt (HMS) activity (a measure of RBC oxidative stress) is only approximately 37% of that induced by primaquine. WR 238,605 is >45X more hemolytic than primaquine. No hemolysis is detectable for WR 250,016, a primaquine metabolite. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

PROJECT 3A161101A91C In-House Laboratory Independent Research

WORK UNIT 123 **Test Systems for Specific Biological Effects of Chemicals**

INVESTIGATORS:

Principal: Dr. Joan E. Jackson

PROBLEM AND OBJECTIVES:

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

OBJECTIVES:

The objective of current research is to investigate the use of a 3-hour, in vitro, radiorespirometric micro-test for rapid evaluation of hemolytic activity of potential antimalarials. The test is used to permit simultaneous quantitation of: a) oxidative; and b) nonoxidative drug-induced red cell damage resulting in hemolysis.

Oxidative drug-induced damage to human erythrocytes is determined using 2 quantitative assays:

1. **METABOLIC:** In the erythrocyte only the enzymes of the hexose monophosphate shunt (HMS) catabolize the C1 of glucose to liberate CO_2. Glucose-6-phosphate dehydrogenase (G-6-PD) catalyzes the initial reaction of the HMS and its activity, therefore, can be utilized as an approximate measure of oxidative stress in normal erythrocytes. During the micro-test, red cells are incubated with D-(1-14C) glucose. The activity of the HMS is measured (after a 30 min. incubation) by trapping $^{14}CO_2$ in $Ba(OH)_2$-soaked filter pads covering microtiter plate incubation wells in which red cells and test antimalarials have been placed.

2. **PROTEOLYTIC:** Oxidative stress of human erythrocytes results in liberation of free tyrosine from RBC protein components. This process can be followed by measurement of tyrosine fluorescence. The assay has previously been shown to be a sensitive test for detection of oxidative damage to red cell integrity. Therefore, drug exposure resulting in oxidative damage to RBC, which may or may not be followed by hemolysis, can be detected and quantified by this method.

To obtain an estimate of nonoxidative drug-induced hemolysis, the microtiter trays are scored after the 30 min. incubaton for percent erythrocyte hemolysis using an F.D.A. approved protocol. Visual judgment of percent hemolysis is estimated based on the appearance of the RBC pellet in the microtiter well. Antimalarials which result in: a) hemolysis, but not b) elevation of the HMS, or c) tyrosine liberation, are considered nonoxidative hemolytic compounds.

RESULTS:

Testing in detail for hemolytic characteristics which might preclude consideration for further studies leading to clinical candidacy have been conducted on four compounds of interest. Results for test compounds are given relative to primaquine (WR 2975) in an assay for determination of the μM/ml of test compound which results in a 50% hemolysis of normal human RBCs. WR 225448 is >120X more hemolytic than primaquine due to one or more nonoxidative lytic mechanisms. WR 242511 is >14X more hemolytic than primaquine although drug-induced elevation of the HMS activity (a measure of RBC oxidative stress) is only approximately 37% of that induced by primaquine. WR 238605 is >45X more hemolytic than primaquine. No hemolysis is detectable for WR 250016, a primaquine metabolite. The radiorespirometric microtest requires only 1/10 the man-hours and 1/8 the cost of supplies

of the standard Welt procedure, a method for measurement of drug enhanced HMS activity in RBC. Further, the micro-test is conducted in vitro, eliminating requirements for more expensive and lengthy in vivo preliminary drug screening tests.

During antimalarial screening, drugs and aqueous solutions are often membrane filter sterilized to prevent error due to bacterial contamination. When membrane filtered drug and aqueous solutions were compared to parallel sterile, unfiltered controls, the following results were obtained:

1. There is a chemical contaminant extractable from cellulose-ester membrane filters with water or aqueous saline. The contaminant has aromatic (benzenoid) uv spectral characteristics (absorbance max at 277 nm).

2. Filtration of drug solutions can result in 99% physical loss of drug from solution. The degree of loss is dependent upon both the filter type and drug filtered.

3. Antimalarial activity of aminoquinoline drugs is markedly reduced following filtration. The loss cannot be attributed entirely to physical drug loss. The drug dose inhibiting 50% of the uptake of labelled hypoxanthine by malarial parasites (ED_{50}) values, corrected for physical loss, are still from 1.0 to >10X greater than those obtained for nonfiltered drug solutions. Chemical contamination, detected and undetected, is suspected as causing drug activity loss.

4. Guidelines for using membrane filters in quantitative malaria drug sensitivity tests are set forth: Drugs should be membrane filter sterilized only at high drug concentrations to both minimize drug loss and the quantity of introduced chemical contamination.

FUTURE OBJECTIVES:

The current study of: a) adverse biological mechanisms of action of new antimalarials; and b) the relationship of hemolytic response to test drug concentration will be expanded. Continued work will determine: a) the range of hemolytic activity within a chemical class of compounds; b) the effect on hemolytic activity of variation of structure within a chemical class; and c) identification of populations at risk if genetically determined metabolic defects are responsible for the adverse effects. Data

obtained in these studies will be used to predict the relationship of chemical changes (addition or removal of side-chain groups) in aminoquinoline structure with reduction in hemolytic activity. This information should prove useful to future antimalarial drug design.

PRESENTATIONS:

1. Baird, J.K., Lambros, C., and Decker-Jackson, J.E. Reduction in aminoquinoline antimalarial activity following membrane filter sterilization. Fifth Int. Congr. Parasit. 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OG 7010	2. DATE OF SUMMARY* 82 10 01		REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 81 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8a. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61101A	3A161101A91C		00	124		
b. CONTRIBUTING							
c. CONTRIBUTING							

11. TITLE (Precede with Security Classification Code)*
(U) Development of specific cell directed antibody-toxin conjugates

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
010100 Microbiology 002600 Biology

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

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	2.0	75
	CURRENT 83	2.0	75

19. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: (202) 576-3759
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS NAME: Robert Seid, Ph.D.
NAME:
NAME:

21. GENERAL USE
Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Toxins; (U) Antibodies; (U) Monoclonal; (U) Cytotoxicity

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

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

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

25. (U) 81 10-82 09 A novel affinity purification technique for ricin with selective binding to N-acetylgalactosamine was developed. Ricin purified by this method had a mouse LD-50 of 5.3 nanograms. N-Bromacetylgalactosamine was synthesized as an analogue for covalent binding to ricin to block its binding sites for galactose. Hybridomas producing monoclonal antibodies specific for B-2 microglobulin were obtained and antibody was purified. Cell lines specifically containing B-2 microglobulin and cells lacking B-2 microglobulin were shown to be sensitive to intact ricin thus establishing a well defined model system. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

PROJECT 3A161101191C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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

Investigators:

Principal: Jerald C. Sadoff, M.D., LTC(P), MC
Associates: Robert Seid, Jr., Ph.D.,
Bennett Kaufman, Ph.D.
Susan Futrovsky

Problem

The general problem is to develop a technique for coupling monoclonal antibody to toxins such that these immunotoxins will efficiently kill only those cells against which the antibody is directed. The specific objectives are to modify certain binding (B) regions of the ricin molecule chemically so that antibody can be coupled to whole toxin. This approach is designed to overcome cell entry problems encountered when enzymatically active A fragments of toxin are coupled to antibody. The objective is to compare whole ricin containing modified B regions coupled to antibody with whole ricin coupled to antibody when used in the presence of lactose in a model system.

Progress

A new technique of affinity purification of ricin on N-acetyl galactosmine columns was developed resulting in ricin with an LD_{50} of 5 nanograms/mouse. This is 10 times more potent than any previously purified ricin. N-Bromoacetyl-galactosmine was synthesized and shown to have the correct composition and structure. This is active site directed compound which will be used to modify the B region of ricin. A model system for testing immunotoxin potency was developed. Monoclonal antibody against B-2 microglobulin, and cell lines lacking B-2 microglobulin, when grown in Methionine free medium demonstrated uptake of ^{35}S-methionine and were shown to be sensitive to ricin. The cells were not sensitive to the antibody alone.

When conditions for optimal cytotoxicity are established in the model system, attempts to kill trypanosomiasis *in vitro* and *in vivo* will be initiated. Mature T cells are responsible for graft vs. host reaction in bone marrow transplants. T cell monoclonal antibodies coupled to ricin will be tested for *in vitro* killing in a human system as a potential therapeutic for bone marrow transfer.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 7011	82 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
81 10 01	H. Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

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

11. TITLE (Precede with Security Classification Code)

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 81	2.0	9
FISCAL YEAR CURRENT 82	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

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

ADDRESS: Washington, D.C. 20012

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

NAME: Roberts, D.R., LTC

TELEPHONE: 202-576-3719

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Bailey, MAJ C.L.

NAME: Linthicum, K.J., CPT

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

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

24. (U) Mosquitoes will be collected in Kenya with a variety of collection methods. Mosquito eggs, larvae, pupae and adults will be collected for taxonomic rearings. The taxonomic series (from rearings) will be employed to detect morphological characters for species descriptions and for constructing identification keys. Adult mosquitoes will be collected and processed for virus isolation attempts. Ecological data will be recorded. Specimens will be collected for colonization and virus transmission attempts.

25. (U) 81-07-81-12 One collecting trip was made to Kenya. A total of 6,483 mosquitoes were collected in 197 trap nights between 23 October and 15 December 1981. From the total, 350 mosquitoes were pinned for taxonomic study and voucher specimens, and 6133 were processed for virus isolation in 379 different pools. No virus isolates were obtained. This work is being continued as an in-house project in Kenya. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sept 82.

*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 65 (FOR ARMY USE) ARE OBSOLETE

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

PROJECT 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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

Investigators
Principal: Donald R. Roberts, LTC, MSC
Associate: Charles L. Bailey, MAJ, MSC; Kenneth J. Linthicum, CPT, MSC

Problem and Objectives

Rift Valley Fever is an enzootic disease in much of the Ethiopian Faunal region and sometimes occurs in epidemic form in parts of the Middle East (a strategically important area of the world). The question as to how this virus is maintained in the enzootic setting has been an important concern of researchers since the virus was discovered and initiating research to try to answer this question was the primary reason for funding this project. A clear understanding of the enzootic cycle to include vector(s), reservoir(s) and ecological habitats of the virus would ultimately facilitate the control of this disease. The objectives for this work unit were to 1) collect taxonomic specimens of potential vector mosquitoes and 2) collect and process potential vector species for Rift Valley Fever Virus in Kenya.

Progress

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

Totals of 326 males, 960 females, 966 pupae and 1900 larvae were collected and returned to the medical entomology project at the Smithsonian Institute. These specimens have been identified and a reference collection has been compiled. A total of 6133 mosquitoes and 384 sand flies were collected and processed for virus isolation attempts. No isolates were obtained for this effort.

Recommendations for the Future

Findings from this work unit were very useful in gaining a grasp of the taxonomic problems of the potential vector species. The lack of success in isolating the virus merely emphasizes the need for a

long-term program of field studies. Although this work unit is terminated, the work that was initiated is being continued under an in-house research project.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | DA OG 9280 | 32 10 01 | DD-DR&E(AR)636

2. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. COMN SECURITY	7. NEGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	128 WWQ9
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 010100 Microbiology 002600 Biology

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

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 82	0.3	18
CURRENT FISCAL YEAR 83	0.3	20

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

20. PERFORMING ORGANIZATION
NAME: AFRIMS
ADDRESS: Bangkok, Thailand
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: BURKE, DONALD S., LTC, MC
TELEPHONE:
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
Foreign Intelligence Considered

POC: DA

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

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

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

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

25 (U) 81 10 - 82 09 In order to identify dengue antibody producing cells in human blood a sensitive test was developed that can be completed overnight. A human myeloma cell line was obtained and the optimum conditions for growth at various rates was determined. Fusions between the myeloma cells and the antibody producing cells are in progress and should provide immortal human cell hybrids that continue to secrete antibodies. For technical report see Walter Reed Army Institute of Research Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

DD FORM 1 MAR 86 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 88 AND 1498-1, 1 MAR 88 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 128: Regulation of the Human Immune Response to Dengue Virus Infection by Auto Anti-Idiotype Antibodies

PRINCIPAL INVESTIGATOR: Donald S. Burke, LTC, MC

1. Regulation of the Human Immune Response to Dengue Virus Infection by Auto Anti-Idiotypic Antibodies

OBJECTIVE: The overall objective of this project was to develop methods for regulation of the human immune response to dengue virus infection by selective use of human anti-idiotypic antibodies.

IMPROTANCE OF THE PROBLEM: Dengue is a historically proven infectious disease problem for U.S. troops in tropical theaters of combat; a major vaccine development program for dengue has been funded by the U.S. Army. The control mechanisms for regulation of the types and rates of antibody synthesis in dengue infections and other acute infections are unknown. Elucidation of these mechanisms could lead to physician-directed modulation of the immune response either to boost immunity during vaccination or to blunt the immune response during acute illness.

RELEVANCE TO THE CORE PROGRAM: A problem in the development of safe and effective live attenuated dengue vaccines is that vaccine immunogenicity is typically directly related to the reactogenicity (disease producting potential) of that strain. Thus the safest virus strains are not very immunogenic while those producing the disease confer solid immunity. A method which could selectively stimulate the immune response to vaccination, without increasing undesirable symptoms, would be a valuable practical step toward insuring troop combat effectiveness in tropical areas.

APPROACHES: (1) To screen human hybridomas for production of naturally occurring anti-idiotypic antibodies directed against idiotypic determinants on anti-dengue immunoglobulins. (2) To produce and purify these anti-idiotypic antibodies in quantity. (3) To purify from serum the corresponding set of anti-dengue antibodies bearing these idiotypic determinants. (4) To develop immunoassays for detection and quantitation of both the set of idiotype-bearing anti-dengue antibodies and the corresponding anti-idiotype antibodies. (5) To determine the kinetics of both the idiotype bearing anti-dengue antibodies and the anti-idiotype antibodies during natural dengue infections in humans. (6) To determine if exeqenously added autoloqous monoclonal anti-idiotype can regulate the production of idiotype bearing anti-bodies by in vitro cultures of peripheral blood monoclonal leukocytes from humans with acute dengue virus infection.

ACCOMPLISHMENTS: (1) A technique for rapid (18 hrs) detection and quantitation of in vitro synthesis of dengue specific IgM, IgG, and IgA by patient blood leukocytes was developed. The technique involves culturing leukocytes in vessels pre-coated with isotype specific anti-sera. (2) Continuous human B cell lines were obtained and their growth kinetics established. (3) Fusions of IgG anti-dengue producing peripheral blood mononuclear leukocytes with human lymphoblastoid cells were attempted but thus far without production of stable hybrids. (4) Evidence was obtained by rate zonal centrifugation of sera from acute flavivirus infected patients for the regular occurrence circulating complexes of IgM and rheumatoid factor IgA anti-IgM.

INTERPRETATION OF ACCOMPLISHMENTS: This project was knowingly submitted as an ambitious, speculative, "high risk for high yield" proposal, requiring large measures of effort and innovation for success. The accomplish-ments to date are modest when compared to the objectives, but are not at all modest when compared to resources expended.

INTENTIONS FOR THE FUTURE OF THE PROJECT: This project is important and feasible. It should continue on ILIR funding for at least one more year.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 00 0281	2. DATE OF SUMMARY* 82 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 81 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8. DISS'N INSTN'N U	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	129
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Protection of Gonadal Function from Cytotoxic Therapy

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology 012900 Physiology 012600 Pharmacology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	1.1	39
FISCAL YEAR CURRENT 83	1.1	25

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Walter Reed Army Institute of Research

ADDRESS:* Washington, DC 20012

RESPONSIBLE INDIVIDUAL

NAME: RUSSELL, Philip K., COL, MC

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

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

ADDRESS:* Washington, DC 20012

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

NAME:* CROSBY, William H. COL, MC

TELEPHONE: (202) 576-3305

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: CUTTING, Mary A.

NAME:

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

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

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

25. (U) 81 10 - 82 09. In pilot work, levels of radiation or cytotoxic chemical exposure which cause significant gonadal damage without mortality have been determined for the mouse model used in these studies. An experiment involving treatment with hormone injections prior to irradiation has been completed. Initial results suggest that hormones do not protect gonads at the level of radiation used. Some tissue from this study is still being processed by histological laboratory; thus, results are incomplete. An experiment investigating possibility of protection against cytotoxic drug is in progress. Serum hormone assays for all experiments remain to be done. The commercial laboratory to whom the contract was assigned was unable to do the assays due to technical problems. (Mouse hormone assays are uncommon and technically difficult). Another laboratory has indicated that it is capable of performing the assays. For technical report see Walter Reed Army Institute of Research Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

Project 3A161101A91C: IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Work Unit 129 Protection of Gonadal Junction from Cytotoxic Therapy

Investigators LTC Ramona Chapman, MC; COL William H. Crosby, MC
Mary Cutting, MS

Description

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

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

Progress

The initial results of studies in radiation-treated mice suggest that hormone treatments at the doses used did not protect the gonads from damage. Experiments with cyclophosphamide-treated mice are in progress at this time. In addition to observing the histological changes due to various treatments, we have collected serum from mice in all experimental groups in order to measure serum levels of follicle-stimulating hormone and luteinizing hormone. This information may help to explain either protective effects or lack of effects of our hormone treatments.

Future Plans

Continuation of this ILIR work unit will include completion of tissue analyses and serum hormone assays from the radiation and cyclophosphamide studies. In addition, long-term experiments using both histological analyses and fertility studies will be completed to investigate the possibility of recovery of gonadal function after injury.

The general concepts and models used in these studies will then be applied to the study of hematopoietic hormones and regulatory mediators and their efficacy in protecting hematopoietic stem cells against cytotoxic influences (work to be continued in Work Unit 228, DA Accession No. DAOG6761).

Publications

1. Chapman, R.M. Doctors and dying (Editorial) JAMA 247:2569-2570, 1982.

2. Chapman, R.M. Emotional care of the cancer patient. In Current Therapy in Hematology/Oncology. Ed: Michael Brain, 1982 (in press).

3. Chapman, R.M. Effect of cytotoxic therapy on sexualty and gonadal function. Seminars in Oncology 9:84-94, 1982.

4. Chapman, R.M., Crosby, W.H. Hodgkin's disease and the pregnant patient. Ann. Intern. Med. 96:681-682, 1982.

5. Sutcliffe, S.B., Chapman, R.M. , Crosby, W.H. Reproductive potential after treatment for Hodgkin's disease (Hr). N. Eng. J. Med. 305:891-892 and 1359, 1981.

6. Sutcliffe, S.B., Chapman, R.M. Pregnancy in lymphomas and leukemias, in Pregnancy and Cancer, 1982 (in press).

7. Vigersky, R.A., Chapman, R.M., Berenberg, J., Glass, A. Testicular dysfunction in untreated Hodgkin's disease. Am. J. Med., 1982 (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA 300028	2. DATE OF SUMMARY: 82 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636

3. DATE PREV SUM'RY: 81 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8A. DISP'N INSTR'N: NL	8B. SPECIFIC DATA - CONTRACTOR ACCESS: ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	130 WWH7
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	A. PROFESSIONAL MAN YRS	B. FUNDS (In thousands)
FISCAL YEAR PRECEDING: 82	0.5	7
FISCAL YEAR CURRENT: 83	0.5	15

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL MC

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20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research
Division of Biochemistry

ADDRESS: Washington, D.C. 20012

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

NAME: Brown, James E., CPT

TELEPHONE: (202) 576-4235

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Gemski, Peter POC: DA

NAME:

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

23. (U) The technical objective of this work unit is to construct and test "immunotoxins" consisting of enzymatic subunits from toxin proteins covalently conjugated to monoclonal antibodies specific for cell surface antigens of parasitic organisms, such as Trypanosoma rhodesiense. The goal would be eventually to provide reagents for adjunctive therapy of parasitic infections constituting a threat to military troops
24. (U) Currently available monoclonal antibodies and toxins will be utilized for experiments. The toxic plant protein ricin will be used. Monoclonal IgG antibody specific for parasitic cell surface antigens will be derivatized with N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) to provide covalent linkage of the antibody to the toxin. The resulting bond is a disulfide linkage which can be easily cleaved intracellularly after uptake of the conjugate. The hybrid protein will be purified by HPLC gel permeation techniques. The resulting purified "immunotoxins" will be available for testing of efficacy and specificity in parasite cell culture. It is expected that numerous preparations will have to be tested since not all cell surface antigens would facilitate receptor-mediated endocytosis of a bound ligand.
25. (U) 81 10 - 82 09 Various experimental conditions were investigated to obtain optimum synthesis of SPDP-immunoglobulin G and SPDP-toxin. These intial experiments were performed using IgG isolated from normal mouse serum. A comparison was made of the utility of using subunit A of ricin versus using the intact toxin protein. It was decided to use the intact toxin. A conjugate species IgG-S-S-Ricin product was examined for molecular weight, subunit composition, degree of cross-linking and for retention of cytotoxicity by the ricin substituent. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

* Available to contractors upon originator's approval

PROJECT: 3A161101A91C IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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

INVESTIGATORS:
Principal: CPT James E. Brown, Ph.D., MSC
Associate: Peter Gemski, Ph.D.

IN COLLABORATION WITH: Klaus M. Esser (CDI)

PROBLEM:

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

PROGRESS:

Preliminary work has been directed toward optimization of reaction conditions to allow maximum recovery of an immunoglobulin G - ricin hybrid cross-linked using N-succinimidyl-3-(2-pyridyldithio) propionate. Because mouse monoclonal antibodies are available in limited quantities, this preliminary work to establish experimental conditions was performed using IgG, isolated by Protein A-Sepharose chromatography from unimmunized mouse serum.

The initial synthetic strategy was as follows: (a) react IgG with SPDP to form 2-pyridyldisulfide-labelled IgG (2-PD-IgG); (b) reduce the single disulfide linkage of native ricin; (c) mix these components to allow displacement of pyridine-2-thione by free sulfhydryl group of ricin, thereby forming a convalent linkage between the two proteins. The first stage of this strategy involved quantitation of the degree of derivatization of IgG at various molar excess amounts of SPDP. As shown in Fig 1, multiple 2-pyridyldisulfide groups may be easily introduced into mouse IgG. A limit to the degree of derivatization was not achieved.

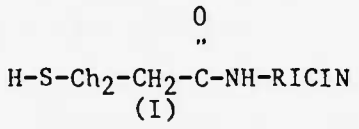
Fig 1. FORMATION OF 2-PYRIDYLDISULFIDE - IgG

Moles SPDP used/mole IgG	Moles residue combined/mole IgG
5.0	1.2; 4.4
15.5	3.3
78.0	6.9
390.0	17.0

The degree of derivatization is measured as the increase in absorbance at 343 nm after the addition of excess dithiothreitol due to the release of pyridine-2-thione. Attempts to link the 2-PD-IgG to dithiothreitol treated

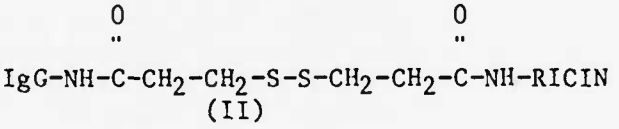
ricin proved unsuccessful. Apparently reformation of the native disulfide linkage is extremely rapid after removal of excess dithiothreitol probably from the close steric proximity in the native protein.

A revised strategy to achieve conjugate synthesis was devised as follows: (a) form 2 pyridyldisulfide IgG; (b) form 2 pyridyldisulfide ricin; (c) test the derivatized ricin with excess ricin to release pyridine-2-thione, thereby forming a reactive species (I); (d) mixture of 2-PD-IgG with the ricin derivation



(I)

(I) to allow formation of a covalent linkage (II), by displacement of pyridine -2-thione from the IgG component. The degree of derivatization



(II)

of native ricin was quantitated at various molar excess amounts of SPDP. As shown in Fig 2, 2 pyridyldisulfide groups can be incorporated into ricin and the degree incorporation is apparently limited to 3-4 residues per ricin molecule. Cytotoxicity of 2 pyridyldisulfide-labelled ricin is approximately equal to that of the native protein.

Two sets of experiments were performed to attempt

Fig 2. . FORMATION OF 2-PYRIDYLDISULFIDE-RICIN

Moles SPDP used/mole ricin	Moles residue combined/mole ricin
2	0.Y6, 2.1, 3.0
5	1.1, 0.9, 4.4, 1.0, 0.4
10	2.0, 2.4, 3.5

formation of the hybrid protein (II). In the first, IgG had 1.2 2-PD-groups per molecule and ricin had 0.41 group per molecule. The degree of conjugate formation was measured by the release of pyridine-2-thione after mixture of the two components. When mixture at a molar ratio of 2.2:1 (ricin: IgG), 76% of the IgG groups were covalently linked to ricin. At a molar ratio of 5.1:1, over 100% reaction was achieved. In the second series of experiments, 2-pyridyldisulfide incorporation was 4.4 groups/molecule with IgG and 1.0 groups/molecule into ricin. Conjugate formation was attempted at three different molar ratios of ricin to IgG: 1.6, 3.3 and 6.6. As estimated by release of pyridine-2-thione, 100% reaction was achieved in all three cases. The formation of conjugate protein was confirmed by application of the reaction mixture to a protein A Sepharose column. Elution with 0.1M glycine-HCl, pH 2.8 buffer was

carried out to recover IgG and IgG derivatives. This eluant was toxic in the HeLa cell microtiter cytotoxicity assay, indicating presence of ricin bound to IgG.

Analysis of the structure of the conjugate species was begun using SDS-polyacrylamide gel electrophoresis and gradient pore polyacylamide gel electophoresis. Initials observations indicate a degree of size heterogeneity in the products formed.

To obtain a reaction product containing approximately one ricin molecule per immunoglobulin molecule a rapid fractionation procedure employing gel permeation HPLC was developed. The system uses a BioRad TSK-400 column joined in series with a Waters Associates I-125 column. Elution at 1 ml/min with 0.1 M sodium phosphate pH 7.0 allows fractionation of components in 30 minutes in the molecules weight range from 10,000-400,000. Thus monomeric ricin-IgG can be separated from higher degree multimeric species. Separation of ricin-IgG from any residual unreacted IgG can be accomplished from applying the sample to a Sepharose 4B column (2 x 5 cm). After washing, bound ricin-IgG hybrids are eluted with 0.1M lactose.

Current studies are using a monoclonal antibody against Trypanosoma rhodesiense, clone WRATaT 1, obtained from Dr. Klaus Esser, Department of Immunology, DCD&I. This IgG antibody, designated 16.3 F1.4, binds to cell surface antigens in the flagellar pocket area, but is a protective antibody when tested in vivo. Hybrids constructed with this antibody can be tested both for capability to bind to the trypanosome and for an in vivo protective effect.

PROJECT 3M161102BS10

RESEARCH ON MILITARY DISEASE INJURY AND HEALTH HAZARDS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6441	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AA	201 WWGA
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7 2:2			

11. TITLE (Precede with Security Classification Code)

(U) Viral Infections of Man

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology 003500 Clinical Medicine

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 82	4.0	351
FISCAL YEAR CURRENT 83	3.0	430

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Virology; (U) Immunology; (U) Arbovirus Infections; (U) Adenovirus Respiratory Diseases; (U) Influenza; (U) Human Volunteer

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

23 (U) To define etiology of acute infectious diseases of special hazard to military personnel, to determine and evaluate factors influencing the occurrence, distribution, severity and medical result of human virus infections, and to develop means for reducing disability due to virus diseases.

24 (U) Contemporary virological and immunological methods are applied to disease problems occurring in troops or in susceptible civilian populations in strategically important areas. New conceptual approaches and methods are developed as needed.

25 (U) 81 10-82 09. A live attenuated dengue-4 vaccine (H-241/PDK-35) was administered to 5 yellow fever immune volunteers in the first human trial of safety and immunogenicity. Only two individuals were infected; both developed viremia in 6 days, mild symptoms, and neutralizing antibody titers greater than 1:250 in 60 days. One year follow up sera were collected from participants in a dengue-2 vaccine (PR-159/S-1) trial at Fort Bragg. In a study of the utility of a C6/36 mosquito cell line for the production of vaccines, a sham vaccine was administered to 12 volunteers. Three people had immediate allergic reactions and five had delayed reactions which probably were IgE mediated. Therefore, the C6/36 cells are not acceptable for the final production steps of vaccines for humans. The IgM immune response to dengue vaccines was greater in recipients without flavivirus (yellow fever) antibody than in yellow fever immunes; however, the latter group has a higher and more prolonged IgG antibody response. The phenomenon of immune enhancement of dengue infections by cross reactive monoclonal antibodies is both virus serotype and antibody dependent and undoubtedly is a major factor in dengue vaccine responses. Immune enhancing antibody was also used to assist primary isolation of flaviviruses. Acute respiratory disease (ARD) rates on basic training remained acceptably low. Surveillance of ARD was improved by utilizing direct data input by individual posts into the WRAIR computer. Electroblot electrophoresis showed that influenza patients develop matrix antibody, which is not found in vaccinees. For technical report, see Walter Reed Army Institute of Research Annual Report, 1 Oct 81 - 30 Sep 82.

* Available to contractors upon originator's approval.

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

Work Unit 201 Viral Infections of Man

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Problems and Objectives

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. Basic research on dengue viruses is directed toward evaluation of the genetic lesions causing attenuation, the enhancement of virus replication by antibody, and their reactivity with type-specific monoclonal antibodies. Human volunteer studies are conducted to evaluate dengue vaccine safety and immunogenicity. Research in respiratory viruses is directed toward the identification of viruses causing acute respiratory disease (ARD), evaluation of ARD incidence rates on basic training posts and the imunogenicity of influenza viral proteins.

Progress

1. Antibody-Mediated Virus Infection Enhancement

The mechanism of infection enhancement of dengue virus replication was shown previously to be due to an 7S (IgG) antibody bridge between the virus and an antibody receptor on the cell surface. Studies with monoclonal antibodies developed at WRAIR

showed that only antibodies directed against cross-reactive hemagglutinating and cross-reactive neutralization determinants on the virus could also form infectious virus-antibody complexes that would infect target cells via Fc receptors. Monoclonal antibodies directed against serotype-specific neutralization determinants or serotype-specific hemagglutination determinants did not enhance virus infection.

Further examination of the phenomenon of antibody-mediated virus infection enhancement (AVIE) has led to the following generalizations:

1. The antibody must cross-react by hemagglutimation inhibition (HI), plaque reduction neutralization test (PRNT) or immunoflorescence (FA) on infected cells to two or more dengue serotypes.
2. All cross-reactive antibodies do not enhance dengue infection.
3. Antibodies that enhance some strains of dengue may not enhance those of another serotype.
4. AVIE is most easily demonstrated with dengue-2 virus.
5. Enhancing antibodies exhibit low cross-reactive neutralizing (N) antibody titers and high cross-reactive HI titers. Antibodies with high cross reactive N titers (>1280) failed to produce AVIE.

2. IgM Antibody-Mediated Virus Infection Enhancement in Cerebrosinal Fluids from Japanese Encephalitis Patients.

Serial spinal fluids and serum specimens from fatal and nonfatal cases of Japanese encephalitis (JE), as well as from Thai cases of non-JE encephalitis were obtained from LTC Donald Burke (Virology Department, AFRIMS). LTC Burke discovered that detectable quantities of JE specific IGM could be found in these specimens taken on the first day of hospitalization, and that rapid and final laboratory identification ofJE as the causative agent of disease could be accomplished by detecting this IgM in an antibody capture enzyme linked immunoabsorbant assay.

We found that JE PRNT tests performed with first.day serum and spinal fluid resulted in approximately 10-fold increases in the number of JE viral plaques (Nakayama strain) on LLC-MK$_2$ monolayers. No viral plaques were observed in serum or spinal fluid alone. Thus, enhancement of the number of plaques suggests that there is additional infectious virus in our seed stock which is not detectable unless it is first complexed to something in spinal fluid or serum (probably non-neutralizing antibody) which in turn attaches to the cell membrane and facilitates virus entry

into the cell. When sera collected 7 to 30 days later were tested, the infection enhancement properties of serum was gone and, in some cases, neutralization of virus was observed. However, the infection enhancing properties of the spinal fluid continued to increase for 7 to 30 days after hospital admission. Infection enhancement titers (the highest dilution of spinal fluid that would increase the viral plaque dose on LLC-MK_2 monolayers) rose to as high as 1/10,240. By 200 days after hospital admission, the sera had low titers of N antibody (1/20 - 1/40); however, there was no neutralization activity in the 200 day spinal fluids and 4 out of 5 still had some infection enhancement activity (1/20 to 1/40). JE infection enhancement was not produced by spinal fluids from people with asymptomatic JE infections, nor by samples from cases of non-JE encephalitis.

The rise of infection enhancing activity in the spinal fluids from JE cases appeared to correlate with the amount of IgM, not IgG, found in the antibody capture ELISA at AFRIMS. We centrifuged spinal fluids through sucrose density gradients and found that the infection enhancement activity was in the 19S region of the gradient, correlating exactly with the presence of JE-specific IgM. In addition, we reacted spinal fluid-virus complexes with antihuman IgM or antihuman IgG. Both antisera blocked the infection enhancement phenomenon in the first experiment using one dilution of spinal fluid, indicating that both IgM and IgG were attached to the virions.

We also investigated the nature of antibody receptors on LLC-MK_2 monkey kidney cell membranes in vitro. We obtained hemolysin (rabbit anti-sheep red blood cell antibody) and showed that the largest proportion of antibodies was of the 19S variety. We also obtained rabbit 7S anti-sheep RBC antibody and confirmed that the preparation had only 7S activity to the sheep RBC. Only the hemolysin-coated RBCs attached to the LLC-MK_2 cells, although both hemolysin and 7S coated sheep RBCs bound in equal numbers to P388D1 mouse macrophage cells. Thus, LLC-MK_2 cells have 19S but not 7S receptors which helps to explain the JE infection enhancement phenomenon produced by cerebrospinal fluids.

3. Dengue Type 4 Vaccine Trials

An initial human trial was performed on a dengue type 4 vaccine candidate (H-241,PDK-35,TD-3,FRhL-3) developed by Dr. Scott B. Halstead, University of Hawaii. The study was carried out in an isolation ward located at USAMRIID Ft. Detrick. Five male volunteers, negative for N antibodies against dengue viruses 1,2,3 and 4 but previously immunized with 17D yellow fever

vaccine, were inoculated subcutaneously with the live, attenuated, candidate vaccine. Two vaccine recipients (40%) developed viremia 5 and 6 days following immunization, which lasted five days in each case. The onset of viremia was followed by the development of headache, myalgia, fatigue, rash and leukopenia (<3000 white blood cells/mm^3). Transient, mild evaluations were observed of serum enzymes (AST, 134-232 [normal = 40]; ALT, 90 [normal = 40] and LDH, 204-405 [normal = 186]. CPK elevations were also seen during the symptomatic period in both viremic volunteers and, while they were mild in one recipient (256 vs normal of 225), the other recipient had levels greater than 1500 for at least 3 days. None of the volunteers developed a vaccine related fever. The symptoms and laboratory values were consistent with a mild dengue infection having associated myositis and transient hepatic dysfunction. Virus was isolated by a direct plaquing technique from nine of the ten viremic sera collected from the two infected volunteers. The virus titers ranged from <1 to 105 PFUs/ml and were considerably higher than the viremias that followed immunization with the dengue type 2 vaccine (PR-159/S-1). Also, although the isolated virus retained the temperature sensitive characteristics of the vaccine virus, the plaque morphology changed. Plaque sizes ranged from very small to medium size. Only the two viremic vaccine recipients developed antibodies to the dengue type 4 virus, both to the parent and the vaccine strains. Antibody titers were relatively high with 60 day dengue-4 HAI titers >5120 and N titers >250 for both volunteers. Studies performed by Dr. Frank Ennis, University of Massachusetts, to determine the effect of the dengue-4 immunization on natural killer cells, indicated increases in activity in four of the five vaccine recipients. However, for two of these, this was the only indication of any vaccine effect. This study indicated the vaccine virus has a degree of genetic instability. More importantly, the immunogenicity was poor, (only 40% of recipients developed N antibody) and the reactogenicity was high.

4. Follow up of Dengue-2 Vaccine Study at Fort Bragg.

During August and September 1982, followup serum samples were collected from recipients of the dengue type 2 (PR-159/S-1) vaccine approximately 18 months after immunization. For 18 infected YFI recipients the geometric mean titers at 6 and 18 months after immunization were 165 and 111, respectively. For three infected YFN recipients, the range of titers at 6 and 18 months was 10-40 and <10 to 30, respectively. The duration of N antibody following DEN-2 immunization is satisfactory.

5. Immune Responses to Dengue Immunization

Rapid waning of antibody, developed following dengue type 2 virus immunization, has been noted in both monkey and human recipients. This phenomenon is often seen in vaccine recipients who had no prior flavivirus antibody and who were not viremic. In collaboration with LTC Donald Burke, AFRIMS, these findings were investigated. Sequential sera, selected from four yellow fever immune (YFI) and eight yellow fever nonimmune (YFN) vaccine recipients were subjected to serotype-specific IgM and IgG antibody capture radioimmune assays. All YFI candidates had prompt N antibody responses to immunization which were maintained at titers greater than 20 for at least three years. The YFN subjects were divided into two groups, three people who showed no N antibody response and five who had a transient response which was no longer detectable by six months after immunization. Initial studies indicated no specific IgM development in the three subjects who did not develop detectable dengue-2 N antibody. IgM was found only in the recipients who seroconverted. In the YFI group, most members showed relatively low P/N ratios of IgM which declined by six months after inoculation. In the YFN group who seroconverted, all subjects showed high P/N ratios for IgM at 30 days after the injection. Here again the IgM antibody had waned by 60 days, although the P/N ratios had fallen as low as those seen for the YFIs. Sera from six volunteers, four YFNs and two YFIs, who were positive for dengue-2 specific IgM antibodies by capture IgM RIA were fractionated by sucrose gradient ultracentrifugation and the resulting fractions were tested for dengue-2 specific IgM and IgG using the capture antibody technique. This showed that essentially all of the RIA and N antibody activity of the YFN responders was 19S RIA and N while that of the YFI group was both 19S and 7S.

6. Evaluation of Aedes albopictus(C6/36) cells as a Vaccine Substrate.

A sham vaccine, prepared using a cell line derived from larval Aedes albopictus, was skin tested on twelve volunteers. While no reactions were observed following prick tests, three immediate reactions did follow intradermal tests. Subcutaneous administration of the C6/36 sham vaccine was initially performed on nine subjects who did not show immediate reactions. Five of these subjects developed delayed reactions at the site of the intradermal test within twenty-four hours after administration of the sham vaccine. A Prausnitz-Kustner (PK) test was performed on the back of one subject using both heated and unheated serum obtained from seven subjects including the three who had immediate

reactions, three who showed a delayed reaction and one who had no response. The PK test led to the the determination that skin reactivity was associated with a heat-labile serum factor probably IgE the C6/36 sham vaccine was deliberately administered subcutaneously to one volunteer who had previously shown an immediate response, he developed an anaphylactic reaction characterized by hives at the site of the subcutaneous inoculation and more distantly. This study demonstrated that the C6/36 cell line is unacceptable as a final allergen substrate unless the skin for vaccine production is identified and removed.

7. Acute Respiratory Disease Surveillance.

In order to increase the efficiency and utilization of data collected in acute respiratory disease on basic training posts, a computerized data processing system was developed with the help of the Division of Biometrics. This program is divided into two parts, ARD rates and etiologies. Information on ARD rates is entered into the WRAIR computer from remote terminals in the Preventive Medicine Offices at the basic training posts. For each post specific ARD rates are calculated by week of training, sex of trainees and training unit. In addition, information on adenovirus and influenza virus vaccine usage are recorded by week. Col Creed Smith at the Letterman Army Medical Center enters data on virus isolations and serological conversions for a subset of hospitalized recruits by post. The program immediately provides cummulative reports of ARD rates and diagnosed etiologies for use by the entering Preventive Medicine Officers, the diagnostic laboratory or the monitoring agencies. In addition, graphic displays are available of weekly, mean monthly or median monthly ARD rates for individual posts or an average of two or more posts. Current ARD rates can be compared to previous years to allow study of overall disease trends.

The computerized surveillance system has allowed, for the first time, "real-time" identification of ARD outbreaks early enough for the implementation of better efforts to define the etiology and/or control the outbreak. Specifically, the system allowed for the identification of unusual increases in hospital admissions for ARD at Fts Bliss, Dix, McClellan and Wood. In the case of Fts Dix and Wood, simultaneous etiological data was being compiled. This identified multiple etiologies, including influenza A, adenovirus type 21, mycoplasma and parainfluenza virus type 2, for the outbreak at Ft Wood in February and March. Adenovirus Type 4 was identified as the most prevalent organism isolated at Ft Dix during a prolonged summer outbreak of ARD. At Ft Dix, saturation immunization against adenoviruses type 4 and 7

was implemented to prevent further rises in ARD rates, seen but not recognized early enough, in previous years. For Fts McClellen and Sill, samples for etiological diagnosis were collected which revealed parainfluenza type 3 and adenovirus type 21 as the most prominent organisms on the two posts, respectively.

In a comparison of the antibody responses to influenza infection and influenza immunization, paired human sera were kindly rovided by Dr. Gordon Meiklejohn, University of Colorado. Representative influenza A viruses (H_1N_1 and H_3N_2) were treated to dissociated the viral proteins by discontinuous polyacrylamide gel electrophoresis. After transferring the proteins to nitrocellulose paper by the Western Blot technique, the protein coated paper strips were incubated with patient sera and antibody which combined was determined autoradiographically. This procedure proved to be highly sensitive for the detection of antibody to matrix protein, but is not useful for hemagglutinin antibodies. The procedure also can be applied to studies of dengue virus infections.

Recommendation:

Continued testing of dengue vaccines are required to define the immunogenicity and safety in recipients who already have dengue antibody. Polyvalent immunization of people should be initiated when at least two vaccines with acceptable levels of infectivity and reactogenicity are available. When the utility and reliability of the ARD surveillance system is adequately established, responsibility for management should be transferred from the research area.

Presentations

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2. Bancroft, W.H. Arbovirus Reagents. WHO Review of Rapid Laboratory Viral Diagnosis Stockholm, Sweden 16-18 1982.

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1. Scott,R.McN., Bancroft, W.H., Hoke, C.H., Jr., Eckels, K.H., Simms, T.E., Jesrani, K.D.T., Summers, P.L. and Russell, P.K. Effect of Previous Yellow Fever Immunization on Clinical and Serological Responses to a Dengue Type 2 (DEN-2) Vaccine . 30th Annual Meeting the American Society of Tropical Medicine and Hygeine. San Juan, P.R. 16-20 Nov 81.

2. Bancroft, W.H., Scott, R.McN., Eckels, K.H., Hoke, C.H., Simms, T.E., Jesrani, K.D.T., Summers, P.L. and Russell, P.K. Safety and Immunogenicity of a Dengue-2 (DEN-2) Virus Vaccine. 21st Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, Ill. 4-6 Nov 1981

3. Gentry, M.K., Henchal, E.A., McCown, J.M. and Brandt, W.E. Development of Monoclonal Antibodies for Identification of Dengue Virus Serotypes. 13th Annual Meeting of the American Society of Tropical Medicine & Hygiene. San Juan Puerto Rico, 18 Nov 1981.

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1. O'Brien, A.W., Binn, L.N., Hall, R.H., Beattie, R.J. and Marchwicki, R.H. Measles Virus Antibodies in a Laboratory Colony of Owl Monkeys (Aotus trivirgatus). Lab. Animals 15:343-345, 1981.

2. Binn, L.N., Marchwicki, R.H. and Eckermann, E.H. Viral Antibody Studies of Laboratory Dogs with Diarrheal Disease. Amer. J. Vet. Res. 42:1665-1667, 1981.

3. Watts, D.M., Harrison, B.A., Niasalak, A., Scott, R. McN. and Burke, D.S. Evaluation of Toxorhynchites splendens (Diptera: Culicidae) as a Bioassay Host for Dengue Viruses. J. Med. Entomol. 19:54-59, 1982.

4. Gentry, M.K., Henchal, E.A., McCown, J.M., Brandt, W.E. and Dalrymple, J.M. Identification of Distinct Antigenic Determinants on Dengue-2 Virus using Monoclonal Antibodies. Amer. J. Trop. Med. Hyg. 31:548-555, 1982.

5. Brandt, W.E., McCown, J.M., Gentry, M.K. and Russell, P.K. Infection Enhancement of Dengue Type 2 Virus in the U-937 Human Monocyte Cell Line by Antibodies to Flavivirus Cross-Reactive Determinants. Infect. Immun, 36:1036-1041, 1982.

6. Henchal, E.A., Gentry, M.K., McCown, J.M. and Brandt, W.E. Dengue Virus-Specific and Flavivirus Group Determinants Identified with Monoclonal Antibodies by Indirect Immunofluorescence. Amer. J. Trop, Med. Hyg. 31:830-836, 1982.

7. Henchal, E.A., McCown, J.M., Seguin, M.C., Gentry, M.K. and Brandt, W.E. Rapid Identification of Dengue Virus Isolates Using Monoclonal Antibodies in an Indirect Immunofluorescent Assay. Amer. J. Trop. Med. Hyg. (in press).

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10. Tingpalapong, M., Whitmire, R.E., Watts, D.M., Burke, D.S., Binn, L.N., Tesaprateep, T., Laungtongkum, S. and Marchwicki, R.H. An Epizootic of Viral Enteritis in Dogs in Thailand. Amer. J. Vet. Res. 43:1687-1690, 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6513	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. RIRD OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'R INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AA	202 WWGB
b. CONTRIBUTING				
c. xxxxxxxx	STOC 80-7,2:2			

11. TITLE (Precede with Security Classification Code)

(U) Mechanisms of Transmission of Hepatitis Viruses

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 82	3.5	225
CURRENT FISCAL YEAR 83	3.0	277

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21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

23 (U) To define the epidemiology of hepatitis in military populations in order to establish methods for reducing disability from hepatitis. Emphasis is on developing and applying sensitive and specific assays for hepatitis viruses, antigens and antibodies and to determine factors important in resistance to disease.

24 (U) New methods for the isolation, identification and comparison of hepatitis viruses are under development. The immune response of patients with viral hepatitis is studied to define sensitive measures of infection and the critical factors relating to immunoprophylaxis. The epidemiology of hepatitis in military populations is described.

25 (U) 81 10-82 09. The cell culture host range of hepatitis A Virus (HAV) is restricted to certain primate species with African Green monkey kidney cells giving the highest titers of released virus. Although no cytopathic effect was observed in HAV infected cells, quantification of propagated virus is possible by either radioimmune assay directly in cell culture tubes or using a new radioimmunofocus assay. Both assays can be modified to detect and titrate HAV neutralizing antibody in vitro. Experimental infection of Aotus monkeys with two different strains of HAV have confirmed that this primate species is an excellent model of human hepatitis A. People infected with hepatitis B virus (HBV) develop IgM antibody to hepatitis B core antigen (anti-HBc). Recovery from infection was found to be associated with the disappearance of 19s IgM anti-HBc, but chronic carriers were found to continue to produce low molecular weight (7s) IgM anti-HBc. Low molecular weight IgM anti-HBc may be a useful prognostic factor in persons who are chronic HBV carriers. HBV vaccine administered subcutaneously by jet injection was sufficiently immunogenic but some recipients developed intradermal nodules up to 3mm diameter at the site of infection. For Technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

* Available to contractors upon originator's approval.

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

Work Unit 202 Mechanisms of Transmission of Hepatitis Viruses

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Problems and Objectives

The hepatitis viruses are among the most common infectious agents responsible for serious diseases among peacetime military forces today. The potential for increased transmission and epidemic spread of some forms of hepatitis, especially hepatitis A, and perhaps some types of non-A, non-B hepatitis, exists during times of mobilization with a possible resultant loss in combat effectiveness of troops. All forms of viral hepatitis may be prevented by interruption of virus transmission or passive and/or active immunoprophylaxis, although effective immunoprophylactic measures have not been fully developed. Current objectives within this work unit include the development of improved methods of specific virus diagnosis, characterization of hepatitis viruses, the study of modes of virus transmission and evaluation of means of preventing viral hepatitis.

Progress

1. Radioimmunofocus assay (RIFA) for hepatitis A virus (HAV)

A new method was developed for the quantitation of hepatitis A virus (HAV) in cell culture, based on the immune autoradiographic detection of foci of infected cells (radioimmunofoci) developing beneath an agarose overlay 14 days

after the inoculation of Petri dish cultures of continuous green monkey kidney cells (BS-C-1). The number of foci developing in each culture was linearly related to the dose of HAV (either HM-175 or PA-21 strain) inoculated. Foci development was prevented by prior incubation of virus with specific antisera, and the specificity of the radio-labeled antibody reaction was confirmed in competitive blocking experiments. This new assay method retains many of the advantages of conventional plaque assays for virus. Compared with existing end-dilution methods for the quantitation of HAV, the RIFA offers greatly improved accuracy and comparable sensitivity, yet is relatively rapid and highly conservative of reagents.

2. Radioimmunofocus inhibition test (RIFIT) for neutralizing antibody to HAV

A new procedure for the detection and quantification of neutralizing antibody to HAV is under development, based on the RIFA method of HAV titration. This procedure, which is analogous to plaque reduction neutralization assays, has been successfully employed in the detection of neutralizing antibody to HAV in Aotus trivirgatus owl monkeys infected with HAV, as well as in naturally infected American soldiers. Testing of early, acute phase sera from patients with naturally acquired hepatitis A by the RIFIT procedure has shown that demonstrable titers of neutralizing antibody to HAV are present in both IgM and IgG immunoglobulin fractions as early as two days after the onset of symptoms. Futhermore the RIFIT procedure is considerably more sensitive in detection of antibody than existing competition radioimmunoassays (HAVAB, Abbott Laboratories, N. Chicago, Il.). Studies are in progress to further characterize the human immune response to HAV in terms of neutralizing antibody, to determine the kinetics of the neutralization reaction, and the degree of antigenic relatedness between various HAV strains in cross-neutralization experiments.

3. Experimental infection of Aotus trivirgatus (owl monkeys) with HAV

Epidemiologic studies have demonstrated the susceptibility of the New World owl monkey (Aotus trivirgatus) to HAV, but have not shown an association between infection and histopathologic or chemical evidence of liver disease. Therefore, in collaboration with MAJ James LeDuc, Medical Division, USAMRIID, and CPT Charotte Keenan, Division of Pathology, WRAIR six sero-negative, colony-bred monkeys were inoculated intravenously with a fecal suspension of PA33 strain HAV, recovered previously from a naturally infected

Aotus in Panama. Six to 17 days after inoculation, viral antigen was shed in the feces of all monkeys, and four to eight days later, serum aminotransferase activities became significantly elevated in each. Liver biopsies obtained 16 to 24 days after inoculation demonstrated mild to moderate histopathologic changes including portal inflammation and random areas of focal necrosis and inflammation extending outward from the portal region. Antibody to virus developed in each monkey by 28 days after inoculation. These data confirm the susceptibility of Aotus to HAV and indicate that infection of this primate provides a useful animal model of human hepatitis A. Subsequent cross-challenge studies of these PA-33 strain-infected Aotus with HM-175 strain HAV (human stool filtrate) revealed a high degree of immunity to re-infection. On the other hand, intravenous challenge of anti-HAV negative Aotus with the HM-175 strain resulted in chemical and histologic changes similar to those previously observed after PA-33 challenge. These studies thus confirm that PA-33 strain HAV, recovered during 1980 from naturally infected Aotus in Panama, is closely related to human strains of HAV, and extend the observations on the natural course of HAV infection in the Aotus monkey.

4. In vitro replication of hepatitis A virus (HAV)

During the past year, further studies on the growth of HAV in cell culture systems have been carried out. The virus has been serially propagated in primary and continuous (BS-C-1 and CV-1) African green monkey kidney and fetal rhesus lung (FRhL2) and kidney (FRhK4, FRhK6 and MA-104) cell cultures. In each of these cells, a persistent infection develops without detectable cytopathic effects. Viral replication has not been observed in cat or dog cell cultures. In cell culture, optimal growth of HAV occurred at 35°C and the presence of fetal bovine serum or 25mM magnesium chloride did not enhance viral replication. In contrast to other studies, HAV antigen was detected in comparatively high levels in supernatant fluids from infected cultures by a solid phase radioimmuno assay (RIA). Previously, the fluorescent antibody test (FAT) was employed to measure the infectivity of HAV cell culture preparations and in neutralization tests. Th FAT is labor intensive and only a small number of samples can be examined. To overcome these difficulties a number of alternate assay procedures are being developed. In each of these procedures, serial dilutions of HAV are inoculated onto susceptible cells (BS-C-1) and incubated at 35°C for 2 to 4 weeks. The infected cells are fixed with acetone, and reacted with specific anti-HAV labeled with I^{125}. After washing, the presence of bound immunoglobulin is determined either by autoradiography or in a gamma counter. In addition to the RIFA,

virus quantitation can be done (1) in test tubes in which the bound I^{125} is counted in the gamma counter thereby providing an estimate of viral antigen content and (2) in microtiter cell culture plates which are examined by autoradiography. All three systems have been used to titrate the virus preparations and provide comparable titers to those obtained by FAT.

5. Production of anti-HAV antibody in animal species

Studies on the antigenicity of cell culture-propagated HAV in rabbits and guinea pigs have been carried out. Guinea pigs given a single dose of $10^{7.5}$ ($TCID_{50}$) of virus developed antibody detectable by RIA (HAVAB). A second dose markedly increased the level of antibody. Rabbits given multiple doses of HAV grown in cell cultures also developed high levels of antibody. Futher studies on the neutralizing capacity of these sera are in progress. These findings suggest that HAV infected cell cultures may produce sufficient antigen to prepare inactivated vaccines.

6. Hepatitis B vaccine administered by jet injection.

A limited trial of the safety and immunogenicity of the Merck Sharp & Dohme inactivated hepatitis B vaccine, Hepatavax-B, was completed during FY1982. A total of 19 volunteers received three immunizations consisting of 20 mcg each alum-absorbed vaccine administered subcutaneously by jet injection. Sixteen recipients developed antibody to hepatitis B surface antigen (anti-HBs) detectable by RIA. This method of vaccine adminstration appears to result in immunogenicity which is comparable to that obtained by intramuscular injection, but did result in the production of transient subcutaneous nodules in several recipients.

7. Epicon investigations of hepatitis A epidemics within the military

During FY1982, the Dept. of Virus Diseases participated in the investigation of two outbreaks of HAV-related disease, one at the U.S. Disciplinary Barracks, Fort Leavenworth Kansas, and one at the Grafenwohr Training Area, in the Federal Republic of Germany. Altogether, over 80 individual cases of hepatitis were studied during these outbreaks. The observations made during the Grafenwohr epidemic are especially pertinent in that they demonstrated a high symptomatic/infected ratio among U.S. soldiers (>95%), and once more underscored the threat posed by this epidemic disease to combat-ready troops. Multiple HAV-antigen-positive fecal specimens were collected during both epidemics, and attempts are underway to isolate the respective causative strains of HAV in cell culture.

Presentations

1. Lemon, S.M., Binn, L.N., Redfield, R.R., Marchwicki, R.H., Gates, N.L. and Bancroft, W.H. Replication of Hepatitis A Virus in African Green Monkey Kidney Cells. 21st Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, Il. 4-6 Nov 1981

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6. Lemon, S.M. Active immunization against hepatitis B virus. Presented at the Annual 7th Medical Command Medical-Surgical Conference, Garmisch, FRG, May 1982.

Abstracts

1. Lemon, S.M., Binn, L.N., Redfield, R.R., Marchwicki, R.H., Gates, N.L. and Bancroft, W.H. Replication of Hepatitis A Virus in African Green Monkey Kidney Cells. Proceedings of 21st Interscience Conference on Antimicrobial Agents and Chemotherapy No. 214, 1981.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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81 10 01	D. Change	U	U		NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AB	203 WWG2
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Foreign intelligence 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) Bacterium

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 military forces. Current emphasis is on control of meningococcal, gonococcal and pseudomonas infections in military forces.

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

25. (U) 81-10 - 82-09 Vaccines against 3 immunotypes of Pseudomonas aeruginosa have been prepared for human use and have been shown to be safe. Two in vivo animal models have been developed which demonstrate that meningococcal outer membrane proteins can confer immunogenicity. Analysis of data regarding the adequacy of antibody response, the reactogenicity, and the composition of a vaccine consisting of group B capsular polysaccharide combined with the serotype proteins of the outer membrane in a non-covalent complex is being carried out before longer trials can begin. A highly sensitive microbore amino acid analyzer and a fully automated sugar analyzer have been constructed. A neutropenic rat model was developed for the study of bacteremia due to Pseudomonas aeruginosa. The chemokinetic factor made by human B cells was found to be a trypsin-resistant and neuraminidase resistant molecule of approximately 30,000 MW. (For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82).

*Available to contractors upon originator's approval

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

Work Unit 203 Bacterial Diseases of Military Importance

Investigators:

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

Problem

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

Progress

A modification of the solid phase radioimunoassay (SPRIA) was developed to characterize the precise specificity of human antibodies to meningococcal outer membrane proteins.

Vaccines against three immunotypes of *P. aeruginosa* have been prepared for human use and shown to be safe and potent in animals. Mouse monoclonal antibodies against *P. aeruginosa* LPS and *E. coli* K1 have been prepared and shown to confer specific protection in animal models.

A technique for rapid purification of Pseudomonas pili utilizing polyethylene glycol has been developed.

Nearly 240 E. coli isolates from blood and spinal fluid were typed by Drs. Orskov in Copenhagen. The most common 0 types were: 06,01,04,016,04,016,018,075 and 08.

A neutropenic rat model was developed for the study of bacteremia due to Pseudomonas aeruginosa.

Two in vivo animal models have been developed which demonstrate the meningococcal outer membrane proteins (MP) can confer immunogenicity upon either polysaccharide or lipopolysaccharide bacterial antigens even when the antigens are from heterologous non-meningococcal organisms.

A highly sensitive microbore amino acid analyzer having several desirable features i.e. low cost, easy maintenance, simple construction, low column pressure, full automation, and high sensitivity in femtomole range using 0-phthalaldehyde as the detecting reagent for primary amino acids has been constructed.

A fully automated sugar analyzer which separates neutral monosaccharides (i.e. rhamnose, glucose, galactose, KDO, fucose, and ribose) by reverse-phase chromatography on a sulfonic and column in the Li^+ form with 90% ethanol as the eluent and tetrazolium blue as the detecting agent has been constucted.

The toxicity of both Pseudomonas aeruginosa and N. gonorrhea LPS by alkaline treatments has been significantly reduced.

N. gonorrhea pili has been chemically cleaved with cyanogen bromide. The resulting peptide fragments were separated. Two large peptides, an insoluble and a water soluble fragment were isolated and were shown to react with antipilus antibody.

Recommendation

1. Continue with the ultimate goal of adding an effective group B vaccine to the existing tetravalent meningococcal vaccine.

2. Develop human monoclonal antibodies against P. aeruginosa, E. coli and E. coli J-5 LPS that protect in animal models and can be used safely in humans.

3. Further define the relationship between the K1 capsule and LPS from specific O types of E. coli.

4. Assess the ability of antibodies to specific antigens of Pseudomonas to modify infection in the rat.

5. Develop meningococcal "protosomes" for use in vivo to enhance antibody production against a variety of microbial antigens of military importance.

6. Interface the amino-acid analyzer and the sugar analyzer with a laboratory computer to handle and maintain the large amount of data generated by these two instruments.

7. Isolation and chemical characterization of the protective determinants of Pseudomonas aeruginosa LPS vaccines.

8. Obtain the shortest peptide that retains receptor binding activity from GC pili.

9. Continue the study of the oligosaccharide components of the LPS of both ser^s and ser^r strains, to determine their chemical composition and structure and identify the epitopes responsible for ser^s, in the normal human serum bactericidal system and the epitopes which function as lethal loci for ser^r organisms in a hyperimmune rabbit serum - exogenous complement system.

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Patents

Method for Producing a Vaccine Against Bacterial Infections Covered by *Pseudomonas aeruginosa*. Pat No. 4,285,936, August 25, 1981.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	3. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6763	82 10 01	DD-DR&E(AR)636

4. DATE PREV SUMRY	5. KIND OF SUMMARY	6. SUMMARY SCTY	7. WORK SECURITY	8. HANDLING	9. DISP'N INSTR'N	9b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

a. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AC	204 WWGC
b. CONTRIBUTING				
c. ~~CONTRIBUTING~~	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Rickettsiae - Host Interactions in Pathogenesis of Disease

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	2.0	206
	CURRENT 83	3.0	244

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

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NAME: Gowda, S., 1LT, MSC POC: DA

21. GENERAL USE

Foreign Intelligence Considered

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

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 characterize subcellular fractions of rickettsiae which have potential as experimental immunogens. Locate and identify the rickettsial surface antigens that affect virulence. Evaluate in mice the immunogenic potential of rickettsial fractions. Investigate alternate techniques to facilitate the early diagnosis of rickettsial diseases and detect rickettsial antigens. These studies will aid in improving the accurate rapid diagnosis of rickettsial infections and in the development of vaccines capable of protecting troops deployed in areas endemic for rickettsial diseases.

24. (U) Isolate subcellular fractions of rickettsial organisms using low ionic strength buffer, ether extraction, and genetic recombination methodologies. Characterize potential immunogens by physiochemical and immunological methodologies. Use antibody prepared against rickettsial surface antigens to evaluate the possible role of the antigens in virulence. Develop a sensitive immunoassay for the detection of rickettsial antigens in body fluids of infected laboratory animals. Isolate and determine the biochemical and immunologic characteristics of these antigens.

25. (U) 81 10 - 82 09 Lymphocyte hybridomas were used to identify and characterize cell envelope antigens in murine immune response to scrub typhus. Both strain-specific and cross-reacting antigenic determinents were demonstrated on antigens separated electrophoretically. Monoclonal antibodies did not affect an infectious neutralization assay for scrub typhus. C3H/HeJ mice were found to be the optimum murine model to study R. conorii infection. "Erythrocyte sensitizing substance"(ESS) of R. conorii was shown to poorly immunogenic. Initial data indicates that rickettsial ESS when coupled to non-rickettsial protein carrier has an increased immunogenicity. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

* Available to contractors upon originator's approval.

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

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

Work Unit 204 Rickettsiae-Host Interactions in Pathogenesis of Disease

Investigators:

Principals: Joseph V. Osterman, PhD; Christine S. Eisemann, MS; 1LT Srinivas Gowda, MSC; LTC Ralph D. Bernier, MC

Associates: Sp4 Peter F. Pond; Sp4 Wilfredo A. Cardona

Problems and Objectives

Efficacious, safe vaccines for rickettsial diseases are needed to preclude severe disruption of military operations when deployed troops are exposed to these organisms. One approach to vaccine development utilizes subcomponents of rickettsiae and has the potential advantage of eliciting protective immunity while minimizing adverse side reactions due to integral endotoxic components of the rickettsiae or contaminating host cell debris. Problems associated with this approach include: 1) isolation and purification of subcellular rickettsial components responsible for eliciting immune protection; 2) localization and identification of rickettsial surface proteins that affect virulence; and 3) antigenic characterization of peripheral proteins of rickettsiae. In the absence of a safe vaccine a method for the accurate, rapid identification of rickettsial infection is needed in order to ensure prompt, effective antibiotic therapy. Historically rapid identification of these infections has not been possible because of the following technical difficulties: 1) the lack of an adequate method to rapidly isolate and identify the rickettsiae; 2) either the absence of a rickettsial specific substance in infected hosts or the lack of an accurate method for detecting such substances; and 3) serologic diagnosis is inadequate because of the time required to develop detectable antibodies.

Progress

Lymphocyte hybridomas were prepared for the three prototype strains of Rickettsia tsutsugamushi and used to identify and characterize cell envelope antigens active in the murine immune response to scrub typhus infection. Antigens were separated by polyacrylamide gel electrophoresis and reacted with monoclonal antibodies in an enzyme linked immunosorbent assay. Both strain-specific and cross-reacting antigenic determinants were demonstrated within single peaks; in addition specific shared determinants were seen in multiple antigens of various molecular

weights. An infectious neutralization assay was developed using polyclonal mouse anti-scrub typhus serum. However, reacting viable organisms with monoclonal antibodies failed to prevent infection of susceptible mice and affect plaque forming assays. A solid phase radioimmune assay which provides a rapid and sensitive method for the detection of rickettsial antibodies was developed. After studying over twenty genetically diverse strains of mice, the C3H/HeJ strain was found to be the optimum animal model for R. conorii infection. This strain is highly sensitive to lethal infection by R. conorii and therefore provides the necessary model for analyzing the immunogenic potential of R. conorii subunit vaccine. Antibody levels following infection, the route of infection, and the ability of rickettsiae to multiply within the infected host cell were studied. Erythrocyte sensitizing substance of R. conorii was demonstrated to be poorly immunogenic. However, initial experiments indicate that when rickettsial antigens are coupled with a non-rickettsial protein (tetanus toxoid), the immunizing potential of the rickettsial antigen is increased. DNA of R. conorii has been successfully isolated. Its molecular size was determined. Attempts to package rickettsial DNA in bacteriophage have begun.

Recommendations

In subcomponent vaccine development, studies aimed at the isolation of rickettsial antigens will be focused on the use of low ionic strength buffer to cause cell fractionation by osmotic shock and the use of recombinant DNA methodologies designed to transfer rickettsial DNA into host E. coli . It is anticipated that rickettsial antigens will then be expressed on the Escherichia coli cell surface. Newly identified subcellular fractions will be tested in mice to determine their potential as immunogens for subunit vaccine development. Studies aimed at potentiating rickettsial antigen immunogenicity through complexing with nonrickettsial substances will continue. Dr. Robert Yolken, John Hopkins University, has recently demonstrated a rickettsial specific substance in the urine of humans infected with R. rickettsii and similar substance in the urine of both humans and dogs Infected with R. conorii . These substances were detected using ELISA technology. Initial studies indicate that these substances can be detected before specific antibodies can be demonstrated. These findings suggest that the development of a methodology capable of detecting rickettsial infection early in the disease process is possible. Initial studies to achieve this objective will focus on the infection of laboratory animals infected with R. conorii .

Formal Presentations

Bernier, Ralph D. 1982. Rickettsial Diseases Clinical Presentation and Diagnosis. Global Medicine Course. USAF School of Aerospace Medicine, 12 April 1982, San Antonio, Texas.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6514	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. RMD 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO		

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AD	205 WWG4
b. CONTRIBUTING				
c. CONTRIBUTING	STOG 80-7.2;2			

11. TITLE (Procede with Security Classification Code)

(U) Vector Transmission of Militarily Important Infectious Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	6.0	459
	CURRENT 83	5.0	553

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Schneider, Dr. I. POC: DA

NAME: Ward, Dr. R.

21. GENERAL USE

Foreign intelligence considered

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

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

23. (U) Develop physiological means of interrupting malaria and leishmaniasis transmission through an understanding of factors affecting parasite infectivity in vivo and in vitro. Refine models of malaria, leishmaniasis and African trypanosomiasis transmission to obtain large numbers of parasites for the study of immune mechanisms. Assess competence of closely related species as malaria and leishmaniasis vectors. Develop method of testing repellents against tsetse flies. Develop a field applicable serological test to detect anophelines infected with falciparum malaria. Realization of objectives may lead to prevention or control of malaria, leishmaniasis, trypanosomiasis and scrub typhus in military troops.

24. (U) Continue producing falciparum sporozoites needed for characterization of monoclonal antibodies. Identify factors that influence the infection process of falciparum malaria in anopheline mosquitoes. Establish colonies of phebotomine sand flies for leishmaniasis transmission studies. Compare susceptibility of different sand fly and anopheline species to leishmanial and malarial parasites respectively. Develop the ELISA test to detect P. falciparum sporozoites in mosquitoes. Refine methods for testing repellents against tsetse flies. Produce large numbers of procyclic trypanosomes for African trypanosomiasis vaccine feasibility studies.

25 (U) 81 10 - 82 09 Methods were developed for routinely infecting An. freeborni with cultured strains of P. falciparum. Monoclonal antibodies to P. falciparum sporozoites have been produced. Colonies of sand flies are being established. Reagents, antibodies and antigens for an ELISA test against malar' sporozoites have been produced and test parameters have been elucidated. Trypanosome procyclics are being produced. For technical report, see Walter Reed Army Institute of Research Annual Report 1 Oct 81 to 30 Sep 82.

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 3M161102BS10 RESEARCH ON MILITARY DISEASE, INJURY AND HEALTH HAZARDS

WORK UNIT 205 Vector Transmission of Militarily Important Infectious Diseases

Investigators
Principal: Donald R. Roberts, LTC, MSC
Associate: John B. Gingrich, MAJ, MSC; Peter V. Perkins, MAJ, MSC; Robert A. Wirtz, CPT, MSC; Ronald A. Ward, Ph.D.; Imogene Schneider, Ph.D.; Thomas R. Burkot, Ph.D. (NRC Fellow); David E. Hayes; Lawrence M. Macken; SP5 Megan G. Dowler; SP4 Sandra Gomez; SP4 Pedro Quintero

Problem and Objectives

Malaria, trypanosomiasis and leishmaniasis are arthropod-borne diseases of military medical importance. Consequently, the development of chemotherapeutic drugs or vaccines for these diseases should be directed against the insect transmitted stages as well as the blood and tissue stages of the parasites. Thus, the use of laboratory cyclic transmission models is an important element of any test system for a drug or vaccine development program. Current research objectives are to 1) develop physiological means of interrupting malaria transmission through an understanding of factors affecting parasite growth, invasion and infectivity in both vertebrate and invertebrate hosts, 2) determine factors influencing infection rates in tsetse flies that would result in making the WRAIR tsetse fly colony more efficient in producing Trypanosoma rhodesiense infections for immunological studies, 3) determine if tsetse flies and/or stable flies can transmit T. rhodesiense by mechanical means, 4) assess the vector competence of closely related anopheline species viz-a-viz malaria transmission and 5) study the mechanisms that determine sandfly susceptibility to leishmaniasis. Realization of these objectives may lead to prevention or control of malaria, trypanosomiasis and leishmaniasis in military troops.

Progress

Anopheles freeborni proved to be more highly susceptible to infection with cultured Plasmodium falciparum gametocytes than were any of the other anopheline species tested, e.g., An. gambiae, An. stephensi, An. albimanus and An. balabacensis, Perlis form. Hence methods were developed for routinely infecting An. freeborni with cultured strains of P. falciparum. Although the percentage of infected mosquitoes per feed has increased approximately threefold during the past year, parasite numbers on the midgut and in

the salivary glands remain low. Procedures have also been developed for producing large numbers of An. freeborni for these studies. Current production exceeds 8,000 weekly which is more than adequate for present requirements.

Falciparum sporozoites, derived from gametocytes produced both in vivo and in vitro have been used to inoculate BALB/c mice for monoclonal antibody production. One mouse has been harvested and the resulting monoclonal antibodies are presently being characterized by immunofluorescent antibody reactions with other stages of the parasite as well as other malaria species. Other sporozoites derived from cultured gametocytes have been used to infect an Aotus monkey (planned) and a man (accidental). Falciparum clones have been produced by the serial dilution method and are being screened for mosquito infectivity. In a cooperative project with NIH, an enzyme-linked immunosorbent assay to detect and identify sporozoites in the vectors is being developed. Preliminary methods with single mosquitoes have been successful and the emphasis is now on modifying these techniques to detect a single infected mosquito in pools of ten mosquitoes.

Sporozoites from infected mosquitoes have been isolated using (1) individual dissections, (2) decapitation followed by compression of the thoraces to extract the glands followed by (3) homogenization and extraction. These whole body extracts are further purified by (4) density gradient centrifugation and (5) DEAE cellulose columns with a buffer gradient. Techniques (3) and (4) result in a 10-15 fold increase in P. berghei sporozoite yield over (2) but the preparations are of lower purity.

Various formulations of culture media, as a serum substitute, were evaluated as a diluent for infecting tsetse flies with red blood cell - Trypanosoma rhodesiense suspensions. The most suitable media produced salivary gland infection rates of 8.2% and 22%, respectively. Mechanical transmission of trypanosomes from an infected to a normal host was studied using Glossina morsitans and Stomoxys (stable flies) In a series of experiments, the former consistently transmitted T. rhodesiense while all attempts with Stomoxys were negative. These results have implications for vaccine development and indicate need for field investigation. Using the in vivo rabbit test and/or the in vitro membrane test, selected repellents are being evaluated for effectiveness against tsetse flies.

Recommendations for the future

The production of P. falciparum sporozoites should be continued as needed for the characterization of monoclonal antibodies. Emphasis should be placed on identifying factors that influence the

infection process of falciparum malaria in An. freeborni mosquitoes. In particular, efforts should focus on increasing parasite numbers per mosquito.

Through genetic selection, develop strains of An. freeborni with varying levels of susceptibility to falciparum malaria parasites.

Increase the yield and purity of mass isolated sporozoites of both P. berghei and P. falciparum through the use of lectin binding techniques, antigen-antibody reactions and/or new column materials.

Establish colonies of phebotomine sand flies for leishmaniasis transmission studies. Compare susceptibility of different sand fly and anopheline species to leishmanial and malarial parasites, respectively.

Continue development of the ELISA test to detect P. falciparum sporozoites in mosquitoes.

Evaluate additional formulations of culture media to find the most appropriate one for producing high levels of salivary gland infection rates in tsetse hosts. Produce large numbers of procyclic trypanosomes for African trypanosomiasis vaccine feasibility studies and for repellent testing. In vitro maintenance of the tsetse colony should be developed to eliminate recurrent nutritive problems that occur with in vivo colony feeding on rabbit hosts.

Formal Presentations

Gingrich, J.B. Trypanosoma brucei rhodesiense: mechanical transmission by tsetse flies, Glossina morsitans, in the laboratory. 5th International Congress of Parasitology meeting in Toronto, CA. 7-14 Aug 1982.

Roberts, D.R. The Influence of DDT sprayed housewalls on the behavior of Anopheles darlingi. Presented to the 1981 annual meeting of the Amer. Soc. of Trop. Med. & Hyg. held in Puerto Rico in November 1981.

Roberts, D.R. Lecture on "The Ecology of Anopheles darlingi in the Amazon Basin" presented at Johns Hopkins University, School of Public Health, Baltimore, MD Feb 1982.

Roberts, D.R. Presentation to the Armed Forces Epidemiology Board on the status of dengue fever in Bolivia. September 1982.

Ward, R.A. Seminar on "Medical Entomology Research at WRAIR and the affiliated Overseas Laboratories" presented at U.S. Department of Agriculture Lab., Gainesville, FL in Feb 1952

Ward, R.A. Lecture on "Ecology of African trypanosomiasis" presented at Medical Entomology Course, Johns Hopkins Univsity, School of Public Health, Baltimore, MD in April 1982.

Publications

Gingrich, J.B., R.A. Ward, L. Macken and M.J. Schoenbechler. 1982. Trypanosoma brucei rhodesiense: factors influencing infection rates of a recent human isolate in the tsetse Glossina morsitans. J. Med. Ent. 19:268-74.

Gingrich, J.B., R.A. Ward, L. Macken and K.M. Esser. 1982. African sleeping sickness: new evidence that mature tsetse flies (Glossina morsitans) can become potent vectors. Trans. Roy. Soc. Trop. Med. & Hyg., 76: 479-81.

Roberts, D.R. 1982. The health of colonists. Science, 217: 484.

Roberts, D.R., W.D. Alecrim, J.M. Heller, S.R. Ehrhardt and J.B. Lima. 1982. Male Eufriesia purpurata a DDT-collecting Euglossine bee in Brazil. Nature 297 (5861); 62-3.

Roberts, D.R., Alecrim, Tavares and MeNeill. 1982. Field observations on the gonotrophic cycle of Brazilian populations of Anopheles darlingi. Root. J. Med. Entomol. (in press).

Ward, R.A. 1981. Culicidae. In: Aquatic biota of Tropical South America. Part I. Arthropoda. (Edited by S.H. Hurlbert et al.) San Diego State Univ. pp. 245-256.

Ward, R.A., N.D. Levine and G.B. Craig, Jr. 1982. Ascogregarina nom. nov. for Ascocystis Grasse, 1953 (Apicomplexa, Eugregarinorida). J. parasitol. 68: 331.

Williams, J.L., B.T. Innis, T.R. Burkot, D.E. Hayes and I. Schneider. Falciparum malaria: Accidental transmission to man by mosquito after infection with culture derived gametocytes. (Submitted to Am. J. Trop. Med. & Hyg.)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OA 6436	82 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
81 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AE	206
b. [illegible]				
c. [illegible]	STOG 80-7.2.2			

11. TITLE (Precede with Security Classification Code)

(U) Microbial Genetics and Taxonomy

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	4.0	596
	CURRENT 83	4.0	715

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Kopecko, D. J.
NAME: Wohlhieter, J. A. POC: DA

21. GENERAL USE

Foreign Intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

23. (U) To define in genetic and molecular terms gene transfer, antigenicity, and virulence of pathogenic enteric bacteria which because of their disease producing capabilities are of importance to military medicine, a major concern of which is the prevention and treatment of enteric infections in Army personnel. The goal is to modify enteric bacteria genetically to produce any desired antigenic structure and level of pathogenicity. Such strains can serve as vaccine strains or as tools to study the infectious process.

24. (U) Genetic recombination between strains of enteric bacteria and recombinant DNA techniques are used for strain construction and modification. Genetic results are extended to include the physical study of the informational macromolecules (i.e., DNA).

25. (U) 81 10 - 82 09 All Shigella sonnei strains carry a 120 Mdal plasmid needed for form I antigen (i.e., O-side chain) synthesis and for epithelial cell penetration. These form I O-side chain determinants have been cloned on a DNA fragment of 15 Mdal. Fragments representing the entire 120 Mdal S. sonnei plasmid have been cloned and are now being examined for additional virulence traits. Similarly, all serotypes of S. flexneri have been found to carry a 140 Mdal plasmid needed for epithelial cell penetration. Fragments of the 140 Mdal S. flexneri plasmid have been cloned and are being examined. A potential oral vaccine strain has been constructed by gene fusion resulting in a Salmonella typhi Ty21a carrying the O-side chain genes of S. flexneri serotypes 2a. Together with the vaccine strain constructed last year, a considerable number of Shigella infections can be prevented assuming these vaccines prove safe and effective. We also cloned the virulence antigen (Vi) genes found in Citrobacter freundii and Salmonella typhi, to study the "genetic switch" that controls the variable expression of this antigen. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 81 - 30 Sep 82.

(A sellable to contractors upon official approval)

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE. * U.S. GPO: 1974-540-843/8691

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

Work Unit 206: Microbial Genetics and Taxonomy

Investigators:
Principal: L. S. Baron, Ph.D.
Associates: J. A. Wohlhieter, Ph.D.; E. M. Johnson, Ph.D.; D. J. Kopecko, Ph.D.; F. A. Rubin, Ph.D.; C. A. Life; N. J. Snellings; M.S.; K. F. Noon, M.S.; SP5 J. N. Coulby, B.S.; SP4 N. Calderon, B.S.

Problem

Enteric bacterial infections have always been a serious health hazard to those entering an area where modern sanitary practices and facilities are lacking. More than 50% of the military personnel involved in field operations frequently are incapacitated by enteric bacterial illnesses. These enteric organisms can produce severe stomach cramps, nausea, vomiting, intestinal ulcerations, bacteremia, dysentery and diarrhea. Such enteric bacterial infections generally occur within several days after personnel enter an area where sanitary conditions are deficient or disrupted. Effective prophylactic field measures do not exist for many of the severe enteric disease agents e.g., Shigella, Salmonella, enterotoxigenic E. coli. Since native populations do develop an immunity to the enteric organisms normally indigenous to their environment, the development and use of effective enteric vaccines should act to augment the level of natural immunity thus reducing the inherent disease level in these areas. Also, effective enteric vaccines would stimulate immunity in military troops who frequently are highly susceptible individuals. Current objectives within this work unit include the development of mono-and multi-valent vaccines against enteric organisms, testing vaccine efficacy in animal models, and using molecular and genetic approaches to study the mechanism of disease pathogenesis resulting in pertinent information that can lead to the development of suitable techniques for the construction of improved vaccines.

Progress

Our research studies, conducted in collaboration with the Dept. Bacterial Diseases, have resulted in the conclusion that large plasmids (120-140 megadaltons in size) are necessary for the virulence of all strains of bacteria that cause dysentery (i.e., all Shigella species and certain Escherichia coli strains).

Specifically, we have shown that all virulent Shigella sonnei strains harbor a 120 Mdal plasmid that encodes the major protective cell surface antigen, the form I somatic antigen, of this species (1,2). Also, virulent isolates of all six serotypes of S. flexneri have been found to carry a 140 megadalton plasmid (3), as do dysenteric strains of E. coli. Animal and tissue culture assays have revealed that these large plasmids encode some virulence property that allows these virulent bacteria to invade epithelial cells, which is the first step in the disease process of dysentery. Recombinant DNA procedures have been developed to clone the plasmid-borne genetic determinants of virulence. At present, the form I antigen genes have been cloned from the S. sonnei plasmid responsible for the production of this antigen.

As reported last year, the galactose epimeraseless S. typhi Ty21a oral vaccine strain of Germanier and co-worker (4) appears to be a potential carrier of many antigens and should be useful in constructing multi-valent oral vaccines that will be protective against many different enteric diseases. To test this hypothesis, we initially transferred the genes for S. sonnei form I somatic antigen synthesis into the S. typhi Ty21a strain (5). The derivative strain stimulates the production of specific intestinal IgA in rabbits (6) and protects mice against challenge by virulent S. typhi and S. sonnei cells (5). Thus, this genetically constructed hybrid vaccine strain should protect against both typhoid fever and shigellosis due to S. sonnei, the most common cause of shigellosis. This bivalent oral vaccine is now being tested in volunteers for safety and efficacy.

During the past year, through genetic manipulations we have transferred the chromosomal genes encoding the somatic antigens of S. flexneri serotype 2a to the S. typhi Ty21a strain, resulting in the construction of a new vaccine strain that should protect against shigellosis due to S. flexneri 2a, a major cause of this disease. Studies to construct a similar hybrid strain protective against S. flexneri serotype 3 are underway. These three oral vaccine strains should protect soldiers against typhoid fever and shigellosis due to S. sonnei and S. flexneri serotypes 2a and 3, which cause greater than 95% of the shigellosis worldwide. Other protential enteric vaccine combinations are also being studied.

Recently, preliminary studies indicate that plasmids are also important for the virulence of certain Salmonella species. Further studies are aimed at determining the precise involvement of these plasmids in Salmonella disease. Other studies underway are directed at genetically isolating, via recombinant DNA technology, the chromosomal genes that are necessary for the virulence of Shigella and Salmonella. The genes encoding the Salmonella and

Citrobacter virulence (Vi) surface antigens have recently been cloned and are being analyzed in detail.

Recombinant DNA technology has been used in collaboration with workers in the Dept. of Bacterial Diseases to isolate the genes that encode the Neisseria gonorrhoeae surface attachment pili. These attachment organelles already serve as the basis for a gonorrhea vaccine. It is anticipated that these cloned genes can be used to study pili gene expression and to amplify the production of pili gene product for vaccine use. Additionally, collaborative studies with the Dept. Rickettsial Diseases and the Dept. Gastroenterology are underway to clone rickettsial surface antigen genes and certain enteric bacterial attachment organelle genes for vaccine use.

Recommendations for the future:

1. Genetically modify the S. typhi Ty21a strain to express the cell surface antigenic determinants of S. flexneri serotype 3. This new strain could be used in combination with the previously developed S. typhi-Shigella hybrid vaccine strains to produce a single multivalent vaccine that will protect against typhoid fever and the three most common causative agents of shigellosis. Also, the recently cloned form I antigen genes will be inserted into the S. typhi Ty21a strain; this derivative should have both increased stability and expression of the form I antigen and should be a better vaccine than our initial construction.

2. Inserting the genes for toxoid antigens into the S. typhi oral vaccine strain to produce a vaccine protective against typhoid fever as well as the enterotoxigenic diseases caused by Vibrio cholera and E. coli. Similar technology is contemplated to produce effective vaccines against still other enteric diseases.

3. Once cloned by recombinant DNA techniques, the Shigella plasmid-borne determinants needed for epithelial cell penetration should serve as an excellent molecular probe with which to rapidly detect dysenteric bacteria in the stools of diseased soldiers/patients. Such a test would be very valuable to the military.

4. Use of genetic manipulations of Shigella and Salmonella species to dissect the steps involved in the respective disease processes, the findings of which should provide basic genetic information and new insights into methods for both prophylactic and chemotherapeutic intervention.

5. Further examination of the specific role of plasmids in the virulence of Salmonella species.

6. New genetic studies directed at creating mutations in Shigella and Salmonella which inhibit the virulence of the organism at various steps in the disease process. The purpose of these mutants is to define new steps in the pathogenic process and to map the new genetic determinants of virulence.

7. Continue collaborative efforts at cloning various surface antigenic determinants of Neisseria gonorrhoeae, E. coli, and Rickettsia conorii for the development of vaccines.

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(D.J. Kopecko)

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Oct 23, 1981 — Use of transposons to identify and manipulate Shigella virulence plasmids. 3rd Internat. Symp. on Drug Resistance in Bacteria. Tokyo, Japan.

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Feb 19, 1982 — The involvement of plasmids in the pathogenicity of Shigella. Dept. Microbiol. Georgetown University, Washington, DC.

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Apr 5, 1982 — Bacterial resistance to antibiotics. Military Veterinary Course, Walter Reed Army Institute of Research, Washington, DC.

Apr 27, 1982 — The identification of Shigella plasmids necessary for intestinal penetration. Dept. Microbiol., Wayne State University, Detroit, MI.

Aug 4, 1982 — Genetic elements in the virulence of Shigella. Gordon Conf. on Microbiol Toxins and Pathogenicity, Plymouth, NH.

Aug 10, 1982 — Shigella virulence plasmids. Symposium entitled "Genetic Studies of Bacterial Virulence." XIII International Congress of Microbiology, Boston, MA.

Sep 1982 — Lecturer in semester course on the Biology of Bacterial Plasmids given annual at the National Institutes of Health Graduate School.

(by J. A. Wohlhieter)
Apr 29, 1982 — Plasmids Associated with Bacterial Virulence. Tropical Medicine Association of Washington.

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PATENT APPLICATION:

L. S. Baron, S. B. Formal and D. J. Kopecko. US Patent Application #289,013 Oral Vaccine for immunization against Enteric Disease.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6445	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AE	207
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Pathogenesis of Enteric Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
59 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	3.0	525
FISCAL YEAR CURRENT 83	2.0	367

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

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NAME: E. Tramont, COL, MD POC: DA

21. GENERAL USE

Foreign intelligence 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) 81 10 - 82 09 Safety tests in volunteers of the Salmonella typhi - Shigella sonnei candidate vaccine strain have commenced. Alterations in the properties of pathogenicity occur in Escherichia coli K-12 following the transfer of shigella chromosomal and plasmid genes. The plasmid encoded outer membrane proteins of Shigella flexneri, Shigella sonnei and invasive Escherichia coli have been compared. The ability of relevant LPS and protein antigens derived from shigellae to elicit IgA and IgG responses in mice has been accomplished. (For technical report see Walter Reed Army Institute of Research Progress Report, 1 Oct 81 - 30 Sep 82).

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

☆ U.S. GPO: 1974—540-843/8691

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

Work Unit 207 Pathogenesis of Enteric Diseases

Investigators:

Principal: Samuel B. Formal, Ph.D.
Associates: Thomas L. Hale, Ph.D.
COL Edmund C. Tramont, M.D., MC
LTC George H. Lowell, M.D., MC

Problem

Diarrheal disease has been a component of military campaigns since biblical times. In recent history these diseases played an important role in the British defeat at Gallipoli and caused significant illness in American troops in North Africa and the South Pacific in WWII, in Korea, in Lebanon and in Viet Nam. The pathogenesis of bacterial infections of the intestinal tract is studied using techniques of biochemistry, genetics, molecular biology, physiology and pathology to establish the factors and mechanisms which are involved in the disease process. The current objectives of this work are to understand the interaction of enteric pathogens with intestinal epithelial cells and to develop vaccines to prevent disease.

Progress

1. Work has continued with the *Salmonella typhi-Shigella sonnei* I candidate vaccine strain. An application for both an IND and a patent has been made. A dose of 1×10^7 cells of the vaccine strain has been fed to 3 volunteers with no unacceptable side effects.

2. The 140 Mdal plasmid, which encodes determinants of virulence in *S. flexneri*, has been transferred to a strain of *E. coli* K-12. The transconjugant gained the ability to invade HeLa cells. Transfer of chromosomal genetic material from *S. flexneri* to the transconjugant resulted in hybrids with additional shigella virulence characteristics. Some of these hybrids had properties similar to those

of wild type shigellae. Invasive E. coli also have a 140 Mdal plasmid. Mutant strains which have lost this plasmid are avirulent. Transfer of the 140 Mdal plasmid from S. flexneri to a plasmid-free E. coli strain restored its ability to invade epithelial cells.

3. It has now been shown that the 140 Mdal virulence-associated plasmids of S. flexneri serotype 3 and 5, as well as the 120 Mdal phase I plasmid of S. sonnei, encode ten outer membrane polypeptides. The 140 Mdal plasmid of an invasive (shigella-like) strain of Escherichia coli encodes a similar complement of outer membrane proteins. Six of the plasmid-coded polypeptides were missing from an avirulent S. flexneri serotype 5 strain which had sustained a large deletion in the virulence plasmid. The data suggest that some or all of these polypeptides may be essential virulence determinants and they are important potential vaccine antigens.

4. The ability of shigella LPS and protein antigens to elicit a secretory IgA response in mice has been analyzed. In addition to the age and strain of mouse, the form of the antigen has been found to be a critical determinant of immunogenicity. This information will be used in the generation of IgA-producing mouse hybridomas.

Recommendations

1. Complete safety testing of S. typhi-S. sonnei vaccine.

2. Prepare invasive E. coli K-12 hybrid strains bearing S. flexneri antigens for use as potential shigella vaccines.

3. Determine the role of outer membrane proteins in the process of epithelial cell invasion by shigellae by attempting to block penetration with monoclonal anti-body.

4. Prepare IgA producing hybridomas against shigella antigens.

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4. Formal, S.B. Pathogenesis, Virulence and Immunity in Shigellosis, NIH, 24 August 1982.

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7. Hale, T.L. The Envelope and Tissue Invasion. University of Aston in Birmingham, Birmingham, England, 29 September 1982.

8. Hale, T.L. Invasive Pathogens. Pasteur Institute. Paris, France. 12 October 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OC 6435	2. DATE OF SUMMARY* 82 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 81 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. RESGRADING*	8a. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AF	208 WWG8
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 30-7.2:2			

11. TITLE (Precede with Security Classification Code)*

(U) Immunity in Protozoan Diseases.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

010100 Microbiology 002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	5.0	439
FISCAL YEAR CURRENT 83	5.0	574

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign intelligence considered

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NAME: Williams, J.L., CPT, MSC POC:DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Antigens; (U) Protozoa; (U) Immunity; (U) Tropical Medicine; (U) Malaria

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

23 (U) To conduct immunological studies of protozoan diseases with emphasis on malaria, to produce P. falciparum malaria antigens by in vitro techniques for immunoassay and immunochemical analysis. These studies will aid in the development of a vaccine to protect soldiers stationed in many areas of the world against a major military disease.
24 (U) The approach used in these studies is to study, in both animal models and through the use of in vitro techniques, the response elicited by the immune system, to determine the role of cellular and molecular mediators in these processes, and to design experimental immunogens which will provide the basis for future vaccine development programs.
25 (U) 81 10-82 09 This year we have identified and are beginning to characterize six labile blood stage (merozoite) antigens, each of which can be stabilized by either growth inhibitory antibodies, or certain protease inhibitors. We are now making monoclonal hybridoma antibodies using mice immunized with these stabilized antigens, and the initial results look promising. We are setting up a lab to use recombinant DNA approaches to producing some of these antigens. Work continues on improving and using parasite culture conditions, synchrony, purification, immunochemical analysis; and in vitro correlates of protective immunity, of strain-specific immunity, and of non-antibody immunity. Gametocytes derived from culture have been used to successfully produce sporozoites in mosquitoes. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 80 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 80 (FOR ARMY USE) ARE OBSOLETE. ☆ U.S. GPO: 1974-540-843/8681

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

Work Unit 208 Immunity in Protozoan Diseases

Investigators:

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LTC Wayne T. Hockmeyer, MSC

Associates: COL Carter L. Diggs, MC
LTC Stephen C. Hembree, MSC
CPT Jeffrey A. Lyon, MSC
CPT James E. Egan, MC
Mr. Andre Toussaint
Ms. Lois A. Simonton
Ms. Teresa Jareed
SP4 David A. Soos
SP4 Lisandro Reyes

Problems and Objectives:

Malaria is a major cause of lost man hours during military operations in tropical and subtropical regions. A vaccine to protect soldiers in the field would have a major advantage over vector control and chemoprophylaxis by not requiring frequent administration in the field. We are investigating the feasibility of such a vaccine by studying the malaria parasite antigens and host immune mechanisms involved in protection against the asexual blood stages and the mosquito-borne sporozoites.

Asexual blood stages:

Major problems: (1) Instability of parasite antigens. (2) Lack of monoclonal antibodies which inhibit parasite growth and which can be used to identify and purify relevant parasite antigens. (3) Ignorance of the number and significance of antigenically differing parasite strains. (4) Requirement for

large amounts of human serum to produce parasites. (5) Need for better ways to produce selected parasite vaccine candidate antigens in quantity.

Objectives: (1) Stabilize parasite antigens. (2) Use stabilized antigens to produce and analyze monoclonal hybridoma antibodies. (3) Develop programs for examining antigenic strain differences. (4) Develop culture media requiring no or only small amounts of human serum. (5) Recruit scientists who will use recombinant DNA technology to attempt to produce parasite antigens in bacteria.

Sporozoites:

Major problems: (1) Lack of a reliable source of P. falciparum gametocytes for experimental purposes. (2) Lack of an in vitro assay which correlates with protection against sporozoite challenge.

Objective: (1) Standardize and optimize procedures for producing gametocytes in vitro which are infective to anophelilne mosquitoes. (2) Sporozoites produced in these mosquitoes will then be used to develop an in vitro assay to measure the development of protective immunity in vivo.

Progress:

Asexual blood stages:

(1) Parasite antigens have been stabilized by two methods: (a) Serum antibody from immune monkey reacts with the surface of merozoites as they are released from schizonts and not only agglutinates them, causing inhibition of reinvasion of red blood cells (reported last year in the Am. J. Trop. Med. Hyg., Chulay et al.), but also stabilizes a set of six metabolically labelled merozoite antigens at 200, 155, 80, 75, 40 and 34 kilodaltons as identified by autoradiography after SDS-PAGE, in comparison with merozoites released in the presence of normal serum (presented by J. Lyon, et al. at the 1982 FASEB Meetings). (b) A mixture of four specific protease inhibitors stabilizes the same set of antigens, plus a new one at 65 kilodaltons not before seen. (Their action appears to be synergistic and although they do not seem to inhibit protein synthesis during the last stages of merozoite maturation, some inhibit the reinvasion process, and some inhibit the development of ring forms into mature trophozoites).

(2) Using protease inhibitor stabilized parasites, and varying the method of antigen preparation, adjuvant, schedule and route of administration, and strain of mice, we have produced mouse sera which inhibit parasite growth in vitro. These mice are being used to produce monoclonal hybridoma antibodies. Thus far, most of the monoclonal antibodies are IgM, which are not good for immunoprecipitation analysis.

(3) COL Hembree developed a protocol which uses an in vitro parasite growth inhibition assay for examining the occurrence of different P. falciparum strain and will implement this in Brazil. Using several known strains of parasites we are screening for strain-specific monoclonal antibodies.

(4) Development of a serum-free culture medium has been impeded due to lack of a full-time scientist to work on this. Nevertheless, some progress has been made using a new 4-day assay in microtitration plates which does not require any media changes. A job description for a civil service position has been announced but has not yet resulted in any highly qualified applicants.

(5) Two scientists have been recruited (one an MSC Officer, the other a Senior visiting NRC Scientist) who will examine the possibilities of cloning parasite cDNA into bacteria in order to produce parasite antigens. They will begin work in November and December 1982.

Other progress: We have confirmed the ability of tumor necrosis factor serum from mice to kill P. falciparum in vitro and are examining this further as a potentially important mechanism of immunity. We continue to improve our methods for detecting antibodies against parasites. We developed an improved method for purifying parasite schizont forms using metrizamide density centrifugation.

Sporozoites:

(1) Mature gametocytes have been produced from several strains and a cloned line of parasites. The gametocytes were infective when fed to three different species of anopheline mosquitoes. Sporozoites derived from infected mosquitoes exhibited normal morphology and were infective to Aotus monkeys. Infectivity of the sporozoites to humans was strongly implicated when a laboratory technician who worked in the

insectary housing the infected mosquitoes accidentally acquired falciparum malaria. Monoclonal antibodies have been produced which react with the sporozoites in the indirect immunofluorescent antibody test. Studies are underway to characterize the monoclonal antibodies.

Recommendations:

Asexual blood stages:

We should actively recruit a scientist to develop improved culture media and methods so that large numbers of parasites can be grown and supplied for the studies requiring parasite antigens, RNA, and DNA. Continue to work on antigen stabilization, monoclonal antibody production, strain differences, and in vitro correlates of protective immunity. Begin work with recombinant DNA methods for producing parasite antigens.

Sporozoites:

Optimization and standardization of gametocyte production is required to insure continued availability of sporozoites. This will require basic studies on the induction of gametocytogenesis and gametocyte maturation. An in vivo model of antisporozoite immunization should be developed using non-human primates, and monoclonal antibodies should be chracterized for ability to confer protective immunity. An in vitro assay should be developed which can quantitate the development of immunity in vivo and the protective ability of monoclonal antibodies.

References cited:

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

2. Lyon, J.A., Haynes, J.D., Pavia, C.A., and Diggs, C.L. 1982. Antibody in cultures of Plasmodium falciparum increases yield of merozoites antigens. Fed. Proc. 41:585.

Presentations:

1. Lyon, J. A., Haynes, J. D., Pavia, C.A., and Diggs, C.L. "Antibody in cultures of Plasmodium falciparum increases yield of merozoite antigens." at the 66th Annual Meeting of The Federation for Experimental Biology, in New Orleans, Louisiana. April 1982. Abstract 1841.

Publications:

1. Howard, R.J., Haynes, J.D., McGinnis, M.H., and Miller, L.H. 1982. Studies on the role of red blood cell glycoproteins as receptors for invasion by Plasmodium falciparum merozoites. Molec. and Biochem. Parasitol. (In Press.)

2. Williams, J.L., Innis, B.T., Burkot, T.R., Hayes, D.E., and Schneider, I. Falciparum Malaria: Accidental transmission to man by mosquito after infection with culture derived gametocytes. (Submitted, Am. Trop. Med. and Hyg.).

3. Pavia, C.S., Diggs, C.L., Haynes, D.J., and Williams, J.L. The use of metrizamide for isopyknic separation and enrichment of Plasmodium falciparum infected schizonts from continues cultures. (submitted)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6440	82 09 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REDRAORG	8a. OMSR INSTR	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	H. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AF	209
b. XXXXXXXX				
c. XXXXXXXX	STOG 80-7.2:2			

11. TITLE (Procede with Security Classification Code)

(U) Parasitic Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING	82	3.0	178
FISCAL YEAR CURRENT			

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Intelligence Considered

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

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

23. (U) To study physiological, biochemical, pathological and epidemiological aspects of parasitic diseases of military importance. To evaluate existing techniques and to develop new techniques for diagnosis, prevention, treatment and control.

24. (U) Culture systems and animal models of parasitic diseases will be developed and used to study the parasites of interest, the parasitic disease process, and the effectiveness of new diagnostic, preventive and therapeutic measures. Studies will emphasize but will not be restricted to malaria, leishmaniasis, trypanosomiasis, and schistosomiasis.

25. (U) 8110-8209. The human macrophage model of leishmaniasis was employed in screening 150 compounds from various collaborative sources for establishment of efficacy of members of chemical classes. A number of investigations were completed and the results were published. These include the susceptibility of antimony-resistant isolates to alternative drugs and the investigation of efficacy of 8-aminoquinolines, purine analogs, imidazoles and liposome-encapsulated drugs. Investigation of requirements of growth of falciparum malaria in long-term culture continued. This work unit is being terminated by consolidation with Work Unit 217. For technical report see WRAIR Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*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 1498-1, 1 MAR 69 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M161102BS10 **Research on Military Disease, Injury and Health Hazards**

WORK UNIT 209 **Parasitic Diseases of Military Importance**

INVESTIGATORS

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

PROBLEM AND OBJECTIVES:

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

PROGRESS:

The human macrophage model of leishmaniasis was employed in the study of the in-vitro susceptibility to antimony of leishmania in isolates of clinically pentavalent antimony-sensitive and -resistant cases. The study was completed and published (1). A study of the susceptibility of antimony-resistant isolates to alternative drugs for treatment of leishmaniasis was completed and published (2). Investigation of possible new agents active against leishmaniasis led to studies of 8-aminoquinolines (Abstract 1), of imidazoles, of encapsulated drugs and of purine analogues (Abstract 2 and Publication 3). This laboratory has become a resource for in vitro screening of putative antileishmanial compounds and 150 compounds from various collaborative sources were screened.

Radiorespirometry for identification of leishmania species and strains was used in the study of 39 leishmania isolates from 18 countries:

1. **Visceral Disease.** Similar radiorespirometric profiles with some evidence of geographic variability were evident in human parasites from France, the Sudan and India, and in a canine isolate from the United States. Three New World L. chagasi were unlike Old World isolates and New World isolates appeared to be more heterogeneous than Old World leishmania. One kala azar isolate from Ethiopia appears to be unique among all 39 isolates tested.

2. **Mucocutaneous Disease.** Parasites from patients in Bolivia, Brazil, Colombia and Peru had similar respirometric profiles and slow catabolic rates distinguishing them from other species and strains.

3. **Diffuse Cutaneous Disease.** Parasites from patients in the Dominican Republic and Ethiopia were distinct from each other and all other leishmania tested.

4. **Cutaneous Disease.** This group is heterogeneous with geographic variation. Isolates of L. tropica from Israel and Turkey were similar but not identical to L. mexicana mexicana from British Honduras. An isolate from Kenya was unique among the 39 isolates. In 13 isolates from U.S. Army personnel with leishmaniasis acquired in Panama, two distinct leishmania were detected.

Developmental studies with long-term cultivation of falciparum malaria continued. Serial passages through membrane filters resulted in decrease of growth in cultures containing plasma, but had no similar effect on growth in cultures containing no plasma but with added serum albumin. The procedure was used in identification of drug susceptibility for clinical application.

FUTURE OBJECTIVES:

Further studies will be conducted in application of the human macrophage technique to determine the susceptibility of visceral isolates to antimony in vitro, to further development of the model system by comparing susceptibility of isolates in human and mouse macrophages, to determination of mechanisms of action of 8-aminoquinolines and in screening of putative antileishmanial agents. The radiorespirometric technique will be applied to

evaluation of relative drug sensitivities of leishmania species and to antimalarial drug development. Identification of growth requirements of falciparum malaria in long-term cultivation will continue.

PUBLICATIONS:

1. Berman, J.D., Chulay, J.D., Hendricks, L.D., and Oster, C.L. 1982. Susceptibility of clinically sensitive and resistant Leishmania to pentavalent antimony in vitro. Am. J. Trop. Med. Hyg. 31, 459-465.

2. Berman, J.D. 1982. In vitro susceptibility of antimony-resistant Leishmania to alternative drugs. J. Infect. Dis. 145, 279.

3. Berman, J.D., and Webster, H.K. 1982. In vitro effects of mycophenolic acid and allopurinol against Leishmania tropica in human macrophages. Antimicr. Agents Chemother. 21, 887-891.

4. Wysor, M.S., Zwelling, L.A., Sanders, J.A., and Grenan, M.M. 1982. Cure of Mice Infected with Trypanosoma rhodesiense by cis-Diamminedichloro-platinum (II) and Disulfiram Rescue. Science 217, 454-456.

PRESENTATIONS:

1. Berman, J.D., and Lee, L.D. 1981. Sensitivity of Leishmania tropica in human macrophages to 8-aminoquinolines in vitro. Am. Soc. Trop. Med. Hyg.

2. Berman, J.D., Webster, H.K., and Dumond, C. 1982. Leishmaniacidal effect and mechanisms of action of Formycin B. Am. Fed. Clin. Res.

3. Decker-Jackson, J.E., Hendricks, L.D., Marsden, P.D., and Walton, B.C. 1982. Radiorespirometric identification of parasites responsible for cutaneous, diffuse cutaneous and mucocutaneous leishmaniases in the New World. Fifth Int. Congr. Parasit.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6444	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. RIRD OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'R INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AH	210 WWH2
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	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:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	3.0	766
FISCAL YEAR CURRENT 83	3.0	787

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: Brown, J.E., CPT

NAME: Rothman, Sara W. POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Toxin; (U) Antigens; (U) DNA; (U) Hybridoma; (U) Immunoglobin

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 develop fundamental information about biochemical and cellular processes related to bacterial, parasitic and viral diseases of importance to the military. Factors associated with diseases such as (1) virulence determinants, toxins, nucleic acids and antigens and (2) products of host responses to disease (immunoglobulins) are being identified and characterized. The mode of action of toxins and structure-function relationships are being studied using concepts and techniques of biochemistry, molecular biology, immunology and cell biology.

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 centrifigation, 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) 81 10 - 82 09 In studies of Shigella toxin, a cell-free activity assay was used to detect toxin activity in Shigella flexneri 2a lysates. The amino acid composition of pure Shiga toxin was determined and full biological characterization was performed. Monoclonal antibodies to toxin were produced and characterized. Pathogenic Clostridium difficile was shown to contain plasmid DNAs and produce a toxin which affects target cell membrane functions. This cytotoxin was partially purified and rabbit antitoxin was produced by popliteal lymph node inoculation techniques. Four new pathogenic bacterial species, Vibrio mimicus, Vibrio damsela, Vibrio hollisac, and Escherichia hermanii were characterized by DNA sequence relatedness analysis. Vibrio mimicus was shown to produce an enterotoxin. For Technical Report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

26. PUBLICATIONS (Furnish publication info for each publication listed)

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 M[illegible]ARY DISEASE, INJURY AND HEALTH HAZARDS

WORK UNIT: 210 Biochemical Research on Military Diseases

INVESTIGATORS:
Principal: Peter Gemski, Ph.D.
Associates: Maria Alvarado, B.A.; SSG Monico Barreiro; CPT James E. Brown, Ph.D., MSC; Anthony Diecidue, B.S.; George R. Fanning, M.S.; SP5 Douglas A. Foret; Mary K. Gentry, M.S.; CPT Darrell E. Griffin, M.S., MSC; Janet Lazere, B.S.; SP6 William Mayo; Sara Rothman, Ph.D.; Mary A. Sodd, M.S.

IN COLLABORATION WITH: D. Brenner (CDC, Atlanta, GA); H. Collins (DCD&I); R. V. Lewis (University of Wyoming); J. A. Wohlhieter, Ph.D. (DCD&I).

DESCRIPTION:

To design and execute research programs that provide fundamental biochemical and molecular definitions of diseases relevant to the military. Factors associated with disease processes such as virulence determinants and toxins of organisms, biochemical and metabolic mechanisms of bacteria and parasites, and products of host responses to disease are being studied through the use of physiocochemical, biochemical, microbiological and immunological concepts and techniques. Such information provides a rational basis for immuological and chemotherapeutic protection against disease and the development of accurate diagnostic procedures.

A. Studies of Shigella and Their Toxins

1. Active Subunits of Shiga Toxin in Shigella Flexneri 2a

When compared to Shigella dysenteriae 1, S. flexneri 2a displays insignificant Shiga toxin cytotoxic activity. We have shown that Shiga toxin, purified from S. dysenteriae 1 inhibits protein synthesis in a cell-free assay. We now show that S. flexneri 2a contains high levels of this cell-free inhibitory activity. These results suggest that S. flexneri produces active subunits of Shiga toxin, rather than the holoprotein. Sterile bacterial sonicates were treated with 8 M urea, 10 mM DTT at 37°C for 30 min. Serial dilutions were tested in a rabbit reticulocyte translation system to determine whether protein synthesis was inhibited. S. flexneri 2a strain M4243 and its isogenic avirulent mutant, 2457-0, both produced 50% inhibition of ^{3}H-leucine incorporation at about 70 µg bacterial protein per ml reaction mixture. S. dysenteriae 1 strain 3818-T and its isogenic avirulent mutant, 3818-0, both produced 50% inhibition at about 30 µg per ml. Inhibition was neutralized by IgG monospecific for Shiga toxin. In contrast, sterile sonicates of S. flexneri 2a were 10,000-fold less active than S. dysenteriae 1 in a microtiter cytotoxicity assay. Moreover, these results suggest that avirulence of S. flexneri 2a strain 2457-0 is not associated with a lack of toxin activity.

2. Amino Acid Composition of Shiga Toxin From Shigella dysenteriae 1.

Shiga toxin, isolated by a combination of ammonium sulfate fractionation,

ion exchange chromatography, gel filtration and preparative isoelectric focusing, has been purified to apparent homogeneity in milligram quantities. The amino acid composition of toxin from three separate preparations was determined after 6 N HCl hydrolysis in the presence of thioglycolic acid. Expressed as mol%, the mean values obtained were: Asx, 13.5; Thr, 12.1; Ser, 7.4; Glx, 9.5; Pro, 1.9; Gly, 10.6; Ala, 4.8; Cys, <0.3; Val, 8.0; Met, 1.1; Ile, 4.1; Leu, 7.5; Tyr, 2.5; Phe, 5.3; His, 1.9; Lys, 6.0; Arg, 3.7. By analytical isoelectric focusing the holotoxin had an isoelectric point of approximately 7.1, suggesting that a major portion of the acid residues exist as the amide derivatives. The lack of detectable cysteine contrasts with our observations that dithiothreitol treatment both enhances cell-free toxin activity and is required for full denaturation during SDS-PAGE.

3. Biological Characterization of Shiga Toxin From Shigella dysenteriae 1.

Shiga toxin has been purified in milligram quantities to near homogeneity from cell lysates of Shigella dysenteriae 1 strain 3818-0. Purification involved an initial ultracentrifugation, ammonium sulfate fractionation, chromatography on DEAE-cellulose and CM-cellulose, gel filtration, and preparative isoelectric focusing in sucrose gradients. The purified toxin was resolved by discontinuous PAGE into a major cytotoxic protein band and a closely-migrating, proteolytically-nicked minor component. Antiserum, generated by immunization with glutaraldehyde-inactivated toxin, was shown to be monospecific against S. dysenteriae cell lysates. This highly purified toxin was cytotoxic to HeLa cells enterotoxic in rabbit ileal loops and lethal to mice. Monospecific antiserum to the toxin neutralized completely these toxin activities in both purified toxin preparations and in crude Shigella cell lysates.

4. Production and Characterization of Monoclonal Antibodies to Shiga Toxin.

Hybridoma cell lines which produce monoclonal antibodies to Shiga toxin from Shigella dysenteriae 1 were prepared by fusion of spleen cells derived from BALB/cJ mice immunized against glutaraldehyde-inactivated Shiga toxin with the P3x63Ag8 mouse myeloma cell line. Preliminary screening for hybrids which secreted antitoxin was based on the neutralization of Shiga toxin cytotoxicity to HeLa cells and on detection of antibodies to Shiga toxin by a solid phase radioimmunoassay. Six hybrid lines producing monoclonal antibodies to Shiga toxin were identified and cloned twice in soft agarose. The antibodies were found to be of the IgG class. Monoclonal antibodies from the six hybrid lines, amplified in mouse ascitic fluids, differed about 50-fold in their ability to neutralize cytotoxicity; the difference among these lines in antibody bound in the radioimmunoassay was about 10-fold. Autoradiographic detection of the binding of monoclonal antibodies to preparations of pure and crude toxin that were electrophoretically fractionated on polyacrylamide gels and transferred to nitrocellulose indicate that the monoclonal antibodies are monospecific for Shiga toxin.

B. Studies of Clostridial Toxins

1. Inhibition of Membrane Functions in Intact HeLa Cells by Clostridium difficile Cytotoxic Culture Filtrates.

The basis for cytotoxicity to intact HeLa cells by cultue filtrates of

Clostridium difficile has been investigated. Decrease in intracellular K^+ levels and inhibition of α-aminoisobutyric acid uptake were detected first after exposure to filtrates, followed by inhibition of macromolecular synthesis. Twenty-five percent of the K^+ remained associated with the cell monolayer, and amino acid uptake and macromolecular synthesis were not totally abolished. These results indicate C. difficile culture filtrates preferentially inhibited membrane functions, either by exhausting ATP supplies or by disrupting the permeability barrier of the cell.

2. Isolation of Plasmid DNA from Toxigenic Clostridium difficile Strains.

Detection and isolation of plasmids from toxigenic strains of Clostridium difficile by established procedures is complicated by (1) the difficulty of lysing these bacteria, (2) the degradation of their plasmid DNA and (3) the comigration of some plasmid DNA with chromosomal DNA during electrophoresis. We have now developed a procedure which overcomes all of these difficulties. To achieve lysis, early log-phase cells were treated sequentially with hyaluronidase (1mg/ml, 37°C, 1hr), lysozyme (1mg/ml, 37°C, 15 min), EDTA ($4x10^{-3}$M, 0°C, 5 min), and N-lauroyl sarcosine (2.5%, pH 12.1). Heat treatment (55°C, 40 min) increased lysis and further degraded chromosomal DNA. Extraction of the mixture by phenol-chloroform denatured and removed proteins; DNA remained in the aqueous phase. Overnight precipitation with 1M NaCl (4°C) removed additional chromosomal DNA. Plasmid DNA was concentrated by precipitation with sodium acetate and ethanol. After agarose gel electrophoresis, plasmid DNA was seen as discrete bands with no contaminating chromosomal DNA. Presence of closed circular DNA in these toxigenic strains of C. difficile was confirmed by electron microscopy. No correlation between toxigenicity and plasmid profile has as yet been demonstrated.

3. Partial Purification of Cytotoxin from Clostridium difficile.

Cytotoxin has been highly enriched from filter-sterilized culture supernatant fluids of Clostridium difficile ATCC 9689. The procedure included high-speed centrifugation, ammonium sulfate precipitation, ion exhange chromatography on DEAE-Sepharose CL-6B, gel filtration on Sepharose 6B, and hydrophobic interaction chromatography on Phenyl-Sepharose CL-4B. Based on HeLa cell cytotoxicity, the enrichment was greater than 1000-fold. In sucrose-gradient isoelectric focusing of a partially purified (post-gel filtration) toxin preparation, the pI was estimated to be between 4.3 to 5.0. The purified preparation was examined by analytical SDS-polyacrylamide gel electrophoresis and non-denaturing polyacrylamide gel electrophoresis. This preparation was tested for lethality in mice and for enterotoxicity in rabbit ileal loops.

4. Popliteal Lymph Node Inoculation to Produce Rabbit Antitoxin Against Clostridium difficile Cytotoxin.

Preparation of antiserum to Clostridium difficile cytotoxin using s.c., i.v. or intradermal routes of inoculation has involved complex protocols usually requiring at least 8 inoculations spanning 6 months or more. We have now produced such antitoxin in rabbits by injection of lethal doses directly into the lymphatic system, with lethality abrogated by coadministration of neutralizing antiserum. Initial s.c. inoculations of 1.2 ml containing 6×10^5 minimum cytotoxic doses (MCD) of C. difficile culture filtrates were given together

with excess anti-toxin. At the same time, the rabbits were primed by injection of 0.2 ml complete Freund's adjuvant into a hind footpad. Thirteen days later the popliteal lymph node in the thigh above the injection site could be palpated. Two tenths ml containing 1 x 10^5 MCD of toxic filtrate plus excess antitoxin was injected into the swollen node. Injection of the same preparation into this node was repeated 20 days later. One week later, neutralizing antibody was detected in serum from these rabbits. Monthly injections of immunogen alone (3-5 x 10^5 MCD) into the node were sufficient to maintain antibody levels.

C. Nucleotide Sequence Relatedness Among Enterobactereaceae

1. Characterization of Biochemically Atypical Vibrio cholerae Strains and Designation of a New Pathogenic Species, Vibrio mimicus.

Biochemically atypical strains classified as Vibrio cholerae were characterized by biochemical reasctions, serology, antibiotic susceptibility testing, and deoxyribonucleic acid relatedness. Strains with the following atypical reactions were shown to be V. cholerae: mannose negative, mannitol negative, lysine decarboxylase negative, no growth in the presence of 5% NaCl, salicin and cellobiose positive. Sucrose-negative strains were shown to constitute a new species, Vibrio mimicus, whose type strain is 1721-77 (AT CC 33653). In addition to its negative sucrose reaction, V. mimicus was differentiated from V. cholerae by its negative Voges-Proskauer, corn oil, and Jordan tartrate reactions and by its sensitivity to polymyxin. V. mimicus was isolated from shellfish and water, as well as from human diarrheal stools and ear infections. Most strains were typable with antisera against V. cholerae. Strains from three serogroups produced either a heat-labile or a heat-stable enterotoxin.

2. Vibrio damsela, a Marine Bacterium, Causes Skin Ulcers on the Damselfish Chromis punctipinnis.

A previously undescribed marine bacterium, Vibrio damsela, was isolated from naturally occurring skin ulcers on a species of temperate-water damselfish, the blacksmith (Chromis punctipinnis). Laboratory infection of the blacksmith with Vibrio damsela produced similar ulcers. Vibrio damsela was pathogenic for four other species of damselfish but not for members of other families of fish. The bacterium has also been isolated from water and from two human wounds and may be a cause of human disease.

3. Identification of Vibrio hollisae sp. nov. from Patients with Diarrhea.

The name Vibrio hollisae (synonym = Special Bacteriology group EF-13) is proposed for a new group of 16 strains that occurred in stool cultures of patients with diarrhea. V. hollisae is a small gram-negative rod, which is motile with a single polar flagellum. No lateral or peritrichous flagella were observed, even when it was grown on a solid medium. Sodium chloride is required for growth, so V. hollisae is a halophilic vibrio. Strains were positive (36°C, 24 or 48 h) for oxidase (Kovacs), indole production, nitrate reduction to nitrite, and fermentation of D-glucose (acid, no gas), L-arabinose, D-galactose, and D-mannose. Strains were negative for the following tests often used in enteric bacteriology: lipase (corn oil); deoxyribonuclease; gelatinase; methyl red; Voges-Proskauer; utilization of citrate, acetate, and malonate; L-lysine

decarboxylase (Møllers); L-ornithine decarboxylase (Møllers); L-arginine dihydrolase (Møllers); growth in KCN medium; and acid production from D-adonitol, D-arabitol, cellobiose, dulcitol, erythritol, glycerol (25% delayed positive at 7 days), i-(myo)-inositol, lactoses, maltose, D-mannitol, melibiose, α-methyl-D-glucoside, mucate, raffinose, Lrhamnose, salicin, D-sorbitol, sucrose, trehalose, and D-xylose. None of the strains was motile (semisolid medium) at 36°C at 48 h, but by 7 days 88% were motile. The strains did not grow within 2 days when plated on thiosulfatecitratebile salts-sucrose (TCBS) agar or MacConkey agar, but they grew on sheep blood agar and marine agar. By DNA-DNA hybridization (75°C, hydroxyapatite with ^{32}P), V. hollisae was only 0 to 4% related to 21 named species in Vibrio and Photobacterium. The type strain is designated ATCC 33564, which has a mean guanine-plus-cytosine content in DNA of 50 mol%. With the disk diffusion method V. hollisae had relatively large zones of inhibition around penicillin, ampicillin, carbenicillin, cephalothin, colistin, polymyxin B, streptomycin, kanamycin, gentamicin, tetracycline, chloramphenicol, and sulfadiazine. Future studies should focus on the isolation of this new vibrio and its ecology and relationship to human diseases.

4. Atypical Biogroups of Escherichia coli Found in Clinical Specimens and Description of Escherichia hermannii sp. nov.

DNA relatedness was used to define the biochemical boundaries of Escherichia coli. A large number of biochemically atypical strains were shown to belong to biogroups of E. coli. These included strains negative in reactions for indole, all three decarboxylases, D-mannitol, lactose, or methyl red and strains positive in reactions for H_2S, urea, citrate, KCN, adonitol, myo-inositol, or phenylalanine deaminase. Frequency and source data are presented for these atypical E. coli biogroups. One group of KCN-positive, cellobiose-positive, yellow-pigmented strains was 84 to 91% interrelated but only 35 to 45% related to E. coli. The name Escherichia hermannii sp. nov. is proposed for this group of organisms that was formerly called Enteric Group 11 by the Enteric Section, Centers for Disease Control, Atlanta, Ga. Twenty-nine strains of E. hermannii have been isolated in the United States from a variety of clinical sources, principally wounds, sputum, and stools. Three additional strains were isolated from food. E. hermannii strains are gram-negative, oxidase-negative, fermentative, motile rods. In addition to yellow pigment and positive KCN and cellobiose tests, the biochemical reactions characteristic of 32 strains of E. hermannii were as follows: gas from D-glucose, acid from D-glucose, maltose, D-xylose, L-arabinose, L-rhamnose, and D-mannitol; no acid from adonitol or inositol; variable acid production from lactose and sucrose; positive tests for indole, methyl red, and mucate; negative tests for Voges-Proskauer, Simmons citrate, H_2S, urea, phenylalanine deaminase, and gelatin hydrolysis; negative or delayed test for L-lysine decarboxylase and negative test for L-arginine dihydrolase; and positive test for ornithine decarboxylase. E. hermannii strains were resistant to penicillin, ampicillin, and carbenicillin and sensitive to other commonly used antibiotics. Wounds account for almost 50% of human isolates of E. hermannii, followed by sputum or lung isolates (ca. 25%) and stool isolates (20%).

5. Choleratoxin and Heat Stable Enterotoxin Production by Vibrio Mimicus.

Vibrio mimicus is a newly described species, previously assumed to be the

Heiberg V biogroup of V. cholerae. It is distinguished from V. cholerae by its negative sucrose, Voges-Proskauer, corn oil and Jordan's tartrate reactions. It has been isolated from shellfish, water, and from human diarrheal stools and ear infections in the United States, Asia, and New Zealand. Most human infections were associated with consumption of oysters or contact with water. Forty-five of the 51 strains in our collection were typable with 18 antisera prepared against non-01 V. cholerae strains. Five strains gave positive results in an enzyme-linked immunosorbent assay for choleratoxin. The presence of toxin genes was confirmed in all of these strains by using a choleratoxin specific gene probe. Four of these strains belonged to the same serotype. One strain from each of three serotypes gave positive results in the infant mouse assay for heat stable enterotoxin. The isolation and enrichment media used for V. cholerae will detect V. mimicus. It is essential, however, to pick sucrose-negative colonies from TCBS in order to isolate this potential pathogen.

6. Vibrio Damsela and V. Hollisae: New Vibrio Species Identified by DNA Hybridization.

The finding of V. cholerae in humans and in the environment stimulated increased interest in characterizing Vibrio species. Strains within each of two previously undescribed groups from human clinical specimens showed more than 70% DNA relatedness (75°C, hydroxyapatite method). There was less 5% DNA relatedness between the groups, and both groups were 7% or less related to described Vibrio species. V. damsela and V. hollisae are the names being proposed for these new species. They are biochemically distinct from each other and from other Vibrio species. V. damsela was first isolated from damselfish and subsequently from human wound infections. V. hollisae has been isolated only from human stools. These species were characterized during a systematic study in which reciprocal DNA hybridization reactions were done on type or reference strains from available species of Vibrio and related genera. Results confirm the extreme heterogeneity of the genus and provide a means to rapidly characterize other Vibrios that will undoubtedly be isolated from human sources.

PUBLICATIONS:

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5. Brenner, D.J., Davis, B.R., Steigerwalt, A.G., Riddle, C.F., McWhorter, A.

C., Allen, S.D., Farmer III, J.J., Saitoh, Y. and Fanning, G.R. 1982. Atypical Biogroups of Escherichia coli Found in Clinical Specimens and Description of Escherichia hermannii sp. nov. J. of Clin. Microbiol., 15: 703-713.

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ABSTRACTS:

1. Fanning, G.R., Hickman, F.W., Farmer III, J.J., Hollis, D.G., Steigerwalt, A.G., Weaver, R.E. and Brenner, D.J. 1982. Vibrio damsella and V. hollisae: new Vibrio species identified by DNA hybridization. Abst. of the Ann. Meeting of the ASM. P108, I86.

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6. Brown, J.E., Schaefer, E.M., Griffin, D.E. and Gemski, P. 1982. Active subunits of Shiga toxin in Shigella flexneri 2a. Abst. of the Ann. Meeting Am. Soc. Micro. P29.

7. Brown, J.E., Lewis, R.V. and Barreiro, M. 1982. Amino acid composition of Shiga toxin from Shigella dysenteriae 1. Abst. of XIII Internal. Congress of Micro. P112.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6744	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. RFCRADING	8a. DISP'R INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AH	211 WWH3
b. CONTRIBUTING				
c. CONTRACTOR/GRANT	XXXXXXXX STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Biochemistry of Parasitic Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 010100 Microbiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	3.0	321
	CURRENT 83	3.0	290

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Owens, Roberta

NAME: POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

23. (U) The objective is to investigate biochemical aspects that influence lipids and membranes of parasitic diseases. The ultimate goal is to formulate appropriate drug carriers for chemotherapy, and carrier modalities that might be utilized as adjuvants or as vaccines. The particular emphases are on the use of liposomes as drug carriers in leishmaniasis, the effects of glycolipids and glycoproteins on the life cycle of the malaria parasite, and the biological effects of various phosphol, pids and endotoxins as adjuvants and macrophage activators that might be benefical in leishmaniasis, malaria viruses, and other diseases. The role of lipid metabolism in liposome effectiveness is investigated. There is military relevance in this research.

24. (U) The approach will be to utilize liposomes as drug carriers in experimental leishmaniasis, malaria, and in normal animals. Liposome-encapsulated drugs will be examined for stability and efficacy. The roles of liposomal phospholipids and endotoxins on arachidonic acid and prostaglandin synthesis will be determined, particularly within macrophages. Appropriate antigens will be incorporated into liposomes along with lipids that have potential for macrophage activation.

25. (U) 81 10 - 82 09 The stability of the antileishmanial drug, WR6026, in liposomes was investigated extensively. Problems of potentially short shelflife were solved by low temperature storage. Lipid A was successfully purified and used as a liposomal adjuvant and potential macrophage activator. Novel effects of liposome phospholipids in malaria were discovered. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

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

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

WORK UNIT: 211 Biochemistry of Parasitic Drugs

INVESTIGATORS:
Principal: Carl R. Alving, M.D., COL, MC
Associates: Roberta L. Richards (Owens), Ph.D., DAC; Marti Jett-Tilton, Ph.D., NRC Associate; Inger Mattsby-Baltzer, Ph.D., NRC Associate; Nabil Wassef, Ph.D., DAC; Earl C. Richardson, DAC; SP4 Ramon Pacheco; SP4 Pearl Burke

DESCRIPTION:

The main objectives of this work were to study means of improving the use of liposomes as delivery vehicles for carrying drugs to treat leishmaniasis and malaria; and use of liposomes as carriers of antigens and adjuvants for vaccines. A particular goal was the identification of substances and conditions that could render liposomes antigenic, and study of the physiological implications of the production of anti-liposome antibodies.

1. Antibodies against liposomes in normal and immune-defective mice.

2. Membrane lipid composition modulates the binding specificity of a monoclonal antibody against liposomes.

3. Suppression of cytotoxicity of diphtheria toxin by monoclonal antibodies against phosphatidylinositol phosphate.

4. Liposome carriers in leishmaniasis chemotherapy with 8-aminoquinoline derivatives.

1. Antibodies Against Liposomes in Normal and Immune-defective Mice.

The immunogenicity of liposomes alone or of liposomes containing lipid A was tested by analysis of the activities of sera obtained from mice injected with liposomes. Immunologically normal mice (F_1 females from crosses of (CBA/N x DBA/2) or (CBA/N x BALB/c)), as well as immunologically defective mice (F_1 males from the same crosses), made anti-liposome antibodies with partial anti-phosphocholine (anti-PC) specificity. Fluorescence-activated cell sorter analysis showed that IgM, but not IgG, antibodies were raised in response to immunization with liposomes. The specificity for PC was demonstrated both by direct binding radioimmunoassay using plates coated with PC-albumin and by antibody-mediated C-dependent release of trapped glucose from liposomes containing phosphatidylcholine. The anti-liposome antibodies (particularly from F_1 males) could be inhibited by PC, but only at high concentrations. The antibodies were also absorbed by liposomes containing DMPC, but absorption was more efficient when liposomes also contained cholesterol or dicetyl phosphate. Liposomes that contained stearylamine instead of dicetyl phosphate absorbed antibodies less efficiently, and liposomes containing phosphatidylethanolamine instead of phosphatidylcholine did not adsorb significant quantities of antibodies. Although lipid A was present in the immunizing liposomes, the antibodies lacked specificity for lipid A, and there was no detectable anti-lipid A specificity that could not be absorbed by liposomes lacking lipid A. These

results suggest that the antigen-combining site on the anti-liposome antibody recognized PC and that the binding of antibody to PC was influenced by other lipids. However, despite their apparent specificity for PC, the anti-liposome antibodies were unusual in that they lacked, or had only minor amounts of, either the TEPC-15 or V_HPC idiotypic markers that are characteristic for murine anti-PC antibodies.

2. Membrane Lipid Composition Modulates the Binding Specificity of a Monoclonal Antibody Against Liposomes.

A hybridoma secreting a monoclonal IgM 'anti-liposome' antibody was produced after infecting a mouse with liposomes containing dipalmitoylphosphatidylcholine, cholesterol, dicetyl phosphate, and lipid A. The antibody was selected by assaying for complement-dependent damage to liposomes lacking lipid A. The monoclonal antibody reacted best with liposomes containing the original immunizing mixture of lipids. Deletion of individual lipid constituents from liposomes diminished the ability of the liposomes to bind (absorb) the antibody. Binding of the antibody was enhanced by including lipid A or galactosylceramide in the lipid bilayer, or by substituting egg phosphatidylcholine for dimyristoyl- (or dipalmitoyl-) phosphatidylcholine. Sphingomyelin could be substituted for dimyristoylphosphatidylcholine without altering the absorption of antibody. Although the monoclonal anti-liposome antibody was completely inhibited by phosphocholine, it was probably not a conventional anti-phosphocholine antibody. The antibody apparently had a partial specificity for phosphate, and was inhibited by glycerophosphocholine, glycerophosphate, sodium phosphate, sodium sulfate, and inositol hexaphosphate, but not by choline or inositol.

3. Suppression of Cytotoxicity of Diphtheria Toxin by Monoclonal Antibodies Against Phosphatidylinositol Phosphate.

The structure and chemical class of the cellular receptor for diphtheria toxin (DT) has not yet been determined. The receptor has been proposed to be a protein, a carbohydrate, or a glycoprotein. Recently it has been demonstrated that DT is a phosphate-binding protein. We have shown that DT binds specifically to the phosphate portion of some, but not all, phospholipids in liposomes. Based on these findings we proposed that the receptor for DT includes a minor membrane phospholipid such as phosphatidylinositol phosphate (PIP). To test this hypothesis we have examinded the ability of monoclonal antibodies directed against liposomal phospholipids to inhibit cytotoxicity of DT. Monoclonal antibodies against PIP inhibited the cytotoxicity of DT.

4. Liposome Carriers in Leishmaniasis Chemotherapy with 8-Aminoquinoline Derivatives.

An improved method is provided for the chemotherapy of leishmanial infections. An 8-aminoquinoline antileishmanial agent is encapsulated within liposomes and the liposome-encapsulated drug is injected into the body. Subject use of a liposome carrier has produced marked enhancement of the effectiveness of the drug against leishmanial parasites in the liver (such as characteristic of infections which are difficult to treat).

PATENTS:

1. Liposome carriers in leishmaniasis chemotherapy with 9-aminoquinoline

derivatives. E. A. Steck and Carl R. Alving, U.S. Patent No. 4,302,459, issued 24 November 1981.

One other U.S. patent is pending.

PUBLICATIONS:

1. Friedman, R.L., Roerdink, F., Iglewski, B.H. and Alving, C.R. 1982. Suppression of cytotoxicity of diphtheria toxin by monoclonal antibodies against phosphatidylinositol phosphate. Biophys. J. 37: 23-24.

2. Banerji, B., Kenny, J.J., Scher, I. and Alving, C.R. 1982. Antibodies against liposomes in normal and immune-defective mice. 1982. J. Immunol. 128: 1603-1607.

3. Banerji, B., Lyon, J.A. and Alving, C.R. 1982. Membrane lipid composition modulates the binding specificity of a monoclonal antibody against liposomes. Biochem. Biophys. Acta 689: 319-326.

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ABSTRACTS AND PRESENTATIONS:

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5. Richards, R.L., Alving, L.I., Moss, J., Banerji, B. and Alving, C.R. 1982. Liposomes as vehicles for vaccines: adjuvanticity and efficacy as protein carriers. J. Am. Oil Chemists Soc. 59(4) Abst 300A-301A.

6. Invited participant and presenter, Biophysical Society Discussion on "Lipid Protein Interactions," Airlie House, Airlie, VA, 4-6 Oct 1981.

7. Invited seminar speaker, NINDC, NIH, Bethesda, MD

8. Invited seminar speaker, Univ. Calif., San Diego, CA, 3 Nov 1981.

9. Invited faculty member and lecturer, Symposium on "Frontiers in Liposome Research," Univ. Calif., San Francisco, CA, 5-7 Nov 1981.

10. Invited seminar speaker, Frederick Cancer Research Center, Frederick, MD, 23 Feb 1982.

11. Invited lecturer, Symposium on "Immunological Aspects of Membranes," Annual Meeting of the American Oil Chemists Society, Toronto, Canada, 3-6 May 1982.

12. Invited participant and consultant, International Scientific Working Group on Leprosy, Leonard Wood Memorial (American Leprosy Foundation), Bellagio, Italy, 31 May-4 June 1982.

13. Invited lectured, 2nd International Conference on Immunopharmacology, Washington, DC 5-10, July 1982.

14. Vice-chairman, 3rd Gordon Research Conference on Drug Carriers in Biology and Medicine, Plymouth, NH, 12-16 July 1982.

15. Organizer and lecturer, First Princeton-Liposome Conference, Princeton, NJ, 22-24 Sep 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6471	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORG'N HIST'R	9a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CG	212 WW16
b. CONTRIBUTING				
c. XXXXXXXXXXXX	STOG 80-7.2	4		

11. TITLE (Procede with Security Classification Code)

(U) Physiology of Systemic Effects of Blast Overpressure

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

017100 Weapons Effects 002300 Biochemistry 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 03	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	3.0	290
	CURRENT 83	3.0	100

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Robert C. Smallridge, LTC, MC

NAME: Pritam Verma, CPT, MSC

21. GENERAL USE

Foreign Intelligence Considered

POC: DA

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Blast Overpressure; (U) Pulmonary Biochemistry; (U) Pulmonary Receptors; (U) Impulse Noise; (U) Biophysical Modeling

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

23. (U) To define the physiologic effects of blast overpressure and to determine the limits of human safety for exposure to impulse noise. To develop a mathematical model of the thoraco-abdominal response to blast waves. There is military relevance.

24. (U) Approach uses biochemical assays and physiologic tests before and after blast and impact injury. Blood is analyzed for enzymes, hormonal markers, elastin and surfactant related products and protein changes detected by 2 dimensional gel electrophoresis. Pulmonary tissue is examined histologically and assayed for hormone receptor concentrations. A finite element model of the sheep and human torso will be developed.

25.(U)81 10- 82 09 The search for a biochemical marker of blast injury has shown CPK, bradykinin and prostaglandins to be unreliable. Research now focuses on elastin and surfactant related moieties in blood and urine and two-dimensional gel electrophoresis as a screening tool for examination of blood, bronchoalveolar lavage, and thoracic lymph. A radioimmunoassay was developed for desmosine (a degradation product of elastin). A thin layer chromatographic assay was developed for dipalmitoyl phosphatidylcholine (a component of surfactant). High affinity thyroid hormone nuclear receptors have been identified in rst lung. After characterization of their properties, they will be studied in the context of blast overpressure effects. A torso model was exposed to blast to validate the computer code defining blast load characteristics. A three dimensional finite element model (3-D FEM) was compared to the established lumped parameter model from Lovelace and found to be comparable. A 3-D FEM of a gastrointestinal tract segment wss developed and run with a typical blast wave input. Results indicate this approach will be useful in determining the physiological variables related to injury, 10ct81-30Sep82.

*Available to contractors upon originator's approval.

DD FORM 1498 1 MAR 86

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

Work Unit 212: Physiology of Systemic Effects of Blast Overpressure

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Robert F. Hoyt, Jr., D.V.M., M.S., MAJ, VC
Andrew J. Young, Ph.D., CPT, MSC

Problem Statement and Objectives

Certain Army weapon systems, some currently in use, others still in development, produce levels of blast overpressure which exceed the limits defined in MIL STD 1474B. The research objective of the Department of Clinical Physiology is to define the risk of non-auditory injury to crewmembers from the blast overpressure produced by these weapon systems. To obtain a general understanding of the interaction of blast waves with crewmembers, a mathematical model will be developed using the inherent biomechanical properties of the human structure. Validation of the model's predictions will be made at frequent intervals to avoid costly development along non-productive paths. Biochemical indicators of non-auditory blast injury will be sought in order to increase the probability of detecting subclinical injury and to reduce the complexity and invasiveness of current techniques for assessing injury.

Progress in FY 82

a. A three year contract for characterization and modeling of the thoraco-abdominal response to blast waves was instituted with JAYCOR, San Diego, CA in the Second quarter of FY 82. The following contract milestones have been achieved:

1. A solid torso model was designed and constructed by JAYCOR. It was then exposed to various levels of blast overpressure at the Lovelace test facility in Albuquerque, NM. Surface pressure measurements from the torso model will be used to validate the computer code defining blast load characteristics.

2. The level of anatomical detail to be incorporated in the three dimensional finite element model (FEM) of the sheep was determined jointly by JAYCOR and WRAIR staff.

3. A simplified, 3-D FEM consisting of 12 elements and given material properties taken from the literature was run using a typical blast wave as input. The predictions of the FEM for intrathoracic pressure were compared to those of the Lovelace lumped

parameter model as well as actual animal data. The output of the FEM agreed well with the other two data sets. This accomplishment establishes the basic validity of the approach and sets the stage for a more detailed anatomical model containing 20 to 50 elements.

4. Static and dynamic material properties of sheep lung parenchyma were measured by the bioengineering department of the University of California at San Diego acting as a sub-contractor to JAYCOR. These laboratory measurements will replace the literature values and estimates now in use by the FEM.

5. JAYCOR constructed and tested a blast exposure chamber for isolated gastrointestinal tract segments. It is hoped that this device will allow experimental evaluation of the role of bubble size and the material properties of the segment contents in producing gastrointestinal injury following blast exposure.

6. A simple 3-D FEM of a gastrointestinal tract segment containing an air bubble and contents of variable density density was run with a typical blast wave as input. The gross deformation of the surface of the segment over the air bubble clearly demonstrated the importance of bubble size and segment contents density in determining local tissue stress and strain. Experimental validation of this FEM is anticipated in FY 83.

b. Blood from sheep which had received significant lung injury from air blast exposure was analysed for the concentration of angiotensin converting enzyme, bradykinin, and prostaglandin E_2. The same measurements were made in matched control animals. There were no significant differences in the levels of these biochemical compounds between the two groups thus indicating that they have little potential as markers of lung injury for our purposes.

c. Two other potential markers of blast related lung injury are desmosine, a degradation product of elastin, and dipalmitoyl phosphatidylcholine, a major component of surfactant. Since there were not any sensitive assay techniques for these compounds available from the literature, our first research task was to develop them ourselves. A major portion of the biochemistry program in FY 82 was devoted to the development of a very sensitive radioimmunoassay for desmosine. A lesser task, though equally important, was the development of a thin layer chromatographic assay for dipalmitoyl phosphatidylcholine. Blood from blast injured sheep will be analysed using these techniques in the second or third quarter of FY 83.

Recommendations and Objectives for FY 83

The major research efforts under this work unit for FY 83 will be:

a. The acquisition of appropriate biomechanical data on sheep-blast wave interaction for validation of computer model predictions.

b. Studies evaluating desmosine and dipalmitoyl phosphatidylcholine as markers of blast-related lung injury.

c. Publication of a request for quotation for a contract to perform 2 dimensional gel electrophoresis on plasma from blast injured sheep. This technique will be used as a screen for unspecified biochemical indicators of blast-related lung injury.

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None

Presentations

None

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Verma, P. S. and Adams, R. G. Simplified method for total kininogen determination by bradykinin radioimmunoassay in human plasma. Clin. Chem. 28:1625, 1982.

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Smallridge, R. C., Rogers, J. and Verma, P. S. Serum angiotensin-converting enzyme: Regulation by thyroid hormone in hyperthyroidism, hypothyroidism, and subacute thyroiditis. Submitted to N. Engl. J. Med.

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Patents Granted

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OC 6451	2. DATE OF SUMMARY* 82 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 81 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8A. DISP'N INSTR'N NL	8B. SPECIFIC DATA* CONTRACTOR ACCESS [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	61102A	3M161102BS10	CD	213 WWJA
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	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 METH'D
76 07	CONT	DA	In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	7.0	580
	CURRENT 83	7.0	674

19. RESPONSIBLE DOD ORGANIZATION

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Division of Neuropsychiatry

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

NAME:* Elsmore, T.F., Ph.D.

TELEPHONE: (202) 576-3037

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Hursh, S.R., MAJ

NAME: Wylie, R.M., Ph.D. POC: DA

21. GENERAL USE

Foreign Intelligence 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) 81 10-82 09 Major Findings: Analysis of the utility of economic concepts for describing behavior was advanced by development of a technique which permits rapid determination of demand functions for single animals. In another economic experiment it was shown that food gathering in gerbils is less elastic than other activities in a multi-response environment. Accuracy of performance of a short-term momory task for monkeys was shown to be dependent on the consequences maintaining observing the stimulus to be remembered. In circadian rhythm experiments it was shown that when animals are required to perform at odd hours of the day, their performance tends to be less cohesive. Monkeys were successfully trained to point at a moving visual stimulus in studies of the neural control of movement. Detailed mapping of the spinal innervation of the gastro-intestinal tract was accomplished using retrograde labeling techniques. For technical report see Walter Reed Army Institute of Research Annual Report 1 Oct 81 - 31 Sep 82.

*Available to contractors upon originator's approval

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

Work Unit: 213 Biological Modulation of Military Performance

Investigators:
Principal: Elsmore, Timothy F., Ph.D.
Associate: Hursh, MAJ S.R.; Raslear, CPT T.G.; Kaufman, CPT L.K.; Petras, J.M., Ph.D.; Wylie, R.M., Ph.D.

Objectives:

The objectives of this project include the definition of the means by which the nervous system mediates bodily responses to stress and injury, and to discern those combinations of physiologic parameters which collectively define the optimal conditions for effective military performance. A major thrust of research in this work unit is the development of animal behavior models that more closely approximate realistic situations outside of the laboratory. Techniques and methods are drawn from a broad spectrum of neuroscience disciplines including psychology, neurophysiology, neuroanatomy, neuropharmacology, and chronobiology.

Progress:

The utility of concepts derived from economics in describing the behavior of laboratory animals has continued to be explored in several experiments. Demand for food was shown to be elastic in terms of choice between two alternative sources of the same food in a single situation, but was also inelastic (i.e. unchanging) across different situations. In a study of substitution and complementarity of commodities, a procedure was developed for determination of demand curves in one month rather than three months, thus permitting a considerable time savings in investigating the impact of different choice procedures upon demand curves. The relative elasticity of behavior motivated by different events is being evaluated in gerbils that are exposed to an environment where food intake, water intake, wheel running, nesting, and time spent in proximity to a conspecific can be monitored. This information is critical in the design of experiments designed to assess the impact of external variables, such as stressors, upon performance.

A series of studies on the impact of time of day upon performance is continuing. The results of several experiments suggest that performance at other than the animals' normally active period tends to be more variable and less stereotyped than performance during the active period. A study of the repeated acquisition of

different response sequences shows that performance is most vulnerable to breakdown during transition from a series of easy tasks to a series of more difficult tasks, and that such breakdown is more likely during the night hours than during the daytime.

Several experiments are directed at developing animal models of short-term memory. A delayed matching-to-sample experiment requires monkeys to observe a sample stimulus (color), then to select that color from a set of comparison stimuli following a delay. Results suggest that accuracy at long delays is improved by reinforcing observation of the sample stimulus. Work has begun with a radial-arm-maze to develop short-term memory procedures in rodents.

Efforts are continuing to develop an understanding of the neural control of limb movement. Earlier results from monkeys performing a weight-lifting task have been confirmed with two additional subjects, and show that sensory input from a limb is important for the animal to respond appropriately in the face of changing load requirements. This work is being extended by the development of a tracking procedure in which monkeys are required to point at a moving spot of light. The animal is instrumented in such a way that limb position, speed, accelleration, and EMGs from relevant muscles are continuously monitored. Several monkeys have been trained in this apparatus, and will be used in studies of how this task is performed both before and after dorsal rhizotomy to eliminate sensory input from the active limb.

Future objectives:

Applicability and utility of economic concepts in the analysis of animal behavior will be further evaluated. Short-term memory models of animal behavior will be evaluated. Procedures for scaling animal sensory processes will be developed and evaluated. Research on the role of kinesthetic sensory input in controlling limb movement will continue.

Presentations

Campbell, C. B. G. On theory-making in comparative neurology. J. B. Johnson Club, Society for Neuroscience, Chicago.

Elsmore, T. F. and Hursh, S. R. Delayed discrimination: variables controlling accuracy on long-delay trials. Association for Behavior Analysis, Milwaukee.

Kaufman, L. W. and Raslear, T. G. The effect of noise on circadian activity patterns in the rat. Eastern Psychological Association, Baltimore.

Petras, J. M. Neurotoxic effects of soman in the cat. New York State College of Veterinary Medicine, Ithaca, NY.

Petras, J. M. Neurotoxic effects of soman in the cat: a comparison with the rat. U. S. Army Medical Research and Development Command Bioscience Review, Aberdeen Proving Ground.

Petras, J. M. The anatomy of spinal autonomic cell groups. Ralph L. Smith Research Center, Kansas Center for Mental Retardation and Human Development, The University of Kansas Medical Center, Kansas City, KA.

Petras, J. M. Neurotoxic effects of soman in the cat and the rat: some preliminary findings. Department of Anatomy, The University of Kansas Medical Center, Kansas City, KA.

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Raslear, T. G. A test of the Pfanzagl bisection model in rats. Eastern Psychological Association.

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Elsmore, T. F. The rise and fall of FR performance. Behavior analysis letters, in press.

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Raslear, T. G. and Keck, L. T. Comment on Foster and White's "Ethnic identity and perceived distance between ethnic categories." Human organization, in press.

Raslear, T. G., Pierrel-Sorrentino, R., and Rudnick, R. Masking and loudness in the rat. Behavioral neurosciences, in press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6755	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CE	214 WWJE
b. CONTRIBUTING				
XXXXXXXXXX	STOG 80-7.2:4			

11. TITLE (Precede with Security Classification Code)

(U) Millimeter Wave Biophysics and Biohazards

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

014100 Radiobiol 012900 Physiol 014000 Rad Chem 017000 Wave Prop

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2.0	299
FISCAL YEAR CURRENT 83	2.0	89

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: J. H. Jacobi

NAME: POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Biophysics; (U) Millimeter Wave; (U) Bioeffects; (U) Permittivity

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

23. (U) The objectives of the millimeter wave bioeffects program are to (1) establish a technology base in millimeter wave instrumentation as needed for biophysical research in this region of the electromagnetic spectrum, (2) to develop millimeter wave exposure systems for use with biological specimens under conditions of both continuous wave and high peak power operations, and (3) to explore biological hazards with special interest in the eye. The military relevance in this research derives from millimeter wave radars.

24. (U) The millimeter wave instrumentation system will consist of a millimeter wave phase locked synthesizer for the range 40-60 GHz. This will serve as the source for a six-port network analyzer that will provide network analysis based description of biological dielectrics in vitro. The continuous wave exposure system will consist of a 35 GHz, 1 kilowatt klystron amplifier, a 10 watt traveling wave tube driver and a 100 milliwatt Gunn diode oscillator. The pulse transmitter will consist of a 35 GHz traveling wave tube amplifier of 30 kilowatts peak power and 3 kilowatts average power. The antenna will consist of a WR 28 feed to an elliptical reflector. The biological hazard studies will emphasize two features: (1) the direct heating action of millimeter waves on the cornea of the eye and (2) the production of thermoacoustic expansion in cornea, lens and retina.

25. (U) 81 10 - 82 09 Development of the 35 GHz pulsed and CW transmitters continued. At the present time, both are ca. 90 - 95% complete. Site preparation is fully completed. Preliminary millimeter wave (37 GHz) measurements of emissivity in vivo indicate a value near 0.90. Spectral scanning equipment development has progressed into the system integration phase. The 40 - 60 GHz six-port network analyzer and digital synthesizer subsystems are completed. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. OD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

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

Work Unit 214: Millimeter Wave Biophysics and Biohazards

Investigators.
Principal: LTC(P) Lawrence E. Larsen, M.D.
Associate: John H. Jacobi, M.S.; Charles N. Rafferty, Ph.D.

Objectives

Millimeter wave spectral region is of great technological interest to the Army. This is a result of the fact that tactical deployment of millimeter wave radars and imaging systems is projected to maintain weapon system operability in battlefield environments designed to render electro-optical components ineffective.

This is a new technology area not only for weapon systems but also for biomedical study. Since these systems are projected to have vast tactical deployment in this decade and little reliable biomedical information exists in this spectral region, we have begun a millimeter wave research program. This program consists of two sections: instrumentation development and exposure system development. Once these stages are completed, biomedical experimentation will begin (projected for completion within FY 83).

Progress

a. Instrumentation development for the 40-60 GHz spectral scanning equipment continues. The subsystem development (six-port network analyzer and digital synthesizer) is nearly completed. Performance tests on the synthesizer's backward wave oscillator indicate successful phase lock over the full 40-60 GHz band using the Henry technique and linear tuning was demonstrated, but phase noise is not yet fully acceptable. The six-port network analyzer packaging has nearly been completed. The major item remaining is the thermoregulation subsystem. The superheterodyne receivers and six-port couplers need further laboratory evaluation for phase tracking. Lastly, the system integration portion has begun with inclusion of a microcomputer for control and processing in a complete data acquisition system.

b. The millimeter wave transmitters have continued development. The 35 GHz pulse system will be completed first with installation due for 1st quarter FY 83. The 35 GHz CW system will follow in the 2nd quarter FY 83. The installation of the transmitter(s) will permit biological experimentation to produce pilot results by the 4th quarter FY 83 since the anechoic chamber, antenna and prime power are complete. Similarly, biological base lines have been established for the projected target organ (ocular cornea) with respect to organ culture methods and SEM/TEM anatomic features.

c. Lastly, a new investigator was hired and we have begun to develop a biophysical chemistry laboratory at Forest Glen. Crossed beam studies with dynamic recording in the microsecond range are planned.

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3. Sanford, S.E., Dukes, T.W., Creighton, M.O., Miller, M., Magy, R., Trevithick, J.R.: Further Studies on Hygromison B Cataracts, Journal of Investigative Opthomology & Visual Science, Vol. 22, pp 154, No. 46, 1982, supplement.

4. Linklater, H.A., Miller, M.E., Creighton, M.O., Layden, R.E., McLeod, H.L., Trevithick, J.R.: Journal of Investigative Opthomology & Visual Science, Vol. 22, pp 156, No. 60, 1982, supplement.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6449	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CD	215 WWJ8
b. CONTRIBUTING				
XXXXXXXXXXX	STOG 80-7.2:4			

11. TITLE (Procede with Security Classification Code): (U) Mechanism of Response to Stress

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
012900 Physiology 002300 Biochemistry 013400 Psychology

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

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	1.0	298
	CURRENT 83	1.0	379

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Kant, G.J., Ph.D.
NAME: POC: DA

21. GENERAL USE
.Foreign Intelligence Considered

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Stress; (U) Neurochemistry; (U) Neurotransmitters; (U) Cyclic nucleotides; (U) Neuropeptides; (U) Kindling; (U) Post-traumatic epilepsy

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 in adaptation to stress and continuous performance. To study neurochemical mechanisms in development of post-traumatic epilepsy, which occurs in 40% of soldiers suffering head wounds, despite anti-convulsant drugs. To provide database for interpretation of military clinical and field studies, and recommendations for prevention and/or treatment in soldiers.

24. (U) Analysis of neurochemical regulation of hormonal response during adaptation to stress. Repeated electrical stimulation of the brain ("kindling") has been selected as the best animal model of post-traumatic epilepsy, because of similarities in time-course. Studies entail brain lesion and stimulation; measurement of neurotransmitters, neuropeptides, cyclic nucleotides and phosphorylation in specific brain regions.

25. (U) 81 10 - 82 09. Stress-induced increases in pituitary cyclic AMP are proportional to the intensity of stressor, and are exaggerated by administration of atropine, a cholinergic blocker. We find sex differences in habituation of biochemical responses to footshock and forced running. In studies of cholinergic systems, we found a diurnal variation in density of muscarinic receptors in brain, and showed that DFP markedly inhibits the stimulated release of endogenous dopamine from brain tissue in vitro. Thyrotropin Releasing Hormone (TRH) increases markedly in seizure-related brain regions (amygdala, hippocampus and cortex) following kindled seizures. We had hoped to increase the level of effort by increasing the number of MSC professional authorized. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 80 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 80 (FOR ARMY USE) ARE OBSOLETE.

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

Work Unit 215 Mechanisms of Response to Military Stress

Investigators:
Principal: Meyerhoff, J.L., M.D.
Associate: Kant, G.J., Ph.D.; Mougey, E.H., M.S.; Collins,, D.R., B.S.; Pennington, L.L., B.S.

Objectives:

Evaluation of neurochemical mechanism of response to stress, brain injury and other factors which produce psychiatric incapacitation or brain syndromes pertinent to military medicine. Included are CNS regulation of pituitary function in acute and repeated exposure to stressors, adaptation to chronic stress, effects of stress on neurotransmitter turnover, neuropeptide and receptor function, as well as studies of neurochemical system interactions (e.g. cholinergic-dopaminergic interactions). These studies are intended to provide a database for interpretation of psychoendocrine studies of stress, and for understanding the mechanisms by which traumatic factors decrement CNS function.

More than 40% of soldiers receiving penetrating missile injuries of the brain will subsequently develop post-traumatic epilepsy. Despite development of anticonvulsant drugs and improvements in aseptic surgical technique, the incidence of post-traumatic epilepsy has not decreased since World War I. Understanding the biochemical mechanisms occurring during the latent period between the injury and development of clinical manifestations of seizures is essential to developing rational preventive measures which might be initiated immediately post-injury.

Progress:

We hypothesized that pituitary cyclic AMP might link stress-induced hypothalamic release of neurotransmitters and releasing factors to observed neuroendocrine changes following stress, e.g. release of ACTH, prolactin or other pituitary hormones. We found that forced running, forced immobilization, or footshock elevated pituitary cyclic AMP. The response occurred in female as well as male rats, but some sex-related differences were seen in the pituitary cyclic AMP response, as well as in the corticosterone and growth hormone responses to stress. Levels of corticosterone rose more rapidly during stress in female rats vs male rats. After 5 min of stress, levels of corticosterone in male rats remained at control levels while corticosterone levels in female rats were maximally elevated. There also were sex differences in growth hormone levels. Mean growth hormone levels were much higher in male rats than female rats and were more sensitive to stress-induced growth hormone suppression. The pituitary cyclic AMP increase was was shown to be proportional to the

intensity of footshock, and a degree of habituation to repeated stress was demonstrated in the first of a series of studies of repeated stress.

Neurotransmitter receptor regulation is an important component in the development and plasticity of the central nervous system. Both the short- and long-term effects of exposure to stressors might be expected to be reflected in changes in receptors and these receptor changes could underlie the behavioral alterations that can result from stress. The discovery of benzodiazepine receptors in brain led us to study the effect of chronic stress on these receptors. Repeated exposure to footshock for 10 days does not appear to alter the number of benzodiazepine receptors in the frontal cortex, striatum, hippocampus, hypthalamus or midbrain.

We have succeeded in employing the "kindling" technique for producing epileptiform seizures. Kindling consists of repetitive, intermittent low intensity electrical stimulation of the amygdala. This results in progressive changes in electric activity and behavior over several weeks, and cluminates in a generalized seizure in response to an electrical stimulus which initially had produced no effect. Kindling seems a particularly good model of post-trauamatic epilepsy because it permits biochemical study of seizure-prone brain tissue without requiring the use of seizure-inducing drugs. Moreover, the latent period seen in the kindling phenomenon is similar to the delay of seizure onset seen in post-traumatic epilepsy. Kindling induced marked increases of TRH in the amygdala, hippocampus, nucleus accumbens, and a 4-fold increase in cortex. No significant changes were observed in the corpus striatum, thalamus, midbrain, brainstem, cerebellum, hypothalamus, or pituitary. These results indicate that kindling can induce significant and prolonged elevations of TRH in specific brain regions after seizures, and that TRH increases occur in regions associated with epileptic foci.

Future Objectives:

We plan to continue to study the mechanism of the pituitary cyclic AMP and other neuroendocrine and neuropeptide responses to stress. Pharmacological experiments will include opioid antagonists as well as adrenergic, dopaminergic, serotonergic purinergic and cholinergic blockers. Endocrine manipulations such as adrenalectomy, adrenal medullectomy and injections of dexamethasone will be employed. We will expose rats pretreated with IBMX (a cyclic nucleotide potentiator) to stressors to determine if release of any particular hormone is augmented. Lesion studies (i.e. hippocampal, amygdalar, septal lesions, etc.) will be initiated to elucidate mechanism of pituitary cyclic AMP response. We also plan to study the interaction between stress responses and pre-treatment with agents related to chemical defense, such as atropine and carbamate cholinesterase inhibitors. We plan to establish the capability to assay noradrenergic receptors in brain, and to study this receptor in stressed animals, effects of hormones on release of neurotransmitters, and effects of stress on glucocorticoid receptors. We plan to extend studies of sex differences in responses to acute and chronic stress.

We plan to continue studies of neurochemical mechanism of development of seizure disorders following trauma, extending neuropeptide studies to include neurotensin, somatostatin, enkephalin and others. Extend studies of novel pharmacologic interventions to prevent development of seizures. Continuation of these studies, however, will require authorization to recruit new investigators to compensate for slots relinquished to facilitate starting a new branch in the department.

Presentations:

1. Kant, G.J., Bunnell, B.N., Lenox, R.H., Pennington, L.L., Collins, D.R., Mougey, E.H., and Meyerhoff, J.L. "Stressors Elevate Pituitary Cyclic AMP in the Rat." Society for Neuroscience, Los Angeles, California (Neuroscience Abstract 1, 333, 1981).

2. Bunnell, B.N., Kant, G.J., Lenox, R.H., Pennington, L.L., Collins, D.R., Mougey, E.H., and Meyerhoff, J.L. "Pituitary cyclic AMP in rats is increased by psychological stress." Society for Neuroscience, Los Angeles, California (Neuroscience Abstract 1, 869, 1981).

3. Lenox, R.H., Kant, G.J., Meyerhoff, J.L., and Annau, Z. "Brain cyclic nucleotide response following central cholinergic activation in rat exposed and chronic level." Society for Neuroscience, Los Angeles, California (Neuroscience Abstract 1, 918, 1981).

4. Meyerhoff, J.L., "The effects of stress, locomotor activity and cholinergic agonists on brain and pituitary cyclic nucleotides." Dept. of Psychiatry Research Seminar. Uniformed Services University of the Health Sciences, Bethesda, Md. 17 March, 1982.

Publications:

1. Kant, G.J., Bates, V.E., Lenox, R.H. and Meyerhoff, J.L. Increases in Cyclic AMP levels in rat brain regions in vivo following isoproterenol. Biochemical Pharmacology 30, 3377-3380 (1981).

2. Kant, G.J., Sessions, G.R., Lenox, R.H., and Meyerhoff, J.L. The effects of Hormonal and Circadian Cycles, stress and activity on levels of cyclic AMP and cyclic GMP in pituitary, hypothalamus, pineal and cerebellum of female rats. Life Sciences 29, 2491-2499 (1981).

3. Meyerhoff, J.L., Kant, G.J., Session, G.R., Mougey, E.H., Pennington, L.L., and Lenox, E.H. Brain and Pituitary cyclic nucleotide response to stress. In: Perspectives in Behavioral Medicine, Vol 2, Williams, R.B., editor (Academic Press, New York, in press).

4. Lenox, R.H., Kant, G.J. and Meyerhoff, J.L. Rapid enzyme inactivation. In: Handbook of Neurochemistry, Vol 2, Lajtha, A., editor, in press.

5. Kant, G.J., Meyerhoff, J.L., Bunnell, B.N., and Lenox, R.H. Cyclic AMP and cyclic GMP response to stress in brain and pituitary: Stress elevate pituitary cyclic AMP. Pharmacology Biochemistry & Behavior, (in press, 1982).

6. Bates, V.E., Kant, G.J., Lenox, R.H., and Meyerhoff, J.L. Cyclic AMP levels in amygdaloid kindled rats. Experimental Neurology, 77:459-464 (1982).

7. Kant, G.J., Bates, V.E., Lenox, R.H., and Meyerhoff, J.L. Effects of acute and chronic desmethylimepramine on levels of cyclic AMP in vivo. Biochemical Pharmacology (in press).

8. Kant, G.J., Lenox, R.H., Bunnell, B.N., Mougey, E.H., Pennington, L.L. and Meyerhoff, J.L. Comparison of Stress Response in Male and Female Rats: Pituitary cyclic AMP and Plasma Prolactin, growth hormone and conticosterone. (Manuscript submitted.)

9. Meyerhoff, J.L., Kant, G.J., and Lenox, R.H. Effects of Cholinergic Agonists on Brain and Pituitary Cclic Nucleotides. In: W. Stavinoh, L. Blank, and Y. Maruyama (editors), Drug Effects on Rapidly Metabolized Compounds in CNS. Pergamon Press (in press).

10. Meyerhoff, J.L., Kant, G.J. and Lenox, R.H. Contribution of locomotor activity to changes in cerebellar cyclic GMP following administration of drugs. In: W. Stavinoha, L. Blank, and Y. Maruyama (editors), Drug Effects on Rapidly Metabolized Compounds in CNS. Pergamon Press (in press).

Publications:

1. Emurian, H.H., Brady, J.V., Meyerhoff, J.L., Mougey, E.H. Behavioral and biological interactions with confined microsocieties in a programmed environment. Grey, J. and Hamden, L.A. (Eds.) Space Manufacturing 4, N.Y. American Institute of Aeronautics and Astronautics, 1981, 407-421.

2. Belenky, G.L., Ruvio, B.A., and Holaday, J.W. Endotoxic shock is accompanied by a naloxone-sensitive increase in nociceptive latencies. Society for Neuroscience Abstracts 7(1981)798.

3. Belenky, G.L., Tortella, F.C., Hitzemann, R.J. and Holaday, J.W. The role of endorphin systems in the effects of single and repeated electroconvulsive shock. In B. Lerer (Ed.) Basic Mechanisms of Electroconvulsive Shock (in press).

4. Belenky, G.L., Cardenales-Ortiz, L., Robles, L, Arday, D., and Holaday, J.W. Amphetamine but not TRH disrupts performance on the radial arm maze. Society for Neuroscience Abstracts 105(1982)105.

5. Belenky, G.L., Newhouse, P., and Jones, F.D. The prevention and treatment of psychiatric casualties in the event of a war in Europe. International Review of the Army, Navy, and Air Force Medical Services 55(1982)303-307.

6. Belenky, G.L. and Jones, F.D. Contemporary Studies in Combat Psychiatry. American Psychiatric Association Abstracts of Annual Meeting (1982).

7. Belenky, G.L. OCONUS Trip Report - Visit to the Mental Health Department of the Israeli Defence Force - June-July 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6473	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CD	216
b. CONTRIBUTING				
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:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	4.0	356
FISCAL YEAR CURRENT 83	4.0	432

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Genser, LTC S.

NAME: Sing, H. C. POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Electrophysiology; (U) Psychophysiology; (U) Psychophysics; (U) Stress; (U) Performance; (U) Human Volunteer

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

23. (U) Objective is the development of non-invasive human psychophysiological monitoring technology in support of field studies of stress in military environments.

24. (U) Approach is to exploit advances in signal acquisition and processing technologies to enlarge the scope of psychophysiological measurements that can be made under field conditions. Techniques are validated in the laboratory prior to deployment in controlled field trials.

25. (U) 81 10 - 82 09 This work provides the technology base for Work Unit 043, Military Stress: Circadian and Ultradian Factors (Accession Number DA OC 6457).

Motor activity data collected over time unobtrusively on freely moving subjects using a wrist-worn self-contained solid state device (actigraph) is considered essential for the evaluation of the effectiveness of alternative rest-activity cycles in maintaining military performance. A four channel analog recorder (Medilogger) was interfaced with an accelerometer mounted in a wrist watch case and worn during a three week continuous operations deployment to Germany (Reforger) to provide the first objective ecological assessment of rest/activity patterns during a military operation. The microcomputer administered Performance Assessment Battery (PAB) has been exported for use in two studies of heat stress in helicopter pilots wearing Chemical Defense gear. In order to configure the PAB so that it is sensitive to the performance effects of a wide variety of stressors and contains a set of tasks that tap the range of neuropsychologically specifiable abilities, a vigilance/discrimination task specifying auditory and visual attention deployment has been developed. The computer based war game permitting the ongoing transparent monitoring of complex cognitive performance has been modified (using contract as well as in-house resources) to permit systematic variation in parameters associated with degree of difficulty and feelings of being under stress. Training modules have been used to derive associated learning curves for the various aspects of strategic and tactical performance. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sept 82.

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

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

Work Unit 216: Military Stress: Non-invasive Monitoring of Health and Performance

Investigators:

Principal: Frederick W. Hegge, Ph.D.

Associates: LTC Sander G. Genser, MC; LTC Daniel Redmond, MC; MAJ Steven Taube, MC; David Thorne, Ph.D.; Stanley Hall, B.A.; Helen Sing, M.S.

Problems and Objectives

This work unit provides the supportive technology base for Work Unit 043 Military Stress: Circadian and Ultradian Factors (Accession Number DA OC 6457) which is designed to address the central psychophysiologic problems of modern combat stress through laboratory and field studies. The technical goals are the exploitation, refinement and application of rapidly improving technologies applicable to physiologic data acquisition and performance assessment. Laboratory studies emphasizing both innovation in instrumentation and data processing techniques are coupled with field studies in military and appropriate civilian environments. The objective is to minimize the intrusion of research into and consequent interference with military operations that are the subject of study.

Progress

A four channel analog recorder (Medilogger) was interfaced with an accelerometer mounted in a wrist watch case to serve as a research actigraph system (RAS) and worn during a two-week continuous operations deployment to Germany (Reforger) to provide (1) the first objective ecological assessment of rest/activity patterns during a military operation designed around anticipated battle scenarios and (2) the first systematic look at artifacts and signal/noise ratios associated with the range of field activities (as vehicular travel) that will define the operating environment for a field deployable actigraph system. A comprehensive review and redesign of the self-contained wrist-mounted motor activity monitor (Actigraph) undertaken with the consultation of the George Washington University Electrical Engineering Department resulted in a proposal for a model with a linearly responsive signal-processing system.

The current Performance Assessment Battery (PAB) is exported for investigating performance deficits resulting from such diverse variables as altitude, heat stress, sleep disruption, chemical defense gear, sickle cell anemia, alcohol and helicopter crew rest schedules. The second generation PAB deletes two tasks shown to be insensitive, allows more precise control over visual stimulus presentation, reduces variability and error in reaction time measurements, provides exact control of randomization procedures, adds 3 new response measures plus a task duration measure, allows task termination to be determined by multiple criteria, makes feedback a selectable option, includes a number of self-checks and safeguards and provides a printout of summary statistics. This latter capability not only helps reduce the delays and costs of data analysis but also allows the experimenter to make informed decisions while an experiment is still in progress. The built in safeguards and other convenience features allows the battery to be used by relatively unsophisticated personnel. PAB version-2 has now been debugged, verified and tested on 18 subjects for approximately 400 runs. Other agencies wishing to use PAB-2 will be provided with documentation, a user's manual, analysis software and normative data, when they are completed.

A new Vigilance Discrimination task requires the subject to respond motorically to a specified stimulus presented randomly within a series of auditory and visual stimuli. The task is tailored to present stimuli as rapidly as the subject is capable of responding. Fatigue, in a 72-hour sleep deprivation study, had the greatest effect upon errors of commission which appeared to have increased linearly with time. Errors of omission reached an early plateau and did not increase until the extremes of fatigue.

STAR, the transparent performance assessment game developed with contract and in-house resources, has been refined, installed and is currently playable on departmental microcomputers. Concurrent with in-house refinement of the game, modification is being undertaken (using contract as well as in-house resources) to permit systematic variation in parameters associated with degree of difficulty and feelings of being under stress. Training modules have been used to derive associated learning curves for the various aspects of strategic and tactical performance.

Future Objectives:

Output from the Medilogger based Actigraph transduction system will be applied in the laboratory and the field to establish the dynamic requirements for a miniature digital system and to perfect techniques for analysis of data of this type. The alternatives provided by the appearance of commercially available actigraph systems (not yet available) will be assessed in light of the aforementioned requirements and the costs and feasibility of proceeding with an internal design. The use of pattern recognition technology to specify actigraphic signatures of events critical to task performance will be explored.

PAB development will be directed towards (1) maximizing sensitivity to the performance effects of a wide variety of stressors and (2) incorporating a set of tasks that parsimoniously taps the range of neuropsychologically specifiable abilities. A database to focus on fatigue will be formed by pooling the Vigilance-Discrimination, Performance Assessment Battery, Lexical and Mood Activation Scale data.

STAR development will be directed towards (1) accumulation of a data base of individual variability in performance parameters, (2) multivariate validation of the current performance taxonomy and (3) consideration of modification for applicability to team performance assessment.

Presentations and Publications

1. Genser, S., Babkoff, H., Thorne, D., Sing, H. and Hegge, F. Cerebral Lateralization, Continuous Performance and Sleep Deprivation. WRAIR Quarterly Research Report, Vol. 3-3, Sep 82.

2. Redmond, D. The Actigraph: In-Process Review. Division of Neuropsychiatry, Walter Reed Army Institute of Research, August 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OB 6448	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. HEADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AG	217 WWMA
b. CONTRIBUTING				
c. XXXXXXXXX	S10G 80-7.2	2		

11. TITLE (Procede with Security Classification Code)

(U) Basic Pharmacological Studies

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012100 Organic Chemistry, 002600 Biology, 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 07	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 82	3.5	289
CURRENT FISCAL YEAR 83	6.0	454

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Provide KEY with Security Classification Code) (U) Malaria; (U) Schistosomiasis; (U) Leishmaniasis; (U) Trypanosomiasis; (U) Parasitology; (U) Medicinal Chemistry; (U) Pharmacology; (U) Toxicology

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

23. (U) To investigate basic chemical, biological, and pharmacological aspects in drug development for use against parasitic diseases of military importance.

24. (U) Chemicals are synthesized, characterized, and analyzed for study, culture systems and animal models of parasitic diseases are developed and used, drug delivery systems and animal models are developed, all to study the efficacy and toxicology of potential drugs. New diagnostic methods are investigated.

25. (U) 81 10-82 09 Preliminary studies are in progress to determine whether the anesthetized rat may be substituted for the anesthetized dog as an experimental model for comparing the cardiac interactions of mefloquine and propanolol. The pharmacological response to an intravenous dose of WR 6026, a candidate leishmanial agent, was studied in dogs. Results were suggestive of histamine release and an assay for plasma histamine was developed to test this hypothesis. Work continued on the development of a hydraulic valve to prevent reduction in bile flow in the canine bile collection model. Larger dogs and catheters with increased diameter have been found to decrease the reduction in bile flow. In addition, an extensive literature search and written summary of the effects of the nerve agent antidote, atropine, upon the cardiovascular system is near completion. A method utilizing commercially available electrodes to monitor the canine ECG during exercise has been developed. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81-30 Sep 82. This work unit has been changed by incorporation of old work unit #209, "Parasitic Diseases of Military Importance."

*Available to contractors upon originator's approval

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

Work Unit 217 Basic Pharmacological Studies

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
Associate: CPT D. Korte, Jr., Dr. H. Lowensohn, LTC B. Schuster, SP6 Norman Wright, MAJ J. von Bredow, Dr. L. Fleckenstein

1. Description.

The work undertaken this year was primarily in the form of methods development. Methods for evaluating the potential cardiac interaction of mefloquine and the antihypertensive agent, propranolol, for assessing the toxicity associated with the intravenous administration of WR 6026, for chronic cannulation of the bile duct in the dog and for determining the effects of chemical agent antidotes on cardiac energetics and exercise tolerance were in varying stages of development.

2. Progress.

Preliminary studies to determine whether the anesthetized rabbit may be substituted for the anesthetized dog as the experimental model for comparing the cardiac interactions of mefloquine and propranolol are in progress. A definitive answer is not available at this time. The pharmacological responses observed after intravenous administration of WR 6026, a candidate antileishmanial agent, are suggestive of histamine release. An assay for blood histamine is being developed so that this hypothesis may be tested. This assay has been shown to be linear for plasma histamine concentrations in the range, 1×10^{-8} to 1×10^{-4} gm/ml, a concentration historically associated with drug-induced histamine release. Work continued on developing a hydraulic valve and to prevent reduction in bile flow in the canine bile collection model. The occlusive cuff and retainer casing for the hydraulic piston are being manufactured commercially. The use of larger dogs and catheters of increased volume has considerably decreased the reduction in bile flow which had hindered model development. An extensive literature search and documentation of the effects of the nerve agent antidote, atropine, upon the cardiovascular system nears completion. It will be used to design a protocol to study the effects of atropine on cardiac energetics and exercise tolerance. An atraumatic method utilizing commercially available electrodes to monitor the canine

ECG has been shown to provide the fidelity required during rigorous exercise testing.

3. Future Work.

Once the appropriate experimental model is established, work will progress on determining the potential interaction of mefloquine with propranolol or primaquine on the electrophysiology of the heart. Studies to establish the reproducibility of the histamine assay are underway. Once the assay is validated, a study to determine whether intravenous administration of WR 6026 to the dog causes release of histamine will begin. Plans are to give intravenous infusions of sodium taurocholate, a promoter of bile secretion, to increase bile output during long-term (12 hrs) bile collections so that a quantitative study to define bile viscosity, osmolarity and ionic strength during prolonged collection period can be made. The ultimate goal of these studies is to develop a chronic unanesthetized canine model to evaluate the effect of the enterohepatic circulation on the pharmacokinetics of candidate drugs. With protocol approval work will begin on a study to determine the effect of atropine on cardiac energetics and exercise tolerance in the dog.

4. Publications.

Heiffer, M.D., Davidson, D.E. and Korte, D.W., Jr.: "Preclinical Testing" in Handbook of Experimental Pharmacology: Antimalarial Drugs, ed. by W. Peters and W.H.G. Richards. Springer-Verlag (in press).

Korte, D.W., Jr., Heiffer, M.J., Ellis, H.V. III, Hacker, M.P., Hong, C.B., Yaun, Y.D., Kintner, L.D. and Lee, C.C.: Chronic Toxicity/Carcinogenicity of Mefloquine HCl (WR 142,490 HCl) in Rats and Mice. Fed. Proc. 41(5):1715, 1982.

Korte, D.W., Jr., and Pamplin, C.L.: A Pharmacokinetic Approach to Selection of dosing Regimens for toxicity Studies: Teratological Evaluation of the Antimalarial, Halofantrine (WR 171,669), in the Rat. Abstract #110, First World Congress on Toxicology and Environmental Health, Washington, DC, May 1982.

Korte, D., Jr., Pamplin, C., Heiffer, M., Reno, F., Voelker, R., Alsakar, R., Trutter, J. and Hagan, W.: Subacute Canine Toxicity Studies with a New Antileishmanial Drug. Pharmacologist 24(3):236, 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6449	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AF	218 WWG7
b. CONTRIBUTING				
c. XXXXXXXX	Stog 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Immunological Mechanisms in Microbial Infections.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 003400 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 08	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2.0	351
FISCAL YEAR CURRENT 83	2.0	381

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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ASSOCIATE INVESTIGATORS
NAME: Gore, Rufus
NAME: Williams, Joseph POC: DA

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Immunity; (U) Antibodies; (U) Antigens; (U) Protozoa; (U) Immunoassays; (U) Animal Model; (U) Leishmania

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

23. (U) The objective of this work unit is to elucidate the mechanisms operative in the natural and artificial induction of immunity to a variety of parasitic infections of military importance. This includes the study of infections in model systems and the development of methodologies for the study of the immune reaction in humans for research as well as diagnostic evaluations.

24. (U) The approaches used for these studies involve the measurement of various parameters of disease and of the immune response to disease in both in vivo and in vitro experiments. A variety of different diseases are also studied.

25. (U) 81 10 - 82 09 Studies have been completed on the development of a mouse model to study visceral leishmaniasis. Twelve strains of inbred mice infected with L. donovani segregate into resistant and susceptible strains. Resistant mice have reduced parasite burdens, minimal hepatosplenomegaly and a capacity to mount CMI responses. Early resistance is known to be under the control of a single lsh gene, phenotypic expression of which is unknown. LK activated macrophages readily kill L. donovani in vitro, and current studies are being done on infected mice to determine whether susceptibility or resistance in vivo is related to a) the capacity of lymphocytes to produce LK and, b) the capacity of macrophages to respond to activation and tiggering signals and kill parasites. Correlation of in vivo and in vitro observations will be crucial to understanding immunoregulation and, ultimately, to control of this disease. Studies begun on immunization and protection of animals against visceral leishmaniasis include: parasite stage, attenuated versus killed, route of inoculation, numbers of organisms, requirement for adjuvants (including type). For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*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 65 (FOR ARMY USE) ARE OBSOLETE.

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

Work Unit 218 Immunological Mechanisms in Microbial Infections

Investigtors:

Principals: LTC Wayne T. Hockmeyer, MSC
LTC Charles N. Oster, MC

Associates: CPT Robert Crawford, MSC
Mr. William H. Hildreth
Mr. Joseph S. Williams
Mr. Rufus Gore
PFC David Wynn
SP4 David Walters

Problem and Objectives:

The study of leishmaniasis is hampered by the lack of a suitable animal model for visceral leishmaniasis that correlates with the pathogenesis of the human disease. The objective of this study is to find an animal model that can be used to elucidate immune mechanisms operative in visceral leishmaniasis. This includes the development of methodologies for the study of the immune reaction by measurement of various parameters of leishmaniasis by both *in vitro* and *in vivo* experiments.

Progress:

Leishmania donovani infections were studied in 12 strains of inbred mice. Early resistance (innate resistance) is controlled by a single autosomal Leishmanial (Lsh) gene. Acquired immunity or resistance developing later in the infection is controlled by a gene or genes with or close to chromosome 17. The phenotypic expression of these genetically controlled traits is not known. Of those parameters studied during the infection, resistance correlates most closely with maintenance of cell mediated immune response, particularly

lymphocyte blastogenesis and delayed type hypersensitivity. Resistant mice possess reduced parasite burdens and limited hepatosplenomegaly.

Inbred mice inoculated with L. tropica (NIH 173) in footpads segregated into resistant (A/J, C3H/HeJ, C3H/HeN, C3HeB/FeJ, C57BL/10J, C57BL/10ScN, C57BL/10ScCR, C57BL/6J, DBA/2J) and susceptible (BALB/cJ, C57L/J, NZW/N, P/J) strains. Resistance was characterized by footpad swelling of <2mm and resolution of lesion in 9-12 wks; susceptibility was characterized by footpad swelling of >4mm, necrosis of lesion, visceralization of infection to liver and spleen, and occasional death by wk 12. Analysis of antimicrobial activities of macrophages from these mice suggested a correlation between susceptibility to infection and lymphokine-induced intracellular destruction of L. tropica. Macrophages from susceptible mice failed to respond in vitro to lymphokines (LK) that induce intracellular killing of L. tropica amastigotes; macrophages from resistant strains of mice responded to lymphokines with 80-90% microbicidal activity. Visceralization of L. tropica in susceptible animals produced systemic disease similar to that described for L. donovani. To analyze whether defective macrophage response to lymphokines also affected resistance to systemic disease, we inoculated the same mouse strains intravenously with L. donovani (WR . 271) and assessed susceptibility by liver parasite burden at 8 weeks. These mice also segregated into resistant (<800 LD units) and susceptible (>800 LD units) strains. There was no correlation between susceptibility to L. tropica infection and susceptibility to L. donovani: mouse strains resistant to L. tropica infection and susceptible to L. donovani were C3H/HeJ, C3HeB/FeJ, and C57BL/6J; C57L/J were susceptible to L. tropica, but resistant to L. donovani. Although macrophage dysfunction in inbred mouse strains correlated with susceptibility to L. tropica, there was no apparent correlation between defective macrophage microbicidal activity and susceptibility to L. donovani. These observations support the contention that control of these two diseases may involve different immune mechanisms.

Recommendations:

Recent work spent on development of a murine model for visceral leishmaniasis and adaptation of an in vitro system to assess the ability of LK activated macrophages to kill L. donovani now allows us to draw the conclusions that immunologic control of visceral and cutaneous disease may in fact be quite different. Since we now know that there is no obvious intrinsic

macrophage defect for the killing of L. donovani in vitro, we will now try and determine whether the difference between resistant and susceptible animals is due to (1) suppression of LK production or (2) failure of macrophages from susceptible infected animals to respond to activation signals. Studies are also being initiated to develop an immunization model using attentuated promastigotes or amastigotes.

Formal Presentations:

1. Hockmeyer, W.T., A.H. Fortier, J.S. Williams, R.W. Gore, M.G. Pappas, and C.A. Nacy. 1982. Influence of macrophage effector activity on resistance of mice to leishmanial infection. Fifth International Congress of Parasitology, Toronto, Canada (August).

2. Hockmeyer, W.T., B.T. Wellde, J.S. William, D.H. Smith, P.A. Kager, P.H. Rees, and R.W. Gore. 1981. Serologic diagnosis of visceral leishmaniasis in Kenya: A comparison of complement fixation (CF) and micro Elisa techniques. Annual Meeting of Tropical Medicine and Hygiene Society, San Juan, Puerto Rico (November).

3. Pappas, M.G., P.B. McGreevy, R. Hajkowski, L.D. Hendricks, and W.T. Hockmeyer. 1982. Serodiagnosis of American cutaneous leishmaniasis by the indirect immunofluorescence test: Evaluation of promastigote and amastigote antigens. Fifth International Congress of Parasitology, Toronto, Canada (August).

4. Oster, C.N., L. Handy, and C.A. Nacy. 1981. Macrophage activation for intracellular killing of Leishmania tropica: Microbicidal activity requires several lymphocytes derived activation signals. Annual Meeting of Tropical Medicine and Hygiene Society, San Juan, Puerto Rico (November).

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1. Hockmeyer, W.T., P.A. Kager, P.H. Rees, and L.D. Hendricks. 1981. The culture of Leishmania donovani in Schneider's insect medium: its value in the diagnosis and management of patients with visceral leishmaniasis. Trans. Royal Soc. Trop. Med. Hyg. 75:861.

2. Kager, P.A., P.H. Rees, B.T. Wellde, W.T. Hockmeyer, and W.H. Lyerly. 1981. Allopurinol in the treatment of visceral leishmaniasis. Trans. Royal Soc. Trop. Med. Hyg. 75:556.

3. Petersen, E.A., F.A. Neva, C.N. Oser, and H.B.Diaz. 1982. Specific inhibition of lymphocyte-proliferation responses by adherent suppressor cells in diffuse cutaneous leishmaniasis. N. Engl. J. Med. 306:387-392.

4. Berman, J.D., J.D. Chulay, L.D. Hendrick, and C.N. Oster. 1982. Susceptibility of clinical sensitive and resistance Leishmania to pentavalent antimony in vitro. Am. J. Trop. Med. Hyg. 31:459-465.

5. Pappas, M.G., C.N. Oster, and C.A. Nacy. 1982. Intracellular destruction of Leishmania tropica by macrophages activated in vivo with Mycobacterium bovis strain BCG. In: Host defenses against intracellular pathogens, T.K. Eisenstein and H. Friedman Eds. (In press).

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7. Pappas, M.G., P.B. McGreevy, R. Hajkowski, L.D. Hendricks, C.N. Oster, and W.T. Hockmeyer. Evaluation of promastigote and amastigote antigens in the indirect fluorescent antibody test for American cutaneous leishmaniasis. (submitted, Am. J. Trop. Med. Hyg.).

8. Berman, J.D., J.D. Chulay, L.D. Hendricks, and C.N. Oster. 1981. Susceptibility of Leishmania from clinically sensitive and resistant lesions to pentavalent antimony in vitro. Intersci. Conf. Antimicrob. Agents Chemother. ABSTR 83.

9. Oster, C.N. and C.A. Nacy. 1981. Kinetics of macrophage activtion for microbicidal activity against Leishmania tropica. Intersci. Conf. Antimicrob. Agents Chemother. ABSTR 82.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DA OC 6480	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	EB	219
b. CONTRIBUTING				
c. ~~XXXXXXXX~~ COORDINATING	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:*

c. TYPE: d. AMOUNT:

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

18. RESOURCE ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	11.0	585
FISCAL YEAR CURRENT 83	7.0	497

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: Olenick, John G.

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Organophosphates; (U) Antidotes; (U) Acetylcholinesterase; (U) Active Sites

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 multi-faceted biochemical research on chemical agents in order to provide the military with a safe and effective prophylactic/therapeutic formulation against chemical agents. These include: the identification of the metabolites and degradation products of chemical agenta and antidotes; the determination of the pharmacokinetics, distribution, transport and metabolism of chemical and antidotal agents; the investigation of the effects of chemical agents and antidotes on enzyme catalysis, specifically on active sites of acetyl- and butyryl-cholinesterases.
24. (U) Analytical aspects of the research will utilize currently-in-use methodologies such as high performance liquid chromatography, gas chromatography and mass spectrometry; other analytical methodologies will be developed as required. Degradation products, as well as the stability, of quinuclidine and oxime nerve agent antidotes will be determined under various conditions of pH and temperature. The effects of organophosphates and nerve agent antidotes on biochemical mechanisms of cholinesterase inhibition and reactivation will be investigated to include a systematic comparison between butyryl- and acetyl-cholinesterases. The active site of cholinesterases will be characterized as a basis for design of protection.
25. (U) 81 10 - 82 09 The stability and degradative fate of HI-6 after exposure to various conditions of pH and temperature were determined. High performance liquid chromatography methodology was developed to perform these analyses. Studies underway are concerned with determining the pharmacokinetics and distribution of 14C-aprophen in various body organs of rats and guinea pigs. Screening of substrate and inhibitor specificities of butyryl- and acetyl-cholinesterases has revealed that inhibitors which preferentially suppress butyrylcholinesterase are frequently components of nerve agent antidotal formulations. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

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

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

WORK UNIT: 219 Biochemical Aspects of Medical Defense Against Chemical Agents

INVESTIGATORS:
Principal: Bhupendra P. Doctor, Ph.D.
Associates: Nesbitt D. Brown, M.S.; John G. Olenick, Ph.D.; Alan D. Wolfe, Ph.D.
Assistants: M. Judith Gemski, B.S.; R. Richard Gray, M.S.; SP4 Bobby Holmes; Leo Kazyak, B.S.; SP4 Margaret G. Stermer-Cox; PFC Jeff S. Verdier

The objective of this work unit is to conduct multifaceted biochemical research on chemical agents in order to provide the military with a safe and effective prophylactic/therapeutic formulation against chemical agents. These include: the identification of the metabolites and degradation products of chemical agents and antidotes; the determination of the pharmacokinetics, distribution, transport and metabolism of chemical and antidotal agents; the investigation of the effects of chemical agents and antidotes on enzyme catalysis, specifically on active sites of acetyl- and butyryl-cholinesterases. The following investigations were conducted:

1. Degradative fate of HI-6 in aqueous solutions at various pH's and temperatures.

2. Studies on inhibitors of butyrylcholinesterase.

3. A collaborative study on the effectiveness of a slow release preparation (microencapsulated) of lidocaine for application to wounds.

1. Degradative Fate of HI-6 in Aqueous Solutions at Various pH's and Temperatures.

HI-6 or 1-(2-hydroxyiminoethyl-pyridinium)-1-(4-carboxyamido-pyridinium) dimethyl either is a bispyridinium oxime that is under consideration for use as an antidote to certain types of organophosphorus poisoning. In formulation and under prolonged storage conditions in different climatic areas, the stability of this oxime will determine its potency and efficacy. In order to study the stability of this compound and its degradation products, a high performance liquid chromatographic (HPLC) method was developed. Experimental samples of HI-6, prepared in distilled water, dilute HCl and NaOH, were analyzed by HPLC. The degradative fate of HI-6 was determined at various time intervals and at various temperatures. Many substituted bispyridinium compounds were obtained as breakdown products in both acid and alkaline solutions. HI-6 was found to be exceedingly unstable at pH's greater than 5 and at temperatures exceeding 25°C. Of noteworthy significance in these stability studies was the observed rapid degradation of HI-6 at physiological conditions (pH 7.4 and 37°C). The degradative by-products are presently being identified by use of mass spectrometry.

2. Studies on Inhibitors of Butyrylcholinesterase.

Butyrylcholinesterase (BCE) is a ubiquitous enzyme of unknown physiological

function. It occurs in mammalian organs, in ganglia, and in blood, and its inhibitor and substrate patterns resemble those of acetylcholinesterase (ACE). Both enzymes possess many inhibitors in common, including organophosphate (OP) threat agents, but preferential inhibition of either enzyme frequently occurs. Among those inhibitors which preferentially suppress BCE activity in comparison with ACE are compounds which exert significant neuropharmacological effects, for example, the phenothiazines, and atropine. Since these compounds are frequently components of OP antidotal formulations, BCE possesses properties of unusual military medical significance. The present research on distinctions between substrate and inhibitor specificities among cholinesterases (CEs) has resulted in discovery of a new, potent BCE inhibitor. In addition, many compounds related to antidote formulation components have been tested, and results suggest that BCE inhibitor potency may be useful as a simple primary screen for candidate protective agents.

Compounds evaluated for their BCE inhibitory potency were selected upon the basis of four criteria: (1) inclusion in formulations designed to protect against OP threat agents, (2) relation to protective formulation constituents, (3) central nervous system influence, and (4) DNA intercalation. The latter criterion was included because phenothiazines are DNA intercalants. All screened phenothiazines, acridines, benzilates, and oximes were inhibitory. The most potent compound tested, however, was the thiaxanthone DNA intercalant, Miracil D. Inhibition was reversible, and Dixon-Webb kinetic analysis yielded a $K_i = 8.8 \times 10^{-8}$ M. Other potent inhibitors included the acridine, proflavine, ($ED_{50} = 3.7 \times 10^{-7}$ M), the phenothiazine, perphenazine, ($ED_{50} = 6.0 \times 10^{-7}$ M), and the benzilate, aprophen ($ED_{50} = 1.3 \times 10^{-6}$ M). Bis-pyridinium oximes were markedly more inhibitory than pyridinium-2-aldoxime hydrochloride. Antimalarials, among them the new phenanthrenemethanol, WR 122,455, inhibited BCE strongly. Results suggest that BCE inhibition is in part a function of molecular space filling properties.

Miracil D is a congener of hycanthone, an antischistosomal drug considered to act though suppression of nucleic acid synthesis. Schistosomal cholinesterases are more sensitive to hycanthone than are human and bovine erythrocyte ACEs. The present discovery invites further analysis of schistosomal CEs and neurotransmitters, but more importantly invites screening of additional thiaxanthones in the hope of identifying other potent BCE inhibitors. BCE inhibitor utility is suggested by the observations that a phenothiazine: (1) protected BCE from OP inactivation, and (2) increased the reactivation rate of diethylphosphoryl-ACE five fold. Formulations which protect against OPs typically contain oximes, tropic acid esters, and benzilic acid esters or phenothiazines. Literature reports and current research have shown these compounds to exert moderate to severe BCE inhibition, and therefore suggest the potential use of BCE as a primary screen for protective agents against OPs.

3. Effectiveness of Microencapsulated Lidocaine for Application to Wounds.

In collaboration with the Biochemistry Section of USAIDR, experiments were conducted to determine the effectiveness of a slow release microencapsulated preparation of lidocaine (xylocaine hydrochloride) for application to wounds, especially facial wounds that result from high velocity bullets. Mass spectrometry analytical assistance was provided in this study.

Preparations of lidocaine (solutions and microencapsulated form) were administered to rabbits i.m. (through an incision) to determine the extent of absorption of the drug and to correlate the distribution of the solution with that of the microencapsulated drug. Analyses of rabbit sera for lidocaine concentrations were performed by gas-chromatography/mass spectrometry. The data indicated clearly that the microencapsulated lidocaine produced levels in the rabbit serum comparable to a 0.5% solution of lidocaine administered i.m. However, this concentration was not adequate to induce sufficient insensitivity to pain. The metabolites of lidocaine (monoethylglicinexylidide and glycine-xylidide) that have been reported in human serum after infusions of the drug into humans were not detected in the rabbit sera.

PROJECTED STUDIES:

Studies will continue on analytical and on enzymological aspects of medical defense against chemical agents. Specifically, these studies will include:

1. Continuation of studies on the determination of degradation products of HI-6 and of metabolites of aprophen.

2. Isolation and purification of acetyl- and butyryl-cholinesterases from various sources including bacteria.

3. Use of highly purified enzymes to study substrate kinetics and inhibitor patterns and to characterize the inhibitory action exhibited by organophosphates and other compounds. It is intended that structure-activity relationships will be revealed by these investigations.

4. A determination of the structure and physiological function of butyryl- and carboxyl-cholinesterases. The structural relatedness and homology of the active site will be studied.

PUBLICATIONS:

1. Brown, N.D., Poon, B.T. and Chulay, J.D. 1982. Determination of chloroquine and its de-ethylated metabolites in human plasma by ion-pair high performance liquid chromatography. J. Chromatogr. 229: 248-254.

2. Brown, N.D., Strickler, M.P. and Whaun, J.M. 1982. A femotomolar ion-pair high performance liquid chromatographic method for determining dansylated polyamine derivatives of red blood cell extracts utilizing an automated polyamine analyzer. J. Chromatogr. (in Press)

3. Wolfe, A.D., Emery, C.E., Verdier, J.S. and Prichard, D.A. June 1982. Studies on butyrylcholinesterase inhibitors. Army Science Conference, West Point, NY.

4. Wolfe, A.D. 1982. Mefloquine, a review. In Antibiotics, Vol. V. Springer-Verlag, Berlin-Heidelberg-New York.

5. Emery, C.E., Stancato, F.A., Brown, R.E., Prichard, D.A. and Wolfe, A.D. 1982. Arylmethanol and thiosemicarbazone influence on plasmodial macromolecular synthesis and cell stability. Am. J. Trop. Med. Hyg.

ABSTRACTS AND PRESENTATIONS:

1. Wolfe, A.D., Emery, C.E., Verdier, J.S. and Prichard, D.A. Studies on butyrylcholinesterase inhibitors. Army Science Conf. June 1982.

2. Emery, C.E., Stancato, F.A., Brown, R.E., Prichard, D.A. and Wolfe, A.D. Some biological properties of selected arylmethanols, thiosemicarbazones, and thiaxanthonones. 5th Int. Congr. of Parasit. Aug 1982.

3. Verdier, J.S., Emery, C.E., Prichard, D. A. and Wolfe, A.D. Partial purification and inhibition of selected cholinesterases. F.A.S.E.B. Apr 1982.

4. Whaun, J.M. and Brown, N.D. Effects of two ornithine decarboxylase (ODC) inhibitors on polyamine synthesis in Plasmodium falciparum. Gordon Research Conference. July 1982.

5. Whaun, J.M. and Brown, N.D. Polyamine inhibition and the malaria infected red cell: A model for studying polyamine metabolism and cell growth. 5th Int. Congr. of Parasit. Aug 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6464	82 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'S	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BD	220 WWI4
b. XXXXXXXXX				
c. XXXXXXXXX	STOG 80-7.215			

11. TITLE (Procede with Security Classification Code)

(u) Pathogenesis of Renal Disease of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012900 Physiology 003500 Clinical Medicine 016200 Stress Physiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	9.0	669
	CURRENT 83	9.0	759

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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NAME: DUARTE, LTC MC C.

NAME: WIESMANN, MAJ MC W.P. POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Renal Failure; (U) Renal Hemodynamics; (U) Heat Stress (U) Shock; (U) Fluid and Solute Hemeostasis; (U) Dialysis; (U) Kidney Function

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

23. (U) To investigate mechanisms for maintaining fluid, electrolyte and hemodynamic homeostasis in response to disease, injury and environmental stress of military significance such as shock, infectious disease, heat stress, gastrointestinal disorders and nephrotoxic drugs in order to provide rational bases for prevention and treatment of renal failure.

24. (U) Clearance methods, micropuncture, membrane transport, tissue culture, radioimmunoassay, enzyme kenetics, isotope dilution, chromatography, and dialysis.

25. (U) 81 10-82 09: A model of acute renal failure induced by combined hemorrhagic hypotension and aortic clamping has been developed within the department which reproduces the clinical and pathologic spectrum of clinical ischemic ARF in man. Hormonal balance studies during the initiation phase of this model suggest a role for endogenous renal catecholamines and prostaglandins in the genesis of renal injury in response to shock. Manipulation of prostaglandin production during the initiation phase of a toxic (gentamicin) ARF indicates a role for these agents in this model as well. Prostaglandin inhibition markedly enhances gentamicin ARF. ARF secondary to cis-platinum toxicity has been shown to be partially ameliorated by the use of sulfhydryl agents at doses compatible with use in man. Two lines of urinary epithelial cells, derived from bladder and kidney, have been characterized in terms of their basal and hormone sensitive transport. Transport has been demonstrated to be sensitive primarily to manipulations of oxidative metabolism in these systems. Studies in uremic RBC have demonstrated high levels of deoxy purine nucleotides which inhibit lymphocyte blastogenesis in vitro. This defect is corrected by use of bypass enzymes and may relate to the impaired immunity seen in uremia. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*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 69 (FOR ARMY USE) ARE OBSOLETE

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

Work Unit 220: Pathogenesis of Renal Disease of Military Importance

Investigators:

Principal:	LTC John P. Johnson, MC
Associates:	COL Donald E. Butkus, MC
	LTC Cristobal G. Duarte, MC
	Mr. John A. Gagnon
	Mr. James S. McNeil
	MAJ (P) William P. Wiesmann, MC

Problems and Objectives

Acute renal failure in combat casualties represents the major renal disease of military importance. Renal injury associated with environmental or toxic stress also represent real or potential sources of military concern. Renal injury may result directly from traumatic insult or accompanying shock or secondarily from environmental insults or from complications of therapy for combat casualties. In either case, mortality and morbidity persist at high levels despite improved methods of patient evaluation, treatment and support (5). Therefore, the major objectives of this study are to delineate those factors of importance in initiating and maintaining renal failure so that measures may be designed to 1) initiate therapy to prevent acute renal failure or reverse early functional injury before cell death and necrosis occur; 2) hasten recovery of renal function if renal failure is established; and 3) reduce mortality from complications of uremia. The major effort of the department has been the development and elucidation of animal models of acute renal failure which resemble the clinical and pathologic spectrum in combat casualties.

Progress

In the past, the major emphasis in studies of acute renal failure has been in the examination of initiating events. Many of these studies have been carried out in easily produced toxic heavy metal models or phamacalogically mediated ischemic models which have little direct relevance to ARF in man. The theoretical basis of such observations has been the notion that renal failure produced by any method will have a final common pathway in terms or pathogentic mechanisms. Speculations based on such observations have produced internally consistent theories primarily revolving about initial vasomotor changes, induced neurally or hormonally,

which mediate decline in GFR. Proposed effectors have included the renin-angiotensin system, the tubulo-glomerular feedback mechanism, participation of kinin or prostaglandin cascades etc. Such theories have proved incapable of explaining the spectrum of renal failure for the following reasons: 1) Numerous lines of evidence suggest that renal vascular resistance and blood flow in the stress state are modulated by the balance of effects of a number of interrelated vasoactive factors poorly studied in the pharmacologically manipulated animal. 2) The maintainance phase of acute renal failure is associated with re-establishment of renal circulation so that the course is most likely determined by metabolic cell damage associated with the initial insult and/or the initial renovascular responses. While the course of acute renal failure may be influenced in its initiation, it is likely that most ARF in combat casualities will be recognized during its mainainance phase. The efforts of the department this year have been devoted to elucidating the course primarily of two models of ARF designed to resemble that seen in man: 1) hemorrhagic hypotension as an ischemic model and 2) gentamicin nephrotoxicity as a toxic model. We have developed or acquired the abilities to examine effectors and therapeutic modulation of these events at all levels of renal function.

Studies in an Ischemic Model of Renal Failure

Extensive studies to date have failed to determine the mechanisms responsible for ischemic acute renal failure (ARF) in man. This may be related to the fact that a model of ARF, strictly comparable to the human counterpart, has yet to be developed. Neither the hemorrhagic hypotension nor renal artery constriction model are suitable paradigms for the study of ischemic acute renal failure (ARF) in the combat casualty. Hemorrhagic hypotension, as produced in the laboratory, while quite analogous to the clinical syndrome, does not provide for selective injury to the kidney in amounts capable of producing severe renal dysfunction without regularly causing the death of the animal acutely. Conversely, renal artery constriction while not as analogous to the clinical syndrome, does induce renal failure without causing the death of the animal. However, renal artery constriction fails to elicit the humoral and neurogenic mechanisms which undoubtedly mediate, at least in part, the vaso - constriction seen in ARF. Moreover, complete lack of renal blood flow prohibits the delineation of the physiologic and hormonal sequence of events leading to renal dysfunction, and may by-pass the usual constrictor mechanisms.

Therefore, a study was designed to develop a model of ARF in the dog which would circumvent the above problems(18,29). To maximize renal injury and yet establish a setting which would allow for the study of events which may contribute to the renal injury and yet not cause the acute death of the animal, the combined insult of hemorrhagic hypotension and supra-renal aortic constriction was induced in a group of 17 anesthetized animals.

Briefly, one group of eight animals (control) were subjected to general

anesthesia and unilateral nephrectomy, after which renal function and systemic hemodynamic functions were monitored for 3 hours. A second group of nine animals (experimental) were anesthetized, subjected to unilateral nephrectomy, followed by 30 ml/kg hemorrhage and simultaneous supra-renal aortic constriction of 3 hrs duration. Subsequently, the constriction was released and the shed blood reinfused. Renal function was monitored for seven days after which the animals were sacrificed and the kidneys examined microscopically.

Four of the nine animals were substantially azotemic seven days after insult. Although the plasma creatinine ranged from 1.1 - 20.5 mg % (5.1 ± 2.2) in nine animals, in the four azotemic animals (BUN 42 - 255 mg %) the plasma creatinine ranged from 3.1 - 20.5 mg % (9.8 ± 4.1). Measurements of the vasoactive hormones during the ischemic insult and seven days later demonstrated initial elevations in plasma renin activity, norepinephrine and PGE^2 without significant alterations in plasma bradykinin. There was no direct correlation noted between the elevations in these substances and the development of ARF. Pathologic examination of kidney tissue removed from the azotemic animals demonstrated focal areas of ischemic necrosis, tubular dilatation and proteinaceous debris occluding tubular lumina. These lesions were characteristic of those seen in acute tubular necrosis in man.

We have thus demonstrated that by utilizing a model designed to selectively injure the kidney, yet allow systemic neurogenic and humoral perturbations characteristic of severe ischemia to remain operative upon the kidney, we can induce ARF similar to that seen in man in approximately 45% of animals at risk. To increase the incidence and/or severity we propose to modify this model by exposing the animals to hemorrhagic hypotension and supra-renal aortic constriction under conditions of arterial hypoxemia which is known to increase renal vascular resistance.

Studies in Anaesthetic Effects on Renal Function

Because of the central role of the kidney in maintaining sodium balance and the frequent use of anesthetics when studying mechanisms of acute renal failure we undertook to examine the influence of thiopental anesthesia on renal tubular sodium reabsorption in the dog (9).

Following administration of the anesthetic renal sodium reabsorption was depressed leading to enhanced excretion of sodium and water. Associated with this response was a decrease in the plasma levels of norepinephrine and epinephrine. Neither renal hemodynamic functions nor the humoral factors, prostaglandin E, plasma renin or arginine vasopressin, appeared to be major determinants fo the natriuresis.

These observations suggest that the administration of thiopental depresses renal sympathetic nerve activity diminishing the renal tubular transport of sodium.

Development of Assay for Vasopressin

A sensitive radioimmunoassay for plasma arginine vasopressin (AVP) was developed within the Division of Medicine for studies of the role of vasopressin in acute renal failure, maintenance of blood pressure and glomerular filtration during shock and in the response to various stress encountered in the military environment.

As a preliminary to testing plasma samples in the experimental situation we are presently examining the responsiveness of plasma AVP, using our assay, to changes in plasma osmolality and intravascular volume induced by water loading, and furosemide administration. Additionally the effect of prostaglandin inhibition on the renal response to vasopressin is also being investigated as AVP has been shown to stimulate renal PGE production.

Preliminary results suggest that the vasopressin assay appears to be sufficiently sensitive to use as a tool for further investigation of the role of vasopressin in disorders of water metabolism hemodynamic and stress.

Studies in Gentimicin Induced Acute Renal Failure

As a model for toxic acute renal failure to study analagously with ischemic acute renal failure, the department has examined gentimicin nephrotoxicity (12). This model may be expected to resemble the renal failure occurring in combat casualities as a secondary consequence of treatment for infectious complications of initiations. Moreover, it will enable us to examine to what extent the intuitions gained from study of ischemic renal failure may be generalized to the entire spectrum seen in man. The course of renal failure following gentamicin administration has been described in an easily produced rat model from initiation through recovery. We have demonstrated biphasic effects on renal concentratry ability, GFR and the levels of such vasoactive agents as renin and prostaglandins. Initial high levels of urinary prostaglandins are associated with a fall in urinary concentrating ability and maintainance of GFR in early induction phase. As prostaglandins fall, plasma renin increases commensurate with a fall in GFR. Altering the balance of vasoactive factors early in this model changes the course of ARF. Inhibition of prostaglandin synthesis markedly enhances renal failure strongly suggesting that vasodilating prostaglandins have an initial balancing or protective effect and that vaosmotor factors are equally important in the initiation phase of toxic as well as ischemic ARF. The past of reproducibility of this model make it a useful one for the study of metabolic events during maintainance ARF as well. Studies are being initiated to evaluate cellular metabolism and relate it to GFR in this model.

Cis-Platinum Nephrotoxicity

Limited studies have been conducted to define a mechanism to prevent the acute nephrotoxicity associated with cis-platinum administration. This represents practically the only significant heavy metal renal failure likely to be seen with any frequency in man. An acute cis-platinum model has been developed and we have demonstrated modification of renal failure by sulfhydryl agents with some apparent specificity: Dimercaptopropane sulfonic acid> dithiotreitol> disulfuram. Correlations are underway with tissue and urinary platinum levels to determine whether this effect represents simple chelation or a protective effect at the membrane level.

Effects of Electrolyte Disorders on Renal Function

Environmental exposure, potential dietary and water deprivation by the combat soldier or casualties may be expected to produced metabolic alterations which may modify their response to renal injury. This year the department has concluded a prolonged metabolic study in alterations of potassium, magnesium and calcium levels on whole animal homeostasis and renal function (7,8,21,28). These studies have demonstrated profound effects of potassium depletion on plasma volume and disposition of major intracellular cations throughout body tissues. The intent of these studies has been to produce and describe physiologically induced abnormalities which may be applied to models of acute renal failure.

Altered Immunity in Renal Failure

Since infection is a major cause of death in acute renal failure, the department has undertaken pilot studies on potential immune defects in uremia (14). Initial observations suggest a disorder in the enzyme adenosine deaminase which results in the accumulation of toxic purine analogs. High levels of these analogs produce, in vitro, similar abnormalities in lymphocyte blastogenesis to those seen in uremia. Moreover, incubation of uremia lymphocytes with bypass enzymes lowers the levels of those toxic byproducts and reverses the lymphocyte defect. Studies we currently underway to examine the development of this disordered purine metabolism during the course of acute renal failure in our animal models.

Studies on Cultured Epithelial Cells

A major effort in the department has been to develop methods of study for cultured epithelial cells and to characterize their functional capacities and suitability as model systems for cellular events related to ischemia or toxic injury. Work has established that cell lines from urinary bladder and kidney demonstrate hormone sensitive transepithelial transport and possess responsive enzyme systems analagous to kidney (10). Characterization of cellular production of vasoactive kallekrein and kinin generation is underway

levels and turnover rates, krebs cyclic enzyme levels) has been undertaken.

Future Plans and Recommendations

During the past year, the department has advanced its knowledge of model systems and controlling conditions with a view to a fully integrated approach to the pathogenesis and modification of the course of acute renal failure. Two new investigators have been acquired and will join the department during fiscal 83. They will provide expertise in isolated tubule and single cell electrophysiology. Studies are designed to examine the role of vasoactive substances and metabolic imbalance in the initiation of ischemic and toxic acute renal failure in whole animals models. Serum and kidney slices from these animals or others run in parallel will be used to examine the development (and potentially modification) of metabolic abnormalities leading to cell death and maintainance renal failure. Accessible serum or urinary markers of renal failure will be sought. Isolated tubules and cultured cells will be used to examine the effects of toxic or anoxic stimuli on cellular function and their reversibility.

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22. Duarte, C.G., Old, C.W., Siedlecki, M., Phillips, R. and Bray, A. Effects of Desoxycorticosterone Acetate (DOCA) on the Hypermagnesemia of K Depletion. Kidney Int. 21:131, 1982.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6753	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. HEDNADING	8a. ONE'N INSTN'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	EA	221 WWJ4
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7.2.1			

11. TITLE (Precede with Security Classification Code)

(U) Neural Mechanisms of Chemical Defense-Related Compounds

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 012600 Pharmacology 012900 Physiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2.0	376
FISCAL YEAR CURRENT 83	2.0	433

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Meyerhoff, J.L., M.D. POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Chemical Defense; (U) Chemical Interactions; (U) Sensory-Motor Processing; (U) Experimental Neuropathology

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

23. (U) Investigations are directed at understanding the effects on nervous system function of chemical defense-related compounds, extrinsic and intrinsic. These studies have both direct and indirect military relevance.

24. (U) Animal experiments are based on anatomic methods for locating critical sites of agent/antidote action; on pharmacologic and biochemical methods for elucidating interactions between agents/antidotes and the body's chemistry; and on physiologic methods for studying the effects of agents/antidotes on neural signal processing.

25. (U) 81 10-82 09 A continuation of work on the neuropathological effects of organophosphate agents on rats reveals similar patterns of neuron death and nerve fiber disruption in cats and rhesus monkeys. Again, selective areas and pathways in the brain are involved. The cerebrospinal fluid of surviving Soman-poisoned animals has been found to contain markedly elevated levels of potassium and tissue enzymes usually used as indicators of tissue damage. DFP and picrotoxin increase the size of receptive fields in the somatosensory cortex of cats, an effect reversed by atropine. Ketamine has been found to have a protective effect equivalent to atropine. The influence of various factors, e.g., environmental temperature, exercise, dehydration, etc. on DFP toxicity are being studied. Exercise appears to have no effect, while dehydration makes rats more susceptible to DFP. DFP inhibits spontaneous dopamine release from certain brain tissues. Chemical defense-related compounds, e.g., neostigmine, are found to produce elevated plasma beta-endorphin levels. Studies on the possible protective effects of naloxone and TRH have been initiated. Extensive data on the hemodynamic effects of DFP have been gathered. For technical report, see the Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

* Available to contractors upon originator's approval.

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

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

Work Unit 221 Neural Mechanisms of Chemical Defense-Related Compounds

Investigators:
Principal: C.B.G. Campbell, M.D., Ph.D., LTC, MC
Associate: C.F. Tyner, M.D., COL, MC; J.L. Meyerhoff, M.D.; J.M. Petras, Ph.D.; G.J. Kant, Ph.D.

Objectives:

The overall objectives of this task are the protection of military personnel from the lethal effects of chemical agents, elimination or reduction of impaired performance, and return to duty of those individuals exposed to sublethal doses of these agents. Emphasis has been placed on determining the sites of action of chemical defense-related compounds, the primary and secondary effects of these agents and their antidotes on nervous tissue, and exploring the possibility of exploiting the intrinsic defense mechanisms of the body as protection against these agents.

Progress:

Last year it was shown that rats with neurological signs, but not necessarily seizures, that survived exposure to soman had extensive areas of neuronal degeneration in patterns consistent from animal to animal. A similar phenomenon has now been found in cats and cynomolgus monkeys. Sixteen cats were exposed to soman in doses ranging from 8.25-13.5 μg/kg. Of the nine survivors, two with seizures and one with no neurological signs had neuronal degeneration in the cerebral cortex, basal ganglia, diencephalon and midbrain. Eight monkeys were injected with soman and four of the six survivors showed neurological signs. All monkeys with neurological signs have neuronal degeneration.

The organophosphate DFP and picrotoxin both increase the size of receptive fields in the somatosensory cortex of cats. The effect is reversed by atropine. Ketamine has been found to have a protective effect essentially equivalent to atropine. Exposure of animals to organosphosphates produces weight loss, apparently a good indicator of the degree of their illness. Some animals are found to recover their weight slowly in spite of a rapid recovery of their ingestive behavior. The possibility of unknown metabolic effects is being examined. The influence of various factors on DFP toxicity are being studied, e.g. environmental temperature, exercise, dehydration, etc. Exercise appears to have no effect, while dehydrated rats are more susceptible to DFP. The cerebrospinal fluid of Soman-poisoned animals that survive the exposure have been found to contain markedly elevated levels of potassium and tissue enzymes usually used as indicators of tissue damage.

DFP, physostigmine, mecamylamine, oxotremorine, and nicotine were tested for effects on the neurotransmitter dopamine. DFP strongly inhibited both spontaneous and potassium-stimulated dopamine release. Oxotremorine decreased and nicotine increased potassium-stimulated release, while nicotine increased spontaneous release as well. It was previously found that survival was increased in rats injected with soman at 2200 hours when compared with rats injected at 1000. Since one possible explanation is that cholinergic receptors undergo circadian rhythms in which more receptors are present to be overstimulated at 1000 than at 2200, muscarinic receptor binding was assayed in the striatum, hippocampus and cortex of groups of rats sacrificed at 1000 and 2200. A 25% increase in the number of striatum muscarinic receptors was found, but no difference in those of the cortex and hippocampus. Atropine sulfate was observed to double the cyclic AMP release by the pituitary after footshock. It also appears to lower pain threshold and increase the cyclic AMP response to forced running, fear and immobilization. A series of studies on the effects of cholinergic drugs on patterns of brain cyclic nucleotide response was begun. These studies are still in progress.

Recommendations for Future Work:

It is planned to contrast sarin with soman in regard to possible neuronal degenerative changes following exposure. Since rats appear to respond in a similar manner to cats and monkeys, rats will be exposed to sarin and their brain tissues examined with routine stains and the experimental silver methods. A correlative light and electron microscopic study of the neuropathogenesis of soman toxicity in the rat is also planned. Research on alterations in the composition of the cerebrospinal fluid following organophosphate exposure as a tool for monitoring CNS damage in patients will continue. Neurochemical studies on the effects of organophosphates on cyclic nucleotides and dopamine, as well as on beta endorphins and endorphin antagonists will be pursued.

Presentations Made

Mougey, E.H., Meyerhoff, J.L. Effect of cholinomimetics and cholinesterase inhibitors on plasma beta endorphin. Society for Neuroscience, 11th Annual Meeting, Los Angeles, CA, Oct 1981.

Petras, J.M. Neurotoxic effects of soman in the cat: a comparison with the rat. USAMRDC Second Bioscience Review, Edgewood, MD, May, 1982.

Publications

Meyerhoff, J.L., Kant, G.J., and Lenox, R.H. Effects of cholinergic agonists on brain and pituitary cyclic nucleotides. In: W. Stavinoha, L. Blank, and Y. Maruyama (editors), Drug Effects on Rapidly Metabolized Compounds in CNS. Pergamon Press (in press).

Meyerhoff, J.L., Kant, G.J., and Lenox, R.H. Contribution of locomotor activity to changes in cerebellar cyclic GMP following administration of drugs. In; W. Stavinoha, L. Blank, and Y. Maruyama (editors), Drug Effects on Rapidly Metabolized Compounds in CNS. Pergamon Press (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6466	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. RSRCH OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DOE'R INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	X ☐ YES ☐ NO	A. WORK UNIT

10. RQ./CODEE:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AI	222 WWP2
b. CONTRIBUTING				
c. XXXXXXXXX ING	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Histopathologic Manifestation of military Diseases and Injuries

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
63 08	Cont	DA	C. In house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	5.0	324
	CURRENT 83	5.0	408

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Roy, Michael, M., Ph.D.

NAME: POC: DA

21. GENERAL USE

Foreign intelligence 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 manifestation of injuries and diseases which have current or potential problems in military personnel. The current efforts are 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 inlcuding histology, histo- and cytochemistry, autoradiography, immunofluorescent microscopy, transmission and scanning electron microscopy are employed. Various immunologic techniques have also been utilized. 25(U) 81 10 - 82 09. The response of mononuclear cells to intraperitoneal infection with Rickettsia tsutsugamushi was defined by scanning electron microscopy. Following sloughing of the infected peritoneal mesothelial cells, mononuclear cells, probably macrophages, migrated in to the denuded areas and appeared to initiate the healing process. The characterisitcs of lymphocytes in the gut lamina propria (GLP) of mice were further defined. In addition to IgM- and IgG-containing B cells, T cells with a variety of functions were identified in the GLP. Expression of immunoglobulins by B cells and the immunoregulatory function of T cells in the Peyer's patches of the gut were further resolved. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

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

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

WORK UNIT: 222 Histopathologic Manifestation of Military Disease and Injuries

INVESTIGATORS:

Principal: Jeenan Tseng, Ph.D
Associate: Michael B. Roy, Ph.D

Problem and Objectives:

The long-term goal of our research is to understand the immunopathological reactions of injuries and diseases occurring in military personnel. To approach these problems, efforts have been directed toward experimental models in animals, which mimic the actual events occurring in humans. Current studies focus on the immunopathological responses of the digestive tract to microbial infections.

A striking feature of the intestinal lamina propria is the preponderant presence of immunoglobulin A (IgA) producing cells. IgA is the substance that plays the major protective role against infectious agents and foreign antigens. The cellular mechanism by which the IgA producing cells become preponderant in the intestinal lamina propria is completely unknown. Because of the fact that the IgA producing cells are derived from precursors in Peyer's patches through migration and that differentiation and maturation of Ig producing cells are regulated by thymus-derived lymphocytes (T cells), it can be suspected that the IgA preponderance in the gut might be due to migration, accumulation, or differentiation of Peyer's patch IgA precursors in the gut lamina propria or that T cells specific for the differentiation and maturation of IgA cells are preponderantly present in Peyer's patches and/or gut lamina propria. To study these possibilities, IgA allotype suppression was induced in mice, a lymphocyte culture system was developed to define the repertoire of Ig producing cell precursors in a lymphoid tissue, and lymphoid cells from the gut lamina propria were isolated to define immunoglobulin expressing capabilities.

Progress:

I. IgA allotype suppression:

Two types of allotype suppression, chronic and acute, were induced in animals. While acute allotype suppression is the best demonstration of acute Ig deficiency due to a temporary depletion of the Ig precursors, chronic allotype suppression is the best for demonstration of the presence of Ig - class specific T cells which regulate the differentiation of Ig producing cells. We tried to induce IgA allotype suppression in mice because a chronic IgA allotype suppression would clearly demonstrate that IgA preponderance in the gut is due to the predominant presence of IgA - specific T cells in gut lamina propria for Peyer's patches. The IgA allotype suppression induced was all of the acute type. The suppression was due to a depletion of IgA precursors in Peyer's patches. T cells isolated from the gut lamina propria and Peyer's patches of suppressed and normal mice did not show any differences in the differentiation of IgM, IgA and IgG producing cells. These results strongly suggest that IgA preponderance in the gut is not due to the preponderant presence of T cells in Peyer's patches or gut lamina propria, but to the migration, accumulation, and expansion of Peyer's patches IgA precursors in the gut lamina propria.

II. Ig expressing repertoire in Peyer's patches:

The results of IgA allotype suppression were further strengthened by the subsequent work on the Ig expressing repertoire of Peyer's patch B lymphocytes (B cells) and T cell regulation of Ig expressions. By testing various mitogens capable of inducing differentiation of B cells into plasma cells, we found that pokeweed mitogen is the best inducer in culture for triggering B cells to mature into IgA plasma cells while concanavalin A (Con A) at submitogenic dose supplemented with bacterial lipopolysacchanide (LPS), also at submitogenic doses, is the best inducer of differentiation into IgM and IgG plasma cells. Employing this culture system, we were able to define the Ig expressing repertoire of lymphocytes from Peyer's patches, spleen and peripheral lymph nodes. Comparing the repertoires, it became clear that Peyer's patches were characterized by possessing precursors predominantly for IgA plasma cells and surface IgA bearing cells, while spleen and other peripheral lymph nodes were characterized by possessing pre-

cursors predominantly for IgM and IgG plasma cells. When T cells from Peyer's patches and spleen or peripheral lymph nodes were examined for the regulation of Ig expressions in the culture system, no differences in the expression of IgA, IgM and IgG isotypes were seen. Thus B cells, rather than T cells, specific for IgA expression are preferentially localized in Peyer's patches.

III. Characteristics of lymphoid cells isolated from gut lamina propria:

The basis for IgA preponderance in the gut was directly studied by isolating lymphoid cells from the gut lamina propria and examining their Ig expressing capabilities. The isolation procedure involved inactivation of intestinal mucous substances with dithiothreital, stripping the epithelium with EDTA, dispersion of lymphoid cells from the lamina propria by collagenase digestion, and purification of the lymphoid cells by Ficoll metrizoate gradient centrifugation. This isolation procedure can be applied in mice, rabbits, rats and guinea pigs. The isolated lymphoid cells from the mouse contained approximately 20% IgA containing cells, 20% B cells, 40% T cells, 10% granulocytes and less than 5% epithelial cells. The B cells were mainly surface IgM bearing cells; surface IgA bearing cells were in large number. When stimulated with mitogens, The B cells mainly differentiated into IgA containing cells which were unable to further divide. When the T cells were examined for their regulatory activity in Ig expressions. They showed no specificity in the d.fferentiation of IgA, IgM and IgG producing cells. Thus, the gut lamina propria accumulates IgA cells and their precursors rather than IgA specific T cells. This accumulation may be due to the specific migration of Peyer's patch IgA cells and their expansion in the gut lamina propria.

Future Objectives:

1. Peyer's patches are the most enriched source of the precursors of the IgA plasma cells in the gut lamina propria. The mechanism for the formation of this characteristic will be further studied.

2. The specific migration of IgA cells to the gut lamina propria is the key mechanism in the development of humoral immunity of the gut and possibly other mucosal tissues. Studies on the mechanism of IgA cell migration should be further pursued.

3. During the isolation of lymphoid cells from the gut lamina propria, granulocytes and macrophages were also isolated. The functions of these cells will be further characterized.

PUBLICATIONS

1. Tseng, J. IgA allotype suppression in mice: A cellular implication for the IgA preponderance in the gut. Cell. Immunol., 65, 247 (1981).

2. Tseng, J. Expression of immunoglobulin heavy chain isotypes by Peyer's patch lymphocytes stimulated with mitogens in culture. J. Immunol., 128, 2719; (1982).

3. Tseng, J. Expression of immunoglobulin isotypes by lymphoid cells of mouse intestinal lamina propria. Cell. Immunol., (1982 in press).

4. Tseng, J. Expression of immunoglobulin isotypes by lymphoid cells isolated from the lamina propria of mouse small intestine. Ann. N.Y. Acad. Sci., (1982 in press).

5. Tseng, J. Expression of immunoglobulin isotypes by lymphoid cells isolated from the lamina propria of mouse small intestine. 5th International Convocation of Immunology, Buffalo, New York. June 15-18, 1982. (Abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AI	223 WWP5
b. CONTRIBUTING				
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11. TITLE (Precede with Security Classification Code)

(U) Pathologic Manifestations of Zoonotic Diseases of Miltary Importance

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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
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FISCAL YEAR CURRENT 83	8.0	422

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME: POC: DA

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(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 trypanosomomiasis and leishmaniasis and the effects of other infectious, toxic, and environmental biol-hazards in a variety of animal hosts. Initiate and provide pathologic studies needed to prevent/control diseases and conditions that impact on quality assurance of the WRAIR-reared and purchased laboratory animals. Provide diagnostic pathology for animals acquiring natural diseases and deaths during quarantine or colonization at the WRAIR. Provide clinical pathology and histopathology support to the WRAIR and other eligible government agencies. All projects are generated from approved protocols and are releated to military medical problems.
24(U) Studies utilized conventional gross and histopathology, clinical pathology, histochemistry, immunohistochemistry, and electron microscopy techniques.
25(U) 81 10 - 82 09. The susceptibility of the owl monkey (Aotus trivirgatus) to hepatitis A virus infection was established. Pathogenetic studies revealed that onset of liver-specific enzyme alterations occurred simultaneously with evidence of necrosis and inflammation in the liver. Review of several histopathologic studies of animals which had been treated with experimental antiprotozoal compounds resulted in further development of these compounds. Preliminary studies revealed unique enzymatic changes in the cerebrospiral fluid of dogs experimentally exposed to nerve gas agents, suggesting that more definitive diagnositic tests can be developed. Results from preliminary studies suggest that species have different patterns of injury in response to casualty-producing blast. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. OO FORMS 1455A 1 NOV 58 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

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

Work Unit 223 Pathologic Manifestations of Zoonotic Diseases of Military Importance

Investigators:
Principal: Lance O. Lollini, LTC, VC
Associate: Ralph M. Bunte, LTC, VC; Charlotte M. Keenan, CPT, VC; Richard E. Long, CPT, VC; Charles B. Clifford, CPT, VC; Isaac J. Hayward, CPT, VC; Samual Lamb, CPT, VC

Description:

To diagnose, define, investigate and compare known and potential diseases common to man and animal, particularly those of military significance. To devise and evaluate means for precise diagnosis, control and/or prevention of inflammation and tissue injury induced by these diseases. To develop new animal models for the study of human diseases. A major effort 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) The histologic changes in rats and dogs associated with candidate antimalarial compounds; (2) The use of the German Shepherd dog as an experimental model for evaluation of human isolates of visceral leishmaniasis; (3) The pathologic and clinicopathologic evaluation of the owl monkey (Aotus trivirgatus) as a model of hepatitis A; (4) The pathologic changes in rabbit esophagi exposed to different concentrations of bile salts, trypsin, and hydrogen ions to develop an animal model of reflux esophagitis; (5) Care of blast-injured casualties; (6) Soman intoxication of carnivors and primates; (7) Assessment of the passage of candidate antitrypanosomal drugs through the blood-brain barrier of imbred mice and rabbits; (8) Evaluation of plasma fibronectin as a factor in wound healing in rabbits; (9) Meclofenamate inhibition of prostaglandin and its effects on gentamicin toxicity in the dog; (10) Correlation between lung water measurements and clinical and pathological data; (11) Evaluation of toxicity of formycin B to hamsters; (12) Assessment of local tolerance

to parenteral administration of various salts of a candidate antitrypanosomal compound (WR163,577) in calves; (13) Pathology in C57BL/Jb mice infected with Trypanosoma rhodesiense; (14) The interaction of vasconstructor and vasodilator hormones released systemically and intrarenally in response to the combined insult of hemorrhagic hypotension and suprarenal aortic constriction in dogs; (15) Pathology of rabbits used as feeders for tsetse flies; (16) Study of gastric emptying by use of 99 MTC - tagged chicken liver in patients with reflux esophagitis; (17) Clinical pathology laboratory support and histopathology laboratory support and collaborative studies.

I. Histologic Changes in Rats & Dogs Associated with Candidate Antimalarial Compounds.

Interdivisional study with the Division of Experimental Therapeutics, WRAIR

Four studies were performed, three have been completed and one is near completion. Lung, heart, spleen, gallbladder, liver, kidney and thymus from dogs receiving compound WR6026.2HCl orally once daily for 28 days were examined microscopically. The incidence of extramedullary hematopoiesis, diffuse congestion, vacuolation of cells in the red pulp, brown pigment, plasma cell infiltration and lymphoid depletion was increased in the spleens of dogs from the high dose groups (4 groups, six dogs each group). Gallbladder submucosa lymphoid foci and mucosal hyperplasia were present at an increased incidence in the high dose groups.

In a similar study utilizing rats (10 males and 10 females each from 4 groups) gavaged with compound WR60626.2HCl for 28 days, lung, heart, spleen, kidney and uterus were examined microscopically. High dose groups had increased amounts of eosinophilic flocculent material and macrophages in pulmonary alveoli. High dose groups also had an increased incidence of lymphocytic infiltrates in the heart, brown granular pigment in the spleen, proteinaceous costs in renal tubules and hydrometra of uterine horns. A subsequent study has revealed that most of these histologic changes are reversible microscopically. Treatment related changes were foamy macrophage infiltration in lungs of all treated groups, vacuolation of splenic lymphoid cells and foamy macrophage infiltration in spleens of all treated rats and renal tubular degeneration in the high dose groups.

Studies on compound WR171,669.MCl are near completion. Four groups of 10 male rats each are being examined for subacute oral toxicity and the effects on

reproductive organs following 16 weeks of treatment. Preliminary findings show drug related changes in various organs of the high dose groups.

II. The Use of the German Shepherd Dog as an Experimental Model for Visceral Leishmaniasis.

Interdepartmental study with the Department of Parasitic Diseases WRAIR.

Visceral leishmaniasis of man and dogs is a disease that is widely distributed geographically. It is endemic in many areas and extensive epidemics can occur with mortality reaching 98% in untreated cases. There is an increasing awareness of the risk of exposure to infection in military units operating in many parts of the world. Treatment with the currently available drugs is prolonged and by no means entirely safe or uniformly successful. While the hamster model has been used successfully for screening of new antileishmanial compounds, additional nonrodent models should be developed. Experimental infection in the dog (Beagles and mongrels) has either been equivocal or incompletely evaluated. It is the objective of this preliminary study to determine if the German Shepherd dog might prove to be an animal model that would develop a uniform infection which when characterized clinically and pathologically would be similar to the infection in man.

In this preliminary study there were six experimental animals-three were infected with 1.7×10^8/kg of Leishmania chagasi and three were infected with 2.8×10^8/kg of Leishmania donovani. All dogs became infected and remained infected throughout the study. This was substantiated by periodic cultures of bone marrow aspirates. Infected animals did not show the weight gain expected for dogs of that size and age. Several dogs exhibited splenomegaly and lymphadenopathy by day 41 post infection. The three dogs infected with L. donovani also developed dermatitis associated with demodectic mange. Funduscopic examinations were done periodically and were unremarkable. Evaluation of the clinical pathology data revealed a mild to moderate anemia, elevated sedimentation rate, elevated total protein, hypergammaglobulinemia, and hypoalbuminemia. Whole blood tryptophan levels were decreased. The histopathology of the lymph nodes and spleens was characterized by follicular hyperplasia, plasmacytosis, and proliferation of macrophages in paracortical areas and medullary cords of lymph nodes and proliferation of macrophages in red and white pulp of the spleens. Clusters of parasitized macrophages were present in other organs, including liver, tonsils, bone marrow, intestine, and lung.

The clinicopathologic findings are consistent with those observed in human visceral leishmaniasis. Two manuscripts have been submitted for publication.

III. Pathologic and Clinicopathologic Evaluation of the Owl Monkey (Aotus trivirgatus) as a Model of Hepatitis A.

Interdepartmental study with the Department of Virolcgy, WRAIR.

Hepatitis A (HAV), hepatitis B and non-A, non-B hepatitis viruses are not readily propagated in vitro and have very limited non-human animal hosts. Laboratory animals are needed for virus production, infectivity detection and assay, studies of virus transmission, pathogenesis of disease and immune responses. In 1979 and 1980, Aotus trivirgatus at WRAIR and newly captured monkeys in Panama were found to be seropositive for HAV. These findings provide suggestive evidence that Aotus monkeys may be susceptible to infection with HAV.

The objective of this initial experimental is to confirm the susceptibility of Aotus monkeys to HAV and to record the clinical, viral, pathological, and serological response to experimental infection. Six seronegative, colony-bred monkeys were inoculated intravenously with a fecal suspension of PA33 strain hepatitis Aa virus, recovered previously from a naturally infected Aotus in Panama. Six to 17 days after inoculation, viral antigen was shed in the feces of all monkeys, and four to eight days later, serum aminotransferase activities became significantly elevated in each. Liver biopsies obtained 16 to 24 days after inoculation demonstrated mild to moderate histopathologic changes including portal region. Antibody to virus developed in each monkey by 28 days after inoculation. These data confirm the susceptibility of Aotus to hepatitis A virus and indicated that infection of this primate provides a useful animal model of human hepatitis A. A manuscript has been submitted for publication.

IV. Pathologic Changes in Rabbit Esophagi Exposed to Different Concentrations of Bile Salts, Trypsin, and Hydrogen Ions.

Interdepartmental study with the Department of Surgical Gastroenterology, WRAIR.

Bile, gastric acid, and probably trypsin play a major role in the development of reflux esophagitis, a significant problem in man. The pathogenetic mechanism(s) of injury is poorly understood.

Two studies were designed by the Department of Surgical Gastroenterology to define the pathogenetic mechanism(s). In one, the changes in transmucosal electrical potential and tissue resistance along with hydrogen ion back diffusion were measured in rabbit esophagi exposed to different concentrations of taurodeoxycholic acid, trypsin, and hydrogen ions. In the second, identical parameters were measured in rabbit esophagi exposed to different concentrations of taurochenodeoxycholic acid, tauroursodeoxycholic acid, and hydrogen ions. In both studies the perfused esophageal segments were removed and examined grossly and microscopically in a blinded fashion to determine histologic changes and any differences there might be in lesion development or evolution between the different substances at varied concentrations. A quantitative scoring system for evaluating tissue changes was developed and used. Correlation of changes in electrical potential, tissue resistance, and hydrogen ion back diffusion with pathological changes for each substance have been completed. The data suggest that electrical resistance is a useful parameter for assessing esophageal mucosal damage, being more rapid and somewhat more sensitive than other measurements. Results from the second study indicated that the bile acids are damaging in the order of deoxycholic > chenodeoxycholic > ursodeoxycholic acid. Abstracts were submitted and published in Gastroenterology.

V. Care of Blast-Injured Casualties.

Interdivisional Study with Division of Surgery.

During the reporting period a major research program, leading to the eventual definition and refinement of diagnostic and therapeutic modalities for blast-injured casualties has been planned and initiated. Early phases will consist of selection of an animal model that will have lesions similar to man and respond similarly to man to various treatment regimens. Although the sheep has been the animal traditionally used in blast biology studies, a pilot study has indicated differences in gastrointestinal lesions observed grossly between sheep and pigs. Conclusions, and choice of an animal model for continuing use await final compilation of data in late 1982, and demonstration of the lesion pattern in a nonhuman primate species after blast exposure, which will severe as an approximation of human injury.

VI. Soman Intoxication of Carnivores and Primates.

Interdepartmental Study with the Department of Medical Neurosciences.

Soman (3,3- dimethyl-2-butylmethylphosphono-flouridate) is an organophosphate that has been shown to produce widespread axon and myelin degeneration in the cerebral cortex, basal ganglia, diencephalon, midbrain, hindbrain, and limbic system. Brain damage has been found in animals sacrificed between 7 and 28 days post-injection.

The objective of this study was to ascertain whether Soman induced brain and peripheral nerve damage could be demonstrated and compared among cats, dogs and macaque monkeys. This was accomplished by the principal investigator from the Division of Neuropsychiatry. The objective pertaining to the Department of Comparative Pathology was to ascertain whether or not Soman could induce lesions in tissues other than the central nervous system. When the central nervous system was not useful to the Division of Neuropsychiatry, it was evaluated by the Department of Comparative Pathology.

Dogs, cats and cynomolgus monkeys were evaluated in separate runs. Soman was injected intramuscularly at an approximate dosage of 90% of the LD_{50} for each species. One animal from each Species was injected with normal saline and served as the control. The postinjection survival time was 6, 12 and 30 days. Necropsies were performed on all animals; however, the central nervous system was removed and evaluated by the investigator from the Division of Neuropsychiatry on all animals that survived at least 12 hours post injection.

Gross and microscopic changes were minimal and fairly consistent for all species. Gross lesions included hemorrhage, edema and congestion in various organs with the most consistent findings occurring in the lungs, liver and kidneys. Microscopic changes also consisted of hemorrhage, edema, congestion and lymphocytic infiltrates involving various organs. The lungs, liver,kidney and occasionally the gastrointestinal tract showed the most consistent changes. The changes were not distinguishable based on survival time. There were no significant gross or microscopic lesions seen in the brain.

Serum and cerebrospinal fluid (CSF) were collected from select dogs injected with Soman that died 3 to 5 hours post injection. There were detectable changes in levels of serum and CSF levels of glucose, potassium, SGOT (AST), LDH, and CPK enzymes.

Both serum and CSF glucose levels were markedly decreased and, in some cases, glucose levels in CSF were higher than in serum. There was a moderate elevation of

SGOT in CSF in a few dogs. Elevation of serum levels to varying degrees occurred in all dogs. There was marked elevation of serum LDH and CPK in all but one dog. Elevation of CSF, LDH and CPK occurred in dogs that survived for at least 3 hours. Levels of serum potassium were elevated; in some cases, levels were twice that of normal. CSF potassium levels were three to five times normal levels. These changes in CSF indicated probable acute damage to the brain. However, these results were based on terminal samples. Additional studies would be necessary to determine the value of enzymes in CSF and serum. There appears to be excellent potential value in more definitive studies of the enzyme, and biochemical changes in CSF over the course of Somand and other organophosphate toxicities in various mammalian species. A protocol involving further studies of clinical laboratory diagnosis in Soman intoxication in cats will be conducted in the next fiscal year.

VII. Assessment of the Passage of Candidate Antitrypanosomal Drugs Through the Blood-Brain Barrier of Inbred Mouse and Rabbit Models.

Interdepartmental Study with the Department of Experimental Therapeutics.

A screening model is being developed to assess the passage of candidate antitrypanosomal drugs across the blood-brain barrier of mice infected with a neurotropic strain of Trypanosoma rhodesiense. Three drugs were used. Melarsoprol is an arsenical which is the drug of choice for treatment of CNS involvement inhumans and was used as a positive control. Pentamidine is a diamidine which has been widely used for treatment without CNS involvement and has been used as a prophylactic. Suramin was the third drug used. It is the drug of choice for treatment of African trypanosomiasis without CNS involvement. However, its use in humans is limited since it passes the blood-brain barriers in only very small amounts. The objective of this study was to evaluate the disappearance of trypanosomes from the brain and bloodstream following treatment of the infected mice with suramin, pentamidine and melarsoprol. Thirty-five mice were used. The brains were removed and one-half of each brain was processed for histology and half for subinoculation. The brains will be reviewed histologically for the presence or absence of encephalitis.

VIII. Plasma Fibronectin as a Factor in Wound Healing in rabbits.

Interdepartmental Study with the Department of Cardiovascular Physiology, Division of Surgery.

Fibronectin is a high molecular weight glycoprotein found in basal laminae and connective tissue matrices in vivo and on the surface of cultured cells in vitro. Fibronectin will bind to collagen, heparin, fibrin, staphlococci and cellular surfaces. It has been suggested that fibronectin plays an important role in cell-cell adhesive contact, in cellular morphology, and in the organization of extracellular matrices.

This study is being conducted to determine the role that fibronectin plays in wound healing, both from the stand point of cell to cell adhesion and its ability to fend off infection due to fostering of phagocytosis by the reticuloendothelial system and macrophages.

Rabbits are being used to test the hypothesis that the depletion of fibronectin delays wound healing and enhances wound infection. The objectives of the study: (1) to determine normal levels of fibronectin in rabbits, (2) to determine progression of wound healing in rabbits who are fibronectin deficient compared to those who are not, (3) to determine incidence of infection in rabbits following a surgical incision.

Wounds will be induced in rabbits with variable plasma fibronectin levels. Skin biopsies of wound sites will be processed for histopathology and stained with hematoxylin and eosin, reticulin stain and with Masson's trichrome stain. The degree of wound healing will be graded according to the amount of granulation tissue present. The pathologist will have no knowledge of treatment groups. The study is now ongoing with seven completed biopsies.

IX. Meclofenamate Inhibition of Prostaglandin and Its Effects on Gentamicin Toxicity in the Dog.

Interdepartmental Study with the Department of Animal Resources, Division of Vet Med and the Department of Nephrology, Division of Medicine.

Gentamicin is an aminoglycoside antibiotic known to cause a rise in the prostaglandin level and nephrotoxicity. Since meclofenamate is an inhibitor of prostaglandin synthesis, it was hypothesized that the nephrotoxic effects of gentamicin would be altered with the concomitant injection of meclofenamate in dogs.

Female mongrel dogs were divided into groups of four each. One dog from each group was injected with normal saline and used as the control. One dog from each group was injected with Gentamicin alone, and two dogs from each group were injected with Gentamicin plus meclofenamate. Four renal biopsies were taken from each

animal using the Vim Silverman biopsy needle and the key hole technique in the following sequence on successive weeks: right kidney, cranial pole; left kidney, cranial pole; right kidney, caudal pole; left kidney, caudal pole. The first biopsy for each animal was taken prior to treatment to assess pre-treatment morphology. Renal biopsies were taken to evaluate kidney morphology based on treatment groups. Biopsy specimen were processed for light and electron microscopy.

The project was done in four runs, each run containing representatives from the principle groups and three of four exams containing controls. At the light microscope level, proximal convoluted tubular nephrosis ranging from minimal to marked was observed in groups of dogs treated with gentamicin alone or with gentamicin plus meclofenamate. Changes included the wrinkling of basement membranes, loss of brush border staining and dilatation or proximal convoluted tubules with cellular necrosis. Control animal biopsies were essentially normal throughout the experiment. There was some variation in the degree of morphologic changes; however, the differences based on treatment received were not significant. It was therefore concluded that, based on this limited study, there was neither ameliorative nor additive effect on gentamicin-type nephrotoxicity in those dogs concurrently treated with meclofenamate.

Selected biopsies have been processed and will also be reviewed with the electron microscope. These additional findings may more clearly define morphologic changes based on treatments received. Findings will be submitted for publication in Nephron.

X. Correlations Between Lung Water Measurements and Clinical and Pathological Data.

Interdepartmental Study with the Department of Cardiophysiology.

This study was conducted to determine the correlations, between lung water measurements and clinical and pathological data. Pulmonary injury was created in dogs by intravenous injection of oleic acid followed by saline. Physiologic data were obtained prior to following administration of saline. The dogs were sacrificed and lungs examined histologically. Lung was also prepared for dry weight determination.

The lesions in the five animals varied amongst individuals with varying severity among sections from the same animals. The lesions were determined to be somewhat similar to those in a previous report of oleic acid injection into the pulmonary artery in that, pulmonary

edema which was the most frequent finding in these lungs with 3 out of 5 animals so affected. However, they differ in that one animal exhibited bronchiolitis, and two animals had infiltration of neutrophilic polymorphonuclear cells into the alveolar septa, one of these animals also had these cells in alveolar spaces. Necrosis of lung parenchyma was also present in one of these two animals. Furthermore, atelectasis, hyaline membranes and thrombi were less extensive in this instance than in the report cited. The regional differences in severity and nature of the lesions in the sections from one dog suggested either a non uniform distribution of oleic acid in the lung, or variable sensitivity to the effects or expression of oleic acid damage in various regions of the lung. Differences between animals can be attributed to varying doses of oleic acid.

XI. Toxicity of Formycin B in Hamsters.

Interdepartmental Study with the Department of Parasitology, WRAIR.

The treatment of choice for leishmaniasis is pentavalent antimony; antimony failures are treated with pentamidine or with Amphotericin B. Lack of knowledge of the mechanism of action of antimony, and of pentamidine, has not permitted development of derivatives of these drugs as new antileishmanial agents. Rather, the antileishmanial efficacy of experimental agents such as purine analogues are being investigated.

Dr. R.K. Robins suggested to us in March 1981 that Formycin B, an inosine analogue, might have antileishmanial activity. Since then, Carson and Chang and ourselves have proven Formycin B to be an attractive antileishmanial agent. We have found that formycin B is 100 x more active than antimony or allopurinol *in vitro* and, confirming the work of Carson and Chang, that a dose of 100 MKD IM for 3 days eliminated 90% of *L. donovani* from hamsters (G=0.46). We have also determined that at least 3 purine synthetic enzymes are inhibited by this drug. Formycin B is therefore an attractive experimental agent because it is active *in vitro* and *in vivo*, and its mechanisms of action have been significantly elucidated.

Four hamsters will be administered Formycin B in 1 ml H_2O P.O. each day for 4 days at the ED_{90} and at 4 times the ED_{90}. Four control hamsters will be administered 1 ml H_2O P.O. for 4 days. On the 4th day, the hamsters will be anesthetized and will be exsanguinated. Hamster blood will then be analyzed to include a complete blood count (white cell differential and reticulocyte count), liver function tests (SGOT, SGPT, total

bilirubin), kidney function tests (BUN, creatinine, phosphate, calcium, total protein, albumin), electrolytes (Na, K, Cl), glucose, alkaline phosphatase, CPK, and uric acid. Because of the possible effect of Formycin B on nucleic acid synthesis in rapidly dividing cells, histopathology of the hemolyphatic system (thymus, spleen, bone marrow,lymph node) and liver will be performed. The broad range of laboratory and pathologic studies is proposed because except for an anecdotal report of leukopenia in dogs, the side effects of Formycin B are unknown. Results of this initial study are under evaluation.

XII. Assessement of Local Tolerance to Parenteral Administration of Various Salts of a Candidate Antitrypanosomal Compound (WR-163,577) in Calves.

Interdepartmental study with the Department of Parasitology, WRAIR.

WR-163,577 is a bisquinaldine with antitrypansomal activity which has been shown to protect mice against Trypanosoma rhodesiense challenge for at least 10 months after one subcutaneous injection. In clinical trials in man, local reactions developed at the sites of injection of the dihydrochloride salt of the compound and the trials were stopped. In mouse studies, of numerous salts of the compound assessed for local and systemic toxicity, the dihydrochloride, acetate, and nitrate salts were found to be the least toxic. In this study designed by the Department of Parasitology, the dihydrochloride, acetate, and nitrate salts wre injected subcutaneously and intramuscularly in calves at a dosage of 25 and 50 mg/kg body weight to assess the local and possibly systemic toxicity. Injection sites were observed daily for approximately 30 days post injection. The calves were then killed and the injection sites were dissected, examined, and measured. In subcutaneous sites the salt residues were indistinguishable from each other and were easily recognized as flattened, irregular islands and cords of bright yellow, dry, crumbly, and flaky material.

The areas involved were approximately 10-20 cm in diameter and involved the subcutaneous fascial planes. There appeared to be minimal absorption of the material and the quantity found generally corresponded to the dosage injected. There was minimal connective tissue response. Moderate edema surrounded the nitrate salt residue.

Intramuscular injection sites contained identical material in fascial planes dissecting between msucle

bundles. Little tissue reaction was noted. There appeared to be minimal absorption of the salts and the quantity observed correlated with the dosage injected. Because of the apparently minimal absorption of the compound injected intramuscularly, there will be significant loss of muscle tissue from condemnation at slaughter. This route of administration, therefore, appears less desirable then subcutaneous injection.

Additional calves were injected subcutaneously with 100 mg/kg body weight of each salt, observed for 30 days, and killed. A large sterile abscess containing approximately 400 ml of serosanguinous fluid was found around the nitrate salt. The acetate and dihydrochloride salts were surrounded by smaller abscesses with approximately 40 ml of fluid each of which contained Staphylococcus aureus.

Microscopic examination has been completed. Data is being evaluated prior to writing of final report and review by a senior staff pathologist.

XIII. Pathology in C57BL/Jb Mice Infected with Trypanosoma rhodesiense.

Interdepartmental study with the Department of Immunology, WRAIR.

There is a need for a practical laboratory animal model for the study of chronic trypanosomiasis. The infection of Trypanosoma rhodesiense in laboratory rats and mice generally leads to an acute fatal disease without the development of the chronic disease or cerebral lesions so much a feature of the disease in man. In recent years there have been some reports of chronic trypanosomiasis with cerebral lesions in mice infected with T. equiperdum and T. brucei. However, a rodent model for the study of chronic trypanosomiasis caused by T. rhodesiense is still lackiing. Initial studies in our laboratories indicated that a chronic infection of trypanosomiasis with significant cerebral lesions could be produced in C57BL/Jb mice infected with a human strain of T. rhodesiense. These findings led to a more detailed study. One hundred and nineteen mice wre inoculated intraperitoneally (IP) with 10^3 T. rhodesiense strain ZVH 18A9. The mice were sequentially killed every two weeks PI with the last surviving infected mice killed at 147 days PI. The infected mice became anemic, hypoglycemic, hypergammaglobulinemic with IgM greatly increased and hypoalbuminemic. Immunofluorescence studies of the kidneys demonstrated an immune complex glomerulonephritis. Lesions histologically similar to those described in chronic trypanosomiasis in man were found in the brain, spleen, lymph nodes, liver, heart,

kidney, pancreas, and epididymis. Data on parasitologic, immunologic and ultrastructural pathology are being compiled. The morphological, hematological, serological and immunological results plus the duration of the infection indicates that the C57BL/Jb mouse infected with this strain of T. rhodesiense makes an excellent model for the study of chronic trypanosomiasis.

XIV. The Interaction of Vasoconstrictor and Vasodilator Hormones Released Systemically and Intrarenally in Response to the Combined Insult of Hemorrhagic Hypotension and Suprarenal Aortic sonstriction in Dogs.

Interdepartmental study with the Deparcment of Nephrology, WRAIR.

Post-traumatic acute renal failure has been recognized as a major complication of combat casualties since WWII. Studies of this problem have been hampered by the lack of an experimental model strictly comparable to humans. This study was designed by the Department of Nephrology to determine the relationships between the vasoconstrictors angiotensin II, norepinephrine, and AVP, and the vasodilators PGE and bradykinin, when renal perfusion pressure is decreased by hypotensive hemorrhage and aortic constriction. Also, the study is designed to determine if the severity of renal dysfunction, as measured by changes in renal blood flow, glomerular infiltration rate, and extraction of PAH, can be correlated to an imbalance between renal vasocontrictors and renal vasodilators. Pathologic studies of kidneys from 18 dogs were performed. One week after the experimental insult, gross and microscopic examinations, including the use of thin sections imbedded in plastic, were conducted to screen for pre-existent morbid changes, to determine the morphologic changes resulting from the experimental manipulations, and to permit correlations of changes in morphologic and physiologic parameters. The combined insult of two hours duration, resulted in a multifocal pattern of lesions in convoluted tubule epithelial cells which varied from individual cell degeneration and regeneration to infarction of large wedges of the renal cortex. Morphologic alterations correlated well with physiologic parameters of renal damage. In order to further document early epithelial cell damage, electron microscopic evaulation is being conducted with the University of Maryland, Baltimore.

XV. Pathology of Rabbits Used as Feeders for Tsetse Flies (Glossina spp).

Interdepartmental study with the Department of Entomology, WRAIR.

Many New Zealand White and Flemish Giant rabbits used as feeders to maintain the WRAIR tsetse fly (Glossina spp) colony have rapidly developed a syndrome characterized by progressive weight loss, unthriftiness, diarrhea, and elevated serum BUN and creatinine. In pathologic studies on approximately 25 rabbits, most have had extensive deposits of amyloid in many organs, most notably in the kidneys, spleen, stomach, intestines and pancreas. Many of the animals have also had bacteremias with associated lesions such as lung and testicular abscesses,, otitis externa and media, septic renal thrombi and infarcts, and septic vegetative endocarditis. The flies feeding on these diseased rabbits have experienced a marked decrease in reproductivity, thus jeopardizing the supply available for research studies. Also, the decreased life-span of the rabbits has increased the number of replacement rabbits needed and the financial costs. Attempts to characterize the composition of the amyloid deposits using fluorescent antibody techniques gave variable and inconsistent results. The possibility of using other histochemical methods are to be explored and possibly employed in attempts to characterize the composition of the amyloid deposits. Currently the data collected during the previous year are being analyzed for publication.

XVI. Study of Gastric Emptying by use of 99mTc - Tagged Chicken Liver as a Marker of Solid Food in Patients with Reflux Esophagitis.

Interdivisional study with the Gastroenterology Service, WRAMC.

Gastroesophageal reflux is a frequently encountered digestive disease of military importance because an incompetent cardioesophageal junction limits the physical ability of otherwise healthy personnel to function in their duty environment. Both the Navy and Army as well as the Air Force, have contributed to the clinical investigation of this disease motivated by the impairment their personnel experience in their respective duty environment.

This study is designed to identify patients with distinct patterns of gastroesophageal reflux (GER) who have delayed gasric emptying of solid food contributing to their symptomatology.

Gastroesophageal reflux is a common problem. Of patients who see physicians, many respond well to conservative therapy of life style alteration (weight loss,

discontinuing smoking, diet alteration), antigravity measures (bed elevation), and antacid use. However, some patients do not respond to these measures, and continue to have daily pyrosis, regurgitation, and often dyspepsia.

At present, little evidence exists on gastric emptying in patients with symptoms from GER. The small amount of data available suggests that liquid emptying is normal, but gastric emptying of a liquid-solid meal (egg salad sandwich) is delayed in 40% with GER. In neither study, was there (1) an attempt to categorize patients with GER, or (2) solid food emptying studies done. In the only study categorizing patients by 24 hour pH monitoring, daytime reflux patients had a faster gastric emptying, and nighttime reflux patients slower gastric emptying than controls. However, this study was done with a nonvalidated mixed liquid-solid radioactive marker technique for gastric empyting.

We propose to carefully categorize patients with GER before the gastric emptying study by routine tests for GER, including 24 hour pH monitoring. Our previous data suggests four different groups of patients can be distinguished within the reflux population on the basis of dyspepsia, pyrosis, and endosceptic esophagitis. Only 2/33 patients studied to date have not been clearly classified into one of four groups by this combination of studies.

The gastric emptying study we will employ is 99mTC-Tagged chicken livers. For many years, 99mTc sulfur-colloid has been used for liver scans in human diagnostic studies. This is based on the fact that after an intravenous injection, the colloid is taken up by the Kupffer cells and distributed uniformly throughout the liver. Chicken livers also take up the colloid. Accordingly, 99mTc-tagged chicken liver can be prepared, incorporated into a meal and used as a marker to quantitate solid food emptying by the stomach. Studies have verified that there is no leeching of the 99mTc by acid or mechanical disruption, therefore making it a reliable test for solid food emptying of the stomach. Tagged chicken livers are prepared by injecting 0.5 mcu of the 99mTc sulfur-colloid into the wing vein of live chicken. This is done by an approved radionuclide administrator. After 30 minutes, the chicken is sacrificed by cervical dislocation. The liver is removed, washed, and cut into small pieces (about 1 cm) which are then mixed with a commercially available preparation of beef stew (7 1/2 oz. Dinty Moore). The liver and chicken carcass are thoroughly examined by the staff veterinary pathologist on duty. The meal is placed under gamma camera (Picher 415) for appropriate calibrations.

After an overnight fast, patients and normal volunteer ingest the meal along with 150cc of water. The patient is then placed under the counter and the stomach imaged. Counts are taken every 15 minutes until T 1/2 for gastric emptying is reached. This is determined by computer analysis of the area of interest, and the 99mTc half-life.

XVII. Clinical Pathology Laboratory and Histopathology Laboratory Support and Collaborative Studies.

The clinical pathology laboratory handled approximately 8,100 requests for hematology and 47,000 determinations for serum biochemistry during the reporting period. The histopathology laboratory processed approximately 6,700 paraffin blocks and 11,695 microslides during the reporting period. These two laboratories support research protocols at WRAIR and its overseas laboratories and other government agencies as well as providing diagnostic support for the Institute's laboratory animal facilities.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6751	82 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'R INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AG	224 WWP4
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7,2:2			

11. TITLE (Precede with Security Classification Code)

(U) Functional and Structural Bases of Blast-Related Tissue Injuries

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 017100 Weapons Effects

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	2.0	95
	CURRENT 83	2.0	132

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME: Sharpnack, Douglas B., CPT, VC POC:DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Functional correlation; (U) Exposure factors; (U) Blast overpressure; (U) Vascular permeability; (U) Vascular ultrastructure; (U) Pathogenesis

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

23(U) To determine the finite structural and functional bases of the pathologic changes classically associated with blast-related injury to various tisues, especially in the respiratory and gastrointestinal systems. Correlate structural and functional pathologic changes with various levels and amounts of blast exposure, emphasizing dose ranges which are near the environmental exposure associated with crew operator positions of large field artillery weapons. Study effects of repeated blast over time periods covering up to 14 days to determine cumulative effects and resolution dynamics. Map the relative sensitivities of airways and vessels in the respiratory system. Compare the fragility of the pulmonary vascular bed with that of blood vessels in other organs and tissues throughout the body. Determine the effects of blast injury on other parameters e.g., susceptibility to infectious diseases of military importance.

24(U) Conventional morphologic techniques including light and electron microscopy will be used. Other procedures will involve use of substances such as carbon particles, ferritin and horseradish peroxidase to determine vascular permeability and clearance functions. Small laboratory rodents, espceially rats and guinea pigs will be the predominant laboratory animals used.

25(U) 81 10 - 82 - 09. Data from previous blast research were assembled into an archival collection for future reference. Scanning and transmission electron microscopy of airways of sheep repeatedly exposed to blast revealed changes suggestive of a residual-type epithelial damage. Studies were initiated using rodents to verify the validity of these findings and to determine their biologic significance. The varying sensitivities of the respiratory and gastrointestinal systems of sheep to a single, high-magnitude blast, as opposed to multiple low-magnitude blasts, were resolved. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*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 65 (FOR ARMY USE) ARE OBSOLETE.

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

Work Unit 224 Functional and Structural Bases of Blast Related Tissue Injuries.

Investigators:
Principal: LTC James B. Moe, DVM, Ph.D.
Associate: CPT Charles B. Clifford, DVM
CPT Douglas D. Sharpnack, DVM, MS

Description

As new weapons systems are developed, it is imperative that consideration be given to the potential effects that these may have on the health and performance of the crews operating these systems. Use of mammals exposed to blast overpressure generated by weapons or other blast-generating devices provides a means of estimating the susceptibility of mammalian tissues to blast overpressures at levels approximating those received by operators of weapons systems. More detailed study of tissues so exposed helps to resolve the biological bases of blast-related injuries. Additionally, the various pathologic structural and functional techniques are useful in determining the complex interaction between blast overpressure and other factors in the modern combat environment.

Problem and Progress

To determine the finite structural and functional bases of the pathologic changes caused by blast-related injury in the various tissues. Of special interest are injuries which result from exposures similar to those received by artillery weapons crews in the field environment. Structural and functional changes are correlated with various amounts of blast overpressure, emphasing dose ranges which are near the environmental exposure associated with crew operator positions of large field artillery weapons. Effects of repeated blasts over time periods covering up to 14 days are studied to determine cumulative damage and resolution dynamics. The relative sensitivities of airways and blood vessels in the respiratory system are mapped. The fragility of the pulmonary vascular bed is compared with that of blood vessels in other organs and tissues of the body. Other functional parameters are investigated.

Conventional morphologic techniques, including light and electron microscopy, as well as special procedures which determine vascular permeability, mucociliary clearance and other functional parameters, are used.

Complex procedures designed to determine the effects of blast overpressure exposure on susceptibility to infectious agents will be adapted as the studies progress.

Results

Results from a large field study of the effects of repeated (50 blasts) exposure to operator-level blast overpressure in sheep were assembled into an archival assembly, including pathology data and photographic documentation. To briefly summarize, this study covered a range of exposures from approximately 4 pounds per per square inch (psi), to 14 psi. At 4 psi, the highest level of blast overpressure to which an operator of any U.S. artillery weapon might reasonably be expected to be repeatedly exposed, there was no substantial evidence of nonauditory tissue injury. At higher levels (7 and 14 psi) there was evidence of injury in the upper airways (larynx and trachea), but not in the lungs. There was also evidence of tissue injury in the gastrointestinal tract at 7 and 14 psi. From these studies it was concluded that there was little risk of substantial injury to nonauditory tissues exposed repeatedly to the maximum blast overpressure generated in operator positions surrounding U.S. artillery weapons. This archival assembly of data and conclusions will be stored at the Walter Reed Army Institute of Research.

The first in a series of experiments designed to use rodents for future blast overpressure research was conducted to determine the feasibility and effectiveness of various fixatives for preservation of rodent tissues. Hamster tissues were fixed by perfusion or immersion in either neutral buffered 10% formalin, 4F1G (4 parts formalin, 1 part glutaraldehyde), or Karnovsky's fixative (glutaraldehyde - parapormaldehyde). 4F1G was found to be the most ideal fixative for all applications. For scanning electron microscopy of airways, immersion was found to be the method of choice for fixation. For light microscopy or transmission electron microscopy of the lungs and other tissues perfusion was preferable. The methacrylate method of tissue processing was developed and adapted to study of rodent tissues. Methods of euthanasia were also evaluated. A second phase of these preliminary experiments involved studying the tissues from normal colony rats supplied by the Lovelace Blast Biology, where it is anticipated that future blast overpressure research work will be conducted. All tissues, especially those of the respiratory system were found to be of excellent quality and free of background disease which would interfere with blast research. The first experiment involving repeated exposure of rodents (rats) to various levels of blast overpressure was initiated. This experiment and antici-

pated follow-on studies will determine feasibility of rodent studies, relative sensitivity of various tissues and organ systems, natural history and resolution of various blast-related tissue injuries, and biomedical significance of these injuries.

Airways of sheep which had been repeatedly exposed to blast overpressure generated by either an artillery weapon in the free field, or by a blast tube examined by scanning electron microscopy. A lesion of a unique nature, whereby respiratory tract epithelial cells were stripped from the underlying layers, was recorded. The validity and reproducibility of this change will be tested in other species. Should this lesion prove to be a consistent one, its impact on potential sequelae to blast exposure, including infection, healing and respiratory function, will be great and will merit detailed investigations.

Collaborative studies with the Department of Clinical Physiology, Division of Medicine have involved consultation and documentation of pathologic aspects of chronic tracheostomy studies, lymphatic duct cannulation studies, isolated gut loop experiments and early development of the waterjet impactor as a tool for blast overpressure research. Preliminary findings have suggested that each of these approaches will have considerable utility and value in the more finite proposed study of blast biology in the future.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 6767	82 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. DIS'N INSTR'N	8b. SPECIFIC DATA* CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BC	225
b. CONTRIBUTING				
c. ~~XXXXXXXXX~~	STOG 80-7.2.5			

11. TITLE (Precede with Security Classification Code)*

(U) Pathophysiology of Blast Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012600 Stress Physiology 017000 Weapons Effects

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	1.0	216
FISCAL YEAR CURRENT 83	1.0	209

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Blast injury; (U) Tissue markers; (U) Serum markers; (U) CPK; (U) LDH; (U) Isoenzymes

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

23 (U) Recent work from this laboratory has shown that serum enzyme systems (particularly CPK) change with bowel infarction. In order to assess properly the changes in various enzyme systems in the peripheral serum subsequent to blast injury, more must be known concerning each enzyme's distribution in the various parts of the GI tract. If a difference in enzyme distribution could be detected, then earlier stages of injury may be detected by assaying the changes in the isoenzymes in the peripheral serum after blast injury. There is military relevance in this research.

24 (U) Our program of serum analyses is being integrated into the program currently being conducted in conjunction with the Department of Clinical Physiology of the Division of Medicine, WRAIR.

25 (U) 81 10 - 82 09

Work this year has centered on studying the distribution of alkaline phosphatase and CPK in the GI tract and related organs. Bowel has less alkaline phosphatase than the pancreas but its monoclonal peak is less electronegative than the majority of the alkaline phosphatase found in the pancreas. This difference could allow differentiation of injuries to these two organs if alkaline phosphatase were released into the blood by them after blast injury. Subsequent work with sera collected from blast exposed sheep received from Clinical Physiology, Division of Medicine, WRAIR, showed that LDH and alkaline phosphatase are relatively stable when stored at -70° whereas CPK shows some variation in results with time. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sept 82.

*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 65 (FOR ARMY USE) ARE OBSOLETE

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

Work Unit 225 Pathophysiology of Blast Injury

Investigator:
Principal: LTC Geoffrey M. Graeber, MC

Background and Objectives:

Blast exposure has been shown to be associated with a special predilection for injuries in specific organs such as the stomach, colon, and the lung. The amplitude and the duration of the blast determines which organs will have a higher probability of being injured. Our work has been directed at delineating potential serum markers which could help in assessing the severity of gastrointestinal injuries seen in soldiers who have been exposed to potentially injurious levels of blast. Work previously completed has shown that analysis of creatine phosphokinase (CPK) and its isoenzymes in the peripheral serum can be used as an accurate indicator of intestinal infarction.[1-3] Simultaneous analysis of lactic dehydrogenase (LDH) and its isoenzymes in the same experimental preparations has shown only minimal changes which would be of lesser importance in evaluating severe intestinal injury.[3] Recent work with strangulated infarction of small bowel confirms these observations.[4]

Work conducted this year has focused on the following areas:

1) Clinical confirmation of the experimental work.

2) Further investigation of the distribution of CPK in the gastrointestinal tract.[5]

3) Starting work on a protocol that would compare the changes in serum alkaline phophatase with the changes in CPK to see which would be a better early marker of severe intestinal injury.

4) Starting evaluation of various methods for determining CPK in the peripheral serum with eventual application for field use.

Progress:

Completion of a three-year study of patients who have had mesenteric infarction has confirmed that the changes seen in our earlier experimental work with mesenteric infarction are relevant

in the clinical realm.[6] This work has shown that peripheral serum CPK and LDH isoenzymes are capable of detecting those patients who have had mesenteric infarction and differentiating them from those patients who have had major, uncomplicated aortic reconstructions and those patients who have had acute myocardial infarctions.

Further studies have been completed on the distribution of CPK in the gastrointestinal tract. These studies have shown that there is a distinct increase in the total amount of CPK present in the antrum of the stomach. This increase is not limited to the seromuscular layer since the mucosa of the antrum appears to have the largest concentration of this enzyme of any mucosa of the gastrointestinal tract. These findings are of particular interest since certain types of blast injury have affinity for the stomach.

A protocol has been initiated to compare the changes in peripheral serum of both CPK and alkaline phosphatase with respect to experimental bowel infarction. Both systems are being evaluated to see which one has more potential to detect severe bowel injury early in its course. On a theoretical basis, CPK will be of more assistance in evaluating transmural injury since it is located predominantly in the seromuscular layer.[5] Alkaline phosphatase will help in evaluating mucosal injury since it is predominantly a mucosal enzyme.[7]

By using serum collected from animals who have had experimental bowel infarctions we have started evaluating two methods of assaying serum CPK which are much more rapid than electrophoresis and would, therefore, be more easily transported to the field and applied. Initial indications are that these tests will perform up to our expectations.

Recommendations For The Future:

Our plans are to complete the above mentioned studies within the next year. All of the above mentioned tests will be transported to the field to do a comparative evaluation of the methods to see which ones will give us the most definitive information concerning early colonic and gastric injury due to blast. We hope to be able to evaluate this in a sheep injury model which has been well established.

In case the two enzyme systems that we are evaluating now do not prove to be as helpful as we think they will be, we are starting to evaluate the hexosaminidases as possible markers of

gastrointestinal injury. This work has just begun and shall be pursued with diligence to see if this group of compounds will be good serum markers of either bowel or gastric injury.

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

Work Unit 225 Pathophysiology of Blast Injury

Literature Cited:

References:

1. Graeber, G.M., Cafferty, P.J., Reardon, M.J., et al.: Elevations of Serum Creatine Phosphokinase (CPK) in Experimental Mesenteric Infarction. Surg Forum 31:148-150, 1980.

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3. Graeber, G.M., Wukich, D.K., Cafferty, P.J., et al.: Changes in Peripheral Serum Creatine Phosphokinase (CPK) and Lactic Dehydrogenase (LDH) in Acute Experimental Colonic Infarction. Ann Surg 194:708-715, 1981.

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5. Graeber, G.M., Cafferty, P.J., Wolf, R.E., Harmon, J.W.: The Disribution of Creatine Phosphokinase in the Gastrointestinal Tract. Submitted for publication.

6. Graeber, G.M., Clagett, G.P., Wolf, R.E., Cafferty, P.J., Harmon, J.W., Rich, N.M.: Alterations in Peripheral Serum Creatine Phosphokinase and Lactic Dehydrogenase Associated With Reconstruction of the Abdominal Aorta, Myocardial Infarction, and Mesenteric Infarction. Submitted for publication.

7. Williams, R.A., Wilson, S.E.: Correlation of Alkaline Phosphatase With Submucosal Blood Flow and Morbid Histology of the Ischemic Colon. Surg Gynecol Obstet 151:412-416, 1980.

Publications:

See Citations 4, 5, and 6 above.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 0G 6768	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BC	226
b. CONTRIBUTING				
c. XXXXXXXXXX	ST0G80-7.2:5			

11. TITLE (Precede with Security Classification Code)

(U) Pathophysiologic Studies of Blast Injury to the Gastrointestinal Tract

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016200 Stress Physiology 008800 Life Support

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2.0	233
FISCAL YEAR CURRENT 83	3.0	211

19. RESPONSIBLE DOD ORGANIZATION

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NAME: SCHWEITZER, E. CPT

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Blast injury; (U) Gastrointestinal hemorrhage; (U) Gastrointestinal perforation; (U) Combat Casualty Management

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

23 (U) Our technical objective will be to study the pathophysiology of the development of mucosal and serosal hemorrhage, which is known to be a consequence of blast injury and which may be an important aspect of combat casualty management in future conflicts. Low level exposure to blast injury is experienced by troops firing weapons. Much higher levels of blast injury is experienced by troops in the vicinity of an explosion, or in a tank which is struck by a projectile. Extremely high levels of blast overpressure would be experienced by troops in the field of a Fuel Air Explosion (FAE) Mine Neutralization System.

24 (U) Observations of the gastrointestinal results of blast injuries over a range of intensities, durations and frequencies will be made in sheep. Sequential laparotomies will be carried out on sheep to observe the natural history of the lesions observed. Gross and microscopic observations will be made. The general physiologic status of the animals will be assessed during these studies by measurements of pulse, respiration rate and white blood cell count.

25 (U) 81 10 - 82 09

During the prior year a report has been prepared reviewing the clinical and experimental literature relating to gastrointestinal blast injury. A pilot experiment was carried out in sheep which showed that sequential laparotomies can be used to assess the abdominal organs of the sheep over time. A SLUFAE explosion by S^3 in San Diego was carried out as well in a pilot study to assess its effects on the GI tract of sheep and pigs. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

* Available to contractors upon originator's approval

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

Work Unit 226 Pathophysiologic studies of blast injury to the Gastrointestinal tract

Investigators:
Principal: John W. Harmon, LTC, MC
Co-Investigator: Keith D. Lillemoe, CPT, MC

Background and Objectives:

With blast overpressure injury, it is common to see gastrointestinal tract injury. The lesions observed acutely range from petechia in mild form progressing to large hematomas in the submucosa and in the most severe cases including perforation of the bowel.[1-6] The hematomas occur most commonly in the stomach and the proximal colon. They also occasionally are seen in the small bowel and retroperitoneum. The natural history of these lesions is not known. A knowledge of the natural history of the lesions is of importance for those who will be managing Blast Injury Casualties. If the lesions resolve over time they are not a significant problem. If, however, they progress to perforation, they are a very major problem. At this time surgeons do not have quidelines for managing non-perforating bowel lesions of blast overpressure.

We are working to develop an experimental approach. The approach we are considering is to use sheep as an experimental animal, to use sequential laparotomies to evaluate bowel injury, and to compare the actual appearance of the bowel with standard laboratory indicators of bowel injury including white blood cell count and temperature, as well as with changes in the serum levels of the isoenzymes of creatine phosphokinase(CPK) and lactic dehydrogenase(LDH). Other studies of ours with CPK and LDH suggest that they may be useful markers for bowel injury.

Progress:

During this fiscal year we carried out a pilot project to assess the feasibility of utilizing sequential laparotomies in sheep to assess bowel injury.

CPT William Seid of the General Surgical Residency at WRAMC performed the animal surgery. Seven sheep completed the experiment. Two underwent 2 laparotomies, on days #1 and #5. Four underwent laparotomies on days #1, #3 and #5. Two sheep expired before completing eight days of follow-up because, of leakage from needle sticks in their stomach which were performed to decompress their very distended stomachs. Adhesions made evaluation of the bowel difficult in the first 2 sheep, but when 3 liter saline irrigation of the peritoneal cavity was added to the regimen the peritoneal cavities were clear at subsequent laparotomies. Baseline CPK and LDH serum valves as well as WBC counts and temperatures were recorded on all these animals to obtain normal ranges. Formal necropsy reports were prepared for all the animals. Photographs of the major abdominal organs were taken at each laparotomy and at necropsy.

Finally LTC John Harmon and ENS Melanie Haluszka, a senior student at USUHS prepared a detailed report reviewing our current knowledge of GI blast injury including the pathophysiology and the reported clinical experience. This report formed the basis for a presentation by LTC Harmon on this subject to the 1982 7th Army Medical Surgery Conference in Garmisch.

Recommendations for the future:

In assessing our experimental approach of sequential laparotomies its advantage is that it allows observation of the abdominal contents directly. Its disadvantage is that is is extremely difficult for personell to perform the surgery and perform post operative care and evaluation so that only a few animals may be studied at a time. We studied 3 groups of 3 animals each, with each group studied for 2 weeks. It is essential to have a good operative and postoperative care facility available for this, to get satisfactory survival. In addition the laparotomies affected the levels of CPK and LDH with elevations in both after the first, but not after subsequent laparotomies. Because of these disadvantages we are reluctant to initiate a program to utilize this technique to study gastrointestinal effects of blast. We are seeking alternative methodologies.

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

Work Unit 226 Pathophysiologic studies of blast injury to the Gastrointestinal tract

Investigators:
Principal: John W. Harmon, LTC, MC
Co-Investigator: Keith D. Lillemoe, CPT, MC

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OH 0383	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
82 02 03 D	Change	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	EB	227
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7.2:1			

11. TITLE (Procede with Security Classification Code)

(U) Chronic systemic Effects of Organophosphate Esters

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Pharmacology 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	1.5	95
FISCAL YEAR CURRENT 83	1.5	95

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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: Nancy E. Whorton, GS-11

NAME: Pritam S. Verma, CPT, MSC

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Cardiac receptors;
(U) Organophosphate esters; (U) Anticholinesterase; (U) Cardiac biochemistry

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

23. (U) To determine the chronic systemic effects of low dose single or repeated exposure of organophosphate esters. Of primary concern is the effect of such agents on myocardial function at a molecular level, specifically in regard to the interaction with cholinergic and other hormonal receptors which modulate myocardial function. There is military relevance in this research.

24. (U) A small animal model will be used to produce sublethal injury using single and multiple dose exposure regimens. Four major areas of cardiac toxicity will be examined (1) cardiac enzymes involved in contractility, (2) myocardial receptors, (3) serum markers, and (4) histopathology. Other tissues known to be under cholinergic influence will also be examined for biochemical alterations.

25. (U) 82 02-82 09 A computer program has been written and is being adapted to the VAX for measurement of myocardial hormone receptors. Studies have begun to establish a data base for activity of a thiol dependent cardiac enzyme in normal animals, after which its activity will be assessed in animals exposed to DFP. A pilot study has demonstrated alterations in the hepatic enzyme T_4-5'-monodeiodinase in rats after a single dose of DFP. Follow-up studies are in progress. A longitudinal study (2 weeks to 52 weeks, after exposure) examining the effects of DFP on pituitary polypeptide secretion has been conducted, and serum samples are being analyzed. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81-30 Sep 82.

*Available to contractors upon originator's approval

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

Project: 3M161102BS10 RESEARCH ON MILITARY DISEASES, INJURY AND HEALTH HAZARDS
Work Unit 227 Chronic Systemic Effects of Organophosphate Esters.
Investigators:

Principal: Robert C. Smallridge, LTC, MC
Associate: Nancy E. Whorton, GS-11
Pritam S. Verma, CPT, MSC
SP4 Irene Gist

A new work unit was established to study the chronic systemic effects of organophosphate esters in a rodent model and on selected organ systems in vitro. The acetylcholinesterase to be used is diisopropylfluorophosphate (DFP). Studies are being performed in three areas.

1 Cardiac Studies: Acute poisoning with organophosphorus compounds can reduce stroke volume and cardiac output, and prolong the circulation time in man (1); large doses of DFP in rats may produce cardiac failure and death (2), DFP also decreases heart rate and contractility in the isolated rat heart, suggesting a direct toxic effect independent of cholinesterase inhibition (2). The mechanism of the deleterious effects of DFP on myocardial contractility is not clear, although an effect on ATPase may be involved as this agent may inactivate ATPase in other tissues (3,4). It is recognized that acute exposure to acetylcholinesterases may produce long-term injury in the central nervous system (CNS). Whether such damage may occur outside the CNS is unknown, although one case of chronic congestive cardiomyopathy has been described (5).

This work unit will examine the effects of DFP on myocardial function at a molecular level. The major emphasis will be on in vivo and in vitro studies of acetylcholinesterase inhibition on cardiac hormonal receptors and cardiac enzymes. The first receptor to be studied will be the solubilized nuclear receptor for thyroid hormone, since this hormone is a potent stimulus for myocardial contractility. In a preliminary study, we have quantitated these receptors in rat heart (6). Progress in the area of receptor physiology this year has been in acquiring and adapting a computer program for the VAX system to enable rapid data reduction. Several enzyme studies are in the planning stages including Ca^{2+}-ATPase prepared from purified myosin and 5'-monodeiodinase, an enzyme studied extensively in this laboratory (7) and which has recently been identified in the heart (8).

2. Pulmonary studies: Central respiratory depression is the usual cause of death after acute exposure to organophosphate esters. The chronic effects of these agents on pulmonary function are unknown. Thyroid hormone receptors in the lung have been identified and partially characterized in our laboratory. Since this hormone influences the production of certain lung phospholipids

and surfactant, lung tissue will be obtained at the time animals are sacrificed for the cardiac toxicity studies. This tissue will be analyzed for tissue receptors and for fatty acid synthetase activity and dipalmitoylphosphatidylcholine (a substrate for surfactant). Progress to date has involved development of the computer program for data analysis. Animal studies will commence in early FY 83.

3. Hormone/metabolic studies: In a pilot study, a single dose of DFP (LD_{20}) produced a significant reduction in activity of the hepatic enzyme T_4-5'-monodeiodinase in rats sacrificed eight weeks after exposure. A confirmatory study, utilizing pair fed control animals to examine the complicating effects of undernutrition after DFP, has been conducted. The samples are currently being analyzed. In collaboration with the Dept. of Neurosciences, a study has been conducted to examine the systemic effects of a single dose of DFP (LD_{20}) on rats sacrified at 2, 6, 9 and 52 weeks after exposure. Body and tissue weights are being examined. Serum was obtained and will be examined particularly for pituitary peptide hormones, since this gland has cholinergic receptors (9) and acute studies have shown cholinergic regulation of several of these hormones (10).

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6761	82 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
81 10 01	D. Change	U	U		NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CD	228 WW17
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:4			

11. TITLE (Precede with Security Classification Code) (U) Regulatory Mechanisms and Pathophysiology of Hematopoiesis Application to Military Hematology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support 002600 Biology 003500 Clinical Medicine 012900 Physiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 82	5.0	260
FISCAL YEAR CURRENT 83	5.0	123

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: W.H. Crosby, COL, MC, A.J. Salvado,

NAME: LTC, MC and R. Meagher, Ph.D.

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede each with Security Classification Code) (U) Leukocytes; (U) Bone Marrow; (U) Hematopoiesis; (U) Marrow Failure; (U) Erythrocytes

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

23.(U) To define the hematologic pathophysiology of bone marrow toxicity from certain families of chemical agents, drugs, radiation, and acute infection; to identify modalities that may protect against hematopoietic stem cell injury; to study basic mechanisms involved in the regulation of hematopoiesis, including iron absorption and to define and purify hematopoietic regulatory mediators. A basic understanding of the regulation of hematopoiesis is very important to the military because of numerous marrow toxic conditions (radiation, drugs, infections, chemicals) to which military personnel may be exposed during their duties.

24.(U) Experimental procedures include biochemical and cell culture techniques, animal models, and the isolation of normal human bone marrow cells. Studies also involve electron microscopic analysis of the ultrastructure of bone marrow tissue during its morphogenesis.

25.(U) 81 10-82 09 Studies of erythropoietin (Ep), a principal hematopoietic hormone important in the regulation of red blood cell production, have proceeded. Techniques for purification of Ep from human serum and urine by high performance liquid chomatography have been refined. Studies of long term in vitro culture of normal human and rabbit marrow have been begun. Animal models have been developed for studying the use of negative feed back regulators of hematopoiesis to protect stem cells from cytotoxic influences. Studies of iron absorption in mice and humans with and without iron loading have continued. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

* Available to contractors upon ord/motor's approval

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

Work Unit 228 Regulatory Mechanisms and Pathophysiology of Hematopoiesis Application to Military Hematology

Investigators MAJ Daniel Wright, MC; LTC August Salvado, MC; COL William Crosby, MC; Dr. Michael Sorrell (Fellow, GWU); LTC John Foxley (Visiting Fellow, British Army Medical Corps); MAJ Ramona Chapman, MC; Mr. Harold Williams, GS-13; Ms. Mary Cutting, MS (Fellow, GWU); Dr. Richard Meagher (IPA Investigator)

Description

Blood cells constitute a complex organ of which normal function requires continuous self-renewal of blood precursor cells within the bone marrow. The demands of blood cell renewal (hematopoiesis) are enormous, and for this reason hematopoiesis is particularly sensitive to the toxic effects of chemicals, drugs, radiation, and acute infections which interfere with cell division or differentiation. The objectives of this work unit are to study basic mechanisms involved in the regulation of hematopoiesis using tissue culture of stem cells and committed hematopoiesis precursor cells from human, mouse, and rabbit marrrow, using leukemic cell lines, and using allogenic transplantation of bone marrow tissue into mice.

Specific studies are designed to study the effects of mediators derived from mature leukocytes and inflammatory fluids upon hematopoiesis, to study the biochemistry and physiologic effects of erythropoietin upon stem cell maturation, and to study basic mechanisms by which iron absorption is regulated and by which iron is utilized by hematopoietic tissues.

Progress

1. Studies of iron and iron absorption

A. An oral iron tolerance test (ITT) has been established as a model for studying iron absorption and iron movement through the plasma, without use of radioactive tracers. The test employs dietary quantities of iron (5-20 mg) in normal and mildly iron deficient men (blood donors). After ingesting small doses of iron, subjects with depleted iron stores show significant elevations in serum iron concentration, while normal men have little or no change. The sensitivity of this ITT is indicated by the following observations: 1) In iron deficient men, different rates of increase and return to normal serum iron levels are associated with varying degrees of iron deficiency. 2) When the ITT is performed on two successive days, the second test shows a diminished response to the iron dose, suggesting that the first dose modifies the absorptive capacity of the intestine. 3) Ascorbic acid given with iron increases iron absorption, causing greater elevations in serum iron. 4) Absorption of iron in fortified cereal or blood cells is less than that of iron alone.

B. We have completed a longitudinal study on the stability of serum transferrin's ability to bind iron. The purpose of this study was to determine the effect long-term storage had on this ability. Serum

samples were stored frozen and lyophilized at -20°C and only lyophilized samples stored at 4°C. Each was analyzed for serum iron and total iron binding capacity. Post-storage values when compared with pre-storage values gave a correlation coefficient of 0.969 for serum iron and 0.965 for total iron binding capacity.

C. Studies of iron absorption in mice using ^{59}Fe and total body counting techniques have been used to identify inbred mouse strains with heritable variations in the control of iron absorption. Studies of this kind in Thalassemic mice have shown that a defect in iron absorption causing excessive iron uptake is a transitory phenomenon that disappears with age (after 3 months from birth). A related mouse model has shown that indomethacin toxicity to the gut requires the animals to be iron replete.

A model involving isolated gut loops created in vivo in mice has been developed to study the relationship between inflammation and inflammatory mediators upon iron absorption. These studies have indicated that Prostaglandin E has a rapid and potent effect of promoting the transfer of intraluminal iron into the body.

2. Studies of long term in vitro culture of normal human and rabbit bone marrow

Techniques have been established for long term human and rabbit bone marrow culture as adapted from the "Dexter" murine system. Normal human marrow specimens are obtained through a collaborative arrangement by which rib specimens are obtained from healthy living-related kidney transplant donors operated on at WRAMC (removal of these rib specimens is part of the routine operative procedure for kidney harvesting). Studies are underway to evaluate spatial cell-cell relationships that are optimal for "test-tube marrow" maintenance in tissue culture using specially made, serrated plastic culture surfaces, and to evaluate the effects of secondary granule proteins from mature human neutrophils on marrow stem cell proliferation and differentiation.

3. Studies of erythropoietin (Ep)

Refinements of techniques to purify Ep from human serum and urine by combining wheat germ column elution techniques with high performance chromatography have been completed and purification quotients determined.

A study of a patient at WRAMC with polycythemic vera who subsequently developed kidney failure, uremia, and profound anemia has been done which indicates that the suppression of erythropoiesis in uremia results from an impaired response of erythroid progenitors to Ep or a reversable alteration of the Ep molecule rendering it biologically inactive, rather than to a primary absence of Ep.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6450	82 10 01	DD-DR&E(AR)636

3. RATE 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	EB	229 WWI8
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2	1		

11. TITLE (Precede with Security Classification Code)

(U) Military Hematology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support 002600 Biology 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
58 05	CONT	DA			C. In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	4.0	118
	CURRENT 83	4.0	80

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Drs. Lucas, Schoomaker, Alving, Kark,
NAME: Salvado, Crosby, Liu and Palmblad

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede each with Security Classification Code) (U) Coagulation; (U) Hematopoiesis; (U) Blood; (U) Marrow Failure; (U) Erythrocytes; (U) Leukocytes; (U) Sickle Cell Trait

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

23.(U) To define the hematologic pathophysiology of trauma, infections, shock, marrow toxic drugs or radiation as related to diseases of military importance; to identify modalities to restore hemostasis, to augment host defense systems against infection; The importance of this basic research to the military is wide ranging and is applicable to both health maintenance of military personnel exposed to unusual environmental, toxic and infectious hazards but also to the treatment of militarily relevant disease.
24.(U) Experimental procedures include biochemical, immunologic, and cell culture methods; in vitro cell-free and membrane-dependent systems; large and small laboratory animal models; and studies of human subjects.
25.(U) 81 10-82 09 a) A plasminogen activator secreted by a human promyelocytic leukemia cell line has been isolated and characterized in order to better understand the role of plasminogen activators in the serious bleeding disorder, disseminated intravascular coagulation b) Studies of fibrinogen in an animal model have elucidated the mechanism for hypofibrinogenemia secondary to L-asparaginase - a pharmacologic tool for understanding the normal regulation of fibrinogen production c) Studies with a human myeloid cell line have shown that guanosine nucleotide synthesis and metabolism is central to the regulation of terminal maturation of myeloid cells into blood leukocytes. d) Techniques have been established to harvest large numbers of leukocytes ($5x10^9$) from normal blood donors by leukapheresis techniques and to purify blood monocytes from leukapheresis preparations by counterflow elutriation. These cells are being prepared to study the regulation of monocyte transformation to macrophages. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 80 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1455A, 1 NOV 58 AND 1498-1, 1 MAR 65 (FOR ARMY USE) ARE OBSOLETE.

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

Work Unit 229 Military Hematology

Investigators MAJ Daniel G. Wright, MC; MAJ Barbara Alving, MC; Dr. Diane Lucas, GS-12; LTC John Kark, MC; Mr. Charles Barr, GS-12; MAJ Phil Baldwin, MC (WRAMC); COL William Crosby, MC; LTC August Salvado, MC; MAJ Eric Schoomaker, MC; Dr. Leo Lui, GS-12; Dr. Jan Palmblad (NRC Senior Fellow); MAJ Robert Knight, MC (WRAMC)

Description

Two distinct research areas have been explored under this work unit in FY82.

1. Studies of coagulation and plasma proteins

Blood coagulation factors, blood platelets, and plasma proteins (e.g. the kinin-kallikrein, fibrolytic and complement systems) are critical for the development and outcome of acute responses to traumatic, thermal and infectious injury. Studies are directed at changes in clotting and plasma proteins and in platelets during trauma, stress, and infection that lead to clinically significant abnormalities of hemostasis. Studies are also directed at understanding the therapeutic potential of intravenous immunoglobulins that have military relevance.

2. Studies of blood phagocytes

Phagocytic blood leukocytes are critical to host defense against bacterial and fungal infections and to the development and outcome of inflammatory responses. Studies of human neutrophil and monocyte function have concentrated upon understanding the secretion of soluble mediators by these cells that influence the immunoresponsive functions of macrophages and lymphocytes and affect connective tissue disorganization and repair. Studies have also been directed at understanding factors that regulate the production of neutrophils in the bone marrow and that influence the distribution and utilization of these phagocytes in peripheral tissues.

Progress

1. Studies of coagulation and plasma proteins

A. Study of the fibrinolytic potential of HL-60 cells and of monocytes infected with dengue.

The HL-60 cell line is derived from a patient with acute promyelocytic leukemia. These cells are known to produce tissue factor; however, their ability to produce plasminogen activator has not been studied. We have grown HL-60 cells and then measured plasminogen activator activity in the medium and cell lysates. This requires purified plasminogen and a plasmin substrate, which is either

[^{3}H]TAME or fibrin. We have quantitated the activator and determined if its concentration is altered when cells are treated with inducers such as when cells are treated with inducers such as DMSO or PMA.

Studies have also been begun to determine influence of dengue infection of monocytes (human) on their production of plasminogen activator (and of tissue factor). This is of importance because patients with this infection may develop disseminated intravascular coagulation and it is not clear if the etiology is due to release of tissue factor, plasminogen activator or to some other mechanism.

B. Effect of L-asparaginase on fibrinogen metabolism

L-asparaginase, a widely used anti-leukemic drug causes hypofibrinogenemia in patients. This has been attributed to decreased fibrinogen synthesis as well as to altered catabolism. We have developed a rabbit model to study the effect of this drug on fibrinogen synthesis and catabolism. This involves pretreating rabbits with the drug and measuring synthesis and catabolism with the radiolabelled amino acid [^{75}Se]selenomethionine. Data indicate that synthesis and not catabolism is altered.

C. Studies of the lupus anticoagulant

During the past year, we have identified phospholipid preparations (liposomes) that are active in the prothrombin time and partial thromboplastin time tests. We have also identified patients with the "lupus anticoagulant" i.e., antibody to phospholipid, which causes prolongation of these tests. Using the liposome preparations, we have begun to determine the phospholipid specificity of the antibody and develop simple coagulation assays through a modification of the activated partial thromboplastin time that can confirm the presence of the anti-phospholipid antibody.

2. Studies of blood phagocytes

A. The regulation of myeloid cell maturation

We have used the human myeloid leukemia cell line, HL-60, as an experimental model for understanding cellular metabolic determinants of myeloid cell maturation. The process of myeloid cell maturation is very important to the homeostasis of blood phagocyte (neutrophils and monocytes) production. We have concentrated upon purine nucleotide biosynthesis in these studies. HL-60 cells are of considerable relevance to these studies because they can be induced in vitro by various compounds to undergo terminal maturation. We have found that when HL-60 cells are exposed to inducing agents, there are consistent decreases in the levels of guanosine nucleotides (GDP and GTP) associated with induced maturation. These changes could be explained by an inhibition of guanosine nucleotide (G-NTD) biosynthesis which is a feature of induction. It was further determined that this inhibition occurred at the level of IMP production and IMP metabolism to form G-NTD. These findings led us to evaluate specific inhibitors of IMP dehydrogenase, the enzyme that mediates the first step in

production of G-NTD. These inhibitors were found to be potent inducers of HL-60 cell maturation. To date these studies have indicated that the production of G-NTD in myeloid precursor cells and the activity of the enzyme IMP-dehydrogenase may be central to the regulation of terminal maturation in myeloid cells.

In related studies of HL-60 cell induced maturation, we have determined that nicotinomide is a potent inducer by itself and also promotes the inducing potential of other inducers. Nicotinomide appears to have this effect by promoting ADP-ribosylation of chromsomal histiene proteins--a hypothesis now being pursued further.

Induced maturation of HL-60 cells was also found to be stimulated by an inhibitor of methylation reactions, 3-deazaaristoeromycin. In studies in collaboration with Dr. Peter Chiang (Div. Biochemistry), this agent has been studied as a probe for evaluating the activity of DNA methyltransferase in control of myeloid cell differentiation.

B. Transformation of human blood monocytes to macrophages

Instruments for continuous flow centrifugation leukapheresis have been set up at WRAMC in collaboration with the Blood Bank. This has permitted us to obtain leukocytes in very large numbers from normal volunteers. Blood monocytes have been purified from these preparations by counter-flow elutriation techniques which were also set up during this year. The cells obtained have been used for ongoing studies of regulatory factors in normal serum and in human neutrophil secondary granules that stimulate the transformation of monocytes to macrophages, a process that takes place at sites of acute inflammation and is necessary for development of functional capacities by macrophages that control antigen recognition, microbicidal events, and tissue repair at sites of inflammation.

Future Plans

Studies of coagulation and plasma proteins and of blood phagocytes will continue in FY83 along lines outlined above. In addition, studies of human and monkey neutrophils will be carried out to evaluate the effects of essential fatty acid deficiency, as occurs in total parenteral hyperalimentation, on neutrophil function and host defense.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OG 9284	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AH	230
b. CONTRIBUTING				
c. ~~XXXXXXXXX~~	STOG 80-7.2.2			

11. TITLE (Precede with Security Classification Code)

(U) Biological Roles of Surface Membrane Components: Parasite Model Systems

19. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 82	2.5	210
	CURRENT 83	2.5	369

10. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Antigenic Variation; (U) Metabolite Transport; (U) Drug Inhibitors; (U) Glycoproteins

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

23. (U) The objective of this work unit is to investigate the biochemistry and molecular biology of cell surface membranes as they relate to processes of antigenicity, survival, nourishment, multiplication and differentiation. Scientific information of a pragmatic, as well as a basic, nature will be sought. For example, membranes will be studied in parasites with a view to revealing and elucidating surface-associated processes that will afford the development of immunoprophylactic and/or chemotherapeutic protection of military personnel against leishmaniasis, trypanosomiasis or other tropical diseases of military importance.

24. (U) Antigenic variation will be studied by application of recombinant DNA, gene cloning, restriction analysis and probe hybridization techniques. Radioactive-labeled metabolic precursors and drugs are employed to determine metabolic pathways, transport processes and surface receptors. 2-D gel electrophoresis combined with selective surface labeling and autoradiography is used to characterize glycoprotein components of isolated membrane preparations. Purification of components is accomplished by gradient centrifugation, affinity chromatography and immunoprecipitation.

25. (U) 81 10 - 82 09 Uptake mechanisms of purine bases and nucleosides by leishmanial promastigotes have been elucidated. Data on the antileishmanial effect of thiosemicarbazone H suggest that binding of adenosine to membrane receptors is inhibited. Mycophenolic acid may owe its antileishmanial activity to selective inhibition of guanylate synthesis via IMP dehydrogenase. Messenger RNA from two trypanosomal antigenic variants has been isolated by guanidine hydrochloride extraction followed by oligo(dT)-cellulose chromatography and then used to direct cell-free protein synthesis in a reticulocyte lysate system. Preparations containing surface glycoprotein-specific mRNA have been identified by specific immunoprecipitation of the translation products. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

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

WORK UNIT: 230 Biological Roles of Surface Membrane Components: Parasite Model Systems

INVESTIGATORS:

Principal: John G. Olenick, Ph.D.
Associates: Ruth Geller, Ph.D.; Brian D. Dansen, Ph.D.
Assistants: SP4 Roberto Ayalta-Medina; SP4 Lynn Decker; John A. Kintzios, M.S.; SP4 Jose Perez-Arbelo

The objective of this work unit is to investigate the biochemistry and molecular biology of cell surface membranes as they relate to processes of antigenicity, survival, nourishment, multiplication and differentiation. Membranes are studied in parasitic protozoa with a view to revealing and elucidating surface-associated processes that will afford the development of immunoprophylactic and/or chemotherapeutic protection of military personnel against leishmaniasis, trypanosomiasis or other tropical diseases of military importance. The following investigations were conducted:

1. The elucidation of purine base and nucleoside uptake mechanisms of leishmanial promastigotes.

2. The antileishmanial effect of thiosemicarbazone H: Inhibition of adenosine receptor binding.

3. The antileishmanial effect of mycophenolic acid: Inhibition of guanylate nucleotide synthesis.

4. Trypanosome variant-specific antigen genes: Analysis using recombinant DNA.

1. The Specificity of Purine Base and Nucleoside Uptake in Promastigotes of Leishmania braziliensis panamensis.

Studies in our laboratory determined that an azacycloheptane derivative of a 2-acetylpyridine thiosemicarbazone exhibited antileishmanial activity due to inhibition of adenosine receptor binding. Before these studies could be conducted, it was essential to characterize the purine base and nucleoside transport systems of the leishmanial promastigote. Promastigotes of Leishmania braziliensis panamensis absorbed the purines adenine, hypoxantine, adenosine and inosine by a combination of diffusion and mediated components. When the uptake rates for these substrates were corrected for diffusion and compared, the purine bases adenine and hypoxanthine were transported at a significantly slower rate than the purine nucleosides adenosine and inosine. Competitive interactions among those purines tested confirmed the presence of mediated and diffusion components and suggested that three transport loci may be operating. The first transport locus, designated Locus 1, transported inosine; Locus 2, the purine bases adenine and hypoxanthine; and Locus 3, adenosine. In addition, adenine and hypoxanthine inhibited the uptake of one another competitively. A comparison of Ki values derived from double reciprocal plots of labeled hypoxanthine and adenine uptake in the presence of the unlabeled substrates as in-

hibitors suggested that adenine has a greater affinity for the transport locus.

2. Antileishmanial Activity of Thiosemicarbazone H: Inhibition of Adenosine Receptor Binding.

This study was conducted following preliminary results that demonstrated an antileishmanial action of thiosemicarbazone H. An azacycloheptane derivative (H) of a 2-acetylpyridine thiosemicarbazone was tested for antileishmanial activity and for an effect upon the binding of adenosine to receptors on the plasma membrane. Adenosine is an important precursor for the parasite for the synthesis of purine nucleotides. Promastigotes of Leishmania braziliensis panamensis (WR008) were exposed to H at varying concentrations and times of incubation and the I50 and the T50 were determined to be 0.1 μg/ml and 14 hr, respectively. Cells were then exposed to H (1 μg/ml for 0, 2, 30 and 60 min) followed by an additional 2 min incubation in the presence of radiolabeled adenosine, 2-deoxyglucose and aminoisobutyric acid. Only adenosine uptake was significantly reduced. In fact, pre-exposure of the cells to the drug for only 2 min reduced the subsequent 2 min adenosine uptake by 40%. Studies were also conducted to determine the effect of the drug on the binding of adenosine to membrane receptors. H significantly reduced the binding of a radiolabeled adenosine agonist (N6-cyclohexyladenosine) and antagonist (1,3-diethyl-8-phenyl-xanthine). These data suggest that thiosemicarbazone H may exert its antileishmanial effect by inhibiting the binding of adenosine to adenosine membrane receptors.

3. The Effect of Mycophenolic Acid on Guanylate Nucleotide Metabolism of Leishmania mexicana mexicana.

We have demonstrated an absence of de novo purine synthesis in Leishmania Spp. These parasitic protozoa satisfy purine requirements via purine salvage pathways unique to the organism. The following study was undertaken to determine the antileishmanial activity of mycophenolic acid, a known inhibitor of the purine salvage enzyme IMP dehydrogenase. Promastigotes and axenic amastigotes of Leishmania mexicana mexicana (WR127) were examined for guanylate synthesis via purine salvage mechanisms and for sensitivity of the latter systems to mycophenolic acid. Promastigotes exposed to the drug (10 μg/ml) over a period of 96 hr significantly reduced cell numbers (T50 = 17 hr) when compared to controls. Moreover, cells exposed to graded concentrations of the drug (0-1 μg/ml) yielded an I50 of 0.37 μg/ml. Similar results were obtained upon exposure of tissue-derived and axenic amastigotes. Both promastigotes and amastigotes readily incorporated 14C-hypoxanthine and 14C-guanine into adenylate and guanylate nucleotide pools via IMP. Although these pool levels were larger in the amastigote, the incorporation of these purine nucleotide precursors occurred at a greater rate in the promastigote. These data suggest a faster rate of metabolism in the promastigote or insect form of the parasite. Upon exposure of these cells to mycophenolic acid (5×10^{-5} M or 3.2 μg/ml), incorporation of the labeled substrates tested was nearly eliminated. Significant increases in total adenylate, but decreases in total guanylate, pool levels were obtained for promastigotes upon exposure to the drug. In addition, the total adenylate to guanylate ratio was significantly increased in the presence of mycophenolic acid. This drug may exert antileishmanial activity by selectively inhibiting guanylate synthesis via IMP dehydrogenase.

4. Variant-Specific Antigen Genes Trypanosoma rhodesiense: Analysis Using Recombinant DNA.

This research intends to employ the methodologies of molecular biology and use recombinant DNA and DNA hybridization analyses in order to uncover and determine the molecular mechanisms regulating the expression of variant-specific antigen genes in African trypanosomes. Variant-specific glycoprotein (VSG), that is present as a surface coat on the trypanosome, was previously shown to be responsible for the unique immunological identity of different variant types in a serodeme of Trypanosome rhodesiense Wellcome strain. The immunological uniqueness of VSG's is the result of extensive variation in the sequence of amino acids comprising the polypeptide chain. Antisera against purified VSG from a number of variants were raised in rabbits. Messenger RNA from two of these variants was isolated by guanidine hydrochloride extraction followed by affinity chromatography via hybridization of the poly(A)-containing RNA to an oligo(dT)-Cellulose column. The mRNA was used to direct a rabbit reticulocyte lysate translation system. Preparations containing VSG-specific mRNA were identified by immunoprecipitation of the translation products followed by SDS-gel electrophoresis, transfer to nitrocellulose by Western blotting and finally autoradiography. The purified VSG mRNA was used as a template for synthesis of single-stranded DNA by AMV-reverse transcriptase. The single-stranded cDNA preparations are currently being used as templates for the synthesis of double-stranded cDNA and as molecular probes into cDNA banks of clones that contain VSG-specific gene sequences.

PROJECTED STUDIES:

Studies on the biochemistry and molecular biology of surface membrane-associated phenomena in parasitic protozoa will continue. Specific aims include:

1. Development of a fluorescent test for viability in leishmanial and trypanosomal organisms utilizing dual staining with fluorescein diacetate (reacts with esterases of viable cells to yield a green fluorescence) and ethidium bromide (intercalates into nucleic acids of dead cells staining them red).

2. Continuation of studies on mycophenolic acid and thiosemicarbazone utilizing purified tissue-derived amastigotes. Of particular interest is the isolation and purification of leishmanial adenosine receptors in order to characterize directly the action of thiosemicarbazone H.

3. Potent antileishmanial activity has been demonstrated for the following purine salvage enzyme inhibitors: 2-azaadenosine, 5'-O-sulfamoyladenosine, tubercidin, nucleosidin, sangivamycin and formycin B. The modes of action of these agents will be examined.

4. Continuation of studies on the molecular basis for antigenic variation in trypanosomes. Double-strained cDNA will be cloned into the PstI cleavage site of the bacterial plasmid pBR322. Families of such clones representing a number of variant-specific glycoproteins will be used to study the organization of the trypanosome genome with respect to surface antigen genes. These clones will also allow the use of trypanosomes as a model system for the study of genome organization, genetic rearrangement and sequential expression of genes in eukaryotes.

5. Development of techniques for characterization of surface membranes using a combination of surface selective radiolabeling and 2-D gel electrophoresis. This will provide protein/glycoprotein "fingerprint" patterns for leishmanial surface membrane preparations, thus facilitating the search for diagnostic and/or protective antigens.

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ABSTRACTS AND PRESENTATIONS:

1. Hansen, B.D. and Webster, H.K. 1981. Purine metabolism in leishmania: Effect of mycophenolic acid on the synthesis of guanylate nucleotides. Am. Soc. Trop. Med. Hyg., Annual Meeting. Abst.

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PATENTS:

Olenick, J.G. and Lorenz, P.E. Float device for density gradient fractionation. U.S. Letters Patent No. 4,346,608, issued 31 Aug 82.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 9282	82 10 01	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. GDRS SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. RD /CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	CH	231 WWIG
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:4			

11. TITLE (Precede with Security Classification Code): (U) Studies of Military Personnel with Sickle Cell Trait (SCT)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
002600 Biology 012400 Personnel Selection and Maintenance

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

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	2.5	75
	CURRENT 83	2.5	109

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: LTC John A. Kark
NAME: COL William H. Crosby

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Sickle Cell; (U) Hypoxia; (U) Thalassemias: (U) Hemolysis

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

23.(U) To determine medical risks to soldiers with sickle cell trait (SCT) who may be assigned to special military roles, such as Army aviation, which require performance during hypoxia. To perform studies of changes produced in their red cells by sickling to determine whether a laboratory test can be devised capable of predicting the relative susceptibility of SCT soldiers to complications of sickling as revealed in altitude chamber studies. These studies are important to Army aviation, because of its unique requirement to carry out missions in hypoxic environments, and because of a lack of data which define the incidence and degree of medical risk to individuals with SCT.
24.(U) A prospective, controlled study of the physiologic effects of hypoxia in an altitude chamber upon SCT aviator candidates and controls, using exposures similar to mission conditions and identical to aviation training. Special procedures will include analysis of hemoglobins, accurate diagnosis of thalassemias, E.M. studies of red cells to identify subtle levels of sickle changes, use of autologous 51-Cr-RBC transfusion to measure hemolysis and splenic sequestration. SCT cells from studied individuals will be examined before and after in vitro sickling for changes in size and density distribution, filterability, 51-Cr-RBC adherence to endothelium, hemoglobin binding by cell membranes, cation and anion permeability, polymerization of Hb S and levels of ATP, 2-3-DPG by NMR studies of intact red cells.
25.(U) Assays necessary for prospective study have been established. Altitude chamber (AFIP) has been adapted for use in this study with installation of monitoring equipment. Initial controls have been studied. Retrospective study of sudden death among troops in basic combat training with SCT has been completed in collaboration with AFIP. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1455A, 1 NOV 65 AND 1456-1, 1 MAR 65 (FOR ARMY USE) ARE OBSOLETE

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

Work Unit 231 Studies of Military Personnel with Sickle Cell Trait (SCT)

Investigators LTC John A. Kark, MC; COL William M. Crosby, MC;
MAJ Daniel G. Wright, MC; MAJ David Posey, MC (AFIP)

Description

Individuals with sickle cell trait (SCT) may now enter previously restricted MOS which involve exposure to hypoxia and other stresses that can cause intravascular sickling in people with SCT and have been infrequently reported to cause medical complications. The frequency and severity of complications in SCT individuals going through usual Army aviation, high altitude parachute, and deep sea diving training and missions is unknown. It is possible that previous infrequent reports of such complications reflected a small percentage of individuals more susceptible to such complications than the usual person with SCT. It is important to be able to identify such a subgroup of SCT individuals at greater risk. Better understanding of events leading to deformed sickled cells and vascular obstruction by sickled cells is needed in order to develop definitive subclinical tests for the risk involved in hypoxia alone or with other stresses.

Army missions involve a unique requirement for performance in specialized military environments which cause hypoxia, at least equivalent to an altitude of 14,000 to 15,000 feet, at times combined with heat exposure, dehydration, and exertional stress. These requirements are evident for Army aviation, high altitude, high altitude parachute corps, and deep sea diving. Entry of individuals with SCT (7-10% of American Blacks) into these duties poses the immediate problem of determining the medical risks resulting from the potential for intravascular sickling, and the impact of such risks on the missions. Required information does not exist to specify appropriate medical regulations. Laboratory tests which could be used to identify the level of risk for given environmental conditions are needed.

Hypoxic exposures in an High Altitude Chamber will be induced in graded levels, to reproduce the aviation training protocol in a prospective, controlled study of the susceptibility of SCT aviator candidates to subclinical and/or clinical complications of hypoxia. Later, these physiologic studies will examine exercise, heat and dehydration, and combination of these stresses with hypoxia, as needed to reproduce mission conditions. Special techniques will include sophisticated E.M. studies of reversible and irreversible sickled cells, studies of survival and splenic sequestration of autologous 51-Cr-RBC, and special studies of renal and pulmonary physiology. Well studied SCT individuals and controls will be stratified by level of SCT-complications, and will serve as a source for intensive study of red cell characteristics which might predict susceptibility to vascular complications of sickling. NMR will be used to measure extent to Hb S polymerization and ATP levels of intact RBC. Changes in RBC density distribution, using a Percoll gradient, RBC size distribution, using a Coulter channellyzer, hemoglobin binding by RBC membranes, cation and anion permeability, filterability, and adherence of RBC to endothelial cultures in response to controlled hypoxia in tonometers will be correlated with the clinical stratification.

Progress

After consultation with the Aeromedical Command at Ft. Rucker, AL, a study was designed to determine the safety and physiologic consequences of NATO altitude indoctrination on aviator officer candidates with sickle cell trait (SCT), by means of a prospective, controlled study of step-wise exposures in an altitude chamber.

A protocol was prepared and defended; equipment and techniques were established, and the following studies were initiated:

"A Preliminary Study of Normal Ear Oximeter Readings at Varied Altitudes in an Hypobaric Chamber"

The principal study of this work unit has also received Clinical Investigation approval:

"A Study of Altitude Chamber Training for Individuals with Sickle Cell Trait" by J.A. Kark, P.G. Tarassoff, D.M. Posey, D.G. Wright, C.U. Hicks, and D.B. Kimball. This is a collaborative effort, administered from Dept. of Hematology, WRAIR involving two other departments at WRAIR, a division at AFIP, Department of Medicine, WRAMC (Hematology, Pulmonary, Nephrology Divisions), Department of Opthalmology, WRAMC, Department of Nuclear Medicine, WRAMC.

Investigators of this work unit were requested by the Preventive Medicine Section, OTSG, to provide consultation concerning the problem of unexpected death in Basic Combat Training, and attended a briefing of Generals Becker, Bender, and Burka. In early July, consultation was provided to Ft. Jackson. The results of this consultation led to issuance of a directive to all Basic Training Centers for the management of soldiers with sickle cell trait, prepared by COL Kimball based on the report by MAJ Posey and LTC Kark.

A retrospective study of sudden death in basic combat training was begun. It is the objective of this study to provide accurate estimates of death rates for SCT individuals in comparison with Black and non-Black individuals without SCT, in basic combat training.

Future Plans

1. Completion of studies outlined above.

2. Design and completion of future studies to define the pathogenesis of SCT related injury in hypoxia and acute physical exertion among physically unconditioned troops, as occurs in BCT.

3. Design of strategies to prevent SCT related hazards that may exist in military environment.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DAOH 0169	3. DATE OF SUMMARY* 82 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
2. DATE PREV SUM'RY 82 04 09	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8A. DISB'M INSTR'N NL	8b. SPECIFIC DATA: CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	EC	232
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:1			

11. TITLE (Procede with Security Classification Code)*

(U) Immunochemistry of Nerve Agents

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

016800 Toxicology 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 04	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	2.5	75
	CURRENT 83	1.0	98

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Bennett M. Kaufman, Ph.D.,

NAME: Robert C. Seid, Jr., Ph.D.

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Immunochemistry; (U) Soman; (U) Monoclonal Antibodies; (U) Nerve Agents

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

23. (U) Development of passive and active immunization for protection against nerve agents in humans. Nerve agents represent a serious threat to combat military personnel.

24. (U) Nerve agents such as Soman and Soman analogues will be covalently coupled to protein carriers such as tetanus toxoid, and adjuvants such as gram negative bacterial membrane proteins, detoxified gram-negative lipopolysaccharides. These vaccines will be tested in mice for potency and used for vaccination of mice to produce monoclonal antibodies. Human monoclonal antibodies of high affinity will be produced by in-vitro vaccination of human lymphoid cells followed by fusion to long term human myeloma lines. Human monoclonal antibodies will be tested for potency. Tissue culture techniques for large scale production of human monoclonal antibody for human use will be explored. Recombinant DNA approaches to large scale production of antibody for human use will be explored.

25. (U) 81-10 - 82-09 Covalently coupled protein hapten conjugates using Chloro G-D have been prepared and initial mouse fusions performed. A human myeloma HAT sensitive cell line has been successfully fused with human spleen and human peripheral blood cells. (For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 81 - 30 Sep 82).

*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 1455-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

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

Work Unit 232 Immunochemistry of Nerve Agents

Investigators:

Principal: Jerald C. Sadoff, M.D., LTC (P), MC
Associates: Carter L. Diggs, M.D., COL, MC
Bennett M. Kaufman, Ph.D.
Robert Seid, Ph.D.

Problem

Development of immunoprophytoxins for nerve agents using hybridoma technology.

Objectives

Develop mouse monoclonal antibodies against SOMAN which are protective in a mouse model. Develop techniques for safe and effective utilization of mouse monoclonals in humans. Develop human monoclonal antibodies against SOMAN for use in humans.

Progress

Conjugates between SOMAN derivatives and a number of adjuvants have been obtained from the U.S. Army Institute of Chemical Defense and/or made by us. These vaccines have been used for immunization of mice. Two human HAT sensitive myeloma cell lines have been obtained and successful fusions with human spleen and human peripheral blood have been accomplished. The equipment and supplies for a human hybridoma laboratory and a mouse hybridoma laboratory dedicated to this project have been obtained.

Recommendations

When mouse monoclonals which protect against SOMAN intoxication are found, these lines should be mutated to produce F(ab) fragments to be used immediately prior to attack. The hypervariable region should be determined by recombinant techniques and placed in human heavy and light chains. *In vitro* stimulation of human cells for production of human monoclonal antibody should be pursued.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300029	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
82 07 01	D. Change	U	U		NI	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	EB	233 WWGV
b. CONTRIBUTING				
c. ~~XXXXXXXXXX~~	STOG 80-7.2:1			

11. TITLE (Procede with Security Classification Code)

(U) Nerve agent antidote screening with invertebrate bioassay systems

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	1.0	50
FISCAL YEAR CURRENT 83	1.0	75

19. RESPONSIBLE DOD ORGANIZATION

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NAME: POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nerve agents; (U) Antidotes; (U) Musca domestica; (U) Atropine; (U) Pyradlioxamine chloride; (U) Sarin; (U) Soman; (U) Tabun; (U) VX

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

23. (U) Develop and evaluate arthropod bioassay systems for screening of potential chemical nerve agent antidotes. Identify the most promising systems, develop testing methodology and conduct comparative evaluations with existing mammalian systems. The development of effective protective measures against chemical warfare agents is a high priority project for the Army and the search for antidotes to the known chemical warfare nerve agents is an important part of that effort. Clearly, realization of the objectives in this work unit may result in development of several rapid, inexpensive arthropod bioassay screening systems for antidotes to CW nerve agents.

24. (U) Conduct a literature review to identify and prioritize the most promising insect systems. Establish new laboratory and insect rearing facilities to support this work. Identify laboratories equipped for CW nerve agent research and obtain permission to conduct research in their facilities. Establish selected insect colonies, develop and refine application procedures, testing methodology and statistical analysis criteria. Conduct tests and evaluate results so that the most useful and effective screening systems can be brought on-line.

25. (U) 81 10 - 82 09 Laboratory space and an insectary area have been obtained. Major supplies and some items of equipment have been ordered. Submitted a job description and request to hire a research invertebrate physiologist. Two laboratories equipped for nerve agent work have been identified and contacted for possible use of their facilities. A bioassay system using Musca domestica has been identified as the method of choice and equipment for microinjection or microapplication has been ordered. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

*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 65 (FOR ARMY USE) ARE OBSOLETE.

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

Work Unit 233 Nerve Agent Antidote Screening with Invertebrate Bioassay Systems

Investigators:
Principal: Donald R. Roberts, LTC, MSC
Associate: Robert A. Wirtz, CPT, MSC

Problem

The Department of Defense is currently placing increased emphasis on the development of defensive measures against chemical warfare nerve agents, classified as acetylcholinesterase (AChE) inhibitors (1,2). AChE is the enzyme which breaks down acetylcholine, the primary neurotransmitter in the mammalian cholinergic nervous system. The blockage of AChE by nerve agents results in the continued presence of excess ACh. This causes repeated firing of target receptors on neurons, muscles and glands which leads to the disruption of neural communication and eventually death (3). Chemical warfare nerve agents with this suspected mode of action include sarin (O-isopropyl methylphosphonofluridate), soman (O-1,2,2-trimethylpropyl methylphosphonofluoridate), tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate) and VX (O-ethyl S-2-diisopropylaminoethyl methylphosphonothioate) (1,2). These compounds are nonreversible inhibitors which rapidly and permanently bind to AChE. They also undergo unimolecular dealkylation, a process referred to as "ageing" (4,5). This ageing results in a nonfunctional enzyme-nerve agent complex which cannot be activated using traditional antidote therapy. The suspected mode of action of nerve agents is similar to that of the organophosphate insecticides, which are also nonreversible AChE inhibitors (3).

Because of the rapid ageing of the nerve agent-enzyme complex, treatment after exposure is usually ineffective. One approach to improving protection against these agents is to administer the antidote prior to exposuure to the nerve agent (6,7). The carbamates (pyridostigmine and physostigmine) also bind with AChE, however they differ from the organophosphosphorus nerve agents in thst the rate of carbamylation, to yield free enzyme, is significantly faster than the rate of dephosphorylation. Administration of these antidotes prior to exposure to the agent results in a "protective" carbamylated-enzyme being present when the nerve agent enters the body. As agent levels are reduced, AChE is released from the carbamate at a rate which will maintain vital neural functions.

Therapeutic agents that are currently available are not completely satisfactory in saving life and in reducing physical and mental decrements. The search for new and more effective therapeutic and pretreatment drugs continues. Because of limitations in conducting efficacy studies in humans when one is dealing with organophosphorous nerve agents, testing is almost entirely carried out with *in vitro* studies or animal models.

As new antidotes or pretreatment drugs are proposed, it will be highly desirable to have a rapid inexpensive bioassay screening system available for drug development. Bioassay systems using intact insects have several distinct advantages over existing models. Most insect systems are rapid and relatively inexpensive with only small amounts of test material and nerve agent required. This can be especially important when candidate antidotes are custom synthesized and available only in small quantities. The use of small amounts of nerve agent, determined on a ug/kg live body weight, is an added safety factor. Most assays can be brought on line quickly, as no complicated equipment or training are usually required. Costly animal rearing and handling facilities and personnel can be eliminated as less expensive mass rearing procedures have been developed for the arthropods currently under consideration for use. The ability to use large numbers of test insects and/or large sample sizes makes these tests particularly applicable to statistical analysis.

The question as to the applicability of tests conducted on insects to mammalian systems is a valid one due to differences in major organ systems and detoxification, activation and transport mechanisms. However, similar modes of action of nerve agents are suspected in both insects and vertebrates and the presence of similar receptors, enzymes and metabolic systems supports this premise. Once test data is available results can be compared to those of existing noninsect bioassay models and *in vitro* data to determine the feasibility of using insect systems for antidote screening.

Progress

This is a new project but progress has been made in getting the work started. Laboratory space and an insectary area have been obtained. Major supplies and some items of equipment have been ordered. A job description has been submitted for the hire of an invertebrate physiologist. Two laboratories equipped for nerve agent work have been identified and contacted for possible use of their facilities. An extensive collection of literature has been compiled and a bioassay system using *Musca domestica* has

been identified as the method of choice and equipment for microinjection or microapplication has been ordered.

Future Plans

Efforts will be continued to fully staff this project, to compile a completed and comprehensive literature search on existing models that might be useful to the objectives of this work unit, to obtain and fully equip the required laboratory space and to test and evaluate a variety of potentially valuable insect bioassay systems.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300294	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. RECHADING	8a. DISP'R INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. PSBE UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	ED	234 WWH9
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code)

(U) Molecular Biology of Medical Defense Against Chemical Agents

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry 002600 Biology 012900 Physiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 82	2.0	300
FISCAL YEAR CURRENT 83	3.0	424

19. RESPONSIBLE DOD ONGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Doctor, B.P.

NAME: Olenick, John G. POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Organophosphates; (U) Antidotes; (U) Mode of Action; (U) Receptors; (U) Enzymes

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

23. (U) The objective of this work unit is to study the medical/chemical defense of military personnel against severely intoxicating chemical agents with a view to defining the molecular basis of inhibited cellular processes.

24. (U) Tissue culture and/or live animal methodology will be developed to study the mode of action of chemical agent poisoning. Pharmacokinetics and metabolic profiles of protective or therapeutic drugs also will be studied. Selective enzymatic assays for chemical agents, antidotes and cellular targets will be developed. The relationship and ontogeny of certain enzymes, receptors and neurotransmitters will be studied. Recombinant DNA and gene cloning procedures will be explored to further define ontogenetic relationships and topology of reactive sites.

25. 81 10 - 82 09 Tissue culture methodology employing a variety of cell systems has been developed to study the mode of action of organophosphates and their antidotes. The antidote aprophen is taken up and metabolized by primary hepatocytes within minutes. The identification of the metabolites is being determined by mass spectrometry. Surprisingly, aprophen is extremely cytotoxic to N4TG1 neuroblastoma cells and NG108-15 neuroblastoma x glioma hybrid cells and is less cytotoxic to clone 9 liver cells and 3T3-C2 fibroblasts. A radioactive assay for acetylcholinesterase hss been developed based on the separation of 14C-acetste from 14C-acetylcholine by a differential absorption of the former on DEAE anion-exchange discs. Incubation of N4TG1 neuroblastoma cells with the orgsnophosphate paraoxon causes a 20-50% increase in the incorporation of 14C-choline into phosphatidylcholine of the cellular phospholipids. However, this increase can be prevented by treating the cells with antidote 2-PAM. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

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

WORK UNIT: 234 Molecular Biology of Medical Defense Against Chemical Agents

INVESTIGATORS:

Principal:	Peter K. Chiang, Ph.D.
Associates:	Bhupendra P. Doctor, Ph.D.; John G. Olenick, Ph.D.
Assistants:	Richard K. Gordon, Ph.D.; SFC Evelyn Moore; SP4 Felipe N. Padilla

The objective of this work unit is to study the medical/chemical defense of military personnel against severely intoxicating chemical agents with a view to defining the molecular basis of inhibited cellular processes. Tissue culture models are developed for investigating the modes of action of organophosphates and their antidotes on the enzymes and receptors of attendant cholinergic systems. The following investigations were conducted:

1. Development of a radioactive assay for acetylcholinesterase using anion-exchange disks.

2. The cytotoxic action of aprophen on various types of cells.

3. Increased incorporation of choline into phosphatidylcholine in N4TG1 neuroblastoma cells exposed to paraoxon.

4. Completion of studies on using S-adenosylhomocysteine hydrolase as a pharmacological target for the inhibition of transmethylation begun by the principal investigator while employed at another Federal research facility.

1. Development of a Radioactive Assay for Acetylcholinesterase Using Anion-Exchange Disks.

A radioactive assay for determining the activity of acetylcholinesterase was developed. The assay is based on the separation of ^{14}C-acetate from ^{14}C-acetylcholine by differential absorption of the former on DEAE anion-exchange (DE81) filter disks. Stacked filter disks were held in a filtration manifold. Three DE81 filter disks bound 88% of the ^{14}C-acetate; more than 95% was bound when four disks were used. When four were used, the ^{14}C-acetylcholine contained less than 2% contaminating acetate. The assay was linear with time and the hydrolysis of ^{14}C-acetylcholine was proportional to the amount of electric eel acetylcholinesterase added. Good agreement of enzyme activities for electric eel acetylcholinesterase was found for both the radioactive assay and the Ellman colorimetric assay under identical conditions. Moreover, when unpurified enzyme preparations (cell supernatants) were assayed, linear steady-state kinetics continued to be observed with this method as contrasted to the nonlinear colorimetric method. This method also permitted the detection in biological samples of low levels of acetylcholinesterase activity which was not detectable by the colorimetric method. A number of cell lines were surveyed for acetylcholinesterase activity using the radioactive disk method. Activity was higher in the N4TG1 neuroblastoma cells than the NG108-15 neuroblastoma X glioma hybrid cells. A clonal cell line of myoblasts from rat heart, H9c2, exhibited relatively low activity. Mouse 3T3-L1 fibroblasts had very low

acetylcholinesterase activity, as did 3T3-C2 fibroblasts, about 600-fold less than that observed in the N4TG1 cells. The present radioactive assay may be conveniently applied to the screening of anticholinesterase agents and the study of acetylcholinesterase or pseudocholinesterase and their roles in membrane function and cellular differentiation.

2. Cytotoxic Action of Aprophen on Various Types of Cells in Culture.

Aprophen is an antispasmodic and cholinolytic agent used in antidolal formulaitons in the treatment of organophosphate poisoning. Because of its high cholinolytic potency and ability to penetrate the blood-brain barrier, it is administered prophylactically and therapeutically as an antidote to nerve agents. The action of aprophen was studied in a variety of cell systems. In primary hepatocytes, aprophen was taken up and rapidly metabolized within minutes. Upon analysis by high performance liquid chromatography, several unknown metabolites were found. Surprisingly, aprophen was very cytotoxic to N4TG1 neuroblastoma cells and NG108-15 neuroblastoma X glioma hybrid cells with I50's in the range of 0.1 and 1 μM. Aprophen was less cytotoxic to clone 9 liver cells and 3T3-C2 fibroblasts (I50's of 25 μM), probably because of their greater ability to metabolize aprophen than the neuroblastoma cells. The nature of the cytotoxic action of aprophen is presently under investigation.

3. Increased Incorporation of Choline into Phosphatidylcholine in N4TG1 Neuroblastoma Cells Exposed to Paraoxon.

N4TG1 neuroblastoma cells were treated with graded doses of paraoxon. After labeling with ^{14}C-choline, paraoxon-treated or paraoxon-free control cells were analyzed for radioactive choline incorporation into aqueous and organic extracts by employing thin layer chromatography. Upon incubation of treated cells, an increase in the incorporation of ^{14}C-choline into phosphatidylcholine of the cellular phospholipids (organic extract) was found, reaching a maximum of approximately 20-30%. The paraoxon dose response increase in choline incorporation was inversely related to the inhibition of acetylcholinesterase activity by paraoxon. Treating the cells with the antidote 2-PAM appeared to prevent the increase in the flux of ^{14}C-choline. In view of the importance of phospholipids in membrane functions, this increase in flux of ^{14}C-choline may alter the fluidity of neuronal membranes, thus resulting in a derangement of membrane functions.

4. S-Adenosylhomocysteine Hydrolase as a Pharmacological Target for Chemotherapy.

Because of the wide spectrum of biological activities of the inhibitors of S-adenosylhomocysteine hydrolase (AdoHcyase), the search for more potent and more specific inhibitors continues. As a result, a new 3-deaza purine analog, 3-deazaaristeromycin (c^{3}Ari), was synthesized. c^{3}Ari was found to have potent antiviral activity in cell culture against a variety of RNA and DNA viruses: vesicular stomatitis, parainfluenza-3, measles, Coxsackie B4, Reo-1, Sindbis, and vaccinia. HSV-1, HSV-2 and polio-1 viruses are minimally affected by c^{3}Ari. Oncogenic transformation of normal rat kidney cells induced by HL-23 type C virus was also inhibited by c^{3}Ari. c^{3}Ari was relatively noncytotoxic at effective antiviral concentrations and is not

subject to phosphorylation or deamination. It acts as a competitive inhibitor with a K_i of x 10^{-9} M for AdoHcyase from hamster liver and a K_i of 3 x 10^{-6} M for the enzyme from beef liver. The antiviral effect of c3Ari can be correlated with the accumulation of AdoHcy and AdoMet in the cells, presumably from the inhibition of AdoHcyase.

Another interesting aspect of c^3Ari is its effect on cellular differentiation. c^3Ari can cause the human promyelocytic leukemia cell line, HL-60, to acquire the characteristics of mature neutrophils. Exponentially growing HL-60 cells in RPMI 1640 and 10% FCS were exposed to varying concentrations of c^3Ari for 7 days. A dose dependent inhibition of cell growth was observed with greater than 90% inhibition occurring in the presence of 5 x 10^{-5} M c^3Ari. A majority of HL-60 cells acquired morphological and functional features of mature neutrophils including the expression of plasma membrane NDA(P)H oxidase and the ability to phagocytize opsonized yeast. Analysis of guanosine nucleotide pools in HL-60 cell extracts by high performance liquid chromatography demonstrated consistent decreases in GDP and GTP in cells exposed to c^3Ari. An accumulation of ^{35}S-adenosylmethionine and ^{35}S-adenosylhomocysteine in c^3Ari-treated HL-60 cells was also observed that was probably due to inhibition of AdoHcyase. The results of these studies indicate that c^3Ari is a potent inducer of maturation for HL-60 cells and provide further support for the concept that guanosine nucleotides may have a regulatory role in cellular maturation. These studies also indicate that maturation of HL-60 cells induced by c^3Ari is associated with perturbations of methylation reactions.

PROJECTED STUDIES:

Studies utilizing tissue culture models to investigate the modes of action of organophosphates and their antidotes will continue. Specific aims include:

1. An eluciation of the mode of cytotoxic action of aprophen. The interplay of cholinergic mechanisms and interactions with catecholamines will be studied in the NG108-15 cells using amphoteric chemical detectors in conjunction with high performance liquid chromatography.

2. Studies will continue to assess the effect of paraoxon poisoning on N4TG1 neuroblastoma cells. Membrane functions will be studied in view of the importance of phospholipids in membrane structure and that an increase in the flux of choline may perturb the fluidity of neuronal membranes. Additional inhibitors of acetylcholinesterase will be utilized to characterize the biochemical alterations resulting in increased choline incorporation.

3. It has been reported by others that acetylcholine receptors can be methylated and that methylation of phospholipids and/or proteins may be involved in neurotransmission. The involvement of phospholipid methylation in cellular events of organophosphate poisoning will be studied.

PUBLICATIONS:

1. Gordon, R.K., Doctor, B.P. and Chiang, P.K. 1982. A radioactive assay for acetylcholinesterase using anion-exchange disk. Anal. Biochem. 124: 333-337.

2. Lucas, D.L., Chiang, P.K., Webster, H.K., Robins, R.K., Wiesmann, W.P. and Wright, D.G. 1982. Effects of 3-deazaguanosine and 3-deazaguanine on the growth and maturation of the human promyelocytic leukemia cell line, HL-60. Proceedings of the IVth International Symposium on Human Purine and Pyrimidine Metabloism. (in press)

3. Montogomery, J.A., Thomas, J.J., Thorpe, M.C. and Chiang, P.K. 1981. Synthesis of S-(3-deazaadenoysl)-L-homocysteine. J. Med. Chem. 24: 1514-1516.

4. Chen, L.T. and Chiang, P.K. 1981. Intrasplenic pH in normal and anemic rats. Am. J. Hematol. 11: 403-407.

5. Montgomery, J.A., Clayton, S.J. and Chiang, P.K. 1982. 1-β-D-Arabinofuranosyl-1H-imidazo[4,5-c]pyridine (Ara-3-dezaadenine). J. Med. Chem. 25: 57-63.

6. Morita, Y., Siraganian, R.P., Tang, C.K. and Chiang, P.K. 1982. The inhibition of histamine release and phosphatidylcholine metabolism by 5'-deoxy-5'-isobutylthio-3-deazaadenosine (3-deaza-SIBA). Biochem. Pharmacol. (in press)

7. Pritchard, P.H., Chiang, P.K., Cantoni, G.L. and Vance, D.E. 1982. Inhibition of phosphatidylethanolamine N-methylation by 3-deazaadenosine stimulates the synthesis of phosphatidylcholine via the CDP-choline pathway. J. Biol. Chem. 257: 6362-6367.

8. Chiang, P.K. and Shattil, S.J. 1982. The pharmacological inhibition of phospholipid methylation. Proceedings of the Second International Symposium of Transmethylation. (in press)

9. Shattil, S.J., Montgomery, J.A. and Chiang, P.K. 1982. The effect of pharmacological inhibition of phospholipid methylation on human platelet function. Blood 59: 906-912.

10. Montgomery, J.A., Clayton, S.J. Thomas, H.J., Shannon, W.M., Arnett, G. Bodner, A.J., Kim, I.K., Cantoni, G.L. and Chiang, P.K. 1982. The carbocyclic analog of 3-deazaadenosine: A novel antiviral agent using S-adenosylhomocysteine hydrolase as a pharmacological target. J. Med. Chem. 25: 626-629.

11. Chiang, P.K. 1982. S-Adenosylhomocysteine hydrolase as a pharmacological target for the inhibition of transmethylation. Proceedings of the IVth International Symposium on Human Purine and Pyrimidine Metabolism. (in press)

ABSTRACTS AND PRESENTATIONS:

1. Chiang, P.K. Second Annual Chemical Defense Bioscience Review, Aberdeen Proving Ground, MD, 14 May 1982.

2. Chiang, P.K. 1982. S-Adenosylhomocysteine hydrolase as a pharmacological target for the inhibition of transmethylation. J. Clin. Chem. Clin. Biochem. 20, P358. Abst.

3. Lucas, D.L., Chiang, P.K. Robins, R.K., Wiesmann, W.P. and Wright, D.G. 1982. Effects of 3-deazeguanosine and 3-deazaguanine on the growth and maturation of the human promyelocytic leukemia cell line, HL-60. J. Clin. Chem. Clin. Biochem. 20, P391. Abst.

4. Chiang, P.K., Gordon, R.K., Brown, N.D., De Clercq, E. and Montgomery, J.A. 1982. 3-Deazaaristeromycin: A novel antiviral agent using S-adenosyl-homocysteine hydrolase as a pharmacological target. Fifth Nucleoside Round Table.

5. Lucas, D.L., Chiang, P.K. and Wright, D.G. 1982. The effect of 3-deaza-aristeromycin, an inhibitor of methylation, on differentiation of human promyelocytic leukemia cells. Fifth Nucleoside Round Table.

6. Chiang, P.K., UCLA-Harbor Medical Center, Torrance, CA, 20 Oct 1981.

7. Chiang, P.K., University of California, School of Medicine, La Jolla, CA, 23 Oct 1981.

8. Chiang, P.K., Transmethylation Conference, Lake Ozarks, MO, 25 Oct 1981.

9. Chiang, P.K, National Institute of Neurological Diseases and Communicative Disorders, NIH, Bethesda, MD, 2 Dec 1981.

10. Chiang, P.K., National Institute on Aging, Gerontology Research Center, Baltimore, MD, 7 Jan 1982.

11. Chiang, P.K., IVth International Symposium on Purine and Pyrimidine Metabolism, Maastricht, Netherlands, 14 June 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 300402	82 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'R INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	X ☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	00	235 WWP3
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

)Ultrastructural Study and Definition of Disease of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2.0	256
FISCAL YEAR CURRENT 83	2.0	285

19. RESPONSIBLE DOD ORGANIZATION

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NAME:

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nutritional; (U) Host-Parasite Relationship; (U) Pathogenesis; (U) Ultrastructural Damage; (U) Repair; (U) Toxins; (U) Trauma

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 ultrastructural bases of the pathogenesis of various diseases of military significance. Of particular interest will be investigation of the development of toxic, traumatic, and nutritional deficiency damage to the respiratory tract and subsequent repair processes. The results of these studies will be useful in determining probable genesis of similar damage in relevant conditions in the human military population. Additional, collaborative studies with other investigators will study the ultrastructural basis of the host-parasite relationship in infectious diseases of military importance.

24(U) Conventional ultrastructural techniques, including transmission and scanning electron microscopy, as well as enzyme histochemistry, immunochemistry and morphometry

25(U) 81 10-82 09 The sequential events of tracheal epithelial repair following mechanical denuding have been characterized utilizing transmission electron microscopy (TEM), autoradiography, and immunocytochemistry. Follow-up studies of surface changes with scanning electron microscopy (SEM) have been initiated. Changes induced in rabbit ileum by purified enterotoxin of Shigella spp. at various exposure times and concentrations have been studied with light and SEM. TEM of the same is now underway. Morphologic aspects of macrophage - Leishmania donovani interaction have been initiated. Ongoing efforts include localization of surface antigens of Trypanosoma rhodesiense, Hepatitis A Virus interaction in vivo and in vitro, and development of diagnostic capabilities in virus and tumor differentiation. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1465A, 1 NOV 65

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

Work Unit 235: Ultrastructural Study and Definition of Diseases of Military Importance

Investigators:
Principal: Kevin Keenan, D.V.M., Ph.D., MAJ, VC
Associate: Tatsuo Hase, M.D.; Douglas Sharpnack, D.V.M., M.S., CPT, VC

Description:

To define histopathologic manifestations of experimentally induced injury or disease which present current or potential problems to military personnel. Of particular interest to this unit are those aspects of injury or disease best studied at the ultrastructural level. Being a central electron microscopy facility for the WRAIR, much of this research is carried on in collaboration with other investigators from throughout the Institute. A multidisciplinary approach including conventional histology, histo-and cytochemistry, autoradiography, immunocytochemistry, scanning and transmission electron microscopy is employed.

Progress:

Progress in original and collaborative studies is presented below:

I. Studies on Tracheal and Bronchial Injury.

Results obtained in conjunction with ongoing investigations of the potential effects of free field artillery blasts on operator personnel have revealed new concepts in the cytodynamics of respiratory epithelial repair. Epithelial regeneration is a basic response to injury and precise definition of this process is fundamental to our understanding of development processes, to maintenance of the adult state, and to many pathological processes including mechanical, toxicological and infectious respiratory injury. An understanding of the proliferative capabilities of epithelial cells, appreciation of the wide spectrum of phenotypic modulations and pathways of differentiation, and determination of the origins of nascent cells during the regenerative process, have widespread significance and implication in pathology.

To explore the regenerative response denuding mechanical injuries of varying severity were made to

maximally stimulate the epithelium and to recruit as many cells as possible into the regenerative process. Syrian golden hamsters (Mesocricetus auratus) were used for these studies because of the striking morphological similarities of hamster tracheal epithelium to that of human bronchus.

The control hamster tracheal epithelium was composed of about 33% cilited cells, 5% secretory cells and 10% basal cells. Proliferative activity, measured with the combined use of colchicine metaphase blockade and single pulse tritiated thymidine (^{3}HTdR) labeling, was low and confined to the secretory and basal cells. Of the cells contributing to the low labeling index (LI=0.63%) and low mitotic rate (MR=0.19), more were secretory cells (LI=0.36%, MR=).14%) than basal cells (LI=0.26%, MR=0.05%)..

Following a focal denuding wound, 31% of the epithelium was lost by 12 hours, but following multifocal wounds, 43% of the epithelium was lost by 12 hours. During these times LI and/or MR remained near control values. Viable cells at the wound margins rapidly changed shape, flattened and migrated into the wounds at about 0.5μ per minute to cover the focal wounds by 12 hours. In addition to migrating, epithelial cells that remained viable demonstrated sublethal changes that included the rapid discharge of mucous granules from secretory cells, internalization of cilia by ciliated cells and evidence of heterophagy.

By 24 hours there was an exponential increase in cell proliferation in the focal wound sites (MR=31.1%). Mitotic secretory cells (MR=19.9%), basal cells (MR=1.4%) and squamous cells - a mixture of flattened secretory and basal cells (MR=9.8%) contributed to this proliferative activity. When only ^{3}HTdR labeled cells were considered as discrete populations at 24 hours, labeled secretory cells had 88% of their number in mitosis and 12% in interphase, compared to labeled basal cells with 45% in mitosis and 55% in interphase. These data indicate that secretory cells passed through the DNA synthesis phase into mitosis at twice the rate of basal cells. Thus secretory cells played the dominant role in tracheal regeneration due to the number of cells involved and their proliferative rate.

This proliferation produced a multilayered epidermoid metaplasia that was best developed at 48 hours. The metaplastic epithelium was largely composed of cells with both secretory (mucous granules) and epidermoid (tonofilament bundles and numerous desmosomes) characteristics. The mitotic activity in this metaplastic epithelium gradually declined and continuous infusion of ^{3}HTdR labeled all these cells.

The peroxidase-antiperoxidase (PAP) method demonstrated a few keratin positive cells in the wound as early as 12 hours, with increasing numbers of cells positive by 24 hours. All cells in the metaplastic epithelium were keratin positive by 48 hours. These data proved that the abundant tonofilament bundles present at 48 hours in the metaplastic cells were composed of keratin. The epidermoid metaplasia was transient in small wounds and persistent in large wounds.

As the MR and LI declined in the wound pre-ciliated and presecretory cells appeared being most evident at 72 hours. These large cells were not labeled by single pulse ^{3}HTdR but were labeled by continuous ^{3}HTdR infusion. Pre-ciliated cells had abundant lucent cytoplasm, large pale nuclei, filiform apical microvilli and evidence of ciliogenesis, similar to that seen during fetal development. Pre-ciliated cells often contained mucous granules, apparently carried over from the parent secretory cell. Later large pale staining ciliated cells, with identical labeling patterns to pre-ciliated cells, were observed budding cilia. With the appearance of these cells the normal pseudostratifiied morphology was rapidly restored in the small wounds.

These data suggest that secretory cells have a greater proliferative potential than basal cells in regenerating tracheal epithelium, and that they contribute to the development of both transient and persistent epidermoid metaplasia and the production of new secretory cells. Moreover, these proliferating secretory cells produce ciliated cells via a transient pre-ciliated cell. These data provide further evidence for the important role of proliferating secretory cells in the histogenesis of epidermoid metaplasia and regeneration of normal morphology following injury. The knowledge gained from these studies provides a firmer basis for the understanding of the histogenesis of other lesions observed in the tracheobronchial epithelium.

Publications:

1. Keenan KP, Combs JW, McDowell EM (1982a) Regeneration of hamster tracheal epithelium after mechanical injury. I. Focal Lesions: Quantitative morphological study of cell proliferation. In press, Virchows Arch B Cell Pathol

2. Keenan KP, Combs JW, McDowell EM (1982b) Regeneration of hamster tracheal epithelium after mechanical injury. II. Multifocal Lesions: Stathmokinetic and autoradiographic studies of cell proliferation. In press. Virchows Arch B Cell Pathol

3. Keenan KP, Combs JW, McDowell EM (1982c) Regeneration of hamster tracheal epithelium after mechanical injury. III. Large and small lesions: Comparative stathmokinetic and single pulse and continuous thymidine labeling autoradiographic studies. In press. Virchows Arch B Cell Pathol

4. Keenan KP, Wilson TS, McDowell EM (1982d) Regeneration of hamster tracheal epithelium after mechanical injury. IV. Histochemical, immunocytochemical and ultrastructural studies. Submitted to Virchows Arch B Cell Pathol

II Studies on the Multiplication of Rickettsia tsutsugamushi in the mammalian host cell.

The ulstrastructure of scrub typhus rickettsia, include a cell wall, a plasma membrane, a prokaryotic type nucleus, a granular cytoplasm, and this resembles certain gram-negative bacteria. However, little is known about the entry and multiplication of rickettsia in the host cell. In our system, in which irradiated L cells were infected with Rickettsia tsutsugamushi, we have observed that multiple progeny rickettsiae appear in host cells without morphologic evidence of rickettsial entry into the cells. The findings indicated that the rickettsial genetic substance is brought into host cells by some forms of infectious particles other than intact rickettsiae, which release the rickettsial genetic substance into the host cell cytoplasm for replication. Morphologic evidence, therefore, indicates that a viral type of multiplication is involved in the proliferation of R. tsutsugamushi in irradiated L cells.

In the rickettsial assembly in the host cell cytoplasm, progeny rickettsiae are first recognizable as small foci where electron-lucent filamentous (f) and electron-dense granular (g) areas differentiate within the amorphous granular cytoplasm. As the assembly progresses, nascent rickettsiae develop into discrete rickettsial bodies, the surfaces of which are covered by a zone of mildly electron-daise material, in which the rickettsial double membrane eventually assembles. Morphologic evidence indicates that the rickettsial assembly involves the formation of filamentous network in the f areas and the manufacture of rickettsial ribosomes in the areas of the rickettsial body and the assembly of double membrane on the rickettsial surface.

Publications:

1. Hase, T. Electron microscopic study on the growth pattern of Rickettsia tsutsugamushi in irradiated L cells. J. Bacteriol. Submitted for publication.

2. Hase, T. Assembly of progeny rickettsiae in the infection of irradiated L cells with Rickettsia tsutsugamushi. J. Bacteriol. Submitted for publication.

III. Studies on Shigella dysenteriae I enterotoxin on the morphology of the rabbit ileum.

Collaborative studies have been initiated with the Dept. Bacterial Diseases, Division of Communicable Disease and Immunology to examine the effects of crude and purified Shigella dysenteriae I enterotoxin at different doses on the morphology of the intestinal mucosa in the rabbit ileal loop models. Light microscopy and histochemistry studies are ongoing and transmission and scanning electron microscopy studies have been initiated. Present findings indicate a dose dependent response of the ileal mucosa to both crude and purified enterotoxin preparations. Initial results suggest that direct cytotoxic damage to the absorptive epithelial cells covering the intestinal villus is the first event in the pathogenesis of the enterotoxins action. This results in sloughing of the absorptive epithelium, areas of microulceration, reduction of the villous length (villous atrophy) and thus a reduction of the absorptive surface area. In contrast to the villous epithelium, the undifferentiated crypt epithelium becomes extremely hyperplastic and this cell population rapidly expands to repair and rebuild the atrophic villi. The initial lesions of villous atrophy and crypt hyperplasia suggest a functional deficit leading to malabsorption. However, since the crypt enterocytes have secretory as well as proliferative capabilities, it appears likely that the overall mechanism of diarrhea and fluid production is the result of a net secretion from the hyperplastic crypts overcoming the absorptive ability of the atrophic villi. Nevertheless, inflammatory changes occurring in the lamina propria further suggests additional factors may be contributing to the pathologic process. Ongoing studies are designed to explore and better define these initial observations.

IV. Morphologic study on the entry of Leishmania donovani amastigotes into mouse macrophages in vitro.

The entry of amastigotes of Leishmania donovani is closely associated with their dissemination in the host animal; accordingly, understanding of the entry mechanism substantially clarifies pathogenesis of the disease and assists in formulating an effective therapeutic measure. At present, entry mechanism of the amastigotes into host macrophages is not clearly known. In this respect, two possibilities exist: one is that the amastigotes enter macrophages actively by their own motility and the other is that the amastigotes are taken up passively by phagocytic actively of macrophages.

Our preliminary study of cultured mouse macrophages infected with the amastigotes by scanning electron microscopy suggests that amashigotes burrow into macrophages actively, with the latter showing little phagocytic activity.

In transmission electron microscopy, amastigotes taken in by macrophages display prominent peripheral microtubules; this may reflect increased motility by the amastigotes. Our preliminary findings, therefore, suggest that the amastigotes enter macrophages actively. Further studies in this line is intended through chronologic observation of the infectious process of cultured mouse macrophages with L. donovani amastigotes.

V. Studies on Hepatitis A Virus (HAV).

In collaboration with the Dept. of Virology, Div. CD&I we have initiated studies on Hepatitis A virus infections, both in vivo in the Aotus monkey model and in vitro in tissue culture systems. In house techniques are being developed for the following:

1) Transmission Electron Microscopy (TEM) of wedge biopsies from infected Aotus monkeys are being processed and studied from ongoing experiments.

2) Rapid diagnosis methods are being developed using negative staining techniques for TEM examination of fecal and tissue culture isolates of HAV.

3) TEM of infected cells from monolayers grown on sectionable petri dishes are being studied.

4) Scanning electron microscopy (SEM) of monolayers are being studied to view surface effects of HAV infection. No demonstrable surface differences have been seen between control and HAV infected cells.

VI. Studies on the Morphological Changes Induced by Ischemia on Renal Proximal Tubule.

Pilot studies in conjunction with protocol development by the Division of Medicine have been completed. Acute ischemic changes are documented within the proximal tubules of affected kidneys. These results have been developed into an extensive protocol by the above collaborating group.

VII. Studies on Localization of Variable Antigen Types on the Surface of Trypanasoma rhodesiense.

Departments of Immunology and Ultrastructural Studies have been successful in attaching latex beads to surface antigen of T. rhodesiense utilizing monoclonal antibody against specific surface antigens. The attached beads were then viewed in the SEM. Tests are now being performed to determine the specificity of these interactions. It is hoped that latex bead binding can serve as a marker for visualization of antigen location.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2532	82 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 INSTN'S	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	AF	236 WWGS
b. CONTRIBUTING	61101A	3A161101A91C	00	113
c. ~~XXXXXXXX~~	STOG 80-7.2.2			

11. TITLE (Precede with Security Classification Code)

(U) Immune Mechanisms in Leishmaniasis

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 82	2.0	126
FISCAL YEAR CURRENT 83	4.0	279

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: NACY, C.A., Ph.D.

NAME: OSTER, C. POC: DA

21. GENERAL USE

Foreign Intelligence 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 is the elucidation of the mechanisms responsible for host destruction of leishmania during active disease or secondary challenge of immunized animals. This information will have a direct bearing on the feasibility of immunization against the disease and will provide methods for assessing immunity. Leishmaniasis extends throughout the tropics on every continent except Australia and is a threat to military operations. U.S. troops are contracting disease during training operations in Panama.

24. (U) The approach will be to examine the capacity of inflammatory macrophages to kill intracellular leishmania and to determine whether or not specifically immunized lymphocyte products can influence macrophage killing of the organisms.

25. (U) 81 10 - 82 09 Leishmania tropica infected macrophages in sterile inflammatory exudates: the parasite entered younger, peroxidase granule-containing macrophages in numbers disproportionate to that expected for random entry into cells. Replication of the parasite once inside the cell was not different from resident peritoneal macrophages. A dissociation of macrophages effector activities occurred in inflammatory macrophages populations: although inflammatory macrophages are more sensitive than resident cells to lymphokines that induce other non-specific effector activities, they were less responsive to lymphokines that induce killing of L. tropica. There was a negative correlation between intracellular killing activity and peroxidase positive macrophages in inflammatory exudates. This incorporates research previously ongoing Project 3A161101A91C, Work Unit 113. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

DD FORM 1498 MAR 88 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 1 MON 68 (FOR ARMY USE) ARE OBSOLETE

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

Work Unit 236 Immune Mechanisms in Leishmaniasis

Investigators:

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

Associates: LTC Charles N. Oster, MC
MAJ David L. Hoover, MC
Mrs. Anne H. Fortier
SP4 Robin R. Henry

Problems and Objectives:

The Leishmania are obligated intracellular protozoan parasites that replicate only within macrophages in mammalian hosts. The successful resolution of cutaneous lesions or systemic disease relies ultimately on the intracellular destruction of parasites by infected macrophages. The major interest of our laboratory is the documentation of parasite interactions with resident and inflammatory macrophages, and the analysis of changes induced in these interaction following nonspecific activation of macrophages by soluble lymphocyte products (lymphokines).

Progress:

To analyze parasite-host cell interactions in vitro, we developed a model assay with Leishmania tropica amastigotes and resident peritoneal macrophages of C3H/HeN mice. The parasite infects and replicates in these cells: infected macrophages maintained as nonadherent peritoneal cells cultures support 6 to 10-fold increases in the number of intracellular amastigotes over 72 hr (1). Addition of lymphokines to these cultures dramatically alters parasite-macrophage interactions, and lymphokine-treated macrophages develop two potent antileishmania activities (2).

The two antileishmanil activities demonstrated in macrophage cultures treated with lymphokines in vitro can also be demonstrated in macrophages activated in vivo. Peritoneal exudate macrophages from C3H/HeN mice infected with Mycobacterium bovis strain BCG are resistant to infection immediately after explantation; these cells are capable of intracellular destruction of L. tropica without further exposure to lymphokines in vitro. In contrast, macrophages from C3H/HeJ mice treated with BCG fail to kill L. tropica unless lymphokines, or one of several other trigger signals, is present in vitro. We can document a priming and triggering sequence for microbicidal activity that is similar to lymphokine-induced events for macrophage nonspecific tumoricidal activity. For priming to occcur in vivo, we also must induce an immune response: macrophage elicited by a variety of sterile inflammatory agents fail to respond to trigger signals in vitro for intracellular killing.

L. tropica does infect macrophages in inflammatory exudates; the parasite enters the younger, peroxidase granule-containing macrophages in numbers disproportionate to that expected for random entry into cells. Replication of the parasite once it is inside the cell, however, is not different from that observed with resident peritoneal macrophages. An interesting dissociation of macrophage effector activities occurs in inflammatory macrophage populations. Although inflammatory macrophages are more sensitive than resident cells to lymphokines that induce nonspecific tumoricidal activity, they are considerably less responsive to lymphokines that induce intracellular killing of L. tropica, There is, in fact, a negative correlation between intracellular killing activity and peroxidase positive macrophages in inflammatory exudates. The inability of inflammatory macrophages to kill intacellular L. tropica, coupled with the increased infection rate of younger macrophages, bring up an intriguing possibility: inflammation may actually contribute to the pathogenesis of leishmanial disease by supplying lymphokine-unresponsive host cells that sequester the parasite from developing immune responses.

Recommendations:

Problems associated with the control of leishmaniasis that will be investigated are: 1) evaluation of host-parasite interactions in vitro and modulation of these interactions with soluble products of immune lymphocytes. 2) evaluation of nonspecific and specifically sensitized lymphocyte products for

immunoprophylactic/therapeutic and diagnostic potential. 3) development of in vitro methods for assessing immunity, and 4) evaluation of nonspecific immunopotentiating agents for control of leishmanial infection.

References cited:

1. Nacy, C.A. and C.L. Diggs. Infect. Immunity 34: 310-313 (1981).

2. Nacy, C.A., M.S. Meltzer, E.J. Leonard, and D.J. Wyler. J. Immunol. 127:2381-2386 (1981).

Formal Presentations:

1. Nacy, C.A. and P. Ralph. 1981. Evaluation of the role of colony-stimulating factor in lymphokine activation of macrophages for antimicrobial activity against Leishmania tropica. Annual Meeting of Tropical Medicine and Hygiene Society, San Juan, Puerto Rico. (November).

2. Oster, C.N., L. Handy, and C.A. Nacy. 1981. Macrophage activation for intracellular killing of Leishmania tropica: Microbicidal activity requires several lymphocyte-derived activation signals. Annual Meeting of Tropical Medicine and Hygiene Society, San Juan, Puerto Rico (November).

3. Nacy, C.A. 1982. Intracellular recognition mechanisms of activated macrophages. International Workshop on the Activated Macrophage, Hilton Head, North Carolina (May).

4. Nacy, C. 1982. Macrophage activation factors derived from a continuous T cell line. 3rd International Lymphokine Workshop. Haverford, Pennsylvania (August).

5. Nacy, C. 1982. Macrophage-parasite interactions. Meeting of the Scientific Working Group on the Immunology and Biochemistry of Leishmaniasis, World Health Organization, Geneva, Switzerland (September).

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Published Papers:

1. Nacy, C.A. and C.L. Diggs, 1981. Intracellular replicaton of Leishmania tropica in mouse peritoneal macrophages: comparison of amastigote replication in adherent and nonadherent macrophages. Infect. Immunity 34:310-313. (October)

2. Nacy, C.A., M.S. Meltzer, E.J. Leonard, and D.J. Wyler. 1981. Intracellular replication and lymphokine- induced destruction of Leishmania tropica in C3H/HeN mouse macrophages. J. Immunol. 127:2381-2386. (December).

3. Nacy, C.A. and S.C. Oaks. 1981. Destruction of rickettsiae. In: Methods for Studying Mononuclear Phagocytes, D.O. Adams, P.J. Edelson, and H. Karen, Eds. Academic Press, New York, p. 725-743. (December).

4. Nacy, C.A. and M.G. Pappas. 1981. Destruction of Leishmania. In: Methods for Studying Mononuclear Phagocytes, D.O. Adams, P.J. Edelson, and H. Karen, Eds., Academic Press, New York, p. 745-758. (December).

5. Nacy, C.A. and M.S. Meltzer. 1982. Macrophages in resistance to rickettsial infection: strains of mice susceptible to the lethal effects of Rickettsia akari show defective macrophage rickettsiacidal activity in vitro. Infect. Immun. 36:1096-1101. (June).

6. Nacy, C.A., E.J. Leonard, and M.S. Meltzer. 1982. Role of activated macrophages in resistance to rickettsial infections In: Phagocytosis - Past and Future, M.J. Karnovski, and L. Bolis, Eds. Academic Press, NY (September).

Papers in press or submitted:

7. Meltzer, M.S., C.A. Nacy, M.M. Stevenson, and E. Skamene. 1982. Macrophages in resistance to rickettsial infections: genetic analysis of susceptibility to lethal effects of Rickettsia akari infection and development of activated cytotoxic macrophages in A and B10.A mice. J. Immunol. (in press, October).

8. Meltzer, M.S., C.A. Nacy, S.L. James, W.R. Benjamin, and J.J. Farrar. 1982. Transient cytotoxic responses of activated macrophages: characterization of signals that regulate cytotoxic activity. In: Proceedings of the Second International Conference on Immunopharmacology, Pergammon Press, Oxford, England (In press, December).

9. Fortier, A.H., D.L. Hoover, and C.A. Nacy. 1982. Intracellular replication of Leishmania tropica in mouse peritoneal macrophages:amastigote infection of resident cells and inflammatory exudate macrophages. Infect. Immun. (In press, December).

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12. Haverly, A.L., M.G., Pappas, R.R. Henry, and C.A. Nacy. 1982. In vitro macrophage antimicrobial activities and in vivo susceptibility to Leishmania tropica infection. In: Host Defenses Against Intracellular Pathogens, T.K. Eisenstein and H. Friedman, Eds. (In press, December).

13. Pappas, M.G., C.N. Oster, and C.A. Nacy. 1982. Intracellular destruction of Leishmania tropica by macrophages activated in vivo with Mycobacterium bovis strain BCG. In: Host Defenses Against Intracellular Pathogens, T.K. Eisenstein and H. Friedman Eds. (In press, December).

14. Nacy, C.A., W.T. Hockmeyer, W.R. Benjamin, J.J. Farrar, S.L. James, and M.S. Meltzer. 1982. Lymphokines from the EL-4 T-cell line induce macrophage microbicidal and tumoricidal activities In: Interleukins, Lymphokines, and Cytokines, J.J. Oppenheim, S. Cohen and M. Landy, Eds. Academic Press, New York (In press, December).

15. Nacy, C.A., A.H. Fortier, M.G. Pappas, and R.R. Henry. Susceptibility of inbred mice to Leishmania tropica infection: correlation of susceptibility with in vitro defective macrophage microbicidal activities (submitted, J. Immunol.).

16. Nacy, C.A., S.L. James, W.R. Benjamin, J.J. Farrar, W.T. Hockmeyer, and M.S. Meltzer. Activation of macrophages for microbicidal and tumoricidal effector functions by soluble factors from EL-4, a continuous T cell line. (Submitted, Infect. Immun.).

17. Hoover, D.L. and C.A. Nacy. Cryopreservation of Leishmania tropica amastigotes for analysis of macrophage-parasite interactions in vitro (submitted, J. Trop. Med. Hyg.)

Published Abstracts

18. Meltzer, M.S., C.A. Nacy, S.L. James, and S.I. Schlager. 1982. Effector activities of lymphokine (LK) activated macrophages. Fed. Proc. 41:769. (April).

19. Scott, P.A., D.L. Sacks,C.A. Nacy, and A. Sher. 1982. Inability of lymphokine to activate macrophages to kill a mucocutaneous strain of Leishmania. Fed Proc. 41:583.

20. Pappas, M.G., A.H. Fortier, and C.A. Nacy. 1982. Infection and replication of Leishmania tropica in macrophages elicited by sterile inflammatory agents. Fed. Proc. 41:963. (April).

21. Fortier, A.H., and C.A. Nacy. 1982. Macrophage activation during Leishmania tropica infection of P/J mice. Fed. Proc. 41:583. (April).

22. Hoover, D.L. and C.A. Nacy. 1982. Microbicidal activity of lymphokine-treated macrophages. Fed. Proc. 41:768. (April).

23. Nacy, C.A. and M.S. Meltzer. 1982. Defective induction of macrophage activity with P/N lymphokines. Fed. Proc. Proc. 41:730. (April).

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PROJECT 3M263750A808

DRUG AND VACCINE DEVELOPMENT

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6481	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RT	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. HEADING	8a. DISP'R INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M263750A808	AA	001 WWMC
b. CONFIGURING				
c. CONTRIBUTING	CARDS 1411A			

11. TITLE (Precede with Security Classification Code)

(U) Phase II Antimalarial Drug Trials

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012600 Clinical Pharmacology 002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 82	3.0	178
CURRENT FISCAL YEAR 83	4.0	295

19. RESPONSIBLE DOD ORGANIZATION

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POC: DA

ASSOCIATE INVESTIGATORS

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NAME: COSGRIFF, LTC T., SCHUSTER, LTC B.

21. GENERAL USE

Foreign intelligence 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 efficacy of new antimalarial drugs in non-immune human volunteers experimentally infected with malaria. Studies are performed in support of the Army Antimalarial Drug Development Program, and are an essential part of each official Investigational New Drug (IND) submission.

24. (U) Normal male volunteers are recruited from the civilian and military (MRVS) population of the greater metropolitan Washington, D.C., area by public advertisement. Each individual receives a thorough medical evaluation and must give his valid, informed consent before being permitted to participate in the study. As a study subject, the volunteer is admitted to an in-patient research facility at Ft. Detrick, inoculated with malaria and treated with the drug or drugs specified in the protocol for each study. Each volunteer is then observed for a sufficient period of time to ensure that he is cured of malaria and is free from any adverse effect from his participation in the study.

25. (U) 81 10-82 09 Studies were continued to determine the lowest dosage regimen of WR 171,669 that produces 100% cure in volunteers experimentally with P. falciparum malaria (Smith strain). These additional volunteers were cured with 1000 mg orally followed by 500 mg in six hours. Studies to determine the lowest dosage of WR 180,409 that produces a 100% cure in similarly infected subjects have been performed. Six volunteers were cured in doses ranging 1500 mg to 1000 mg given in divided doses. Three patients continued to receive WR 638 for cystinosis in a study performed in conjunction with the NIH. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

Project 3M263750A808 DRUG AND VACCINE DEVELOPMENT

Work Unit 001 Phase II Antimalarial Drug Trials

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
Associate: LTC C. Pamplin, LTC T. Cosgriff, COL C. Canfield, COL R. Dimond, LTC B. Schuster, Dr. L. Fleckenstein, MAJ M. Shmuklarsky, SP5 P. Barr

1. Description.

Phase II clinical studies involve evaluating the efficacy of candidate antimalarial drugs in a limited number of patients subjected to a controlled clinical infection with malaria. These studies are an essential bridge between tolerance studies in healthy, noninfected volunteers and a wide scale study of the curative potential of the new drug in malaria patients. A major aspect of Phase II studies is determination of a curative dose. Pharmacokinetic evaluations of the candidate drugs in man are also performed as they are an essential prerequisite of dosage selection.

2. Progress.

Human efficacy studies for treatment of blood-induced Smith strain P. falciparum malaria treated with WR 171,669 hydrochloride (halofantrine) were completed at USAMRIID. An additional three subjects were treated with 1000 mg orally, followed by 500 mg in 6 hours, and were cured. The average parasite clearance time for the eight subjects treated at the lowest 100% curative dose level (1500 mg) was 53.1 hours, and the average fever clearance time was 53.4 hours.

A clinical protocol for the evaluation of the efficacy of WR 180,409 against blood-induced infections of Smith strain P. falciparum malaria was begun. The purpose of this protocol is to determine the lowest total dose which is 100% effective in treating this multi-drug resistant malaria. A total of seven subjects were enrolled in the study and treated with 500 mg q 12 hrs x 3 (3 subjects), 500 mg at 0 hrs and 12 hrs followed by 250 mg at 24 hrs (3 subjects), and 500 mg at 0 hrs followed by 250 mg at 12 hrs and 24 hrs. The drug has been 100% effective in the first seven subjects.

WR 638 is being studied as a treatment regimen for cystinosis in conjunction with the Institute of Child Health and Development at the National Institutes of Health. Currently, three patients

receive WR 638 on a daily basis (dose of 17 mg/kg/day, 32 mg/kg/day and 139 mg/kg/day) and are doing well.

3. Future Work.

Efficacy studies of WR 180,409 will continue. A second clinical protocol for the testing of efficacy of WR 180,409 will be instituted at the Walter Reed Army Hospital. The three patients will cystinosis currently receiving WR 638 will be continued on WR 638 during the next year.

4. Publications.

Cosgriff, T.M., Hodgson, L.A. and West, J.V.: Antithrombin III content of blood collected with and without heparin. Vox. Sanguinis (in press).

Cosgriff, T.M., Boudreau, E.F., Pamplin, C.L. III, Doberstyn, E.B., Desjardins, R.E., and Canfield, C.J.: Evaluation of the antimalarial activity of the phenanthrenemethanol halofantrine (WR 171,669). Am. J. Trop. Med. Hyg. (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 2527	82 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
81 10 01	D. Change	U	U		NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M263750A808	AC	002
b. CONTRIBUTING				
c. XXXXXXXXXX	CARDS 114F			

11. TITLE (Precede with Security Classification Code)

(U) Evaluation of New Antiparasitic Drugs and Vaccines in the Tropics

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	6.0	1,111
	CURRENT 83	9.0	1,440

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Malaria; (U) Mefloquine; (U) Halofantrine; (U) Monkey; (U) Human Volunteer

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

23. (U) The objective of this task is to establish the efficacy of new drugs for both prophylaxis and treatment of tropical infectious diseases of military importance. Particular emphasis is placed on malaria, a disease of worldwide endemicity and resistance to conventional drugs, which continues to cause high attack rates (up to 50%) in unprotected troops. The effect of conventional and experimental antimalarials in treatment, prophylaxis and transmission of drug resistant falciparum malaria will be determined.

24. (U) Army investigational antimalarial drugs are compared with standard drugs and new combinations of standard drugs in the treatment and prophylaxis of drug resistant falciparum malaria in hospitalized human volunteers. Advanced development and field testing of new techniques supporting this task will be accomplished. Candidate antimalarial drugs will be evaluated using simian malaria, as a model for human malaria.

25. (U) 81 10-82 09 Malaria treatment trials with a combination of quinine-tetracycline have demonstrated it is highly effective when six days of each drug are given. Present studies are attempting to decrease the number of days required for quinine. Studies on the pharmacokenetics of quinine, and the combination of quinine and tetracycline are being done in collaboration with the Div of Experimental Therapeutics. A malaria treatment trial using halofantrine in comparison to mefloquine is in progress. A RII mefloquine failure has been well documented. In collaboration with local investigators, Thai medicinal plants are being examined for their antimalarial properties. The effect of 2'-deoxycoformycin in a primate malaria model is being studied. In vitro drug sensitivity testing using a radioisotope uptake technique has been established. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81-30 Sep 82.

* Available to contractors upon originator's approval.

Project Number: 3M263750A808
Title: Evaluation of New Antiparasitic Drugs and Vaccines in the Tropics
Work Unit Number: 002

Investigators: LTC(P) Michael W. Benenson, MC; LTC Kenneth E. Dixon, MC; MAJ Ellen F. Boudreau, MC; MAJ Horace K. Webster, MSC; LTC George S. Ward, VC; MAJ Michael R. Elwell, VC; MAJ Richard G. Andre, MSC; CPT Michael A. Ussery, MC; LTC Donald S. Burke, MC; Aanada Nisalak, M.D.; Katchrinnee Pavanand, M.D.; Markpol Tingpalapong, VC

1. The treatment of P. falciparum Malaria with a Combination of Quinine and Tetracycline

PROBLEM: With increasing resistance to quinine treatment of P. falciparum malaria in Thailand, previously effective courses of seven to ten days of oral quinine therapy are failing and new combination drug regimens are in need of efficacy testing. The Department of Medicine at AFRIMS conducted a study in Phrabuddhabat, Central Thailand from September 1980-January 1982, which showed that a seven day course of quinine had a cure rate of 60 percent in 15 patients and that a ten day course cured 69 percent of 13 patients (1).

At Mahidol University, School of Tropical Medicine, Dr. Tranakchit Harinasuta reported only a 52 percent cure rate in a 40 patient series originating from the Kampuchean border and treated with three days of quinine in combination with seven days of tetracycline (2). These patients were hospitalized in Bangkok for their entire 28 day follow-up thus excluding the possibility of reinfection.

In 1981 the malaria division of the Thai Ministry of Public Health treated 28 patients with three days of quinine and seven days of tetracycline and achieved a 100 percent cure rate in Kalasin province northeast Thailand (3).

The vanguard of resistant strains of falciparum malaria continues to emerge from the Kampuchean border. This is a pressing problem for the Thai military due to the significant effect of malaria morbidity on troop strength invested to guard that border.

PROGRESS: One hundred patients have been treated from November 1981 to October 15, 1982 at Sa Kaeo Malaria Research Center. Nine different quinine and tetracycline treatment regimens have been compared against mefloquine. These groups have ranged in various combinations from seven and seven to three and six of quinine and tetracycline respectively.

The two treatment regimens which are of current interest are six days of quinine and six days of tetracycline (Q_6T_6) and three days of quinine and six days of tetracycline (Q_3T_6). The first treatment group

(Q_6T_6) had a 100 percent cure rate in 21 patients completing a 28 day follow-up period. The second treatment group (Q_3T_6) had a 72 percent cure rate in 18 patients followed to 28 days. As a control group mefloquine had a 100 percent cure rate in nine patients to date.

In the Q_3T_6 group the mean parasite clearance time (PCT) for the 14 sensitive patients was 91 hours, whereas the mean PCT for the five recrudescent patients was 110.9 hours.

Also in the Q_3T_6 group the mean fever clearance time (FCT) for the 14 sensitive patients was 73 hours and the the five recrudescent patients was 108 hours. There was no vomiting in the recrudescent group, which might explain the poor drug response. However, two of the five recrudescent patients returned to camp at day 14, so had been exposed to reinfection for seven days prior to their 21 day parasite count.

Comparing the 23 patients to date in the Q_6T_6 treatment group to the Q_3T_6 group, the mean parasite clearance time of the longer regimen was 86.6 hours and the mean fever clearance time for Q_6T_6 was 63.4 hours. Parasite clearance time was not significantly different but fever clearance time was 10 hours shorter in the longer treatment regimen.

Mefloquine as the standard for malaria treatment in Thailand had a mean PCT in nine patients of 62.9 hours and a mean FCT of 33.7 hours in that same patient group.

The mean initial parasite counts in the mefloquine, Q_3T_6, and Q_6T_6 treatments groups were 12,516, 22,316 and 19,780 respectively.

FUTURE OBJECTIVES: Future plans include phasing-out the Q_3T_6 treatment group and evaluating only three groups Q_3T_7, Q_6T_6 and mefloquine in the next year.

2. Treatment of an Acute Case of Plasmodium Malariae Malaria with Mefloquine

PROBLEM: Mefloquine hydrochloride has been shown to be an effective drug in the therapy of infection due to

P. falciparum (4-7) and P. vivax (8,9). P. malariae occurs in Thailand, but is much less common than the other two species. There are no recorded cases of P. malariae malaria treated with mefloquine.

PROGRESS: A 30 year old Thai male presented with a history of fever, chills, headache, backache and dizziness for 12 days. In his work, he travelled throughout Thailand and had been frequently exposed to malaria vectors for the previous month. He was free of symptoms, except for slight dizziness, at the time of admission and his temperature at that time was 36.7°C. He weighed 124 lbs. He denied taking any antimalarials since the onset of symptoms and his serum contained no detectable levels of quinine or sulfa. His initial parasite count was 5220/cu. mm. A diagnosis of vivax malaria was made and the patient was entered into the vivax study and treated with mefloquine 1500 mg.p.o.

Examination of additional slides established the correct diagnosis of P. malariae. This was confirmed in all slides, including those obtained on admission, by the presence of numerous band forms of trophozoites, lack of enlargement of parasitized red cells, absence of Schuffner's stippling, the presence of course pigment typical of P. malariae and the low numbers of merozoites (6-12) per mature schizont. Although the patient remained relatively free of symptoms, his parasite clearance time was 166 hours and his fever clearance time was 93 hours. This is much higher than comparable figures for vivax malaria treated with mefloquine in the same study (mean PCT = 59 hours and mean FCT = 28 hours).

FUTURE OBJECTIVES: Study is complete.

3. The Comparative Bioavailability and Renal Clearance of the Combination of Quinine and Tetracycline Given Simultaneously or Sequentially

PROBLEM: Over the last ten years in Southeast Asia, with the growing resistance of Plasmodium Falciparum to chloroquine and fansidar, the drug combination of quinine and tetracycline has been widely utilized (10). Published recommendations advise simultaneous use of quinine and tetracycline (11, 12, 13). Although quinine provides a rapid clearance of parasites, the tetracycline

portion of the drug combination produces a radical cure (14). Therefore any factors which would hamper absorption of tetracycline should lead to treatment failures.

It has been postulated that since quinine is a base, it might decrease the absorption of tetracycline which is known to have poor bioavailability in the presence of alkalis (15,16) and food, both of which result in an elevated gastric pH. This pharmacokinetic study was designed to analyze the amount of both tetracycline and quinine available in the blood after two methods of administration.

PROGRESS: From 15 June to 15 October 1982, fifteen patients have been treated under this protocol. Six subjects have received sequential treatment with quinine sulfate 650 mg q hr p.o. for three days followed by tetracycline HCl 250-250 mg-500 mg given q 8 hr p.o. for a seven day course. Nine patients have received quinine sulfate 650 mg q 8 hr p.o. x three days administered simultaneously with tetracycline HCl 250 mg-250 mg and 500 mg q 8 hr p.o. for a total of seven days.

Five of the total patients in both treatment groups fell into the high parasite count group, > 20,000/mm^3 (mean parasite count 55,265), and ten of the remaining fifteen patients were in the low parasite count group, <20,000/mm^3 (mean parasite count 5201). The goal is to have equal numbers of high and low initial parasite counts in each treatment group. Twenty-eight day follow-ups have not been obtained on these patients since it was initially designed as a pharmacokinetic study. There were no RII or RIII drug resistance patterns encountered in this study.

One patient returned with recurrent P. falciparum malaria on day 42 having only been in Saraburi and Hua Hin since his hospital stay. He denied any excursions to malaria transmission areas. He could be classified as an RI treatment failure.

Only one patient had any persistantly abnormal lab values in the first seven days of his treatment course. He was QTK 10, a 46 year old male, who had a slightly elevated SGOT between 59-66 on day 0, 3 and 7 of his hospital course. His other liver function studies returned to normal as his disease resolved.

Mean parasite clearance time in the sequential treatment group was 88 hours and in the simultaneous treatment group was 93.4 hours. Mean fever clearance time in the sequential treatment group was 64 hours and in the simultaneous treatment group was > 75 hours. This project is targeted to have 25 patients at completion and we have treated 60 percent of the patients to date.

The serum and urine collected on each patient at timed intervals throughout the 7-10 day hospital course have not yet been analyzed for quinine and tetracycline levels. The WRAIR collaborator on this protocol is Dr. Lawrence Fleckinstein of the Division of Experimental Therapeutics.

FUTURE OBJECTIVES: In the remaining ten patients in the study we plan to have them return weekly for parasite counts for a 28 day follow-up period.

We are also examining each patient's serum protein electrophoresis and serum glycoprotein electrophoresis for changes in the normal pattern with acute malaria as previously reported in experimentally induced malaria in a non-immune population (17, 18). If there is a significant shift in globulins one might expect greater drug binding and decreased availability of the active drug.

4. The Pharmacokinetics of Intravenous Quinine in Patients with Naturally Acquired Falciparum Malaria

PROBLEM: Quinine remains the most useful drug for treating severely ill malaria patients or those with parasites resistant to other drugs. The success of quinine treatment depends in large measure on achieving adequate drug concentrations in the blood, and there are known to be large inter-individual variations in plasma quinine levels following oral or intravenous therapy (19,20). Previous bioavailability and pharmacokinetics studies have not used analytical methods specific for quinine (19,20,21). This investigation will use the highly sensitive and specific high performance liquid chromatography to measure pharmacokinetics parameters such as quinine clearance, volume of distribution and protein binding with the goal of

identifying major factors accounting for variability in quinine disposition in patients undergoing therapy.

PROGRESS: Since the study started in January 1982, two female and 12 male patients have been admitted. Plasma from the first 10 patients was sent to WRAIR in May 1982 and plasma from the second four patient series was sent in August 1982 for determination of quinine levels. The age distribution of the patients to date is 10/14 have been 20-30 years of age, 2/14 have been between 30-40 years of age and 2/14 patients have been in the 40-45 years of age group.

The mean initial parasite count in patients to date is 29,149. The mean parasite clearance time currently is 100 hours and mean fever clearance time is 90 hours. One patient failed to clear and was classified as an RII resistant case.

FUTURE OBJECTIVES: This study is the first of other anticipated future investigations of quinine pharmacokenetics and the factors contributing to the variability of steady state quinine levels. The ultimate goal of these studies is to improve antimalarial drug therapy with quinine by identifying factors affecting quinine disposition and to improve the predictability of drug levels. We shall also test the pharmacokinetics of quinine and tetracycline (this combination is the only effective, commercially available therapy for drug resistant malaria in Thailand) when given simultaneously or sequentially.

5. The Treatment of Plasmodium falciparum Malaria with Halofantrine a Phenanthrenemethanol

PROBLEM: Due to rapidly emerging drug resistant strains of Plasmodium falciparum malaria in Thailand, the development and clinical testing of new antimalarial classes of drugs is of utmost importance. For effective outpatient treatment of malaria, single dose or short term therapy with a cure rate approaching 100 percent is the goal of the Army Drug Development Program. Halofantrine WR 171,669, a 9-phenanthrenemethanol completed Phase II drug trials with experimentally-induced malaria in healthy volunteers in 1981. It was tested in 27 non-immune subjects infected with Vietnam Smith strain of P. falciparum and three other non-immune

subjects, one infected with Cambodian Buchanan strain of P. falciparum and two infected with Chesson strain of P. vivax (22). At that time, all patients were cured except two of four patients on 1500 mg single dose and one of three patients at 1000 mg single dose. Three of three patients were cured at 250 mg q 6 hr x four and at 500 mg q 12 hr x two. Eight of eight patients were cured at 1000 mg followed six hours later by 500 mg. Therefore to be conservative, in the Army's first field trial of the drug, the higher 1500 mg split dose over one day was chosen as the drug regimen to be compared in a double blind efficacy trial against mefloquine as a 1500 mg single dose.

PROGRESS: From June 28 to October 15 1982, at Ft. Taksin, Chantaburi, in a population of Royal Thai Marines, 19/38 patients were treated with halofantrine and 19/38 patients were treated with mefloquine for P. falciparum malaria. Of those patients receiving halofantrine in a dose of 1000 mg followed six hours later by 500 mg. 13/19 were sensitive, 1/19 experienced an RII pattern of resistance and 5/19 experienced an RI pattern of resistance (23). In the second group of patients receiving mefloquine as a 1500 mg single dose 18/19 were sensitive and 1/19 exhibited an RII pattern of drug resistance.

The double-blind design of the study was interrupted after 30 patients were treated and six recrudescences had occurred. It was vital to know if both drugs were failing or if the majority of treatment failures were in the halofantrine drug group.

Mean parasite clearance time for halofantrine was 79 hours and for mefloquine 90 hours. Mean fever clearance time for halofantrine was 56.7 hours and for mefloquine 62 hours.

The incidence of post dosing symptoms in the first twenty four hours after halofantrine was 2/19 with headache, 4/19 with nausea, 5/19 with diarrhea, 4/19 with vomiting, 3/19 with abdominal pain and 5/19 with no symptoms.

The number of patients with post dosing symptoms in the first 24 hours after mefloquine administration were as follows: 4/19 with vomiting 5/19 with nausea,

6/19 with diarrhea, 7/19 with abdominal pain and 3/19 patients with no symptoms.

The cure rate for mefloquine was 95 percent in this patient population, while cure rate for halofantrine was 74 percent.

Due to previous treatment success in the Phase II human trials with halofantrine (22,23,24), good predicted efficacy by the aotus model (25), and low in vitro inhibitory doses required with halofantrine in the multi-drug resistant Smith strain (26) and in three strains from various areas of Thailand currently (27), we are attributing this poor cure rate to poor drug absorption.

FUTURE OBJECTIVES: In an effort to improve the cure rate with halofantrine, we plan to amend our current treatment regimen and to give halofantrine in three or four 500 mg doses spaced six hours apart.

A bioassay of the drug in the patient's plasma against parasitized red cells in the hypoxanthine microtiter plate in vitro model will be used to document absorption of the drug.

This method will not give biochemical levels of the drug but it will give the additive growth inhibition to the parasite of both drug and its active metabolite at various sampling times in the patient's course.

6. In vitro Antimalarial Drug Sensitivity Testing

PROBLEM: In Thailand, Plasmodium falciparum is now resistant to conventional antimalarial drugs. This resistance varies from almost complete in the case of chloroquine and other 4-aminoquinolines and pyrimetha-mine/sulfadoxine to moderate but increasing for quinine. Quinine at high therapeutic dose continues to be effective when combined with tetracycline. Two new antimalarial drugs, mefloquine and halofantrine, have been introduced into Thailand and are now in various stages at field evaluation. In vitro antimalarial drug sensitivity testing provides an objective means of quantifying dose-response characteristics for individual drugs and thus the identification of resistance patterns in Thailand.

PROGRESS: A radioisotope microdilution technique has been adopted to test antimalarial activity in vitro under field conditions. The technique was standardized in the central Bangkok laboratory. The technique is based on incorporation of (^{3}H) hyposanthine by parasitized RBC in microculture. Inhibition of uptake of (^{3}H) hyposanthine by the parasites serves as an indicator of antimalarial activity. This technique has proven more sensitive and precise than traditional microscopic methods. It also permits large scale testing with fewer personnel. At present four drugs are being tested in vitro as part of an antimalarial drug efficacy study at Chantaburi in cooporation with the Royal Thai Navy and Marine Corps. The drugs used for sensitivity testing are mefloquine, halofantrine, quinine and chloroquine. At Chantaburi about 50 cases will be studied. Data from this study will be compared to data from other geographical areas of Thailand. The project will accomplish the following (1) provide in vitro/ in vivo correlation of antimalarial drug response; (2) establish base-line quantitative data (ID 50); (3) permit identification and collection of drug resistant malaria strains; and (4) allow comparative testing of malaria strains from treatment failures when they occur. Comparative studies are also being done to determine whether cryopreserved strains can be directly used for testing by the radioisotope technique. A case of RII mefloquine resistance has been observed at Chantaburi in which sensitivity testing confirmed a decreased susceptibility to mefloquine.

FUTURE OBJECTIVES: Antimalarial drug resistance is an on-going problem. It is essential that antimalarial drug sensitivity testing be done on a continuing basis. The question of whether cryopreserved malaria strains can be used directly in the radioisotope technique needs to be answered since this will permit use of a centralized testing facility. Studies will also be initiated to permit the addition of other antimalarial drugs to the testing scheme.

7. Effect of Antimalarial Drugs on Human Lymphocyte Response to Mitogenic Lectins

PROBLEM: Since immunosuppression is a characteristic of malaria infection the possibility that an antimalarial agent may itself compromise immune responsiveness

becomes an important clinical consideration. A drug induced decrease in host immune capacity during malaria infection could result in a prolonged parasite clearance time and subsequent delayed recovery from the disease. Similarily, the compromise to the patient may result in increased susceptibility to intercurrent illness. There is also the concern for malaria endemic populations where suboptimal chemoprophylaxis may combine with the disease itself so as to compromise vaccine employment - especially a prospective malaria vaccine.

PROGRESS: Mitogenic lectin induced lymphocyte blast transformation provides an established assay for evaluation of cellular immune responsiveness. We have standardized an *in vitro* mitogenic lectin assay to assess whether selected antimalarial drugs suppress cellular immune responsiveness in human lymphocytes. Preliminary studies show that the new antimalarial drugs, mefloquine and halofantrine, suppress normal lymphocyte response to phytohemaglutinin (PHA), Concanavalin A (Con A) and pokeweed mitogen (PWM).

FUTURE OBJECTIVES: These preliminary observations need to be confirmed in a larger population sample. These studies are currently underway. Studies are also being done to assess whether these antimalarial drugs have an effect in mixed lymphocyte culture (MLC).

8. Hypoxanthine Metabolism by Human Malaria Infected Erythrocytes: Focus for the Design of New Antimalarial Drugs

PROBLEM: The development of resistance to almost all conventional antimalarial drugs by *Plasmodium falciparum* makes critical the need to discover new chemotherapeutic agents. Truly rational approaches to antimalarial chemotherapy have been hampered by a lack of basic biochemical understanding of host-parasite relationships in human malaria infection. We have used malaria culture techniques along with novel chromatographic procedures (28) to study purine metabolism during the intraerythrocytic (IE) growth cycle of *P. falciparum* Identification of differences in host-parasite purine metabolism could present appropriate targets for design of new antimalarial drugs.

PROGRESS: We have identified the major metabolic pathways used by IE *P. falciparum* to synthesize both

guanosine and adenosine nucleotides from the precursor base, hypoxanthine (29,30). We next selected inhibitors specific for the purine enzymes associated with these essential nucleotide pathways (31). Hadacidin, but not alanosine, blocked synthesis of adenosine nucleotides from hypoxanthine via IMP. Hadacidin and alanosine are known to inhibit adenylosuccinate synthetase. The lack of inhibition by alanosine may be due to an inability to form the active metabolite, L-alanosyl-AICOR, which requires an active de novo purine pathway. We have shown that P. falciparum does not synthesize purines de novo (32). Bredinin and mycophenolic acid interferred with synthesis of guanosine nucleotides from hypoxanthine via IMP. These agents are inhibitors of IMP dehydrogenase. These studies confirm the importance of hypoxanthine as a precursor for synthesis of purine nucleotides and nucleic acids by malaria infected erythrocytes.

FUTURE OBJECTIVES: These studies identify biochemical targets associated with the human malaria parasites' metabolism of hypoxanthine which are essential for synthesis of purine nucleotides and nucleic acids. Specific focus on these unique features of parasite purine metabolism and the classes of inhibitors effective against them could lead to the design of new antimalarial drugs. We plan to continue assessment of specific purine enzyme inhibitors with special emphasis on the salvage enzyme, hypoxanthine phosphoribosyltransferase.

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

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

PROGRESS: An intensive chemical analysis was made on Eurycoma longifolia, a Thai medicinal plant exhibiting strong in vitro schizonticidal activity on natural isolates of P. falciparum. Five different crude chloroform extracts obtained were tested and only one fraction (fr.a) was found with strong schizonticidal effect (1.5653 µg/ml blood suspension). Further chemical isolation of this major fraction was made and four different compounds were obtained. Different concentrations of each compound isolated were tested for in vitro inhibitory effect on P. falciparum development. Results revealed that compound "a" was the only component of Eurycoma longifloria that exhibited schizonticidal activity (0.3945 µg/ml blood suspension).

Chemical characterization and structural formula elucidation were available on two compounds. The compound "a" with strong in vitro schizonticidal activity was a lactone. The other compound was a scopolatin with no activity.

FUTURE OBJECTIVES: An attempt to confirm the anti-malarial activity of Eurycomalactone on different strains of P. falciparum is being made. The evaluation of in vitro inhibitory effect by morphological comparison is a tedious and laborious technique as compared to the parasites' uptake of the labelled nucleic acid precursor-hypoxanthine. This technique is being standardized and will make possible large scale studies for in vitro comparison of different compounds on various strains of P. falciparum.

10. Evaluation of Plasmodium cynomolgi Sporozoites Induced Infections of Captive Born Macaca fasicularis

PROBLEM: India has ceased exportation of rhesus monkeys which are used in the Plasmodium cynomolgi antimalarial compound testing model. A systematic evaluation of captive born Macaca fasicularis has not been completed to determine if this species could be used to supplement scarce rhesus monkeys.

PROGRESS: Sporozoite infection of 18 cynomolgus monkeys with Plasmodium cynomolgi has been completed. Three groups consisting of low dose (<3 million), high dose (>3 million); and high dose-splenectomized were infected IV with sporozoites. Preinfection and weekly acute

infection period CBCs were performed. Parasitemia curves are being followed for a period of 120 days. When gametocytes of both sexes were present, mosquitoes were fed to determine if sporozoite production and thus a complete monkey-mosquito-monkey cycle could be maintained. The low dose intact monkeys did not develop a substantial or persistant parasitemia. An appreciable percentage of the high inocular intact monkeys did not develop parasitemias of sufficient persistance to make them a useful model. Additionally, only one of eight developed a gametocytemia which resulted in successful sporozoite production. Every monkey in the high dose, splenectomized group produced respectable gametocytemias and all mosquito feedings resulted in oocyst or sporozoite production. Some splenectomized high inocula monkeys developed parasitemias indistinguishable from parasitemia patterns in intact rhesus. The other splenectomized monkeys produced satisfactory initial parasitemias followed by persistant but very low parasite numbers. These late parasitemias may not be of sufficient magnitude to be useful. An extremely high leukocytosis was observed in the high dose, splenectomized group during high parasitemias.

FUTURE OBJECTIVES: Determine persistance of parasitemias in high inocula splenectomized cynomolgus monkeys following chloroquine administration. Also, primaquine will be given at less than curative doses to evaluate tissue stage persistance. All monkeys will be followed for a minimum of 120 days.

11. Evaluation of the Efficacy of Ribavirin and Triacetyl Ribavirin in Japanese encephalitis Infections

PROBLEM: Japanese encephalitis virus (JEV) is endemic in Southeast Asia with case fatality rates between 10 and 90 percent. JEV infection is a serious threat to local populations and military forces deployed anywhere in this region. The ability to select within 24 hours a subpopulation of patients at highest risk has made the use of an effective antiviral drug more attractive. Previous studies have shown activity of ribavirin against JEV infections *in vitro* but its *in vivo* efficacy is limited by its inability to pass the blood-brain barrier. Therefore other derivatives such as triacetyl ribavirin as well as intrathecal ribavirin treatment regimens are being evaluated.

PROGRESS: The most significant development in these studies has been the detection of ribavirin in serum and CSF of treated monkeys by high performance liquid chromatography (HPLC). Previously pharmacokinetic data could only be obtained by using radioactive drugs or by performing biological assays requiring expensive logistical support and yielding results of low accuracy. This technique is currently being used to determine the ability of triacetyl ribavirin to pass the blood-brain barrier of treated monkeys. Experimental JEV infections have been performed and LD_{50} determined.

Toxicity studies have been completed with intrathecal ribavirin in monkeys and a subtoxic dose will be evaluated to determine ribavirin efficacy by this treatment.

FUTURE OBJECTIVES:

1. Promising ribavirin derivatives should be given to monkeys i.v. to determine their ability to pass the blood-brain barrier.

2. The derivative that is most effective at entering the CNS should be tested for efficacy in monkeys with experimental JE.

Project Number: 3M263750A808
Title: Evaluation of New Antiparasitic Drugs and Vaccines in the Tropics
Work Unit Number: 002

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6448	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. HDGRADING	8A. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M263750A808	AB	003 WWGG
b. CONTRIBUTING				
c. CONTRIBUTING	CARDS 1413A			

11. TITLE (Precede with Security Classification Code)

(U) Advanced Vaccine Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
58 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	4.0	633
	CURRENT 83	3.0	735

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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TELEPHONE: 202-576-3551

20. PERFORMING ORGANIZATION

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

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

NAME: Berman, S., PH.D.

TELEPHONE: 301-427-5208

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Altieri, P.L.

NAME: Powell, C. POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede Each with Security Classification Code) (U) Biological products; (U) Pseudomonas vaccines; (U) Typhoid-Shigella hybrid vaccine; (U) E. coli vaccines; (U) Leishmania diagnostic skin test antigen; (U) Meningococcal B protein-polysaccharide vaccine; (U) Bioassay; (U) Freeze-drying

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (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) 81 10 - 82 09 Investigations on the development of new and improved biologics for military use have continued. 1. Purified polysaccharides Pseudomonas (strains 134VA and 1244) vaccines have been prepared and made available for human studies. 2. A Salmonella typhosa-Shigella sonnei, live oral, freeze-dried vaccine has been made available for human studies. 3. A J-5 strain Escherichia coli fluid, whole cell vaccine has been prepared and tested and work is continuing on developing production techniques for an oral Escherichia coli vaccine derived from the pili of the microorganism. 4. Production of certified seed lots of a strain of Leishmania has been initiated. 5. Production of meninogoccal protein-polysaccharide vaccines from cultures of the 8047 and Norway strains has been started incorporating a revised growth medium and modifications to the previous purification procedures. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

* Available to contractors upon originator's approval.

PROJECT 3M263750A808 DRUG AND VACCINE DEVELOPMENT

WORK UNIT 003 ADVANCED VACCINE DEVELOPMENT

Investigators:

Principal: Dr. Sanford L. Berman

Associates: Mrs. Patricia Altieri
Mr. Calvin Powell

Problem and Objectives

This work unit is concerned with conducting research on the development of manufacturing methods and the subsequent production of new biological products for use in diagnosis, prevention and treatment of infectious diseases of military importance. The unit is also concerned with investigating modifications of existing biological products to increase effectiveness and to reduce reactivitiy, to afford greater stability and to minimize logistic requirements. These studies included work on the development of vaccines derived from cultures of several strains of Pseudomonas aeruginosa, from cultures of a typhoid-shigella hybrid, from fluid preparations of the J-5 strain of Escherichia coli, from the pilus component of Escherichia coli, from purified proteins and polysaccharides derived from cultures of the B strain of Neisseria meningitidis and a skin test antigen for the Leishmania parasite.

Progress

Purified lipopolysaccharides suitable for human use, derived from cultures of Pseudomonas aeruginosa, strains 1244 and 134VA have been prepared, tested and made available for field studies. The purity, potency and safety tests on a lot of a typhoid-shigella vaccine were completed and this product is available for human trials. Studies on a mouse potency assay for this product were successfully completed and it is currently used for testing other hybrids suggested as potential vaccine strains. Sub-lots of a fluid preparation of Neisseria gonorrhea were received from a contractor, pooled, bottled, tested, and made available for a planned trial in humans. In addition, a lot of a formalin-saline solution was prepared to serve as a placebo in this study. A lot of vaccine made from fluid cultures of the J-5 strain of Escherichia coli has been prepared and tested, and is available for testing in humans. Developmental studies on producing oral vaccines derived from the pilus component of Escherichia coli have continued. The purification procedures to date have resulted in an insoluble end product

contaminated with a spore forming organism resistant to sterilization by an irradiation level not destructive to the pilus protein. Changes in the production and purification procedures are currently under investigation and are directed toward eliminating these two problems. Experimental production runs with the 8047 and Norway B strains of Neisseria menigitidis were completed. On the basis of these studies, production of B strain meninogoccal protein-polysaccharide vaccines from cultures of these strains and suitable for human use have been initiated incorporating a revised growth medium and modifications to the previous purification procedures. Developmental studies were also initiated on techniques for producing a diagnostic skin test antigen for the diagnosis and prognosis of Leishmaniasis. Current efforts are directed toward producing stable, tested seed preparations of the Leishmania parasite.

Recommendations

Developmental studies on the techniques for the production of a sterile E. coli soluble pilus protein vaccine suitable for human use should continue and based on the results a lot of vaccine made available for field studies. Production of B meningococcal protein-polysaccharide vaccines with the 8047 and Norway strains of N. meningitidis should continue and lots of the vaccines made in sufficient quantities for subsequent testing in humans. Work should continue on developing the methods required for producing stable, certified seed materials for subsequent use in making a skin test antigen for the diagnosis and prognosis of Leishmaniasis. Freeze-drying support will continue to be provided to other investigators at WRAIR as required.

Bibliography

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2. Sadoff, J., Seid, R., Altieri, P.L., Collins, H., Sidberry, H., Futrovsky, S., and Berman, S.L. 1981. Non-toxic lipopolysaccharide vaccines for Pseudomonas aeruginosa. Abstracts ASM, No. S-58, page 64.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 7009	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M263750A808	AB	004
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	Cards 1413A			

11. TITLE (Precede with Security Classification Code)

(U) Gonococcal Vaccine Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
81 06	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	4.0	80
	CURRENT 83	4.0	500

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: (202) 576-3601

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: D. McChesney, CPT, MSC,Ph.D

NAME: J. Boslego, MAJ, MD,MC, M. Piziak,

NAME: CPT, MSC

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Neisseria; (U) Gonorrhoeae; (U) Gonococcal Vaccine; (U) Antigen; (U) Immunity

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

23. (U) To develop a gonococcal vaccine. Gonorrhea has reached epidemic proportions in field troops in some areas (20 cases/1000/day) and gonococcal strains have developed resistance to penicillin as well as second line drugs.

24. (U) The general approach is to study and determine the immunologic response to naturally occurring gonococcal infections, determine the gonococcal antigen(s) responsible for that immunologic response, correlate these studies with natural disease, and then develop that antigen(s) as a vaccine candidate. Gonococcal pili which function to attach the gonococcus to epithelial mucosal cells, have been isolated and purified. Antibodies directed against gonococcal pili block the attachment of gonococci to epithelial cells. A prototype gonococcal pilus vaccine has been tested in humans and has been found to be safe and immunogenic. Field studies to determine vaccine efficacy, antibody level correlates and antigenic variation are planned. It is anticipated that a multi-antigen vaccine will eventually be developed.

25. (U) 81-10 - 82-09 The amount of vaccine and the route of immunization have been optimized. An ELISA assay has been established. The rates of gonorrhea in populations projected to be offered the vaccine have been determined and the PPNG rates calculated. Antibiotic sensitivities for eight have been established. The IEA assay has been improved using an acridine-orange stain. The iron requirement for growth of approximately 30 strains of Neisseria gonorrhea and their sensitivity to penicillin have been determined. A system using whole gonococci and either polyclonal or monoclonal antisera to the homologous organisms from which the vaccine was made is under development. Gonococcal proteins I and II have been isolated and purified. (For technical report use Walter Reed Army Institute of Research Annual Report, 1 Oct 81 - 30 Sep 82).

*Available to contractors upon originator's approval

Project 3M263750A808 DRUG AND VACCINE DEVELOPMENT

Work Unit 004 Gonococcal Vaccine Development

Investigators:

Principals: Edmund C. Tramont, M.D., COL, MC
Associates: John W. Boslego, M.D., MAJ, MC
Raymond Chung, M.D., MAJ, MC
Daniel McChesney, Ph.D., CPT, MSC
Myron Piziak, MA, CPT, MSC

Problem

The highest attack rate of infection with N. gonorrhoeae are in persons between the ages of 15 and 30. More than 80% of individuals in the military are less than 30 years old. American military personnel are deployed in foreign countries where the indiscriminate use of antibiotics has become widespread. As a result, increased resistance of the gonococcus to antibiotics has steadily developed. Furthermore, gonorrhea is the most frequently reported communicable disease in the United States today. Clearly, the present methods of control of gonorrhea have not been successful. A gonococcal vaccine offers the most promising means of decreasing the incidence of that disease.

Antigenic heterogenicity of gonococcal pili is a major obstacle to a broadly effective gonococcal pilus vaccine.

Objectives

1. Optimize the dose and route of immunization within the time constraints during which the projected populations will be available for vaccination.

2. Establish an assay for determining the amount of anti-gonococcal antibody present in serum and genital secretions. This assay must be accurate, rapid, reproducible and the data must be acquired in a manner that allows automated data storage and computation.

3. Determine current rates for gonorrhea and the percent of PPNG in the population projected to be offered the vaccine.

4. Improve the inhibition of attachment assay to increase reading speed and lessen user fatigue.

5. Determine if a relationship exists between a N. gonorrhoeae strain's ability to take up iron and its sensitivity to penicillin.

6. Establish a system that will allow the relatedness between strains of whole gonococci to be determined.

7. Determine the importance of gonococcal proteins I and II in the pathogenicity of gonococcal infection and the antibody response to these proteins in natural disease.

8. Develop monoclonal antibodies specific for gonococcal pili.

9. Examine pili from diverse strain and compare to vaccine strain (P32).

10. Establish system to examine strains from vaccine failures from upcoming field trial, and determine if failure is due to antigenic heterogenicity of pili.

Progress

1. The amount of vaccine and the route of immunization have been optimized through two trials involving approximately 80 human volunteers, 5 dosage levels and 4 immunization schedules. The optimum dose was determined to be two 100 g injections given 1 day apart.

2. An ELISA assay has been established to determine antibody levels to any suitable test antigen (protein, LPS, polysaccharide, whole organisms) using serum, genital secretions, mouse ascites fluid and tissue culture supernatant. This assay is capable of determining all class specific antibodies as well as subclass antibodies. The test meets all of our

requirements. The raw data is transmitted directly from the ELISA reader to an Apple personal computer where it is stored and calculated.

3. The rates of gonorrhea in the populations projected to be offered the vaccine have been determined and the PPNG rates calculated. We have also provided laboratory support to a Division of Preventive Medicine, EPICON, study in Korea by providing personnel to help collect specimens in Korea and by determining antibiotic sensitivities for eight drugs commonly used in treatment.

4. The inhibition of attachment assay is important in determining the functional ability of antibodies raised in response to vaccination. This test is performed by counting the number of gonococci stained by Gram's method attached to epithelial cells. This is tedious and time comsuming. The test has been improved by using an acridine-orange stain and a fluorescent microscope. This improvement has increased the speed with which a slide can be read and has reduced technician fatigue.

5. The iron requirement for growth of approximately 30 strains of N. gonorrhoeae and their sensitivity to penicillin have been determined. While no definite conclusions are possible at this time it appears that those strains that can effectively scavenge iron are the most sensitive to penicillin.

6. In order to be able to fully analyze gonococcal vaccine failures, a system of differentiating gonococcal strains is required. A system using whole gonococci and either polyclonal or monoclonal antisera to the homologous organisms from which the vaccine was made is under development. This system has worked in small preliminary studies but problems have arisen with quantitating the amount of pili on the organism.

7. Gonococcal proteins I and II have been isolated and purified.

8. BalbC mice were immunized with P32 pili and whole bugs and then boosted with either homologous pili/whole bugs or heterologous (135) pili/whole

bugs. A fusion was performed using mouse myeloma cell lines p3, NS1, and 653. Hybridoma cells were produced which secreted monoclonal antibodies specific for cell wall antigens from P32, 135 and other strains of gonococci. About 10 monoclonal antibodies were identified with different specificities.

9. Pili were purified from strains of N. gonorrhoeae. About 15 different pili-types have been purified.

10. An absorption assay was established in which pili on whole bugs are used to absorb monoclonal antibodies and then reduce binding in a ELISA system when vaccine strain pili are used as antigen on the plate.

Recommendations

1. A trial involving a minimum of 15 volunteers per group should be conducted giving two 100 g injections of gonococcal pilus vaccine either 2 weeks or 4 weeks apart. Either of these schedules would be more logistically feasible with the newly projected test population.

2. Software to support the ELISA reader should continue to be developed for data computation. This will increase the usefulness of this instrument.

3. Support for the Division of Preventive Medicine studies on sexually transmitted disease should be continued.

4. Work in the areas of iron uptake should be continued. Work on establishing the relatedness between strains of gonococci and on the importance of Protein I and II in pathogenicity should be continued with a very high priority.

5. More pili types need to be purified.

6. Monoclonal antibodies should be used to determine the degree of relatedness among pili strains.

7. The absorption system should be further expanded and utilized to examine wild GC strains in preparation for the field trial.

8. Utilize the monoclonal Ab to compare pili from vaccine failures in the GC study with the gonococcal vaccine pili.

9. Examine functional attribute of monoclonal antibodies (inhibition of attachment, opsonic activity).

Formal Presentations

COL Edmund C. Tramont, M.D., MC
October 1981 - Seoul, Korea; Wayne State Medical College
November 1981 - International Symposium STD, San Juan, Puerto Rico
December 1981 - University of Pittsburgh
January 1982 - Tripler Army Medical Center, Hawaii
25th Infantry Division, Scofield Barracks, Hawaii; Rutgers Medical School
February 1982 - Harvard University Medical School
University of Maryland Medical School
March 1982 - Johns Hopkins Medical School
April 1982 - Manila, Philippines
Clark Air Force Base
Subic Bay
Okinawa
Seoul, Korea
May 1982 - University North Carolina
June 1982 - Fort Dix, New Jersey
August 1982 - Symposium STD, Montreal, Canada

Bibliography

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2. Takafuji, E.T., Greenup, R.L., Gann, V.J., McChesney, D.G., Sadoff, J.C., Tramont, E.C. Epidemiologic Patterns of Gonorrhea in a Military Community. Submitted for publication.

3. Tramont, E.C., Antigenic Determinants and Immune Responses: N. gonorrhoeae. Presented 18 November 1981, 1st Sexually Transmitted Diseases World Congress, San Juan, Puerto Rico.

4. Woodbury, C., McChesney, D., Kopecko, D., Dececco, B., Tramont, E.C. Iron Utilization and penicillin Sensitivity in Clinical Isolates of Neisseria gonorrhoeae-ASM, 1982, Atlanta, GA.

5. Karney, W., Correacoronas, R., Zajtchuk, R., Schwartz, J., Smith, L.P., Tramont, E.C. Ceforanide Versus Cephalothin Prophylaxis in Cardiac Surgery. ICAAC, Miami Beach, Florida, October 1982.

6. McChesney, D., Morales, J., Piziak, M., Woodbury, C., Brinton, C., Tramont, E.C. Cross-Reactivity of Gonococcal Pilus Vaccine. ICAAC, Miami Beach, Florida, October 1982.

7. Tramont, E.C.-Book Review-Sexually Transmitted Diseases: A Guide to Diagnosis and Treatment. Medical Experimentation Publishing Co., 1981, for Military Medicine.

8. McChesney, Daniel G., E.C. Tramont, J. Ciak, J. Boslego, C. Brinton and S. Wood. Studies on the pilus-mediated adhesion of Neisseria gonorrhoeae. ASM Conference on Bacterial Adhesion in Pathogenesis, 1981.

PROJECT 3M162770A870

RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	3. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DAOG 6764	82 10 01	DD-DR&E(AR)636

2. 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AC	071
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7.2.2			

11. TITLE (Procede with Security Classification Code)*

(U) Biosystematics of Arthropods of Military Medical Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology 005900 Environmental Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	3.0	178
	CURRENT 83	3.0	

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Walter Reed Army Institute of Research

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TELEPHONE: 202-357-1856

SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: Harbach, CPT R.E.

NAME: Zavortink, Dr. T.

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Biosystematics; (U) Disease Vectors; (U) Arthropods; (U) Mosquitoes; (U) Epidemiology; (U) Malaria; (U) Arboviruses

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

23. (U) Conduct biosystematic research of important arthropod groups in support of epidemiological studies and disease control strategies of importance to the military. Disease vector groups currently under investigation are (1) Anopheles of Orient and Middle East (malaria), (2) Culex (Culex) of Middle East (arboviruses and filariasis), (3) Aedes (Stegomyia) of Afrotropical Region (arboviruses) and (4) Trichoprosopon of the Neotropics (arboviruses). Build a computer data base from over 1,000,000 mosquito specimens and collection records in the Smithsonian Institution.

24. (U) Comparative morphological study of medically important mosquito groups in regions of military interest, with biological, cytogenetic, electrophoretic and cross-mating studies of vector populations, and correlation of all data to provide (1) descriptions and illustrations of species, (2) development of effective identification keys, and (3) information about medical importance of the species. File biological data for museum mosquito specimens in SELGEM computer program to facilitate biosystematic research and to understand vector behavioral patterns.

25. (U) 81 10 -82 09 Published one large monographic revision (subgenus Paraedes of genus Aedes), a pictorial key to the mosquitoes associated with yellow fever in Africa and karyotype differences for 3 species in Balabacensis Complex. Five new species in the African Luteocephalus and Africanus complexes of Aedes (Stegomyia) and 2 new species in the Balabacensis Complex of Anopheles (Cellia) were discovered. Incorporated 2,627 collection forms into the computer data base. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sept 82.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1455A 1 NOV 65 AND 1455-1, 1 MAR 55 (FOR ARMY USE) ARE OBSOLETE.

Project 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

Work Unit 071 Biosystematics of Arthropods of Military Medical Importance

Investigators:
Principal: Bruce A. Harrison, LTC, MSC
Associate: Michael E. Faran, CPT, MSC; Kenneth J. Linthicum, CPT, MSC; E. L. Peyton; Y.-M. Huang, Ph.D.; Thomas J. Zavortink, Ph.D; SP4 Richard Soltero; Thomas V. Gaffigan; James E. Pecor

Problem

Epidemiological studies and disease control strategies involving arthropod-borne diseases are dependent upon biosystematic research support to provide accurate identifications of arthropod vectors and reservoirs. The objectives of biosystematic research of medically important arthropod groups are (1) to describe and illustrate all the species in these groups, (2) to resolve any systematic problems, (3) to develop effective keys for identifying all life stages of the species under study, (4) to provide basic biological and ecological data useful in understanding the epidemiology of diseases and in the control of vector species, (5) to provide data concerning the medical importance of each species, and (6) to train personnel in field studies and systematic research. Current studies are focused on the following important mosquito vector groups (1) malaria vector-groups of the Oriental Region [Leucosphyrus Group of *Anopheles (Cellia)*], and of the Middle East (genus *Anopheles*), (2) arbovirus and filariasis vectors of the Middle East and Afrotropical regions, *Aedes (Stegomyia)* and the *Culex (Culex)*, and (3) arbovirus vectors of the Neotropical Region, genus *Trichoprosopon*. In conjunction with the study of these mosquito-vector groups, a computer based master file of detailed systematic and ecological data is being prepared from over 1,000,000 mosquito specimens and their collection records. This latter study is directed at providing easily accessible, coordinated ecological and vector data to the military, public health organizations and other scientific and environmental agencies concerning vector species of mosquitoes.

Progress

Collaborative research continued with AFRIMS group and Mahidol University, Bangkok, Thailand, toward a revision of the Leucosphyrus Group of *Anopheles (Cellia)*, with particular

emphasis on resolving sibling species problems within the Balabacensis Complex. Two undescribed morphologically distinct forms previously recognized from Thailand and Malaysia have now been confirmed as distinct species by morphologic, cytogenetic, electrophoretic and cross-mating studies. A paper describing the distinctive karyotype of one of these species (Perlis Form) and separating it from 2 other members of the Balabacensis Complex (dirus and takasagoensis) was published in late 1981. AFRIMS and Mahidol personnel collected feral specimens of the Perlis Form in early 1982, providing the first feral specimens of this species upon which a taxonomic description and a type-series can be based. Previous to these efforts the Perlis Form was known only from colonies. A manuscript describing the second undescribed species, the Fraser's Hill Form, is nearing completion. With the recognition of these 2 new species, 5 distinct species of the Balabacensis Complex are now recognized as occurring in Thailand, where only one, balabacensis balabacensis, was recognized as recently as 1978. The epidemiological implications of these sibling species on malaria transmission in Thailand are currently under investigation by AFRIMS personnel. A reexamination of the Anopheles species of the Middle East was initiated and the preparation of revised keys, biological notes and distribution notes for the malaria vector species are underway. The manuscript for a revision of the Argyritarsus Section of the Anopheles (Nyssorhynchus) is undergoing final corrections before publication. The revisionary study of the arbovirus vector groups of Aedes (Stegomyia) in Africa made considerable progress during this period. Examination of specimens in the Africanus Complex (4 species and the Luteocephalus Complex (2 species) has revealed an additional 2 new species in the Luteocephalus Complex. The taxonomic differentiation of these species is essential for understanding the epidemiology of human and primate dengue transmission, as well as other arboviruses, in Africa. French researchers in Senegal have already determined that one of the new species of the Africanus Complex probably is not involved in dengue virus transmission. This species has very different habitat requirements from the "africanus" species in Senegal known to be involved in dengue virus transmission. Taxonomic descriptions of these 5 new species are in preparation. A pictorial key for the identification of Aedes (Stegomyia) mosquitoes associated with yellow fever in Africa was published in January 1982. Studies on the Pipiens Complex of Culex (Culex) reported last year have been expanded to a comprehensive study of the Culex (Culex) of North Africa and South Asia. This study covers approximately 25 species, including members of the Pipiens Complex, Univittatus Complex, tritaeniorhynchus and several other species that have been incriminated in the transmission of Rift Valley Fever, West Nile, JE and/or other viruses in the study

area. The type-specimens of approximately 65 normal taxa involved in this study were examined in the British Museum (Nat. Hist.), the Pasteur Institut and the Paris Museum (Nat. History) during Aug-Sept 1982. In addition, several thousand specimens were borrowed for study from other repositories and institutions. This study is directed toward the production of a handbook for the Culex (Culex) of North Africa and Southwest Asia. With the hiring of a new professional, a revision of the genus Trichoprosopon of the Neotropics was reactivated. This study was previously heavily subsidized by U. S. Army funds and is approximately 75% completed. At least 4 new species in the genus have been identified and are being prepared for description. Certain species in this genus are major human biters in Neotropical forests, and VEE as well as other viruses have been isolated from several species. In addition to the above research, consultants of the Biosystematic Unit published or have in press 2 large works. These treat the subgenus Paraedes of the genus Aedes, and a guide to the genera of mosquitoes of Thailand.

Three field trips were conducted during May-June 1982. These trips resulted in the collection of valuable specimens from Bolivia, Senegal and Suriname. The Bolivian collection was conducted in conjunction with arbovirus studies and resulted in the collection of 7000 adult mosquitoes identified and pooled for virus isolation. In addition, 1189 adult specimens (85% with associated immature skins) were pinned for taxonomic studies. A total of 58 species of mosquitoes of 14 genera were collected during the trip. The Senegal trip resulted in the examination of hundreds of Aedes (Stegomyia) specimens in the ORSTOM collections in Dakar, a sizeable loan of material being handcarried back to the U.S. and a good collaborative arrangement being established with the French Scientists of ORSTOM in several West African countries. The Suriname trip resulted in very valuable specimens of 6 genera from a country very poorly represented in museum collections.

A total of 20,267 specimens were received by the unit as gifts, transfers, loans, etc., which represents a major accession of scientific specimens. During the same period 5891 specimens were shipped out as loans, exchanges, or gifts.

During the year 2,627 collection records involving 108,093 specimens were added to the computer data base. These entries nearly completed the geographic file for Mexico and Central America, with only records from Mexico and a small number from Costa Rica remaining for entry. A standard query form was developed to handle requests for epidemiological-ecological information about mosquito species in any computer program. World

Data Bank II was obtained from the National Technical Information Service. This program will produce computer-digitized maps for collection sites and species distribution maps at any desired scale, based on the specimens in the data base.

Future Plans

Research will continue on the Leucosphyrus Group of Anopheles (Cellica), the genus Anopheles of the Middle East, the Aedes (Stegomyia) of the Afrotropical region, the Culex (Culex) of North Africa and Southwest Asia, and the genus Trichoprosopon of the Neotropics. Publication of the revision of the Argyritarsis Section of Anopheles (Nyssorhynchus) and several small papers describing new species in groups under study is anticipated in the near future. Pending the completion of several studies, work will begin on the ground pool breeding Aedes of Africa, which are suspected to be involved in the transmission of Rift Valley Fever Virus. Plans are in progress for field collection trips to Egypt, Israel, Turkey, India and several African countries.

Formal Presentations

Faran, M.E. 1981. Computerized information and collection management system for Systematic Research and Medical Entomology. Presented at Annual Meeting of Entomological Society of America. Nov. 29, - 3 Dec, 1982, San Diego, CA.

Harbach, R.E. 1981. Mosquitoes of the Pipiens Complex of Culex; Structure of the Male phallosome and the DV/D. Presented at the Annual meeting of Entomological Society of America, Nov. 29- 3 Dec, San Diego, CA.

Harrison, B.A. 1982. Malaria in the Middle East. Presented at BiAnnualEntomology Training Course, 9 February, Fort Sam Houston, TX, and at Armed Forces Pest Management Board, WRAIR, 4 Mar, Washington, D.C.

Faran, M.E. 1982. Organized and presented Introduction to Symposium entitled "Mosquito Systematics: Determination and Recognition of a Disease Vector" Presented at Annual Meeting of American Mosquito Control Association, Apr. 18-22, Sacramento, CA.

Linthicum, K.J. 1982. The Role of morphological systematics in a project to determine the enzootic cycle of Rift Valley Fever in Kenya. Presented at a Meeting of the American Mosquito Control Association.

Burnett, C. 1982. Computerized information and collection management system for Systematic Research and Medical Entomology (Diptera: Culicidae). Presented at Computers in Research at the Smithsonian Symposium, Apr 6-7, Washington, D.C.

Publications

Baimi, V., B.A. Harrison and L. Somchit. 1981. Karotype differentiation of three anopheline taxa in the Balabacensis complex of Southeast Asia (Diptera: Culicidae). Genetica 57: 81-86. (Nov. 1981).

Harbach, R.E. and K.L. Knight. (1981) 1982. Corrections and additions to Taxonomists' Glossary of Mosquito Anatomy. Mosq. Syst. 13: 2011-2217. (Jan. 1982).

Reinert, J.F. 1981. Medical Entomology Studies - XV. A revision of the subgenus Paraedes of the genus Aedes (Diptera: Culicidae). Contrib. Am. Entomol. Inst. 18(4): 1-911. (Nov. 1981).

Huang, Y.-M. and R.A. Ward. (1981) 1982. A pictorial key for the identification of the mosquitoes associated with yellow fever in Africa. Mosq. Syst. 13: 138-149. (Jan. 1982).

Klein, T.A., B.A. Harrison, I. Inlao and P. Boonyakanist. 1982. Colonization of Thailand strains of Anopheles nivipes and Anopheles philippinensis. Mosq. News.. 42(3): pp 374-380.

Rattanarithikul, R. 1982. A guide to the genera of mosquitoes (Diptera: Culicidae) of Thailand with illustrated keys biological notes and preservation and mounting techniques. Mosq. Syst. In press.

Linthicum, K.J. 1983. Mosquito Studies (Diptera Culicidae). A revision of the Argyritarsis Section of the subgenus Nyssorhynchus of Anolpheles. Contrib. Am. Entomol. Inst. (in press).

Faran, M.E., C. Burnett, J.J. Crockett and W.L. Lawson. 1983. Computerized information and collection management system for systematic research and medical entomology. (Diptera: Culicidae). J. Med. Entomol. (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OB 6489	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISB'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AA	072
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	STOG 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: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	3.0	68
	CURRENT 83	3.0	204

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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Washington, DC 20012

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

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TELEPHONE: (202) 576-3553

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Takafuji, Ernest T., LTC, MC

NAME: Kirkpatrick, James W., LTC, MC POC:DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Epidemiology; (U) Infectious Disease; (U) Risk Assessment; (U) Data Bases

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

23. (U) To identify, define, and study known and potential causes of disability in military populations using relevant, existing epidemiologic techniques and developing appropriate new methodology. To apply this information to the assessment, prevention and control of infectious diseases in military populations.

24. (U) Contemporary epidemiologic methods are applied to causes of disability in military populations. Multi-disciplinary collaborative approaches are utilized and new methods developed as required.

25. (U) 81 10-82 09. Analyses of the following studies are in progress: assessment of risk of coccidioidomycosis at Ft. Irwin; long-term follow-up of soldiers with hepatitis B; development of better methods for leishmaniasis diagnosis; assessment of risks of infectious diseases to the Rapid Deployment Force; evaluation of reduced dosages of Human Diploid Cell Rabies Vaccine given via jet injector; routes of transmission of hepatitis A in US Disciplinary Barracks and field units; assessment of disease acquisition during OCONUS Special Forces deployments; etiology of gastroenteritis during an outbreak at Ft. Belvoir; experimental designs for gonococcal vaccine efficacy trials; prevalence of penicillinase-producing Neisseira gonorrheae in US Army populations, epidemiology and prevention of leptospirosis in jungle trainees at Ft. Sherman, Panama; surveillance of disease risks to US troops deployed in the Sinai Peninsula; prevalence of tuberculosis skin test reactivity at Ft. Dix; etiologies of conjunctivitis at Ft. McClellan; risk of schistosomiasis in an engineer unit training in Puerto Rico. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81-30 Sep 82.

*Available to contractors upon originator's approval

Project 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS

Work Unit 072 Assessment of Infectious Diseases of Military Importance

Investigators.
Principal: COL Richard N. Miller, MC
Associate: LTC James W. Kirkpatrick, MC
LTC Ernest T. Takafuji, MC
MAJ Mary K. McKenna, ANC
MAJ Ronald E. Prier, MC
MAJ Wayne M. Lednar, MC
CPT Jose L. Sanchez, 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: In October 1981, a brigade from Ft. Benning, GA deployed to Ft. Irwin Military Reservation for several weeks of training was screened for coccidioidomycosis by means of the spherulin skin test. The unit was skin tested two weeks prior to deployment and 6 weeks following desert training. The skin test conversion rate was less than 3%. Although no cases of clinically apparent disease were identified in this unit, 3 cases of coccidioidal meningitis with a history of exposure to Ft. Irwin have occurred in the last two years. The low skin test conversion rate and the unpredictability of disease in units have made routine spherulin screening impractical.

Surveillance for suspicious respiratory illnesses with a history of exposure to Ft. Irwin continues.

2. Hepatitis: Sixty-four cases of acute hepatitis A occurred among 1492 inmates confined at the U.S. Disciplinary Barracks, Fort Leavenworth, KS between 15 March and 1 June 1982. No cadre or civilian employees became ill. The outbreak appeared to have been common source with a minor contribution of interpersonal transmission. Over 1000 sera were evaluated by the Department of Virus Diseases, WRAIR, for HAV and HBV markers. The attack rate was 10% among susceptibles. Risk factors included living or working in certain parts of the prison during late March to mid-April 1982, and factors indicating interpersonal contact with affected inmates. Contact with raw sewage may have been the most important risk factor. Subsequent to the initial EPICON investigation of 6-20 May, a follow-up visit and case-control study were performed to confirm the importance of various risk factors. Epidemiologic analyses continue. Thirty-five cases of HAV occurred in the 1/30 Field Artillery stationed in Augsburg, FRG. Soldiers became ill while at Grafenwoehr training area during the period 12-26 July 1982. A clear origin for the common source outbreak has not yet been identified. Numerous field sanitation deficiencies were noted at Grafenwoehr. The causative exposure most likely occurred in mid-June while training in the Augsburg area. Approximately 500 sera were evaluated for recent HAV infection. The attack rate was 9.6% among susceptibles. Both of these outbreaks reveal that HAV in adults is a primarily symptomatic disease with symptomatic to asymptomatic ratios ranging from 6:1 to 8:1. Twenty-one cases of HAV occurred among two U.S. Army Reserve units training at Fort McCoy, WI in a third outbreak during the period June to July 1982. An EPICON investigation is currently underway collaboratively with the State Epidemiologists of Iowa and Minnesota to identify cases and risk factors for infection.

3. Special Forces Surveillance: Pre- and post-deployment sera and post-deployment morbidity questionnaires are obtained on most OCONUS deployments from Ft. Bragg. Results are used to augment other information on specific disease risks in the areas as well as to assist with planning for medical support and estimates of personnel strength.

4. Gastroenteritis - Fort Belvoir - July, August 1981: An outbreak of acute infectious, non-bacterial gastroenteritis occurred in the Ft. Belvoir hospital staff involving a total of 22 individuals. No common source of transmission was identified but a direct association between degree of close interpersonal contact, presence of young household members and household size was found. No specific viral etiologic agent(s) could be isolated from stools of affected individuals.

5. Leishmaniasis: Efforts are continuing with the Divisions of Communicable Disease and Immunology and Experimental Therapeutics in the development of a leishmanin skin test antigen that could be used in the screening of soldiers deployed to geographical areas endemic for leishmaniasis, or as a useful tool in the diagnosis of disease. Medical surveillance of units deployed to Panama is continuing to identify suspect cases of infection. Epidemiological data on cases treated at WRAMC are being collected, and will be analyzed in the near future.

6. Gonococcal Vaccine Efficacy Trial: The previous protocol was rewritten and submitted for review and approval by the three services as well as DOD. Pending these approvals, detailed planning is ongoing for a large-scale efficacy trial to be conducted in CY 1983.

7. Penicillinase-Producing Neisseria gonorrheae (PPNG) and Syphilis in U.S. Personnel in Korea: Studies of the prevalence of PPNG in this population showed almost half the gonorrhea to be penicillinase-producing. This determination supported a change in the recommended therapy for gonorrhea to spectinomycin. Close monitoring of the incidence of syphilis was instituted to detect any increase in this disease resulting from the change in therapy of gonorrhea.

8. Leptospirosis: During the fall of 1981, three battalions that were deployed to Panama for jungle training experienced febrile illnesses that were later confirmed to be due to leptospirosis. Attack rates of 5-9% were documented, and occurrence of illness appeared to be correlated to rainfall and jungle water exposure. These outbreaks resulted in a Leptospirosis Workshop held at WRAIR on 28 May 1982 to address issues on diagnosis, therapy, prevention, and surveillance for

this disease. Protocols have been developed for the prevention and therapy of leptospirosis using doxycycline. The studies will commence in October 1982. Surveillance for febrile illnesses related to jungle training is continuing.

9. RDF/Bright Star 82 Surveillance: Personnel from this Division accompanied each segment of troops participating in overseas deployments during this exercise to observe morbidity, obtain clinical specimens, and provide some guidance to the units. Results of these studies have been provided to RDJTF to assist with contingency planning.

10. Multi-National Peace Keeping Force and Observers (MFO) Surveillance: Surveillance of troops deployed to the Sinai is being conducted in collaboration with the local medical support provided. Surveillance has incorporated the collection of pre- and post-deployment blood samples and the administration of questionnaires addressing illnesses experienced. The surveillance began in January 1982 and will extend through December 1982, after which time a report summarizing the experiences will be written.

11. Tuberculosis at Ft. Dix: An investigation of an outbreak of active tuberculosis in an engineer company assessed the degree of spread of infection within this unit. A related project involved the conduct of a skin test reactivity survey of a systematic sample of the Ft. Dix permanent party population. The result was an estimate of the prevalence of skin test reactivity (9%) throughout this group.

12. Conjunctivitis - Fort McClellan - July, August 1982: A reported outbreak of Trachomal conjunctivitis at Fort McClellan, Alabama was investigated by the EPICON team from WRAIR. No difference in incidence rates between 1982 and 1981 was detected, nor could C. trachomatis account for any of the 39 cases seen. Allergic, bacterial and other etiologies were thought to be the most probable responsible agents in this situation. Heightened clinical awareness of methods of Trachomal conjunctivitis diagnosis was emphasized among the responsible hospital staff.

13. HDCV immunogenicity: A study of the immunogenicity of 0.1 ml of Human Diploid Cell Vaccine was done in 104 volunteers from Special Forces units at Fort Bragg, North Carolina. All subjects receiving 3 doses achieved protective antibody levels. Analysis of data is continuing.

14. Schistosomiasis risk for 548th Engineer Battalion training in Puerto Rico - July 1982: An assessment of the risk of acquiring schistosomiasis by troops training in Puerto Rico was addressed by administering a post-deployment questionnaire to a total of 185 engineer troops involved in a 3-week training exercise at Roosevelt Roads Naval Station and Vieques Island. Only a minority of people (7%) reported any contact with fresh water. Hematologic screening of exposed individuals for eosinophilia revealed no evidence of recent schistosomal infection. No symptoms suggestive of acute schistosomiasis could be detected.

Formal Presentations:

1. "Bright Star 82 and The Medical Threat in the Middle East" FORSCOM Surgeon's Conference, Atlanta, GA, 20 April 1982, MAJ Ronald E. Prier, MC.

2. "Infectious Disease Threats to the RDF" Preventive Medicine Symposium, 25 May 1982, MAJ Ronald E. Prier, MC.

3. "Disease Surveillance of Units Deployed for Overseas Operations" Preventive Medicine Symposium, 25 May 1982, MAJ Ronald E. Prier, MC.

4. "Recent Outbreaks of Leptospirosis Associated with Jungle Training in Panama" Preventive Medicine Symposium, 26 May 1982, LTC Ernest Takafuji, MC; CPT Benedict Diniega, MC; CPT Samuel Ruben, MC; CPT Jose Sanchez, MC.

5. "Hepatitis B Virus Markers in a Marine Unit Deployed to the Middle East" Preventive Medicine Symposium, 26 May 1982, CPT James Hilburn, MC.

6. "Emerging Penicillinase Producing Neisseria Gonorrhoeae (PPNG) Among Army Personnel" Preventive Medicine Symposium, 26 May 1982, LTC James Kirkpatrick, MC.

7. "The Risk of Coccidioidomycosis at the National Training Center" Preventive Medicine Symposium, 26 May 1982, COL Richard N. Miller, MC.

8. "Controversies in the Use of Antibiotics for the Prophylaxis of Haemophilus influenza" Preventive Medicine Symposium, 26 May 1982, CPT (P) Wayne M. Lednar, MC.

9. "Hepatitis Outbreak - Fort Leavenworth, KS" Preventive Medicine Symposium, 26 May 1982, CPT Lorrin Pang, MC.

10. "Computers in the Preventive Medicine Activity - The Future is Now" Preventive Medicine Symposium, 26 May 1982, COL Richard N. Miller, MC.

11. "Leptospirosis in Panama and Recent Outbreaks" Leptospirosis Workshop, 28 May 1982, LTC Ernest T. Takafuji, MC.

12. "An Epidemiology Consultant Service (EPICON) Investigation of a Tuberculosis Outbreak at a TRADOC Post" Phyllis J. Verhonick Research Symposium, San Antonio, TX, 6-11 June 1982, MAJ Mary K. McKenna, ANC.

13. "Recent Studies of PPNG in Korea" Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD, 3 September 1982, LTC James W. Kirkpatrick, MC.

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1. McKenna, M.K., Hales, P.W. "Application of the Nursing Process to Improve the Quality of Nursing Service Provided to a Military Community in Germany," Military Medicine, Vol 147, No. 7, July 1982.

2. Lemon, S.M., Lednar, W.M., Bancroft, W.H., Cannon, H.G., Benenson, M.W., Park, J.H., Churchill, F.E., Tezak, R.W., Erdtmann, F.J., Kirchdoerfer, R.G., Lewis, P.G., James, J.J., and Miller, R.N. "Etiology of Viral Hepatitis in American Soldiers." American Journal of Epidemiology, 116 (3): 438-450 (September), 1982.

3. Benenson, M.W., Takafuji, E.T., Lemon, S.M., Greenup, R.L., and Sulzer, A.J. "Oocyst-transmitted Toxoplasmosis Associated with Ingestion of Contaminated Water." New England Journal of Medicine, 307: 666-669 (September 9), 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	3. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 2527	82 10 01	DD-DR&E(AR)636

2. 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
81 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A870	AB	073
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)

(U) Threat Assessment of Diseases of Military Importance in the Tropics

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	11.0	677
	CURRENT 83	9.0	900

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)

NAME: BENENSON, M.W., LTC

TELEPHONE: (02) 281-7776

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS NAME: ANDRE,R.G.,MAJ: ROSENBERG, R.M.,CPT; ECHEVERRIA,P.D.,LTC; BURKE, D.S.,LTC; USSERY,M.A.,CPT

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Malaria; (U) Diarrhea; (U) Chancroid; (U) Vectors; (U) Dengue

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

23. (U) The technical objective is to assess the risk of various tropical diseases to military troops and operations, and to determine the potential mortality and morbidity of military personnel undertaking operations in the tropics.

24. (U) This requires defining the ecology, epidemiology, and etiology of various tropical diseases through the development of new or improved technologies related to field studies, in vitro cultivation, microbiological assays, vector colonization, serological procedures, and other necessary approaches.

25. (U) 81 10-82 09 Studies on the malaria susceptibility of members of the mosquito species complexes previously identified are continuing. Colony reared mosquitoes are fed on naturally infected patients and the susceptibility to infection and detrimental effect of the infection are being determined. A circum-sporozoite antibody technique is being developed and tested to determine species of malaria and infection rates of wild caught mosquitoes. The DNA probe for toxogenic E. coli has been used extensively in the field and has proved to be an effective tool. Separate studies on the role of pigs and of water on the transmission of E. coli, and the etiology of diarrhea in a major Bangkok Hospital, have been done using the probe. Patients with penile lesion are being cultured for H. ducreyi to determine the prevalence and the importance of chancroid as a cause of venereal disease in Thailand. The dengue hemorrhagic fever and FUO study continues to develop data on the prevalence of each of the dengue serotypes and the importance of other causes of FUO. A study on acute encephalitis in Bangkok is determining the importance of flaviviruses in this disease. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81-30 Sep 82.

* Available to contractors upon originator's approval.

Project Number: 3M162770A870 RISK ASSESSMENT OF MILITARY DISEASE HAZARDS
Title: Threat Assessment of Diseases of Military Importance in the Tropics
Work Unit Number: 073

Investigators: LTC(P) Michael W. Benenson, MC; MAJ Richard G. Andre, MSC; CPT Ronald M. Rosenberg, MSC; LTC Peter D. Echeverria, MC; LTC Donald S. Burke, MC; CPT Michael A. Ussery, MC; LTC George S. Ward, VC; MAJ Michael R. Elwell, VC; Chiraphun Duangmani, M.D.; Ananda Nisalak, M.D.; Markpol Tingpalapong, DVM

1. Epidemiology of Enterotoxigenic E. coli in Thailand: Application of a Colony DNA Hybridization Assay to Detect These Pathogens

PROBLEM: To define the epidemiology of enterotoxigenic E. coli (ETEC) with a DNA hybridization assay.

PROGRESS: AFRIMS has recently established and tested the DNA hybridization assay to detect ETEC in diarrheal stolls (1) and water (2). Four populations have been studied in the environment. A pig farm in Sri Racha has been screened to determine if pigs are a source of ETEC for man. Two villages are being surveyed daily over one year to define the importance of ETEC as a cause of diarrhea and environmental sources of these pathogens. Over 200 children who are seen with diarrhea at Children's Hospital, Bangkok and over 900 patients with diarrhea seen at Soongnern Hospital, Soongnern have been investigated. Homes of diarrhea patients and control homes have been examined with the DNA hybridization assay.

FUTURE OBJECTIVES: The two year long study of the villages is in its eighth month and the Soongnern Hospital is in its tenth month; other populations will be examined to further determine sources of ETEC.

A new ST probe which detects ST which is only detected in pig loops will be used to determine the enteropathogenicity of this organism for man.

2. Isolation and Characterization of Haemophilus ducreyi in Thailand

PROBLEM: Chancroid is the second most common venereal disease in Asia (3). Previous attempts to isolate Haemophilus ducreyi from penile ulcers have been unsuccessful and therefore it has been impossible to determine the in vitro sensitivity of this organism so that rational antimicrobial therapy can be given (4).

PROGRESS: In the last two years we have been able to isolate these organisms from only three of over 300 patients. In the last two weeks we have improved our methods and are now isolating Haemophilus ducreyi from 60 percent of men with penile ulcers. Our increased success is due to two factors. One, we have included

deactivated fetal calf serum in rabbit and horse blood in Gibco G-C base agar. Two, we have managed to reduce the fungal and bacteria contamination on the plates. Sodium azide has been included in water used to create an atmosphere with high humidity and candle jars have been washed daily with disinfectant. Normal saline used to moisten swabs was also frequently contaminated with gram negative organisms. Sterile bottles of normal saline are currently changed daily.

FUTURE OBJECTIVES: To study 100 patients with penile ulcers and culture them for H. ducreyi. Darkfield examination and VDRLs could also be performed to rule out syphilis and Dr. Chaninthorn of Thai Component will culture patients for Herpes simplex. This study should determine what proportion of penile ulcers are associated with H. ducreyi in Bangkok.

Isolates of H. ducreyi will be tested for antibiotic sensitivities to various antibiotics. Plasmids coding for antibiotic resistance will also be characterized.

Chromosomal DNA has been purified from an isolate of H. ducreyi and will be nick translated to incorporate α-^{32}P. This DNA will be used as a probe to identify this organism in pus collected from penile ulcers smeared and fixed on nitrocellulose paper. This method will be compared to routine culturing. Hopefully a method of labelling such a probe with fluorescence will have been developed in the next year which may lead to a simple rapid method of identifying H. ducreyi.

3. Mosquito Cytogenetic, Electrophoretic and Cross Mating Studies

PROBLEM: To define and delimit the taxa in the vector Anopheles species complexes by cytogenetic, electrophoretic and cross mating techniques for the following reasons: (a) to check against current morphological species concepts; (b) as an accurate determination of chromosomal polymorphisms and genetic variations in natural populations of malaria vector species and/or suspected vector species; and (c) to correlate genetic variations in natural populations of the vector species with habitat differences, innate susceptibility to the human malarias, and behavioral patterns that may facilitate more effective control measures in the future.

PROGRESS: Preliminary analysis of salivary gland chromosomes reveals that the autosomes of Anopheles dirus (5), An. takasogoensis, An. balabacensis Perlis form and Fraser's Hill form appear to be very similar. Nevertheless, the X chromosome seems to be different in length, with different banding sequences in zone 6. Analyses of mitotic and meiotic karyotypes of these species and forms of the An. balabacensis complex show that metaphase chromosomes, especially the sex chromosomes of An. dirus, An. takasogoensis and An. balabacensis Perlis form are significantly different. These karyological differences are very useful in differentiating these taxa, particularly the Perlis form, and support the concept of these taxa being elevated to species status (6).

An attempt to find biochemical markers for interspecific differences between An. dirus, An. balabacensis Perlis form and Fraser's Hill form has been carried out using starch and polyacrylamide gel electrophoretic techniques. The latter seems to yield better resolution. In the Balabacensis complex, esterases 2 and 3 show polymorphic bands with fast, intermediate, and slow electromorphs for each locus. Differences were noted between the members of the complex when testing for the enzymes ODH, XDH, and PGI-1.

Forced mating crosses in both directions between An. balabacensis Perlis form and Fraser's Hill form produced only small numbers of viable F1 hybrid offspring in one direction and no viable offspring in the other direction. In contrast, crosses between Fraser's Hill form and An. dirus produced normal hybrid offspring through the 3rd generation.

These cytological, electrophoretic, and crossing data, together with morphological studies, clearly indicate that An. balabacensis is actually a sibling species complex consisting of at least four full biological species. Similarly, preliminary results of studies on An. maculatus and An. nivipes (7) show that there are at least three and two sibling species of these mosquitoes, respectively, in Thailand.

FUTURE OBJECTIVES: These studies on the sibling species complexes of vector anophelines in Thailand will continue during the coming year. Emphasis will be placed

on natural mosquito population determinations correlated with formal genetic studies using cytogenetic and electrophoretic techniques.

4. Comparative Susceptibility of Known and Suspected Species/Strains of Anopheles to Plasmodium Parasites

PROBLEM: The objectives of this investigation are as follows: (a) To determine and compare the susceptibility of primary and potential secondary vectors of malaria to Plasmodium parasites; (b) to delineate the development of malaria parasites in Anopheles spp. with varying degrees of susceptibility; and (c) to observe the feeding behavior of colonized vectors of human malaria under laboratory conditions.

PROGRESS: During this reporting period, attempts were made to find a partially refractory anopheline to Plasmodium cynomolgi (8) and/or P. knowlesi. Of eight species or strains tested, most were less susceptible than An. dirus, but none which developed oocysts failed to go to the sporozoite stage.

Human susceptibility studies were initiated at the start of this fiscal year in Tha Muang, Kanchanaburi. Over 75 falciparum patients and 100 vivax patients have volunteered for the study. Unfortunately, less than 40 percent of the feeds infected mosquitoes. An. dirus was used as the control mosquito species and was more susceptible to both Plasmodium species than were the other mosquito species tested. Of the experimental mosquito species, An. balabacensis Perlis form was the most susceptible and An. maculatus Huai Kuum strain the least. All species developed some infected salivary glands by Day 14.

FUTURE OBJECTIVES: Due to the high number of negative feeds and the number of species/strains of mosqitoes left to be tested, this study will continue for at least another year. Attempts to correlate negative feeds with seasonality or prior drug usage will be made.

5. Identification of Field-Collected Sporozoites

PROBLEM: Three different tests are available to potentially identify Plasmodium sporozoites in mosquito

salivary glands: (1) circum-sporozoite precipitin test; (2) immunofluorescent antibody test; and (3) radioimmuno assay test. The objective of this study is to evaluate these three tests in the laboratory, and then adapt the tests to use in the field to identify natural infections in vector anophelines.

PROGRESS: Laboratory-reared mosquitoes were fed on patients infected with gametocytes of Plasmodium vivax or P. falciparum. Other mosquitoes were fed on monkeys infected with P. cynomolgi or P. knowlesi. Infected salivary glands were later crushed, and the sporozoites were inoculated into rabbits for antibody production. At least four boosters were given to each rabbit over a period of several months. Known sporozoites were added to antiserum samples, and then were examined microscopically following incubation. The circum-sporozoite precipitin reaction (9) was readily visible with some sporozoites and appeared to be species specific.

FUTURE OBJECTIVES: During the next year, the immunofluorescent antibody test and the radioimmuno assay test will be evaluated in collaboration with NIH. An attempt will be made to also develop an ELISA test for use in the field.

6. Mosquito Survey and Taxonomic Studies

PROBLEM: To elucidate the mosquito fauna of Thailand and Southeast Asia. Primary emphasis is put on the determination of diagnostic characters that separate the vector species and groups containing vector species that transmit parasite detrimental to humans.

PROGRESS: Morphological studies on the Leucosphyrus Complex (10), the Maculatus Complex, and the Anopheles philippinensis-nivipes group were continued during the past year. A manuscript confirming a separate species status for An. philippinensis and An. nivipes has been cleared for publication (11). Work on the taxonomic revision of the Kochi group of Aedes (Finlaya) continued. Descriptions and keys for this group have been completed and manuscript prepared; however, many illustrations of the various stages of these species remain to be done. The surveillance of vector species densities and distributions in Thailand and Malaysia revealed the potential of several important vector-human parasite interactions. The guide to the genera of mosquitoes

occurring in Thailand, along with illustrated keys, biological notes, and mounting techniques, was revised and is now in press.

FUTURE OBJECTIVES: During the next year primary emphasis will be placed on delineating usable morphological markers to identify natural populations of the sibling species complexes of An. balabacensis and An. maculatus. Illustrations for the Aedes (Finlaya) manuscript will be finalized. Preparation of keys specific to human disease vectors in Southeast Asia is proposed.

7. Detrimental Effects of Plasmodium Infections on the Survival Rate of Anopheles dirus

PROBLEM: The objectives of this study are as follows: (a) to determine if the longevity of mosquitoes infected with Plasmodium is different significantly from that of uninfected mosquitoes; (b) to determine if the longevity among mosquitoes with heavy or light infection rates is significantly different; and (c) to determine if the longevity of mosquitoes infected with different species of Plasmodium is different significantly among groups.

PROGRESS: Manuscripts describing the results of studies on the effects of Plasmodium cynomolgi on Anopheles dirus were revised this year and sent for publication. The first manuscript covers differences found in the mortality rates of non-infected versus infected groups, and this manuscript has appeared in print (12). The correlation of differences in the survival rates of mosquitoes infected with various densities of parasites is discussed in the second manuscript which is in press (13).

An investigation of the effects of human malaria parasites on the longevity of An. dirus was begun this year. One hundred lots of mosquitoes have been fed on vivax patients and 75 on falciparum patients. Only 40 percent of the feeds were infective to the mosquitoes. Lightly infected mosquitoes and controls live two months; whereas, many of the heavily infected mosquiotes died during the first 30 days. These studies will continue for another year so that the results can be statistically analyzed.

8. Serosurvey and Virus Isolation from Rodents to Determine the Hantaan Virus Presence in Thailand

PROBLEM: Recent studies have found Hantaan virus to be the causative agent of Korean hemorrhagic fever (KHF), a syndrome of significance in Korea and Manchuria and of potential significance in the USSR, the Balkans, parts of Western Europe and Scandinavia (14). Evidence has recently been obtained in Seoul, Korea that urban Rattus also are chronically infected with Hantaan virus. Cases of KHF in man have now been linked temporarily and geographically to infected wild rats in urban Seoul and Osaka, Japan (15). In addition, antibodies to Hantaan virus have been found in Rattus captured near the docks in Japan, Korea and the United States. Chronic infection, rats and international shipping thus provide a likely chain which may have disseminated this virus worldwide. Thus the potential for this agent to cause human disease may be far greater and more geographically diverse than is presently appreciated.

The objectives of this study are (1) Identify areas of Thailand where Hantaan virus antibody positive rodents exists and test human sera in those areas; (2) isolation of Hantaan virus from infected rodents.

PROGRESS: In a collaborative effect with MAJ LeDuc, USAMRIID, Ft. Detrick, a serosurvey of 100 rodents was completed at the Klong Toey port in Bangkok. Five positive sera were found and four of these were from one specific area at the port. A follow up trapping of rodents at this location has been completed and tissue samples have been frozen for Hantaan virus isolation at USAMRIID. Serum samples are currently being collected from people working in the vicinity of the rodent trapping area.

FUTURE OBJECTIVES: A serosurvey and tissue collection for virus isolation from rodents will begin next month at the Bang Pacong port area to determine if Hantaan virus is present there.

9. Epidemiology of Encephalitis in Thailand

PROBLEM: Encephalitis in general, and Japanese B encephalitis (JE) in particular, have been recognized as diseases of major epidemiological risk in Thailand.

In 1969 and 1970, a detailed analysis of JE in Northern Thailand was performed, but no review has been done since then. A primary goal of field investigation teams is to supply information which is directly useful in the control of disease. Commitment of resources can be directed only by detailed knowledge of the epidemiological importance of a disease. Therefore, encephalitis surveillance data collected by the Center for Disease Control of Thailand has been reviewed in order to ascertain the population most likely to benefit from attempts to control JE.

PROGRESS: Reported numbers of cases of encephalitis are tabulated by the Center for Disease Control and population data by the Department of Vital Statistics. 1970 census data was used for estimation of age specific rates. Overall, an average of 1598 cases (range = 986 in 1970 to 2413 in 1980) were reported each year, with an average case fatality ratio of 23 percent. A general upward trend in reported cases has occurred. In each year the peak number of cases occurred in July, though in three years the peak encompassed two months (July plus either June or August). Cases were reported in all months. Each year, the Northern region contributed the largest number of cases (34 percent on the average). Within the Northern region, the provinces of Uttaradit (46 cases/100,000), Phayao (21), Chiang Rai (19), Chiang Mai (11), and Nan (26), reported the highest rates in 1980. The lowest rates have been from Southern provinces. Age specific attack rates were about 30/100,000 in children age 1-17 in Uttaradit, and about 18/100,000 in similar age groups in Chiang Mai. These data show that the northern region remains the area of highest risk for encephalitis, and that certain provinces consistantly report higher rates than others. Children under 14 are at highest risk. Risk estimates are consistant with earlier published data.

FUTURE OBJECTIVES:

1. Further study should be under taken to ascertain the proportion of encephalitis which is due to JE.

2. If substantial numbers of cases are not due to JE, efforts should be directed at determining actual etiology.

3. Programs to evaluate measures to reduce JE transmission should be undertaken. These surveillance data can be used to effectively guide such studies.

10. Acute Encephalitis at Bangkok Children's Hospital

PROBLEM: Acute encephalitis contributes a large number of cases each year to the patient load at Bangkok Children's Hospital (16) and has been a threat to American soldiers stationed in Southeast Asia (17). To assess the magnitude of encephalitis as a problem, determine the flavivirus etiology of such cases, and ascertain the population at risk, surveillance of encephalitis has been maintained at Children's Hospital for four years.

PROGRESS: Since 1979, all cases with encephalitis have been seen and interviewed. Clinical and epidemiological data, paired sera and cerebrospinal fluid (CSF) were collected and tested using HAI for flaviviruses and IgM and IgG capture (MAC and GAC) ELISAS for Japanese B encephalitis (JE) (18). Diagnosis of JE required a four fold HAI rise or a MAC ELISA OD reading of greater than the average reading for a known weak positive specimen in either serum or CSF.

Eighty-three cases of encephalitis were seen at Children's Hospital between January 1979 and September 1982. Fifty-eight percent of these were due to Japanese B encephalitis by either test, an average of 12 cases per year. Twenty percent of JE cases and seventeen percent of Non-JE cases were fatal during the initial hospital admission. Thirty-four percent of the JE cases were male. Their mean age was 6.9 years (range one year to 14 years). Most cases came from the provinces surrounding Bangkok although 27 percent had Bangkok addresses. Cases occurred between March and December. No cases were seen in January or February, while 33 percent of cases occurred during June (eight percent expected) the largest percentage of any month. Agreement between HAI and ELISA diagnosis occurred in 46 of the JE cases completely studied (20 JE and 26 not JE). Approximately 50 percent of encephalitis was not due to JE or dengue. No cases were HAI positive and ELISA negative. However, ten cases were ELISA positive and HAI negative. Analysis of these ten cases revealed that six had stable HAI titers of $\geq$ 80. Of these five

had anti-JE IgM in both sera. Three had anti-JE IgM in CSF and the other three were not tested. These six were probably due to JE. By contrast, the other four of the ten patients with conflicting results were HAI seronegative but had a single serum (3) or two sera (1) positive for anti-JE IgM. All three CSF's tested from these four patients were negative for anti-JE IgM. These four cases may have been infected by JE, but their encephalitis was probably of another etiology, pointing out the importance of studying CSF by ELISA before diagnosing JE encephalitis. Only one patient had a serum ELISA positive for dengue, and he had a broad 2° flavivirus response. Thus, one case of encephalitis may have been due to dengue virus.

FUTURE OBJECTIVES:

1. Continue surveillance of encephalitis seen at Children's Hospital.

2. Attempt to ascertain the etiology of Non JE encephalitis.

3. Should anti-viral chemotherapy, such as ribavirin, prove promising in animal models, design protocols to evaluate its use in treating human cases.

11. Dengue Hemorrhagic Fever and Pyrexia of Unknown Origin at Children's Hospital

PROBLEM: Previous studies have linked pre-existing dengue antibody with the occurrence of hemorrhagic fever and shock during second infections. This hypothesis has important implications not only for understanding the pathogenesis of hemorrhagic fever, but also for the development of dengue vaccines. Also, pyrexia, undiagnosed on admission (PUO) been shown to be frequently due to dengue infection in both Thai children and U.S. soldiers stationed in Southeast Asia. Finally, surveillance data on dengue cases has been relied upon by local health authorities to guide anti-mosquito spraying efforts. Therefore, surveillance of dengue hemorrhagic fever (DHF) and PUO's at Children's Hospital in Bangkok has been maintained to study these conditions. Data collected in Bangkok during the previous 20 years has shown that the four dengue virus types do not cause DHF or dengue fever with the same frequency and do not appear in the community at the same time. The identification of dengue virus isolated during each year has allowed prediction of the virus type of future isolations.

PROGRESS: During calendar year 1981, 198 cases of DHF were seen at Children's Hospital. HAI studies of paired sera showed that 10 percent of these were primary and 80 percent secondary infections. Five percent were not dengue and five percent were unavailable for follow up. Thus far, virus isolates have been obtained from 32 cases. D1 was obtained in four primary and five secondary infections. D2 was found in one primary and 17 secondary infections. No virus isolates were obtained from the nine non-dengue cases. Data from 42 PUO patients seen between January and September showed that 12 percent were primary, ten percent secondary, 62 percent not dengue, and 16 percent were lost to follow up. Through September, 131 cases of hemorrhagic fever were seen in 1982. Of these 14 percent were primary dengue infection, 72 percent secondary flavivirus infection and 11 percent were not dengue infections. Only three isolates have been obtained thus far, and all are D2.

These data confirm previous data which show (1) a large fraction of PUO's are due to dengue infections (22 percent), (2) DHF is associated with a secondary flavivirus antibody response, and (3) D2 is rarely the etiology of primary DHF, but is the most commonly isolated strain from 2° cases.

FUTURE OBJECTIVES:

1. The PUO portion of this study was complete and was terminated in September of 1981.

2. DHF surveillance will continue in order to increase knowledge of viruses of likely risk to seronegative individuals.

3. Isolation attempts will continue on remaining sera.

4. DHF surveillance should be expanded to include other centers in Southeast Asia and the Western Pacific in order to gain broader knowledge about the spread of dengue viruses throughout this region. The circulation of multiple dengue strains in the Caribbean region, the recent occurrence of DHF in that area, and of dengue in the continental United States mandate that we maximize our opportunities to learn about the spread, pathogenesis and prevention of dengue infection.

Project Number: 3M162770A870
Title: Threat Assessment of Diseases of Military Importance in the Tropics
Work Unit Number: 073

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PROJECT 3M162770A871

PREVENTION OF MILITARY DISEASE HAZARDS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DAOG 6764	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AC	122 WWGW
b. CONTRIBUTING				
c. XXXXXXXXXX	STOG 80-7.2 2			

11. TITLE (Procede with Security Classification Code)

(U) Biosystematics of Arthropods of Military Medical Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 005900 Environmental Biology

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

17. CONTRACT/GRANT

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

18. RESOURCES ESTIMATE	A. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	3.0	178
FISCAL YEAR CURRENT 83	3.0	295

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research
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RESPONSIBLE INDIVIDUAL NAME: Russell, COL P. K.
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Harbach, CPT R.E.
NAME: Zavortink, Dr. T. POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Biosystematics; (U) Disease Vectors; (U) Arthropods; (U) Mosquitoes; (U) Epidemiology; (U) Malaria; (U) Arboviruses

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

23. (U) Conduct biosystematic research of important arthropod groups in support of epidemiological studies and disease control strategies of importance to the military. Disease vector groups currently under investigation are (1) Anopheles of Orient and Middle East (malaria), (2) Culex (Culex) of Middle East (arboviruses and filariasis), (3) Aedes (Stegomyia) of Afrotropical Region (arboviruses) and (4) Trichoprosopon of the Neotropics (arboviruses). Build a computer data base from over 1,000,000 mosquito specimens and collection records in the Smithsonian Institution.

24. (U) Comparative morphological atudy of medically important mosquito groups in regions of military interest, with biological, cytogenetic, electrophoretic and cross-mating studies of vector populations, and correlation of all data to provide (1) descriptions and illustrations of species, (2) development of effective identification keys, and (3) information about medical importance of the species. File biological data for museum mosquito specimens in SELGEM computer program to facilitate biosystematic research and to understand vector behavioral patterns.

25. (U) 81 10 -82 09 Published one large monographic revision (subgenus Paraedes of genus Aedes), a pictorial key to the mosquitoes associated with yellow fever in Africa and karyotype differences for 3 species in Balabacensis Complex. Five new species in the African Luteocephalus and Africanus complexes of Aedes (Stegomyia) and 2 new species in the Balabacensis Complex of Anopheles (Cellia) were discovered. Incorporated 2,627 collection forms into the computer data base. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sept 82.

* 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 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 122 Biosystematics of Arthropods of Military Medical Importance

Investigators:
Principal: Bruce A. Harrison, LTC, MSC
Associate: Michael E. Faran, CPT, MSC; Kenneth J. Linthicum, CPT, MSC; E. L. Peyton; Y.-M. Huang, Ph.D.; Thomas J. Zavortink, Ph.D; SP4 Richard Soltero; Thomas V. Gaffigan; James E. Pecor

Problem

Epidemiological studies and disease control strategies involving arthropod-borne diseases are dependent upon biosystematic research support to provide accurate identifications of arthropod vectors and reservoirs. The objectives of biosystematic research of medically important arthropod groups are (1) to describe and illustrate all the species in these groups, (2) to resolve any systematic problems, (3) to develop effective keys for identifying all life stages of the species under study, (4) to provide basic biological and ecological data useful in understanding the epidemiology of diseases and in the control of vector species, (5) to provide data concerning the medical importance of each species, and (6) to train personnel in field studies and systematic research. Current studies are focused on the following important mosquito vector groups (1) malaria vector-groups of the Oriental Region [Leucosphyrus Group of *Anopheles (Cellia)*], and of the Middle East (genus *Anopheles*), (2) arbovirus and filariasis vectors of the Middle East and Afrotropical regions, *Aedes (Stegomyia)* and the *Culex (Culex)*, and (3) arbovirus vectors of the Neotropical Region, genus *Trichoprosopon*. In conjunction with the study of these mosquito-vector groups, a computer based master file of detailed systematic and ecological data is being prepared from over 1,000,000 mosquito specimens and their collection records. This latter study is directed at providing easily accessible, coordinated ecological and vector data to the military, public health organizations and other scientific and environmental agencies concerning vector species of mosquitoes.

Progress

Collaborative research continued with AFRIMS group and Mahidol University, Bangkok, Thailand, toward a revision of the Leucosphyrus Group of *Anopheles (Cellia)*, with particular

emphasis on resolving sibling species problems within the Balabacensis Complex. Two undescribed morphologically distinct forms previously recognized from Thailand and Malaysia have now been confirmed as distinct species by morphologic, cytogenetic, electrophoretic and cross-mating studies. A paper describing the distinctive karyotype of one of these species (Perlis Form) and separating it from 2 other members of the Balabacensis Complex (dirus and takasagoensis) was published in late 1981. AFRIMS and Mahidol personnel collected feral specimens of the Perlis Form in early 1982, providing the first feral specimens of this species upon which a taxonomic description and a type-series can be based. Previous to these efforts the Perlis Form was known only from colonies. A manuscript describing the second undescribed species, the Fraser's Hill Form, is nearing completion. With the recognition of these 2 new species, 5 distinct species of the Balabacensis Complex are now recognized as occurring in Thailand, where only one, balabacensis balabacensis, was recognized as recently as 1978. The epidemiological implications of these sibling species on malaria transmission in Thailand are currently under investigation by AFRIMS personnel. A reexamination of the Anopheles species of the Middle East was initiated and the preparation of revised keys, biological notes and distribution notes for the malaria vector species are underway. The manuscript for a revision of the Argyritarsus Section of the Anopheles (Nyssorhynchus) is undergoing final corrections before publication. The revisionary study of the arbovirus vector groups of Aedes (Stegomyia) in Africa made considerable progress during this period. Examination of specimens in the Africanus Complex (4 species and the Luteocephalus Complex (2 species) has revealed an additional 2 new species in the Luteocephalus Complex. The taxonomic differentiation of these species is essential for understanding the epidemiology of human and primate dengue transmission, as well as other arboviruses, in Africa. French researchers in Senegal have already determined that one of the new species of the Africanus Complex probably is not involved in dengue virus transmission. This species has very different habitat requirements from the "africanus" species in Senegal known to be involved in dengue virus transmission. Taxonomic descriptions of these 5 new species are in preparation. A pictorial key for the identification of Aedes (Stegomyia) mosquitoes associated with yellow fever in Africa was published in January 1982. Studies on the Pipiens Complex of Culex (Culex) reported last year have been expanded to a comprehensive study of the Culex (Culex) of North Africa and South Asia. This study covers approximately 25 species, including members of the Pipiens Complex, Univittatus Complex, tritaeniorhynchus and several other species that have been incriminated in the transmission of Rift Valley Fever, West Nile, JE and/or other viruses in the study

area. The type-specimens of approximately 65 normal taxa involved in this study were examined in the British Museum (Nat. Hist.), the Pasteur Institut and the Paris Museum (Nat. History) during Aug-Sept 1982. In addition, several thousand specimens were borrowed for study from other repositories and institutions. This study is directed toward the production of a handbook for the Culex (Culex) of North Africa and Southwest Asia. With the hiring of a new professional, a revision of the genus Trichoprosopon of the Neotropics was reactivated. This study was previously heavily subsidized by U. S. Army funds and is approximately 75% completed. At least 4 new species in the genus have been identified and are being prepared for description. Certain species in this genus are major human biters in Neotropical forests, and VEE as well as other viruses have been isolated from several species. In addition to the above research, consultants of the Biosystematic Unit published or have in press 2 large works. These treat the subgenus Paraedes of the genus Aedes, and a guide to the genera of mosquitoes of Thailand.

Three field trips were conducted during May-June 1982. These trips resulted in the collection of valuable specimens from Bolivia, Senegal and Suriname. The Bolivian collection was conducted in conjunction with arbovirus studies and resulted in the collection of 7000 adult mosquitoes identified and pooled for virus isolation. In addition, 1189 adult specimens (85% with associated immature skins) were pinned for taxonomic studies. A total of 58 species of mosquitoes of 14 genera were collected during the trip. The Senegal trip resulted in the examination of hundreds of Aedes (Stegomyia) specimens in the ORSTOM collections in Dakar, a sizeable loan of material being handcarried back to the U.S. and a good collaborative arrangement being established with the French Scientists of ORSTOM in several West African countries. The Suriname trip resulted in very valuable specimens of 6 genera from a country very poorly represented in museum collections.

A total of 20,267 specimens were received by the unit as gifts, transfers, loans, etc., which represents a major accession of scientific specimens. During the same period 5891 specimens were shipped out as loans, exchanges, or gifts.

During the year 2,627 collection records involving 108,093 specimens were added to the computer data base. These entries nearly completed the geographic file for Mexico and Central America, with only records from Mexico and a small number from Costa Rica remaining for entry. A standard query form was developed to handle requests for epidemiological-ecological information about mosquito species in any computer program. World

Data Bank II was obtained from the National Technical Information Service. This program will produce computer-digitized maps for collection sites and species distribution maps at any desired scale, based on the specimens in the data base.

Future Plans

Research will continue on the Leucosphyrus Group of Anopheles (Cellica), the genus Anopheles of the Middle East, the Aedes (Stegomyia) of the Afrotropical region, the Culex (Culex) of North Africa and Southwest Asia, and the genus Trichoprosopon of the Neotropics. Publication of the revision of the Argyritarsis Section of Anopheles (Nyssorhynchus) and several small papers describing new species in groups under study is anticipated in the near future. Pending the completion of several studies, work will begin on the ground pool breeding Aedes of Africa, which are suspected to be involved in the transmission of Rift Valley Fever Virus. Plans are in progress for field collection trips to Egypt, Israel, Turkey, India and several African countries.

Formal Presentations

Faran, M.E. 1981. Computerized information and collection management system for Systematic Research and Medical Entomology. Presented at Annual Meeting of Entomological Society of America. Nov. 29, - 3 Dec, 1982, San Diego, CA.

Harbach, R.E. 1981. Mosquitoes of the Pipiens Complex of Culex; Structure of the Male phallosome and the DV/D. Presented at the Annual meeting of Entomological Society of America, Nov. 29- 3 Dec, San Diego, CA.

Harrison, B.A. 1982. Malaria in the Middle East. Presented at BiAnnualEntomology Training Course, 9 February, Fort Sam Houston, TX, and at Armed Forces Pest Management Board, WRAIR, 4 Mar, Washington, D.C.

Faran, M.E. 1982. Organized and presented Introduction to Symposium entitled "Mosquito Systematics: Determination and Recognition of a Disease Vector" Presented at Annual Meeting of American Mosquito Control Association, Apr. 18-22, Sacramento, CA.

Linthicum, K.J. 1982. The Role of morphological systematics in a project to determine the enzootic cycle of Rift Valley Fever in Kenya. Presented at a Meeting of the American Mosquito Control Association.

Burnett, C. 1982. Computerized information and collection management system for Systematic Research and Medical Entomology (Diptera: Culicidae). Presented at Computers in Research at the Smithsonian Symposium, Apr 6-7, Washington, D.C.

Publications

Baimi, V., B.A. Harrison and L. Somchit. 1981. Karotype differentiation of three anopheline taxa in the Balabacensis complex of Southeast Asia (Diptera: Culicidae). Genetica 57: 81-86. (Nov. 1981).

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Reinert, J.F. 1981. Medical Entomology Studies - XV. A revision of the subgenus Paraedes of the genus Aedes (Diptera: Culicidae). Contrib. Am. Entomol. Inst. 18(4): 1-911. (Nov. 1981).

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Rattanarithikul, R. 1982. A guide to the genera of mosquitoes (Diptera: Culicidae) of Thailand with illustrated keys, biological notes and preservation and mounting techniques. Mosq. Syst. In press.

Linthicum, K.J. 1983. Mosquito Studies (Diptera Culicidae). A revision of the Argyritarsis Section of the subgenus Nyssorhynchus of Anolpheles. Contrib. Am. Entomol. Inst. (in press).

Faran, M.E., C. Burnett, J.J. Crockett and W.L. Lawson. 1983. Computerized information and collection management system for systematic research and medical entomology. (Diptera: Culicidae). J. Med. Entomol. (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6538	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AA	151
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Precede with Security Classification Code)
(U) Characteristics of Attenuated Dengue Viruses

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 07	CONT	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2.0	246
FISCAL YEAR CURRENT 83	3.0	268

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Harrison, Venton R.
NAME: Summers, Peter L. POC: DA

21. GENERAL USE
Foreign intelligence 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) 81 10 - 82 09 1. A dengue-4 vaccine (H241 PDK 35-TD3) was prepared and tested and an IND was submitted to the Bureau of Biologics, FDA, so that clinical testing could begin. Five human subjects, all yellow fever immune, were vaccinated with the candidate dengue-4 vaccine. Two of five volunteers who were viremic and had mild clinical reactions, also produced antibodies to high titer. Virus isolates from blood specimens were phenotypically changed with an increase in plaque size. 2. A clone (C6/36) of Aedes albopictus mosquito cells was found to be useful for growth, cloning, production, and stabilization of dengue-2 and dengue-3 attenuated virus clones. Virus-free supernatant culture fluids were inoculated intradermally in human subjects and found to stimulate an immediate hypersensitivity-like reaction in some subjects. Rhesus monkeys had similar hypersensitivity reactions to the C6/36 sham vaccine and may be used to test for purification of vaccines prepared in the C6/36 cell line. 3. Detection of enhancing antibodies in human subjects prior to and following dengue-2 and dengue-4 vaccination are being evaluated. A rapid and sensitive technique using a short incubation period of virus in a human monocyte cell line followed by infectious center formation in monkey kidney cells was used to assay enhancing antibodies. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*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 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

WORK UNIT 151 CHARACTERISTICS OF ATTENUATED DENGUE VIRUSES

Investigators:

Principal: Kenneth H. Eckels, Ph.D.

Associates: Doria Dubois, Ph.D.
Venton R. Harrison
Peter L. Summers

Problem and Objectives

The project involves the development, production, and assay of live-attenuated vaccines against various strains of dengue viruses. Isolates of dengue 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. Immunogenic clones will be used for the production of experimental lots of virus seed and vaccine. Selected clones will be tested in small groups of human volunteers to measure reactogenicity and efficacy.

Progress

A dengue-4 vaccine (H241 PDK 35-TD3) and production seed were prepared by the Department of Biologics Research after receiving a cloned and tested master seed from a contractor (Dr. Scott Halstead). An IND was submitted to the Bureau of Biologics, FDA, for clinical testing of the vaccine. Five human subjects, all with previous yellow fever vaccination but free of dengue antibodies, were vaccinated with the candidate dengue-4 vaccine. Two of the five volunteers responded to the vaccine by stimulating antibodies to high titer. These same 2 vaccine recipients also had some mild clinical reactions including an erythematous rash. Both individuals were viremic prior to symptoms and immune responses. Virus recovered from the blood appeared to be phenotypically changed with an increase in plaque size.

A clone (C6/36) of Aedes albopictus mosquito cells was evaluated for attenuation and growth of various dengue virus serotypes. Safety tests in laboratory animals and in cell cultures indicated the absence of adventitious microbial agents. Human safety was evaluated by intradermal inoculation of virus-free cell culture

supernatant fluids. Reactivity at the injection site in a number of the recipients indicated an immediate hypersensitivity type reaction. It has been found that rhesus monkeys can be used to test immediate hypersensitivity reactions and this model will be used to monitor allergenic activity of experimental vaccine preparations.

A series of dengue-2 and dengue-3 virus clones have been developed using the C6/36 mosquito cell line. The clones will replicate in the FRhL cell line with a minimal reduction in virus titer so that a final vaccine product can be made in these cells. All clones appear to be genetically stable, are uniformly small plaque and temperature sensitive.

A dengue-2 seed (PR-159, unattenuated) was prepared in FRhL cells under conditions suitable for human products. The unattenuated seed will be titrated in monkeys and tested for adventitious agents prior to use as a "challenge" virus to test vaccine efficacy in humans.

Recommendations

Currently, results from the dengue-4 vaccine (H241) clinical trial are being compiled and evaluated. Future trials with this vaccine are under consideration. Another alternative is the development of an additional dengue-4 vaccine using a Caribbean strain of this virus.

Use of the C6/36 cell line for vaccine preparations will require characterization and removal of the allergenic protein from these experimental vaccines. Monkeys will be used to measure reactivity by either a Prausnitz-Kustner test or by a skin test based on the monkey's naturally-acquired state of hypersensitivity. Procedures for separating the allergenic protein from viral antigens will include gradient centrifugation, membrane dialysis, and column chromatography.

Dengue-2 and dengue-3 clones, previously passaged and purified in C6/36 cells will be further evaluated. Monkey immunogenicity and attenuation will be tested using virus grown in C6/36 or FRhL cells. Due to allergenic reactivity in C6/36 supernatant culture fluids, final vaccines will probably be prepared in the FRhL cell line. Efforts will be made to evaluate these clones as fully as possible using laboratory markers which include temperature sensitivity, replication in U-937 (lymphoid) cells, and the capacity for enhancement using heterologous or homologous antibodies. A mouse potency assay using BALB/c mice is being evaluated for use in conjunction with or as an alternative to monkey testing.

Unattenuated dengue-2 and dengue-4 seeds will be titrated in monkeys and tested for adventitious agents so that vaccine efficacy can be tested in human subjects.

Detection of enhancing antibodies in human subjects prior to and following dengue vaccination will be evaluated. A rapid and sensitive technique using a short incubation of virus in the U-937 cell line followed by infectious center formation on LLC-MK_2 cells can be used to assay enhancing antibodies.

Formal Presentations

Eckels, K.H., D.R. Dubois, S. Berman, and P.K. Russell. Evaluation of mosquito cell lines for the preparation of dengue virus vaccines. American Society of Tropical Medicine and Hygiene annual meeting, San Juan, Puerto Rico, 1981.

Patents, IND Approvals

Dengue vaccine (Type 4) live attenuated virus strain: H241 PDK 35-TD3. IND submitted 26 Jan 82 to BOB, FDA and subsequently approved.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | DA OG 6766 | 82 10 01 | DD-DR&E(AR)636

5. DATE PREV SUM'RY	6. KIND OF SUMMARY	7. SUMMARY SCTY	8. WORK SECURITY	9. HEADING	9A. DISP'N INSTR'N	9B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. RO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63750A	3M263750A808	AB	
b. CONTRIBUTING	62770A	3M162770A871	AB	152
c. [redacted]	CARDS 1413A			

11. TITLE (Precede with Security Classification Code)
(U) Role of Polysaccharide Antigens in Immunity

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
010100 Microbiology 002600 Biology

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

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 82	1	185
CURRENT FISCAL YEAR 83	2	486

19. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: (202) 576-3344
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: B. Brandt
NAME: J. Boslego, MAJ, MD, MC
NAME: W. Zollinger, Ph.D.

21. GENERAL USE
Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Vaccines; (U) Human Volunteers; (U) Meningococci; (U) Pseudomonas

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

23. (U) Infectious diseases continue to be a threat to military operations. Effective vaccines are a means to control infections, and several have reached the stage of development which requires preliminary testing in human beings for safety and antigenicity. Current emphasis is on the testing of meningococcal vaccines. Preliminary safety and antigenicity studies in human beings of experimental vaccines are necessary before efficacy studies of experimental vaccines can be undertaken.

24. (U) Experimental vaccines, consisting of living attenuated bacteria, killed bacteria, or purified products extracted from bacteria, are prepared in pilot lots by the Department of Biologic Products, WRAIR. These are tested for safety and antigenicity in the laboratory. Following review by the SGO and the Bureau of Biologics, FDA and with the consent and cooperation of Field Commanders, these experimental products are tested in soldier volunteers for safety and antigenicity.

25. (U) 81-10 - 82-09 Laboratory analysis of sera from 300 volunteers immunized with an experimental tetravalent (A,C,Y,W135) polysaccharide vaccine (45 micrograms, 90 microgram doses) was completed. The bactericidal antibody response was excellent and independent of dose. Reactions to the vaccine appears more dependent on the number of polysaccharide components in vaccine rather than total dose of polysaccharide. Four hundred volunteers at Ft. Dix were immunized with either 60 micrograms or 200 micrograms of commercially prepared tetravalent meningococcal vaccine (Connaught Laboratories). Additional laboratory studies are continuing. Preparation of new lots of group B meningococcal polysaccharide/protein vaccine was begun. These new lots will contain serotype proteins currently responsible for major outbreaks of group B disease. (For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 81 - 30 Sep 82).

*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 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 152 Role of Polysaccharide Antigens in Immunity

Investigators:

Principal: Samuel B. Formal, Ph.D.
Associates: Brenda L. Brandt, MS
Wendell D. Zollinger, Ph.D.
John Boslego, M.D. MAJ,

Problem and Objectives

1) To develop a tetravalent (A,C,Y,W135) meningococcal polysaccharide vaccine to be administered to all Army basic training recruits. 2) To determine the lowest dose at which the tetravalent meningococcal vaccine can be administered and remain immunogenic. 3) To develop an immunogenic polysaccharide-protein vaccine to prevent group B meningococcal disease in the Army. 4) To develop an effective pentavalent meningococcal vaccine (Tetravalent + the Group B polysaccharide protein).

Progress

Laboratory analysis of sera from 300 Army recruit volunteers (Ft. Dix, 1980) immunized with an experimental tetravalent (A,C,Y,W135) meningococcal polysaccharide vaccine was completed. The study involved immunization of groups of 100 volunteers at two doses of tetravalent vaccine (45 and 90 µg total polysaccharide) and an additional 100 volunteers with the standard divalent (A,C) vaccine at a dose of 100 µg total polysaccharide (Connaught Laboratories). Results from this trial showed that binding antibody (1) responses did not differ between the two doses for groups A,C and W135; however, for group Y the higher dose was more immunogenic. For bactericidal antibody (2), responses to the two doses were equivalent for all four polysaccharides. These data confirm the observation (3) that the dose response for bactericidal antibody is flat for group Y and W135 and suggest that this may be the case for groups A and C, as well. When the group A and C responses to the two doses of

tetravalent vaccine were compared with responses to several lots of commercially prepared divalent vaccine (Connaught Laboratories), we found that lot-to-lot differences among the vaccines were more important in determining antibody responses than was the dose of vaccine.

Reactogenicity data revealed that the number of components in the vaccine is more important than is dose in determining local and systemic reactions. We concluded from these data that while dose does not appear to be an important variable in reactogenicity, the number of components in the vaccine does.

In August, 1982, a trial was conducted at Ft. Dix in Army recruit volunters. This study was to confirm with a commercially prepared vaccine that a low dose (60 μg total polysaccharide) was as immunogenic as the "standard" vaccine containing 50μg of each polysaccharide (A,C,Y and W135). Both the 60 and 200 μg doses were well tolerated by the volunteers. Laboratory analysis is now in progress.

Preparation of new lots of group B meningococcal polysaccharides-protein vaccines is under way. These new lots will contain serotype proteins currently responsible for major outbreaks of group B disease. These lots will be tested in volunteers at both WRAIR and Ft. Dix in 1983.

Recommendations

In August 1982, the Army began routinely administering a tetravalent meningococcal vaccine (A,C,Y and W135) to all incoming basic training recruits. This vaccine is being administered at a 200 μg total polysaccharide dose (50 μg each). If the results of the trial begun in August with the commercially prepared tetravalent vaccine confirm our previous observations (3) that a lower dose of vaccine is as immunogenic as the 200 μg dose, we would recommend lowering the dose of tetravalent vaccine to 60μg total polysaccharide.

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2. Artenstein, M.S., Brandt, B.L., Tramont, E.C., and Zollinger, Ph.D. 1976. Immune response to Neisseria meningitidis, p274-279. In N.R. Rose and H. Frieman (ed) Manual of Clinical Immunology. American Society for Microbiology, Washington, DC.

3. Griffiss, J.M., Brandt, B.L., Broud, D.D. 1982. Human Immune Response to Various Doses of Group Y and W135 Meningococcal Vaccines. Infect. Immun. 37:205-208, 1982.

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1. Griffiss, J.M., Brandt, B.L., Altieri, P.L., Pier, G.B. and Berman, S.L. 1981. Safety and Immunogenicity of Group Y and Group W135 Meningococcal Capsular Vaccines in Adults. Infect. Immun. 34:725-732.

2. Griffiss, J.M., Brandt, B.L., and Broud, D.D. 1982. Human Immune Response to Various Doses of Group Y and Group W135 Meningococcal Polysaccharide Vaccines. Infect. Immun. 37:205-208.

3. Griffiss, J.M., and Brandt, B.L. 1983. Immunologic relationship between the capsular polysaccharides of serogroups Z and 29E Neisseria meningitidis. J. Gen. Microbiol., In Press.

4. Griffiss, J.M., Brandt, B.L., Altieri, P.L., Pier, G.B. and Berman, S.L. 1983. Safety and Immunogenicity of group 29E meningococcal capsular polysaccharide vaccine in adults. Infect. Immun., In Press.

IND Approval: Approval to conduct phase IV of BB-IND 335, Purified Meningococcal Polysaccharide Vaccine (Multivalent). Study was completed between August and September 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	3. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 644	82 10 01	DD-DR&E(AR)636

2. 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
81 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	871AC	153
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2			

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:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	4.0	407
FISCAL YEAR CURRENT 83	4.0	550

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS NAME: Jerrells, T.J.; Rice, R.M., Kelly, D.J., and MacMillan, J.G.

NAME:

POC: DA

21. GENERAL USE

Foreign intelligence 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 to include subhuman primates; determine the sequence of events leading to immunity following vaccination or infection. These studies are aimed at development of vaccines that will protect deployed military troops, and development of immunoassays to evaluate the extent of immunity induced by vaccination.
24. (U) Gamma irradiation of rickettsiae to produce attenuated immunogens. Evaluate tissue culture-propagated strains for use as immunogens to provide protection against scrub typhus infection. Analyze correlates of lymphocyte recognition to determine the adequacy of immune response. Correlation of subhuman primate response to that seen in the murine model. Determine the genetic basis of resistance and sensitivity of the mouse model to scrub typhus infection.
25. (U) 81 10 - 82 09 Karp strain rickettsiae grown in tissue culture stimulated protective immunity in mice that was paralleled by the development of cellular immunity. Mice receiving yolk sac derived, irradiated rickettsiae responded with a cellular immunity as evidenced by in vitro splenic lymphocyte proliferation (LP) and production of lymphokines. In congenic strains of C3H mice both fixed and inflammatory macrophages were shown to be important in genetic resistance to scrub typhus infection and in the development and expression of immune clearance. Both genetically susceptible and resistant strains of mice developed delayed-type hypersensitivity (DTH) after challenge. DTH, LP, and the production of lymphokines were shown to be mediated by thymus derived lymphocytes and to be good indicators of the development of protective immunity. Production of antibody in scrub typhus infection was shown to require T-helper cells. Methodologies used to evaluate the immune response in mice have been successfully transferred to the subhuman model. For technical report see Walter Reed Army Institute of Research Progress Report. | Oct 81 - 30 Sep 82.

[redacted]

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. OO FORMS 1498A 1 NOV 68 AND 1498-1, 1 MAR 75 (FOR ARMY USE) ARE OBSOLETE

* U.S. GPO: 1974—540-843/8691

PROJECT 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 153 Rickettsial Diseases of Military Personnel

Investigators:

Principals: Joseph V. Osterman, PhD; LTC Ralph D. Bernier, MC; Thomas R. Jerrells, PhD; MAJ Robert M. Rice, VC; MAJ Daryl J. Kelly, MSC; CPT James G. MacMillan, VC

Associates: Sp4 Miriam R. Pedersen; Sp4 Bradley R. Oelmann; PFC Lisa P. Smith; PFC Stephan E. Platt

Problems and Objectives

These investigations are aimed at the production of rickettsial experimental immunogens which will provide safe, efficacious rickettsial vaccines designed to protect troops in areas of endemic disease. An important aspect of this work is to define the pathogenesis of rickettsial infections in laboratory animals with particular interest in determining the sequence of events leading to immunity following vaccination or infection. Gamma irradiated rickettsiae are used as immunogens. Immunologic studies employ both embryonated egg and tissue culture grown rickettsiae. Immunogens specifically designated for vaccine development are grown in primary chick embryo tissue culture. Immunological studies will be focused mainly on the effector mechanisms involved in immunity to rickettsial infections in mice. Methodologies for studying immune mechanisms developed in the murine model have been adapted to a subhuman primate model and investigation of these effector mechanisms in the subhuman primate model will be started. Adaptability of other tissue culture systems suitable for human vaccine development are under consideration. Development of an accurate, sensitive immuno assays to evaluate the extent of immunity induced by vaccination is an essential element in these studies. Of equal importance is the development of an accurate assay to determine rickettsial antigens in vaccine preparations.

Progress

Karp strain Rickettsia tsutsugamushi has been grown in primary chick embryo tissue culture. Studies to evaluate the requirements for optimum growth of the rickettsiae with reference to type of media, nutritional supplements, CO_2 and buffer requirements, temperature and type of flask were completed. Investigations to determine the optimum number of infecting

rickettsiae per monolayer surface area were also performed. Preliminary studies suggest that tissue culture grown rickettsiae do stimulate a protective immunity in mice which is paralleled by the development of an antigen-specific cellular immunity.

Cell-mediated immunity of inbred mice immunized with irradiated yolk sac propagated scrub typhus immunogen was evaluated. It was found that mice receiving established doses of the Karp strain of scrub typhus rickettsiae responded with a demonstrable cellular immunity as evidenced by *in vitro* splenic lymphocyte proliferation and production of the lymphokines migration inhibition factor and immune interferon. Immunized animals also exhibited a delayed-type hypersensitivity when tested with homologous antigen. Levels of rickettsemia were lower in immunized mice compared to nonimmune mice following intraperitoneal challenge with one thousand viable Karp rickettsiae.

In vitro lymphocyte proliferation using spleen lymphocytes was used to evaluate the development of cross reactive immunity following immunization with a single strain of scrub typhus rickettsiae. Lymphocyte proliferation was found to reflect the development of a population of antigen responsive thymus derived lymphocytes, which in general exhibited maximal responses to the homologous immunizing rickettsiae but extensive reactivity was noted to heterologous strains of rickettsiae. The development of lymphocyte proliferative response in mice immunized by sublethal infections with the Karp or Kato strains were delayed compared to the responses noted after immunization with the less pathogenic Gilliam strain of rickettsiae. This raises the possibility of immunoregulatory imbalances as a consequence of a relatively severe although nonlethal infection with the Karp and Kato strains of rickettsiae. The development of lymphocyte proliferative responses was demonstrated to be a good indicator of the development of protective immunity in the murine model after immunization with viable and irradiated scrub typhus immunogens.

Immune (gamma) interferon (IFN) production by antigen responsive lymphocytes was evaluated in C3H/He mice immunized subcutaneously with 1000 MLD_{50} of Gilliam strain, *R. tsutsugamushi* . After twenty eight days the immunized mice were challenged with various doses of purified, irradiated Gilliam rickettsiae given intravenously. At various intervals after antigen administration, mice were bled and serum IFN titers were determined by protection of L929 cells against cytopathic effect produced by vesicular stomatitis virus. Peak interferon production occurred four hours after antigen injection. The interferon produced was sensitive to pH 2.0 treatment, stable at

56°C, and not neutralized with rabbit anti-alpha/beta interferon (criteria for immune interferon). Antigen specificity was demonstrated by a lack of IFN production by nonimmune mice after challenge with 5mg of Gilliam antigen and a lack of IFNproduction by mice immunized with a normal yolk sac preparation. Parallel studies using spleen cells obtained from both immunized and nonimmunized mice demonstrated IFN production occurred only in response to the specific immunizing rickettsial antigen and only in lymphocytes recovered from immunized mice. The IFN producing cells were shown to possess the Lyt 1.2 surface antigen. These cells also produced migration inhibition factor (MIF) in response to specific immunizing antigens. The close correlation of IFN and MIF production and the ease of quantitative assessment of IFN production suggests IFN production as a reliable measure of antigen sensitive lymphocytes.

Using mice immunized after a sublethal infection with scrub typhus rickettsiae, the development of delayed-type hypersensitivity was evaluated as a parameter of immunity to rickettsial challenge. Both genetically susceptible and resistant strains of mice were found to develop after challenge. The DTH response declined to unreactive levels in susceptible mice prior to death suggesting a state of anergy develops in acute, fulmanent experimental scrub typhus infection. Delayed-type hypersensitivity was found to be a good indicator of the development of protective cell-mediated immunity after immunization with scrub typhus rickettsiae.

The role of inflammatory macrophages and resident fixed macrophages in genetic resistance to lethal scrub typhus was evaluated using congenic strains of C3H mice differing in susceptibility to Gilliam strain of scrub typhus rickettsiae. It was found that both strains of mice possessed an innate rickettsiacidal mechanism which protected against intravenous infection. This mechanism was sensitive to silica or low levels of irradiation (200-300 RADS) in the sensitive C3H/He mouse, but was eliminated only by a combination of silica and irradiation in the resistant C3H/RV mouse. This suggests a combination of fixed (silica sensitive) and inflammatory (radiation sensitive) macrophages mediate innate resistance to intravenous challenge. The resistance to intraperitoneal infection exhibited by the C3H/RV strain of mice was found to be sensitive to irradiation but not to silica, suggesting that resistance was mediated only by an inflammatory macrophage process. This was further suggested by a monocytosis in C3H/RV mice following challenge with Gilliam rickettsiae.

In studies designed to evaluate the role of antibody in immunity to scrub typhus rickettsiae, athymic ("nude") BALB/c mice

were infected with Gilliam strain of R. tsutsugamushi. In contrast to thymus bearing BALB/c mice, which are genetically resistant to Gilliam infection, athymic mice were susceptible to intraperitoneal infection. The athymic mice, when placed on antibiotic therapy, produced antibodies to R. akari, R. conorii, and R.typhi rickettsiae, but not to R. tsutsugamushi . The antibody produced by the athymic mice to these rickettsiae was of the IgM type and reacted primarily with soluble antigens. When athymic mice were reconstituted with immune T-lymphocytes, a demonstrable antibody response to R. tsutsugamushi infection was observed. Additional studies in which Gilliam immune mice were injected with TNP-Gilliam rickettsiae, TNP-akari rickettsiae, and TNP-BSA demonstrated that the antibody response in thymus bearing mice to R. tsutsugamushi is dependent on the production of rickettsia specific T-helper cells after immunization. This is in contrast to the immune response seen with representative members of the typhus group and the spotted fever group rickettsiae, which are T-helper cell independent.

Studies have been initiated to establish the cynomolygus monkey as the model system for determining the presence of cellular and humoral immune responses to R. tsutsugamushi antigens after infection and vaccination. In the first phase of these studies, monkeys were immunized with the protein antigens tetanus toxoid (TT) and keyhole limpet hemocyanin (KLH). Immune responses to these antigens were followed using the methodologies developed in this laboratory for evaluating the development of a specific cell-mediated immunity (CMI), as well as the development of a humoral antibody response in the murine model.

Recommendations

Production of tissue culture grown immunogen will focus on improved rickettsial yield and biochemical and biological characterization of vaccine preparations. The goal is development of methodology which allows for predictable, consistent rickettsial yield from the tissue culture system. Specific objectives are production of vaccine lots to support Phase III of subhuman primate studies. Of particular importance is the development of a plaque purification procedure for scrub typhus in primary chick embryo tissue culture. Efforts will be directed to evaluation of immunity after vaccination of mice and monkeys with tissue culture grown scrub typhus immunogens. Methodology will be developed to assess the antigenic potency of various lots of tissue culture derived immunogen to allow standardization of these immunogens. The role of circulating antibody in the prevention of rickettsemia suggested by previous studies will be explored in depth. If antibody is an important factor in prevention of rickettsemia, enhancement of the immunogenicity of irradiated

rickettsiae, which are poor inducers of antibody production, will be attempted using mild adjuvants applicable to subhuman primate and human trials. Cell cooperation in the development of immune responses to rickettsiae of various species will be evaluated in order to better understand the totality of the immune response to rickettsial antigens. These studies will allow a rational approach to immunization.

The role of lymphokines as both mediators of effector mechanisms and as reliable monitors of protective immunity will be explored. Emphasis will be placed on immune interferon based on ease of assay and historical importance of interferon in macrophage activation, role in clearance of intracellular pathogens, and the emerging idea of interferon as an important immunoregulatory molecule. Emphasis will be placed on transfer of technology developed in the murine model for examination of immune responses to rickettsial antigens to subhuman primates. It is anticipated that these in vitro correlates of immunity will be later employed to assess the levels of immunity in subhuman primates and ultimately humans after immunization with rickettsial immunogens. These assays should also allow evaluation of populations of individuals for the presence and persistance of immune respones following natural infection.

Formal Presentations

1. Jerrells, T.R.: Characterization of T-lymphocytes mediating delayed-type hypersensitivity and protective immunity to R. tsutsugamushi in mice. 21st Midwinter Conference of Immunologists, Pacific Grove, California, January, 1982.

2. Jerrells, T.R. and Osterman, J.V.: Role of fixed and inflammatory macrophages in resistance of mice to R. tsutsugamushi infection. 66th Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, Louisiana, April, 1982.

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4. Jerrells, Thomas R., Bennie A. Palmer, and Joseph V. Osterman. Gamma-Irradiated Scrub Typhus Immunogens: Development of Cell-Mediated Immunity after Vaccination of Inbred Mice. (In press, Infect. Immun.)

5. Jerrells, Thomas R., and Joseph V. Osterman. Development of Specific and Cross-Reactive Lymphocyte Proliferative Responses During Chronic Immunizing Infections with Rickettsia tsutsugamushi. (submitted, Infect. Immun.)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	3. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OB 6531	82 10 01	DD-DR&E(AR)636

5. DATE PREV SUM'RY	6. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AH	154
b. CONTRIBUTING				
XXXXXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Procede with Security Classification Code)

(U) Prevention and Treatment of Plague

15. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	1.0	306
	CURRENT 83	1.0	338

19. RESPONSIBLE DOD ORGANIZATION

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TELEPHONE: 301-427-5176 or 5110

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Harrison, Daniel N., Ph.D.

NAME: POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Yersinia pestis; (U) Plague; (U) Diagnosis; (U) ELISA; (U) RIA; (U) Serology; (U) Antigenemia; (U) Vaccines; (U) Immunization; (U) Mastomys

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

23. (U) Develop capabilities to diagnose, prevent, treat and control plague to protect troops against pneumonic and bubonic disease.

24. (U) Rapid diagnostic tests are developed and evaluated using clinical specimens. New plague vaccines and therapeutic drugs are assayed for efficacy in animal models. Strains of Y. pestis are examined for virulence and antibiotic susceptibilities.

25. (U) 81 10 - 82 09 An ELISA to detect specific F1 antigen of Y. pestis was developed for rapid diagnosis. Using this test, antigenemias of 4-8 micrograms per ml were found in sera of patients with acute bubonic plague. RIA procedures to detect antigens or antibodies were established for use to measure relative sensitivities of ELISA and other procedures. ELISA and staphylococcal radioimmune precipitation techniques for measuring plague antibody were found to be of comparable sensitivity. Work on monoclonal antibodies demonstrated that recently developed anti-F1 monoclones precipitate the F1 antigen produced by Y. pestis strains isolated in 11 countries on 4 continents. Counter-current immunoelectrophoresis was investigated for rapid diagnosis of plague but proved less sensitive and more difficult to control for specificity than ELISA or RIA. Several commercially available systems for identifying bacteria isolated from clinical materials were examined for reliability in recognizing Y. pestis. Potentials for misidentification of variants of Y. pestis were discovered. An improved procedure was developed for isolating atypical Y. pestis from clinical specimens using laboratory bred African multimammate mice (Mastomys coucha). Laboratory rats vaccinated with an experimental killed plague vaccine produced from avirulent organisms developed serologic responses similar to those derived from the U.S.P. vaccine currently used to immunize troops. For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

PROJECT 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

WORK UNIT 154 PREVENTION AND TREATMENT OF PLAGUE

Investigators:

Principal: MAJ James E. Williams, MSC

Associates: CPT Michael W. Hastriter, MSC
Daniel N. Harrison, Ph.D.
SSG Carol A. Braden
PFC Larmon Smith

Problem and Objectives

Plague remains a threat to unvaccinated and possibly to vaccinated military and civilian populations in many parts of the world, especially in catastrophic or wartime situations that preclude application of modern sanitary practices. In addition to typical plague, there exist potentials for plague from variant bacilli that are resistant to the more efficacious drugs used in therapy and from nonencapsulated strains that are not readily detected by current diagnostic tests. The objective is improved capabilities for protecting troops against pneumonic and bubonic plague. Current research program focuses on three requirements for reducing the impact of plague on soldiers in combat: i) rapid and sensitive procedures for use in the field to diagnose plague infections, to evaluate drug susceptibilities for effective therapy, and to characterize new strains of the pathogen for military intelligence, ii) a forecasting system to rapidly determine where and when plague surveillance or control activities are necessary, and iii) improved production techniques for plague vaccine to insure that sufficient supplies could be prepared during emergencies.

Progress

An ELISA capable of detecting 0.4 nanogram of specific F1 antigen of Y. pestis was developed for rapid diagnosis. The procedure requires 2 1/2 hours. Using this test, F1 antigenemias of 4-8 micrograms per ml were found in sera of patients with acute bubonic plague. RIA procedures to detect antigens or antibodies were established for use to measure relative sensitivities of ELISA and other diagnostic procedures, for use as research tools, and for use when providing base-laboratory diagnostic support for DOD activities. ELISA and staphylococcal radioimmune precipitation (St-RIP) techniques for measuring plague antibody were found to be of comparable sensitivity.

Some progress was achieved in efforts to improve the sensitivity and specificity of latex agglutination as a simple and rapid procedure to establish a presumptive diagnosis of plague under field conditions. Staphylococcal protein A was found suitable as a reagent in ELISA and RIA for diagnosis of human disease but of no value in testing rodent sera for serological surveillance of rodent plague. Work on monoclonal antibodies demonstrated that recently developed anti-F1 monoclones precipitate the F1 antigen produced by Y. pestis strains isolated in 11 countries on 4 continents. Counter-current immunoelectrophoresis was investigated for rapid diagnosis of plague but proved less sensitive and more difficult to control for specificity than ELISA or RIA. Several commercially available test systems for identifying bacteria that are isolated from clinical materials were examined for their reliability in recognizing Y. pestis. Serious potentials for misidentification of variants of the plague bacillus were discovered. An improved procedure was developed for isolating atypical bacilli from clinical specimens using laboratory bred African multimammate mice (Mastomys coucha). Experiments in laboratory rats vaccinated with a killed plague vaccine produced from avirulent organisms demonstrated that the new vaccine illicits serologic responses similar to those derived from the U.S.P. vaccine currently used to immunize troops. In the area of forecasting techniques, work was started on a simplified version of the computer model formerly developed for documenting plague in the CONUS.

Recommendations

Additional work is needed on tests for rapid diagnosis of plague by the detection of specific Y. pestis antigens. The ELISA for measuring F1 envelope antigen can identify infections with typical plague bacilli. It requires field-testing. A rapid diagnostic capability to recognize infections with atypical, nonencapsulated plague bacilli should be developed. Easily portable field-kits to quickly characterize suspect isolates and to establish drug susceptibilities should also be developed and tested. Evaluations of new antibacterial drugs for chemotherapeutic value against plague should be undertaken. Work on computerized techniques to improve forecasting capabilities should be continued.

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5. Williams, J.E. Plague. IN: Tropical Medicine and Medical Parasitology. Goldsmith, R.S. and Heyneman, D. (eds.). Lange Medical Publications, Los Altos, California (Submitted).

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9. Williams, J.E. and Cavanaugh, D.C. Chronic infections in laboratory rodents from inoculation of nonencapsulated plague bacilli (Yersinia pestis). Submitted to Experientia.

10. Williams, J.E. and Cavanaugh, D.C. Differential signs of plague in young and old California ground squirrels (Spermophilus beecheyi). Submitted to the Journal of Wildlife Diseases.

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12. Wright, J.D., Hastriter, M.W., and Robinson, D.M. Observations on the ultrastructure and distribution of Rickettsia tsutsugamushi in naturally infected Leptotrombidium (Leptotrombidium) arenicola (Acari: Trombiculidae). Submitted to the Journal of Medical Entomology.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6536	82 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
81 10 01	H. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AF	155
b. [redacted]				
c. [redacted]	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. DATE/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	3.0	435
	CURRENT 83	3.0	882

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME: FLECKENSTEIN, Dr. L.

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Pharmacodynamics; (U) Pharmacokinetics; (U) Toxicology; (U) Antimalarial Drugs; (U) Preclinical Pharmacology; (U) Quantitation Methodology

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

23. (U) Research objectives are the development of in vivo and in vitro models to study the pharmacodynamics, metabolism and pharmacokinetics of antiparasitic compounds being developed for use in man. These studies are designed to provide the developmental rationale for the introduction of candidate drugs to man as well as satisfying all regulatory requirements for granting an IND for clinical trial of the candidate drugs. These compounds are developed to permit maximum utilization of military personnel in areas where parasitic diseases are endemic.

24. (U) Studies are performed in non-infected, healthy animals to determine the manner in which the candidate drug is metabolized by the animal in addition to determining how the drug produces its effect. These studies are necessary to predict human tolerance to the candidate drug (Phase I). Pharmacokinetic analysis of the drug actions using analytical techniques specifically developed for each candidate drug provides a rational basis for dosing during human studies.

25. (U) 81 10-82 09 Technical management continued for 13 extramural pharmacology contracts. Administrative direction and support was continued or initiated for 8 IND's in an active status and two potential IND compounds. The metHb production caused by the 8-aminoquinolines WR 2975 (primaquine), WR 238,605, WR 225,448 and WR 242,511 was studied in beagle dogs. The elimination half life C-14 labeled WR 6026 was found to be five days in the rat and 6 days in the beagle dog. In vitro metabolism with WR 6026 by rat liver microsomes produced two metabolites. The major metabolite is 2 to 3 times more active than the parent compound. Twelve metabolites of WR 6026 have been found in rat urine and some of these have been submitted for in vitro antileishmanial screening. When WR 6026 was added to a culture of leishmania parasites, 2 metabolites were found. For technical report see WRAIR Annual Progress Report, 1 Oct 81-30 Sep 82. This work unit has been incorporated into work unit # 157.

*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 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 155 Determination of Pharmacological Effects of Antimalarial Drugs

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
Associate: MAJ J. von Bredow, Dr. H. Chung, Dr. L. Fleckenstein, CPT Dr. Korte, Jr., CPT J. Anders, CPT A. Theoharides, LTC C. Pamplin, Dr. H. Lowensohn, SFC J. Baker, J.H. DiGiovanni, PFC S. Ivory

1. Description.

Studies undertaken by the department in support of the Army Drug Development Program have continued in two major areas. The first area is concerned with how the body affects the action of the candidate drug and includes studies to determine the pharmacokinetic and metabolic profiles of the drugs. The second major area is concerned with how the candidate drug affects the body or the pharmacodynamics of the drug. Sensitive drug assay methods and in vitro techniques necessary for the study of the drugs continue to be developed. In addition, the department continues to direct extramural contract work in support of active IND's and those compounds being groomed for IND status.

2. Progress.

During FY 1982, the Biochemical Pharmacology Branch, Department of Pharmacology, investigated the methemoglobinogenic potential of 8-aminoquinoline anti-infectious drugs, such as primaquine (WR 2975) and its analogs. A large portion of time was devoted to the disposition, pharmacokinetics, biotransformation, metabolite identification and toxicity of WR 6026 2HCl, a very promising antileishmanial agent.

Methemoglobinogenic potential of some 8-aminoquinolines in beagle dogs. Primaquine (WR 2975), WR 238,605, WR 225,448 and WR 242,511 were given to male beagle dogs (3 dogs/drug) for 4 consecutive days at 0.016 mmole per kg which was based on 3.0 mg (base) of primaquine/kg. Methemoglobin (MHb) baseline was monitored for a week prior to dosing. Blood samples were taken twice daily for 31 days after dosing for MHb level determinations. Primaquine produced approximately 5-6% MHb at day 4, WR 238,605 10-25% at day 4, WR 225,448 15-30% at day 5, and WR 242,511 produced 45-50% MHb at day 5 also. The MHb elimination half-life was long for all four compounds with WR 225,448

approximately 9 days and about 5 to 6 days for the other three compounds. According to area under the curve (AUC), primaquine produced modest levels of MHb of approximately 300 mg MHb/ml/hr while WR 242,511 produced the most at 2500 mg MHb/ml/hr. WR 238,605 and WR 225,448 produced 920 and 1610 mg MHb/ml/hr, respectively.

The determination of elimination half-life of WR 6026 in rats and beagle dog. Elimination half-life ($T_{1/2}$) of WR 6026, a candidate antileishmanial compound was determined after a single oral and IV dose (10 mg base/kg) of the C-14 labeled drug was administered to male rats and a single IV dose was administered to male beagle dog. The elimination $T_{1/2}$ for total radioactivity in the rats was approximaely 5 days for both PO and IV administration and approximately 6 days for IV administration in the beagle dog.

Pilot 28 day toxicology study of WR 6026 in the rats. The elimination $T_{1/2}$ for WR 6026 in oral administration to rats determined by this laboratory was used to calculate a dosing scheme for this 28 day toxicology study. The dosing scheme included six dose levels of drug preceded by three daily loading doses (4, 3 and 2 times the maintenance dose) to achieve steady state levels throughout the dosing period. There were 3 rats per dosing level (23.7, 17.8, 15.4, 13.3, 11.6 and 8.7 mg(base)/kg dosed by daily gavage for 28 days). The maximum tolerated dose was considered to be 15.4 mg/kg. The loading dose was well tolerated at the nonlethal dosage levels and there were no toxic signs during or immediately following dosing.

The determination of methemoglobin potential of WR 6026 2HCl in human hemoglobin in vitro. WR 6026 2HCl was tested in vitro for its methemoglobinogenic potential by the method developed by H. Chung in this laboratory. A range of concentrations of WR 6026 2HCl were tested. However, a concentration as high as 1×10^{-3} M of this drug did not cause any production of methemoglobin in comparison with the control. Therefore, it was concluded that WR 6026 2HCl did not appear to be methemoglobinogenic by this in vitro test method.

In vitro metabolism of WR 6026 by rat liver microsomes. Rat liver microsomal metabolism studies showned that WR 6026 is metabolized to a major and a minor metabolite which are more polar than WR 6026. Studies with inducers of cytochrome P-450 monooxygenase (Arochor 1254 and phenobarbital) demonstrated that the formation of the major metabolite is dramatically increased by pretreatment of animals with these inducers. This major metabolite is two to three times more active than its parent

compound in an in vitro antileishmanial screening assay. Studies are now in progress to identify these metabolites.

In vivo biotransformation study of WR 6026. Urinary extracts from rats treated with ^{14}C-WR 6026 were used for the in vivo biotransformation study. These studies showed 12 different peaks of radioactivity present utilizing HPLC with gradient chromatography. Two of these peaks correspond chromatographically with those observed in vitro with liver microsomes. These metabolites are currently being isolated for testing in the in vitro antileishmanial screening system.

The study of the metabolism of WR 6026 by leishmania promastigotes. A preliminary study was performed on the metabolism of WR 6026 by leishmania promastigotes. The results of this study show that these organisms metabolize WR 6026 into four polar compounds, two of which are chromatographically identical to those observed in the urine extract of the rats treted with WR 6026 and those observed in vitro in rat liver microsomes.

HPLC analysis of mefloquine. A new technique for the analysis of mefloquine in blood or plasma has been developed which utilizes a more complete sample purification step followed by a simplified chromatographic procedure. Utilization of these refined samples makes possible the optimization of the chromatography resulting in well defined peaks of short retention time. The HPLC system is coupled with an improved ultraviolet detector capable of absorption at 220 nm, the wavelength of greatest absorptivity for mefloquine. This new analytical technique has been validated for the analysis of mefloquine in the range of 15 ng/ml to 1400 ng/ml blood or plasma.

3. Future Work.

Identification of the minor metabolites of WR 6026 will be performed. The efficacy of these major and minor metabolites will be tested. Pharmacokinetic studies of WR 6026 in rats will be performed. In vitro and in vivo studies of the methemoglobin potential of newly developed 8-aminoquinolines will be continued. Study of biotransformation of new drugs by isolated hepatocytes will be initiated.

4. Publications.

H. Chung, V.R. Jimmerson, R.S. Rozman and J.E. Sanders: The disposition of the diastereoisomer of mefloquine in mice. Pharmacology 24:267-274, 1982.

J.C. Anders, J.R. Baker and H. Chung: Methemoglobin production of primaquine diphosphate and three other 8-aminoquinoline candidate antimalarial drugs. (Submitted to The Society of Toxicology for presentation.)

J.C. Anders and H. Chung: Improved technique for the cyanomethemoglobin portion of the Austin and Drabkin sulfmethemoglobin assay. (Submitted to The Society of Toxicology for presentation.)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6495	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	9b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	H. Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AG	156
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	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	82 09 30	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	5.0	343
	CURRENT 82	5.0	466

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Canfield, Craig J., COL MC
NAME:

21. GENERAL USE

Foreign intelligence 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) 81 10-82 09 New synthesis efforts in the acridinedione-acridinedioneimine series, pyrimethamine analogs, and indoloquinolines will be terminated in the first quarter of the next fiscal year. Evaluation of these series is not yet complete, and several members will undergo advanced screening in the next fiscal year. Quassinoides, which show significant in vitro activity, have shown only low levels of in vivo activity. Follow-up on this lead is intended. Rhesus testing of tissue schizonticides has resumed, and data for 8-aminoquinolines are being gathered for structure-activity studies. Efforts in the areas of schistosomiasis, leishmaniasis, and trypanosomiasis remain low, however, novel heterocyclic quaternary compounds are showing promising antitrypanosomal activity. Conversion of the data processing system and files to the new in-house computer is on schedule. Approximately 518 samples were submitted from the synthesis program. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81-30 Sep 82. This work unit is being terminated by incorporation into old work unit #157, "Development of Anti-Parasitic Disease Drugs."

*Available to contractors upon originator's approval.

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Project 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 156 Synthesis of Antiparasitic Drugs

Investigators:

Principal: LTC Robert O. Pick, Ph.D.
Associates: COL Craig J. Canfield, MC; Bing T. Poon, Ph.D.; Daniel L. Klayman Ph.D.; Nancy A. Roth, Ph.D.; MAJ John P. Scovill, Ph.D.; A.J. Lin, Ph.D.; Hikmat A. Musallam, B.S.

Efforts in this work unit were in four main areas described below.

1. The Research Contract Synthesis Program

During this period, active contractual programs devoted to the synthesis of potential antiparasitic agents were divided as follows: malaria 3, leishmaniasis 1½, trypanosomiasis 2½. Two preparations laboratories and a radiolabel synthesis contract also supported the program. A portion of these last three contracts also supported the Chemical Defense Program. The decreased antiparasitic contractual effort is in line with the lack of activity. Several new proposals have been reviewed, but work in new areas will not be effected until FY 83.

The 8-aminoquinolines continue to show good blood as well as tissue schizontocidal activity. Efforts continue in two principal areas: (a) investigate chain length variation and branching in the 5-OR series; and (b) investigate the effect of substitution in the 2- and 3-positions instead of, and, in addition to, a CH_3 group in position-4.

The naphthalene analogs of the 8-aminoquinolines have not shown tissue schizontocidal activity, hence efforts will be discontinued in this area. Synthetic efforts in the areas of amodiaquine analogs, pyrimethamine types, and acridinoneimines will be discontinued after the first quarter of FY 83, but testing and data analysis will continue. Many of these compounds are very active, and prophylactic and toxicity work must be done.

In leishmaniasis and trypanosomiasis, pyridine bis-amidines and oxime precursors continue to show activity. In the area of purine metabolism, efforts are shifting from the sulfamoylated compounds to variations in inosine analogs.

2. Data Processing

The conversion of the Chemical Information Retrieval System (CIRS) to the new in-house computer has continued on schedule, and the verification of the chemistry data base has been completed. This should greatly aid the data base conversion effort to the new system.

3. Acquisition of Compounds

The following table summarizes the various classes of compounds received for screening/testing during FY 82.

	Originals	Duplicates	Total
Purchased	26	33	59
Gifts	146	65	211
Synthesized	363	73	436
Discreet	2867	170	3037
Prep Labs	46	36	82
	3448	377	3825

4. Organic Synthesis Section

About 100 compounds were synthesized for biological evaluation during the past year, virtually all of which are new to the chemical literature.

The construction of a series of thiosemicarbazones derived from 1- and 3-acetylisoquinoline has been completed, and biological results are available now for some of the members of the series. Antimalarial activity does not appear to have been appreciably raised by these structural modifications.

Based upon the observation that a thiosemicarbazone of 2-acetylpyrimidine had significant antimalarial activity, the synthesis of 2-acetylpyrimidine has been refined, and the preparation of thiosemicarbazones derived from it has been initiated.

A series of 2-acetylpyridine N-oxide thiosemicarbazones was prepared and their antimalarial and antitumor activities were evaluated. No significant promise is indicated in this field. The synthesis and testing of 2-acetylpyrazine thiosemicarbazones was also completed. These compounds were appreciably less active than the corresponding pyridine analogs. A

thiosemicarbazone derived from 2-acetylthiazole has exhibited promising antileukemic activity, however, the remainder of the members of this class do not display encouraging antimalarial activity. Steps have been taken to develop water soluble thiosemicarbazones as a means of improving the therapeutic efficacy of this class of compounds.

Thiosemicarbazones developed by this section continue to show promising in vitro and in vivo activity against herpes simplex viruses I and II. A licensing agreement is being negotiated to allow development of this lead by a commercial firm.

Analytical Services

Collaborative efforts with the Department of Pharmacology of this division have been initiated into the study of the metabolism of the antileishmanial drug, WR 6026. Efforts are directed at isolation, structural identification and synthesis of the metabolites, in order to satisfy FDA drug development requirements and with a view towards the possible discovery of metabolites with enhanced antileishmanial activity.

Analysis and identification of a number of foreign pharmaceuticals was accomplished for medical intelligence services.

Papers Published

1. Klayman, D.L., and Copeland, E., "Radioprotective Agents," Kirk-Othmer Encycloped. of Chemical Technology, John Wiley and Sons, 1982.

2. Collins, F.M., Klayman, D.L., and Morrison, N.E., Activity of 2-Acetylpyridine and 2-Acetylquinoline Thiosemicarbazones Tested in vitro in Combination with other Antituberculous Drugs, Am. Rev. of Respiratory Diseases, 125, 58, (1982).

3. Scovill, J.P., Klayman, D.L., Franchino, C.F., 2-Acetylpyridine Thiosemicarbazones. 4. Metal Complexes as Potential Anti-Malarial and Antileukemic Agents, J. Med. Chem., 25, 1261 (1982).

4. Collins, F.M., Klayman, D.L., and Morrison, N.E., Correlation between Structure and Antimycobacterial Activity in a Series of 2-Acetylpyridine Thiosemicarbazones, J. General Microbiology, 128, 1349 (1982).

5. Wysor, M.S., and Scovill, J.P., Chemotherapeutic Activity of Silver Sulfonamides against Trypanosoma rhodesiense. Drugs in Expt. and Clinical Res., 7, 155 (1982).

6. Lambros, C., Childs, G.E., Notsch, J.D., Klayman, D.L., Davidson, D.E., and Scovill, J.P., Antimalarial Activity of 2-Acetylpyridine Thiosemicarbazones. Presented at 5th International Congress of Parasitology, Toronto, Ont. 7 Aug 1982.

7. Scovill, J.P., and Klayman, D.L., 2-Acetylpyridine Thiosemicarbazones. Effects of Isosteric Modifications upon Antimalarial Activity. Presented at the North American Medicinal Chemistry Symposium, Toronto, Ont. 20 Jun 1982.

8. Scovill, J.P., and Klayman, D.L., 2-Acetylpyridine Thiosemicarbazone-Transition Metal Complexes as Potential Chemotherapeutic Agents. Presented at 16th Great Lakes Regional Meeting of the American Chemical Society, Normal, IL. 7 Jun 82.

9. Klayman, D.L., and Lin, A.J., Facile Synthesis of N^4-Mono and N^4,N^4-Disubstituted 2-Acetylpyridine Thiosemicarbazones via Transamination. Presented at the 1st Symposium on Pyridine Chemistry, Indianapolis, IN. 21 Oct 1982.

10. Dobek, A.S., Klayman, D.L., Dickson, E.T., Jr., Scovill, J.P., Inhibition of Clinically Significant Bacterial Organisms. In vitro by 2-Acetylpyridine, 2-Acetylquinoline, and 1- and 3-Acetylisoquinoline Thiosemicarbazones. Presented at the 182nd Annual Meeting of the American Society for Microbiology, Atlanta, GA. 7 Mar 1982.

Papers Submitted for Publication or in Preparation

1. Klayman, D.L., Scovill, J.P., Bartosevich, J.F., Bruce, J., 2-Acetylpyridine Thiosemicarbazones. 5. 1-[1-(2-pyridyl)ethyl]-3-Thiosemicarbazides as Potential Antimalarial Agents. J. Med. Chem., in the press.

2. Lambros, C., Childs, G.E., Notsch, J.D., Scovill, J.P., Klayman, D.L., Davidson, D.E., Jr., In Vitro Assessment of 2-Acetylpyridine Thiosemicarbazones against Chloroquine-Resistant Plasmodium falciparum, Antimicrob. Agents Chemother., in the press.

3. Klayman, D.L., Scovill, J.P., Bartosevich, J.F., Bruce, J., Massie, S.P., Grant, S.D., Gonzalez, A., 2-Acetylpyridine Thiosemicarbazones. 6. Derivatives of 2-Acetylquinoline as Potential Antimalarial Agents. Ms. in preparation.

4. Klayman, D.L., Scovill, J.P., Bruce, J., Bartosevich, J.F., Roth, N., 2-Acetylpyridine Thiosemicarbazones. 7. Derivatives of Isoquinoline as Potential Antimalarial Agents. Ms. in preparation.

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6. Pick, R.O., New and Prospective Developments in Drugs for Malaria, Scand. J. Infect. Diseases, Suppl. (1982). In press.

Patents Awarded or Applied For

1. Klayman, D.L., Scovill, J.P., Bartosevich, J.F., Mason, C.J., Griffin, T.S., 2-Acetyl and 2-Propionylpyridine Thiosemicarbazones. U.S. Patent No. 4,317,776. 2 Mar 1982.

2. Klayman, D.L., Massie, S.P., Grant, S.D., Gonzalez, A., Scovill, J.P. Novel 2-Acetylquinoline Thiosemicarbazones and Preparation Thereof. U.S. Patent Application No. 348,462. 12 Feb 1982.

3. Klayman, D.L., Scovill, J.P., Franchino, C.F., Transition Metal Complexes of 2-Acetyl and 2-Propionylpyridine Thiosemicarbazones and Their Selenium Analogs. U.S. Patent Application No. 364,089. 31 Mar 1982.

4. Klayman, D.L., Scovill, J.P., 2-Acetyl and 2-Propionylpyridine Selenosemicarbazones, Their Preparation and Use. U.S. Patent Application No. 364,085. 31 Mar 1982.

5. Klayman, D.L., Scovill, J.P., Bartosevich, J.F., Mason, C.J., Griffin, T.S., Method of Treating Bacterial Infections with 2-Acetyl and 2-Propionylpyridine Thiosemicarbazones. U.S. Patent Application No. 311,369. 14 Oct 1981.

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7. Klayman, D.L., Scovill, J.P., Bartosevich, J.F., Mason, C.J., Griffin, T.S., The Use of 2-Acetyl and 2-Propionylpyridine Thiosemicarbazones as Antimalarials. U.S. Patent Application No. 311,368. 14 Oct 1981.

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9. Shipman, C., Jr., Klayman, D.L., Smith, S.H., Drach, J.C., Antiviral Activity Against Herpes Simplex Virus by 2-Acetylpyridine Thiosemicarbazones. U.S. Patent Application No. 363,723. 10 Mar 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6535	B2 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
81 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AF	157
b. CONTRIBUTING				
XXXXXXXXXX	STOG 80-7.2:2			

11. TITLE (Procede with Security Classification Code)

(U) Development of Anti-Parasitic Disease Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

021600 Pharmacology 002600 Biology 012100 Organic Chemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING	82	6.9	215
FISCAL YEAR CURRENT	83	15.0	442

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Intelligence Considered.

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Drug Development; (U) Parasitic Disease; (U) Chemical Synthesis; (U) Biology; (U) Pharmacodynamics; (U) Drug Metabolism; (U) Quantitative Methodology

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

23. (U) To develop new drugs with chemoprophylactic or chemotherapeutic activity against parasitic diseases of military importance.

24. (U) Potentially active antiparasitic drugs are identified and obtained by synthesis or purchase. Active compounds are identified by testing candidate drugs for activity in laboratory model systems of the disease. Studies are performed in animals to determine metabolism of the compounds, mechanism of drug effect, pharmacokinetics and tolerance. Information is used in guiding new drug synthesis and in selecting candidate drugs for clinical trials.

25. (U) 8110-8209 Screening tests were done by in-house and contractor laboratories in malaria, leishmaniasis, schistosomiasis and trypanosomiasis. Approximately 3000 compounds were tested for antimalarial activity in the primary screens and of approximately 200 active compounds approximately 100 were selected for advanced study against P. berghei in mice, P. falciparum in Aotus monkeys and P. cynomolgi in rhesus monkeys. A new series of arsenical compounds were developed which have efficacy at extremely low dosages against trypanosomiasis in mice in both acute and chronic disease models. Approximately 700 compounds were tested for activity against P. falciparum in vitro and 400 more tested against T. rhodesiense in vitro. Approximately 900 compounds were tested against leishmaniasis. This work Unit is changed by consolidation with Work Units 155 and 156, with title change from "Experimental Drug Development". For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

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

PROJECT 3M162770A871AF Prevention of Military Disease Hazards

WORK UNIT 157 **Development of Antiparasitic Disease Drugs**

INVESTIGATORS:

Principal: COL David E. Davidson, Jr., VC
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Dr. Gerald J. McCormick

Associate: CPT Chris Lambros, MSC
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PROBLEM AND OBJECTIVES:

In many parts of the world where U.S. military personnel may be deployed, diseases such as malaria, leishmaniasis, schistosomiasis and trypanosomiasis are endemic. Prevalence of both falciparum and vivax malarias is increasing because of failing control and eradication efforts in many countries. In many of these areas, falciparum malaria has become resistant to currently available drugs. Current chemotherapy of leishmaniasis, schistosomiasis and trypanosomiasis is inadequate. There are no drugs available for prophylaxis, and those that are available for therapy have limited efficacy and dangerous side-effects. The objective of this work unit is the discovery and development of new drugs for prophylaxis and treatment of these diseases in military personnel. In-house research is complemented by and coordinated with contractor laboratory drug testing and research.

PROGRESS:

Screening tests in animal models were performed by in-house and contractor laboratories for antimalarial efficacy at approximately the same rate as in the previous year. Approximately 3000 candidate compounds were tested in the primary screen and of these approximately 200 had activity. Approximately 100 of these were selected for secondary studies against resistant strains of P. berghei in mice, against human P. falciparum in Aotus monkeys and against vivax-like P. cynomolgi in rhesus monkeys. Approximately 700 compounds were evaluated against chloroquine-sensitive and -resistant strains of P. falciparum in an in vitro test system. Structure-activity relationships were

studied in series of 2-acetylpyridines and thiosemi-carbazones, dihydrotriazines and 8-aminoquinolines. Induction of resistance to mefloquine *in vitro* was achieved in two strains of *P. falciparum*.

Approximately 2000 compounds were screened against *T. rhodesiense* in mice. Several novel arsenical and amidine compounds had exceptional activity. An *in vivo* system was developed for assessment of ability of drugs to cross the blood-brain barrier and a series of melaminylthioarsenites were evaluated in it for efficacy against CNS involvement in mice with chronic African trypanosomiasis.

In the topical prophylactic antischistosomal drug screen, 126 compounds were tested; 111 of these were initial tests. Ten of the new compounds provided 90% or better protection even after the treated skin surface was washed with water for one-half hour. These compounds represented several chemical classes: 4-aminoquinolines (3), hexachlorophene analogues (5), thioureals (1) and miscellaneous aromatic compounds (1). An additional seven compounds were active but were readily removed from the skin by water washing. Follow-up tests performed on active compounds included three on the levels of protection afforded by different concentrations of active solute in the treatment solution, eight on the persistence of protection through extended water washes and a study on the persistence of protection when both treatment age and water wash duration were varied. Wipe application of 1.25% hexachlorophene three days prior to exposure to cercariae still provided better than 90% protection even after a three-hour water wash.

Approximately 900 compounds were tested against visceral leishmaniasis in hamsters and cutaneous leishmaniasis in mystromys and mice. Identificaton of strain and follow-up of clinical care were done for personnel with leishmaniasis contracted in Panama during jungle warfare training exercises.

FUTURE OBJECTIVES

Screening capability will be maintained to support the search for new, active classes of antiparasitic drugs and to guide synthesis of more efficacious and less toxic analogues among classes of compounds with activity against malaria, leishmaniasis, schistosomiasis and trypanosomiasis. Attempts will be made to study the *in-vitro* mefloquine-resistant strains in terms of morphology, biochemistry and susceptibility to clinical antimalarial drugs and their analogues.

PUBLICATIONS:

1. Lambros, C., Childs, G.E., Notsch, J.D., Scovill, J.P., Klayman, D.L., and Davidson, D.E., Jr. 1982. In vitro assessment of 1-acetylpyridine thiosemicarbazones against chloroquine-resistant Plasmodium falciparum. Anti-microb. Agents Chemother. In press.

2. Childs, G.E., Lambros, C., Notsch, J.D., Ager, A., Pamplin, C.L., and Davidson, D.E. Comparison of in vitro and in vivo activities of 9-phenanthrenecarbinols. Submitted for publication.

3. Childs, G.E., Lightner, L., McKinney, L.A., Groves, M.G., Price, E.E., and Hendricks, L.D. Inbred mice as model hosts for cutaneous leishmaniasis. I. Resistance and susceptibility to infection with Leishmania braziliensis, L. mexicana and L. aethiopica. Submitted for publication.

PRESENTATIONS:

1. Lambros, C., Childs, G.E., Notsch, J.D., Scovill, J.P., Klayman, D.L., and Davidson, D.E., Jr. Antimalarial activity of 2-acetylpyridine thiosemicarbazones. Fifth Int. Congr. Parasit. 1982.

2. Childs, G.E., Lambros, C., Notsch, J.D., and Davidson, D.E. Factors influencing antimalarial activity of 9-phenanthrenemethanols. Fifth Int. Congr. Parasit. 1982.

3. Baird, K., Lambros, C., and Jackson, J.E.D. Reduction in aminoquinoline antimalarial activity following membrane filter sterilization. Fifth Int. Congr. Parasit. 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

AGENCY ACCESSION	DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6765	82 10 01	DD-DR&E(AR)636

1. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. OTHER INSTR'N	8A. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AF	158
b. CONTRIBUTING				
c. ~~XXXXXXXX~~	STOG 80-7.2:2			

11. TITLE (Procede with Security Classification Code)

(U) Exploratory Vaccine Development Against Parasitic Diseases.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Micribiology 002600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	5.0	337
FISCAL YEAR CURRENT 83	2.0	237

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: Barbaro, J.F.

NAME: Jackson, P.R. POC:DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede each with Security Classification Code)

(U) Antigens; (U) Immunoassays; (U) Parasitic Diseases; (U)Immunity; (U) Vaccines

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 isolate and characterize antigens particularly those of malaria, trypanosomiasis and leishmaniasis, and the evaluation of them as potential immunogens in experimental animals for the development of a safe and effective vaccine. These diseases impede military performance whenever troops are deoloyed in endemic areas emphasizing the need for a suitable vaccine to facilitate military operations.

24 (U) The approaches used in these studies are to develop techniques for the isolation of parasitic antigens; to use standard biochemical and immunochemical procedures to characterize and purify these antigens; to develop quantitative in vitro immunoassay to monitor the purity of these isolated antigens; and to determined the effectiveness as vaccines of these antigens in experimental animals.

25 (U) 81 10 - 82 09 New technological procedures to analyze kDNA of leishmania with restriction endonucleases are extremely sensitive and can detect differences between species and strains of the parasite. They are more sensitive than previous methods of DNA bouyant density, excretion fraction serotyping, and isoenzyme studies. However, this method, requires several weeks before an identification can be provided to the clinician. Preliminary Southern blot hybridization studies indicate that nucleotide sequences are shared among leishmania causing visceral disease. A visceral parasite does not share nucleotide sequence homologies with cutaneous isolates. This method can provide clinical results within 24-48 hr, and based on preliminary observations, material can be isolated directly from lesions, obviating the need for parasite culturing. The monoclonal antibody program is continuing with 2 strains of L. donovani used either to infect or immunize donor mice. An IFA screening assay is now in place. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

PROJECT 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 158 Exploratory Vaccine Development Against Parasitic Diseases

Investigators:

Principals: Peter R. Jackson, Ph.D.
LTC Wayne T. Hockmeyer, MSC

Associates: John F. Barbaro, Ph.D.
Mr. John M. Stiteler
SP5 Perry J. Sayles

Problem and Objectives:

The problem under study is the development of vaccines against the human parasites which cause leishmaniasis, trypanosomiasis and malaria. These diseases impede the military performance of troops deployed in endemic areas, necessitating effective vaccination procedures. Parasite identification is crucial to vaccine production and the current goal is the development of Leishmania identification methods based on both hybridoma monoclonal antibodies (MAbs) and kinetoplast DNA (kDNA) analysis. Specific objectives are to determine if: (1) the methods differentiate species and strains and correlate with clinical presentation; (2) the extant Leishmania identification techniques produce comparable results when applied to the same isolates; (3) the strains from within a geographic area can be identified and related. MAbs also will be used to isolate antigens for evaluation as immunogens in experimental animals.

Progress:

Leishmania characterization by restriction endonuclease digestion of mitochondrial (Kinetoplast) DNA (kDNA) is continuing. New protocols have reduced kDNA purification time from 70 to 6 hours. Sufficient kDNA is obtained from 50-100 ml of in vitro cultivated promastigotes (about 2×10^7 cells/ml) for about 5 restriction enzyme digests. The enzymes used are Hpa II and/or Taq I. Both 3-10% linear gradient polyacrylamide and 2% agarose gels separate kDNA fragments sufficiently to differentiate Leishmania. The linear gradient polyacrylamide

gels produce more complex kDNA fragment profiles than agarose gels. Photographs of an isolate's kDNA fragment pattern can be obtained 2 days after sufficient parasites are cultivated. A Nucleic Acid Analyzer System has been purchased which is being used for the micro-computer-assisted storage of the number and molecular size of the kDNA fragments as determined by gel electrophoresis. Computer programs which will allow for the automated comparison of restriction enzyme results of unknowns with characterized Leishmania, are being devised. Electron microscopy was used to show the kDNA is intact and undamaged by the new isolation procedure, but after restriction endonuclease digestion, the kDNA is reduced to thousands of small fragments. This visually confirmed gel electrophoresis results concerning the physical state of intact and enzyme digested kDNA. New Leishmania isolates have been examined with the following results: At least 2 genetic types of Leishmania cause human cutaneous leishmaniasis in Panama. This has not been reported before and the species responsible are being investigated. Five of 6 infected military personnel who returned from Panama had Leishmania with almost identical restriction enzyme produced kDNA profiles. This supports the idea that Leishmania from an endemic area may be genetically related. Two isolates of Leishmania from patients who acquired cutaneous disease in Africa, were also very similar in kDNA fragment patterns. None of the Panamanian or Kenyan isolates had kDNA restriction fragment profiles similar to cutaneous parasites from other parts of the world. Southern blot hybridization indicated that kDNA nucleotide sequences are shared among Leishmania responsible for visceral disease (L. donovani) in Khartoum, Kenya and India, (L. infantum) in France, and (L. chagasi) in Honduras. However, no nucleotide sequence homology was found between L. donovani from Khartoum and parasites responsible for cutaneous disease in Panama (L. braziliensis panamanensis) or Kenya (L. aethiopica). It is possible, therefore, that a technique for genetically typing Leishmania and predicting the pathology they may cause, can be developed using kDNA hybridization procedures. Long term cultures of Leishmania, using cloned and uncloned isolates, did not reveal major changes in the kDNA restriction enzyme fragment patterns. The 5 L. donovani clones were found to have kDNA fragment patterns similar to the original isolate. When compared to other methods of Leishmania characterization, restriction endonuclease analysis of kDNA is extremely sensitive and can detect differences between species and strains. The technique appears capable of detecting even minor differences among isolates and is thus more sensitive than DNA bouyant density, excretion fraction serotyping, and isoenzyme studies.

The production of monoclonal antibodies (MAb) to Leishmania is in progress. A great deal of preliminary work has been completed so that now 2 stains of L. donovani have been used to: (1) infect mice with amastigotes; (2) immunize mice with irradiated promastigotes; (3) infect mice with promastigotes. Leishmania antibodies were detected in all mice listed above by an indirect fluorescent assay using amastigotes and promastigotes as antigens. MAb development is now underway using these animals and in vitro cultivated murine myeloma cells.

Recommendations:

1. Examine additional isolates by restriction endonuclease digestion and ^{32}P-hybridization methods to determine genetic relationships of Leishmania. Attempt to relate results with specific diseases through the use of well characterized marker strains.

2. Develop ^{32}P blot hybridization procedure into a field test for detecting infection in man, animals and insect vectors.

3. Coordinate with Division of Biometrics for computer programs for automatic matching of kDNA fragments from Leishmania using data from NA2 Nucleic Acid Analyzer. Attempt to relate fragment patterns to disease type caused by parasite.

4. Develop more monoclonal antibodies against Leishmania for use in parasite identification and antigen analysis studies.

Formal Presentations:

1. Jackson, P.R., J.A. Wohlhieter; W.T. Hockmeyer. 1982. Leishmania Characterization by Restriction Endonuclease Digestion of Kinetoplast DNA. Abstract 306. Fifth International Congress of Parasitology August 7-14, 1982. Toronto, Canada.

Bibliography:

1. Jackson, P.R. 1980. Surface Saccharides of Trypanosoma rhodesiense: Changes with Bloodstream Trypomastigote Antigenic Type and Parasite Growth Stage. Abstract. 31. Thirty-third Annual Meetings, Society of Protozoologists, Washington, DC.

2. Jackson, P.R., J.C. Jackson, D.E. Raney. 1981. A Sterile Leakproof Plastic Vial for Cell Cryopreservation in

Liquid Nitrogen: Application to Parasitic Protozoa. Cryobiology 18: 608-611.

3. Jackson, P.R: J.A. Wohlhieter; W.T. Hockmeyer. 1982. Leishmania Characterization by Restriction Endonuclease Digestion of Kinetoplast DNA. Abstract 306. Fifth International Congress of Parasitology, August 7-14, 1982. Toronto, Canada.

4. Jackson, P.R., and C.L. Diggs. 1982. Trypanosoma rhodesiense Bloodstream Trypomastigote and Culture Procyclic Cell Surface Carbohyrate. Submitted to Journal of Protozoology.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 0G 2527	2. DATE OF SUMMARY* 82 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUMRY 81 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8a. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AH	159
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7.2.2			

11. TITLE (Precede with Security Classification Code)*

(U) Prevention and Treatment of Military Important Diseases in the Tropics

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine 010100 Microbiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	8.5	490
FISCAL YEAR CURRENT 83	6.0	720

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U)Malaria; (U)Dengue; (U)Dengue Hemorrhagic Fever; (U)Leptospirosis; (U)Japanese Encephalitis; (U)Rabies

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

23. (U) The technical objective is to develop new approaches to the prevention and treatment of tropical diseases of military importance. Malaria and dengue are emphasized because of severity and for propensity to cause high attack rates shortly after the onset of military operations.

24. (U) Approaches include characterization of the cellular immune response in patients infected with malaria, epidemiologic/ecologic studies to determine vectors and hosts in order to develop control methods, and studies to determine the etiologic factors of dengue hemorrhagic fever, and the etiology of hepatitis.

25. (U) 81 10-82 09 Studies are continuing on identifying changes in the cell mediated response of patients with naturally acquired malaria. The effect of antimalarial drugs on human lymphocyte response is being investigated. Metabolic studies of the malaria parasite and the host cells are being conducted to determine potential target areas for the design of new antimalarial drugs. Dengue enhancing antibody studies have developed a primate model for DHF. The antibody captive technique for rapid diagnosis of dengue and Japanese encephalitis is well established and is being extended to early detection of rabies infection. Electrophoretic techniques are being developed to identify members within the sibling complexes for mosquito vectors of malaria so rational control methods can be instituted. Antiviral drugs have been studied in tissue culture and will be extended to primate models. Studies on the pathogenesis of cerebral malaria have begun using primates to develop techniques for comparing cerebral transit time of infected and non-infected red cells. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81-30 Sep 82.

*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 Number: 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS
Title: Prevention and Treatment of Military Important Diseases in the Tropics
Work Unit Number: 159

Investigators: LTC(P) Michael W. Benenson, MC; LTC Donald S. Burke, MC; LTC George S. Ward, VC; MAJ Michael R. Elwell, VC; MAJ Richard G. Andre, MSC; CPT Ronald M. Rosenberg, MSC; MAJ Horace K. Webster, MSC; CPT Micheal J. Gilbreath, MSC; Katchrinnee Pavanand, M.D.; Ananda Nisalak, M.D.; Markpol Tingpalapong, DVM

1. Adenosine Deaminase in Human Malaria Infection

PROBLEM: Hereditary deficiency of the purine enzyme, adenosine deaminase is associated with severe combined immunodeficiency disease (SCID) - a condition in which both T-and B-lymphocyte function is impaired. Partial restoration of lymphocyte function can be achieved in SCID patients by enzyme replacement therapy involving whole blood or packed RBC transfusion. It thus appears that the ADA in normal RBC is sufficient to correct in part the purinogenic defect in ADA deficient lymphocytes. The precise mechanism for this effect is not understood although it may involve the role of the red cell mass in systemic adenosine metabolism in a way that influences purine metabolism in lymphocytes. Acute human malaria infection is characterized by immune suppression. In particular there is a decreased functional responsiveness of mononuclear cells - especially T-lymphocytes. It is also known that the intraerythrocytic malaria parasite produces major changes in the purine metabolism of the host red cell mass.

PROGRESS: We have studied purine metabolism in erythrocytes and lymphocytes from Thai adults naturally infected with P. falciparum. Heparinized whole blood was used to prepare lysates for enzyme studies and perchloric acid extracts for purine nucleotide profiles. Adenosine deaminase (ADA) was measured by a radio-chemical method involving an automated HPLC system. Nucleotide profiles were determined by an anion-exchange gradient HPLC method. Adenosine deaminase activity was increased in P. falciparum infected erythrocytes from individuals with naturally acquired infection. Nucleotide profiles on individuals with low parasitemias (<0.5 percent) showed an increased in erythrocyte adenosine triphosphate (ATP) levels. Adenosine which is not deaminated by ADA would be available for phosphorylation to ATP by adenosine dinase. Nucleotide profiles on lymphocytes revealed a decrease in ATP levels. This suggest that the energy potential of lymphocytes in malaria infected individuals is reduced and that the lymphocytes' ability to respond metabolically is depressed.

FUTURE OBJECTIVES: These studies suggest what may be a biochemical correlate of immune dysfunction in human malaria infection. The perturbation in erythrocyte

adenosine metabolism caused by the malaria parasite may produce a defect in lymphocyte purine metabolism which renders this cell functionally defective. Work is currently underway to confirm these preliminary observations in a larger population study. Specific emphasis is being given to studies on malaria lymphocyte adenosine metabolism, adenosine receptors and cyclic nucleotides.

2. Adenosine Deaminase in Malaria Infection: Effect of 2'-Deoxycoformycin in vivo

PROBLEM: Purine nucleotides are required by the rapidly proliferating malaria parasite for both energy metabolism and nucleic acid synthesis. The malaria parasite cannot synthesize purines de novo and depends for its intra-erythrocytic (IE) growth and development on salvage of purine bases from the host RBC and extracellular environment. We have shown with Plasmodium falciparum, in vitro, that hyposanthine is utilized as a purine base precursor for parasite synthesis of adenosine and guanosine nucleotides and that specific inhibition of these synthetic pathways leads to parasite destruction. Whether hypoxanthine is the malaria parasites preferred substrate in vivo is not known. An increase in adenosine deaminase (ADA) activity, however, is an obvious means for production of IE hypoxanthine. Increased availability of hypoxanthine would be a natural consequence of adenosine metabolism in the mature erythrocyte (viz: $\xrightarrow{ADA}$ inosine $\xrightarrow{PNP}$ hypoxanthine) since this cell lacks the enzyme xanthine oxidase. Conversely, inhibition of ADA activity could act to deprive the rapidly growing IE malaria parasite of a readily accessible hypoxanthine pool for purine nucleotide synthesis. It was, therefore, of interest to determine whether specific inhibition of ADA activity in vivo using the tight binding inhibitor, 2'-deoxycoformycin, interferred with the malaria parasites IE growth and development.

PROGRESS: Adult male rhesus monkeys with no previous exposure to malaria infection were experimentally infected with Plasmodium knowlesi. Samples of whole blood were collected at selected times during the IE infection cycle and at various levels of parasitemia. Lysates were prepared from washed RBC. The ADA assay was done by a radiochemical HPLC methods which measured the conversion of (^{14}C) adenosine to (^{14}C) inosine.

There was a 3.6 fold increase in RBC ADA activity of infected rhesus with a mean parasitemia of 6.2 percent parasitized RBC (1.91 ± 0.18 vs 6.95 ± 0.70) nanomoles/min/mg protein, control vs infected RBC; n = 12). ADA levels were observed to increase with increasing parasitemias in serially sampled animals. Malaria parasitized RBC (PRBC) have been shown by starch gel electrophoresis to contain a distinct parasite ADA enzyme. It is apparent, therefore, that IE malaria parasite growth and proliferation is associated with an increase in PRBC ADA activity. 2'-Deoxycoformycin (DCF) (single i.v. dose, 250 mg) effectively inhibited RBC ADA activity in malaria infected monkeys at six and 24 hours. Parasitemias were decreased at six hours and continued to fall over the ensuring 24 hours. Microscopic examination of six hour DCF treated PRBC revealed parasite nuclear and cytoplasmic deterioration. By 24 hours the majority of PRBC contained degenerate parasite forms. DCF thus appears to produce a potent antimalarial effect *in vivo*.

FUTURE OBJECTIVES: Adenosine deaminase appears to be a potential metabolic target for the design of new antimalarial chemotherapy. Work is currently underway to more fully understand the action of 2'-deoxycoformycin on the malaria parasite and the role of ADA in parasite development and proliferation.

3. Antibody Secretions of Malarious Individuals (Immuregulation in Malaria)

PROBLEM: To examine the synthesis and secretion of total IgM, IgG and IgA during 12 days of *in vitro* culture, by peripheral blood mononuclear cells from malarious Thais.

PROGRESS: The presence of autoantibodies is often taken as an indication of an alteration in immunoregulation. Studies on the nature of antibody synthesis and secretion by MNC from malarious patients are of importance because of the ability of *in vitro* antibody production to serve as a uniquely sensitive indication system for studying immunoregulatory pathways as well as delineating functional and qualitative alterations in both the humoral and cellular components of the immune response during malarial infection.

We utilized a solid phase system, with commercially obtained rabbit anti-IgA, IgM or IgG covalently bound to a cross linked polyacrylamide bead. Specificity was tested with purified immunoglobulins with no cross reactivity detected. The IgG, IgA, and IgM synthesized and secreted into the media were measured by separate solid phase radioimmunoassays for each immunoglobulin class with specific rabbit anti-immunoassays and 125I-labelled immunoglobulins. The assays were performed in 96 well, round bottomed, microtiter plates. To each well was added 10u of culture supernatant or standard, 50u of radio labelled IgG, IgA, or IgM (25,000 CPM), and 50u of appropriate solid phase antisera. After overnight incubation at 25°C, the wells were resuspended and harvested on glass fiber filter strips using a Bellco microharvester. Individual discs were counted using a gamma counter. Presently, we are standardizing the antibody synthesis assay.

FUTURE OBJECTIVES: Studies on the immunoregulation to malarial infection are of importance. Increasing resistance of the parasite and the developmental work on malarial vaccine emphasizes the importance of these studies.

4. Subpopulations of T Cells (Tg and Tm) in Patients with Malaria

PROBLEM: To quantitate subpopulations of T cells (Tg suppressor and Tm Helpers) in the peripheral blood of patients with malaria.

PROGRESS: In the present study we utilized rosetting techniques to enumerate the putative suppressor (Tg) and helper (Tm) T-cell subpopulations in the peripheral blood of adult Thais with malaria. A lower percentage of both Tg and Tm subpopulations and a lower number and percentage of total T cells was found in these patients during the acute period of infection than in the peripheral blood of healthy donors. However, the percentages of total T, Tg and Tm cells were higher during the convalescent period and were comparable to the value found in the peripheral blood of healthy donors. No correlations were found between the percentages of these T-cell subpopulations and the level of parasitemia or the hematocrit.

FUTURE OBJECTIVES: Further studies should be conducted to assess the sequential development of the host (human and primate) immune responses to malaria infection.

5. Examination of Sera from Indonesians with Malaria Splenomegaly Syndrome in Assays of Blastogenic Responsiveness to Mitogenic Lectins and Cell Surface Antigens

PROBLEM: To examine the effect of sera from Indonesians with Malarial Splenomegaly Syndrome on the cellular immune function of human mononuclear cells using the mitogen induced lymphocyte transformation assay and the mixed leukocyte culture system.

BACKGROUND: We have previously shown that the mitogenic responsiveness of normal peripheral blood mononuclear cells was markedly reduced to both PHA and Con A when 20 percent pooled or individual sera from patients with P. falciparum and P. vivax malaria were added to the mononuclear cells. Sera from patients also displayed an inhibitory effect on the normal bastogeneic response to allogeneic cell surface antigens in vitro. In a collaborative study with the NAMRU 2 laboratory we are examining sera from individuals with tropical splenomegaly syndrome in an effort to delineate whether inhibitory characteristic are present and can be associated with clinical findings.

METHODS: Methods have been previously described in detailed (2).

6. The Effect of Anti-coagulants on Cold Reactive Anti-lymphocyte Activity in the Blood of Patients Naturally Infected with Malaria

PROBLEM: To compare lymphocytotoxicity in malarious patients plasma and serum.

PROGRESS: The effect of three different anti-coagulants on the level of cold-reactive anti-lymphocyte activity (ALA) in the peripheral blood (PB) of malarious individuals was assessed to determine if plasma could be substituted for serum in assays designed to characterize ALA. Plasma was obtained from PB previously treated with Heparin, acid-citrate dextrose (ACD), or ethylenediamine tetraacetic acid (EDTA). An equivalent level of ALA was found in the serum and plasma obtained

from ACD or EDTA treated blood, however, ALA in the Heparin treated blood was substantially lower. Thus, it appears that plasma obtained by treating PB with ACD or EDTA, but not heparin can be used instead of serum to investigate the role of anti-lymphocyte factors in malarial infections. The major practical advantage of this procedure is the higher yield of MNC and plasma to investigate the interactions of lymphocototoxic factors and autologous MNC.

FUTURE OBJECTIVES: This study is complete.

7. Kinetics of Japanese encephalitis (IgM and IgG in Human Serum and CSF

PROBLEM: Japanese encephalitis (JE) is endemic in Southeast Asia and has a high case fatality rate. Existing methods of diagnosis (Hemagglutination inhibition-HAI) are adequate for retrospective studies but results cannot be obtained early enough to affect treatment of the disease.

PROGRESS: Thirty-two patients with a clinical diagnosis of encephalitis have been studied during the 1981 epidemic season in the provincial hospital at Kampangphet, a province with a high rate of JE. JE could be quickly diagnosed by the demonstration of specific anti-JE IgM in the CSF by IgM antibody capture (MAC) immunoassays. CSF samples from 25 patients with other diseases with possible CNS involvement were negative of JE IgM.

Five siblings of encephalitis cases with demonstrable asymptomatic JE had JE IgM in their serum but not in their CSF.

FUTURE OBJECTIVES:

1. JE MAC immunoassays should replace conventional HAI serology for diagnosis of JE.

2. JE MAC immunoassays should be used to select patients for trial of promising antiviral drugs.

8. Production of Flavivirus Temperature Sensitive Mutants

PROBLEM: The existence of a battery of temperature sensitive (ts) mutants of flaviviruses and the

demonstration of complementation would allow investigation of the biochemical functions of nonstructural virus specified proteins, the identification and characterization of the lesion in candidate vaccine viruses and in the field isolates, and determination of the relationship of protein function to virus virulence.

PROGRESS: The heat resistent strain of Japanese encephalitis virus (JEV) has been treated with two additional mutagens N-methyl-N'-Nitro-N'-Nitrosoguannidine (NG) and Fluorouracil (FU). Virus treated with NG has been cloned and 422 clones have been tested for ts character. Of these clones eight were stable ts mutants. Virus grown in the presence of FU has been cloned and 248 clones have been tested for ts character. Of these clones nine were stable ts mutants.

FUTURE OBJECTIVES:

1. These stable mutants are being characterized as to mutant function and should be analyzed for complementation with other mutants.

2. If complementation can be demonstrated, field isolates of JEV should be tested to determine the frequency of naturally occurring ts mutants, the nature of their lesion and its relationship to pathogenesis.

9. A Primate Model for Hemorrhagic Dengue

PROBLEM: To attempt to develop an animal model for DHF to determine the role of enhancing maternal antibody.

PROGRESS: We collected dengue type 2 virus isolates from Thai children less than one year old hospitalized in Bangkok with hemorrhagic fever and also collected serum specimens from the mothers of these infants. Mothers' sera were screened for their ability to enhance growth of the corresponding infant's virus in vitro in cultures of a continuous mouse macrophage cell line. One maternal serum-infant virus pair which showed strong in vitro antibody dependent enhancement (ADE) of virus growth was selected for use in primate inoculations. The virus chosen had been isolated from a fatal case of DHF. The "DHF maternal sera" selected produced maximal ADE of virus growth in vitro at a 1:1000 dilution.

Colony born infant primates, ages eight to 18 months, were the study subjects. Clinical signs were monitored, and bloods were drawn daily for measurement of hematocrit, white blood cells, platelets, viremia, and antibody response. All monkeys were inoculated with 10^4 or 10^5 plaque forming units of the test dengue 2 virus strain intravenously. Experimental monkeys received "DHF maternal sera" intravenously 24 hours prior to virus injection while control monkeys received similar injections of non-immune human sera.

Six infant cynomolgus monkeys were inoculated with serum doses calculated to give a 1:3000 or 1:10,000 dilution of the "DHF maternal serum" in the primate's extracellular fluid (ECF) space. No disease was observed, although both the viremia and antibody response occurred earlier in the monkeys that received the "DHF maternal serum."

Four infant rhesus monkeys were inoculated with serum doses calculated to give a 1:500, 1:1000, 1:2000, or 1:5000 dilution in the ECF space. The monkeys pretreated with the two highest doses of "DHF maternal serum" developed profound (<30,000 platelets per mm^3) thrombocytopenia five to 10 days after virus inoculation. The monkey receiving the 1:1000 serum dose developed a positive tourniquet test, multiple spontaneous bleeding sites, and died of hemorrhagic shock. The two monkeys receiving lower doses of "DHF maternal serum" and all four monkeys which received pretreatment with normal non-immune sera all remained well without thrombocytopenia.

FUTURE OBJECTIVES: Early results indicate a feasible model. Further work should be done studying the parameters of the model.

10. Ectoparasite and Rickettsia tsutsugamushi Studies in Thailand

PROBLEM: The goals of this research are to establish and describe ectoparasites that are or are potential vectors of human parasites or pathogens of human disease in Thailand, and to delineate the distribution of natural populations of larval mites infected with Rickettsia tsutsugamushi in Thailand.

PROGRESS: Collaborative studies between the Department of Medical Entomolgoy, AFRIMS, and the USAMRU, Kuala Lumpur, Malaysia, have shown that several strains of Rickettsia tsutsugamushi occur in nine different mite species in various parts of Thailand (3). Some of these species are new and are being or have been described (4).

Collections of ectoparasites were made from rodents collected in attempts to isolate Hantann virus in the port area of Bangkok. Several species of lice, ticks, fleas, and mites were found to heavily infest the rats occurring in the vicinity of the warehouses and foodstalls. Hantann virus was isolated from a rat captured near a large foodstall.

After some revisions The Checklist of the Ticks of Thailand has been accepted for publication is currently in press(5).

FUTURE OBJECTIVES: With the discovery and identification of at least four new species of chiggers that were found to contain Rickettsia, future collaboration with the laboratory in Malaysia is planned in order to determine the role these mites play in human disease transmission. Temporal and spatial relationships between scrub typhus and its vectors will be investigated where human cases have been contracted.

11. Leptospirosis in the Non-Human Primate Model: Chemoprophylaxis and Early Diagnosis of Infection

PROBLEM: Leptospirosis is a common zoonotic disease found throughout the world. The clinical features in man range from an influenza-like illness to a more severe disease form manifested by continued fever with meningitic symptoms and signs (6,7). In some cases infection can lead to renal and hepatic failure, jaundice, and even death (6,8). Leptospirosis is frequently found in the tropical areas of the world (9,10) and recent attention has focused on several outbreaks in soldiers training in jungle areas (11). Symptomatic treatment and antibiotic therapy is used in the acute illness. However, once symptoms are evident the beneficial effect of antibiotics is questionable (7). The relatively long recovery period, even with treatment, suggest that prevention is the practical approach in solving the

problem of leptospirosis. It is difficult to prevent direct contact with leptospira contaminated water in a tropical environment, especially during military maneuvers. Immunization against specific serovars of leptospira can protect animals but immunization of man is not practical unless the serovar endemic to the area is identified or a vaccine with broad antiserovar activity is developed.

The current objectives are:

1. To characterize clinical leptospirosis in the non-human primate model.

2. To determine the efficacy of antibiotic treatment as a disease prophylaxis for the acute infection.

3. To determine if an ELISA method for detecting leptospira antibody or antigenemia is a useful means for obtaining rapid early diagnosis of leptospirosis.

PROGRESS: A pilot study has been completed in Macaca mulatta and Macaca irus monkeys infected with a human isolate of Leptospira bataviae, a strain commonly isolated from patients in Thailand with clinical leptospirosis. Following intraperitoneal injection of 10^7 organisms, a leptospiremia was detected in four of five M. irus and four of four M. mulatta for a period of one to six days after infection. Some CSF cultures were also positive for Leptospira. A febrile response was present in these monkeys on days two, three, and four. All monkeys survived the infection. Serum and cerebrospinal fluid samples were obtained for future testing for antibody and leptospiral antigen.

FUTURE OBJECTIVES:

1. Doxycycline, given daily as a prophylactic measure will be tested in monkeys given Leptospira bataveae.

2. If the antibiotic prophylaxis is successful in preventing bacteremia and fever, this treatment will also be tested in the weaning hamster infected with Leptospira. This infection in hamster is usually lethal. The minimum inhibitory concentration of doxycycline for Leptospira in vitro will also be determined.

12. The Diagnosis of Canine Rabies Infection Using the "Antibody Capture" Solid Phase Elisa Method (Acelisa)

PROBLEM: Rabies is endemic in Thailand. In a recent report over a ten year period, the yearly range was 237-[illegible]2 cases in man and 871-3286 cases in dog (12). With such a high incidence of disease, a reliable, rapid method of early diagnosis of infection is important. Many laboratory techniques have been developed for the diagnosis of rabies. The Seller's stain for Negri bodies, the Fluorescent Antibody technique (13), the radio immunoassay (14) and detection of IgM after vaccination by immunoperoxidase method have all been used for the diagnosis of rabies infection in man and animals. Each of the tests have required either considerable time or sophisticated equipment to obtain the result. The detection of low level (IgM) in human serum has been reported at the 3rd and 4th day post exposure to rabies antigen (15).

The antibody capture solid phase enzyme linked immunosorbent assay (ACELISA) has one of a unique properties that is gross specific immunoglobulins are functionally concentrated onto the solid from the liquid so that very low concentration can be detected (16). We propose to use this method to detect the acute rabies infection in the dog by measuring the level of IgM in CSF as well as in serum. This method may provide a means for early diagnosis of the canine rabies infection.

The current objectives are:

1. To determine the onset of detectable IgM in the CSF and sera of dogs with acute rabies infection.

2. To determine whether the rabies virus is shed into the CSF sera during an acute phase of illness.

3. To develop a simple, rapid, reliable technique for the diagnosis of canine rabies infection that does not require long quarantine or killing of the dog for testing.

4. To compare the result of the ACELISA technique with other established methods for the diagnosis of acute rabies infection.

PROGRESS: Serum and CSF were collected from 18 rabid and 29 non-rabid dogs. All serum and CSF will be tested for rabies antibody titer by ACELISA and REFIT.

13. Antibody Capture RIA for Diagnosis of Rabies

PROBLEM: Previous work has demonstrated that Japanese B cencephalitis can be reliably diagnosed early in an illness by determining the specific activity of anti-JE IgM in CSF and serum. The concept that locally synthesized specific antiviral IgM in the CSF might allow diagnosis of other forms of viral encephalitis required further evaluation. The presence of a privately supported study of the clinical management of rabies encephalitis provided the opportunity to study sera and CSF from several well-diagnosed local cases of encephalitis. Ultimately, this study will describe the kinetics of IgG and IgM anti-rabies antibody in serum and CSF in a manner similar to that used for Japanese encephalitis.

PROGRESS: During the summer of 1982, sera from five patients receiving Merieux Institute B-propionolactone inactivated vaccine were used as pilot specimens. An IgM capture (MAC) RIA was designed. Rabbit anti-human IgM was bound to the plates, the serum specimen washed over it, Merieux vaccine antigen applied, and finally ^{125}I tagged, purified IgG from a rabies hyperimmunized rabbit added. The Merieux vaccine was superior to rabies infected neuroblastoma or BHK cells. The optimum dilution of antigen was a 1/4 dilution of vaccine. Sera from 14 days post-immunization bound 2.7 times more tagged antibody than pre-immunization sera. Post vaccination sera were clearly distinguishable from pre-vaccination sera when tested for IgM. The IgG antibody capture (GAC) assay is presently under development.

FUTURE OBJECTIVES:

1. Continue to determine optimum conditions for rabies MAC and GAC.

2. Proceed to test sera and CSF from patients with diagnosed rabies.

3. Ascertain the kinetics of anti-rabies antibody production in serum and CSF, especially in relationship to pathogenesis of rabies encephalitis.

Project Number: 3M162770A871
Title: Prevention and Treatment of Military Important Diseases in the Tropics
Work Unit Number: 159

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'R INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AH	160
b. CONTRIBUTING				
c. [redacted]	S10G 80-7.2	2		

11. TITLE (Precede with Security Classification Code)

(U) Field Studies of Rickettsioses and Other Tropical Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	5.0	106
	CURRENT 83	7.0	117

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Scrub typhus; (U) Rickettsia tsutsugamushi; (U) Human volunteers; (U) Leptotrombidium; (U) Mites; (U) Chiggers; (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) This work unit has as its ultimate goal the elimination of scrub typhus, a disease endemic in the Asiatic-Pacific region, as a military medical problem. Specific problems relating to the epidemiology, pathogenesis, diagnosis, treatment, and prevention of this disease are being studied.

24. (U) 1. Test sera from acutely ill scrub typhus patients, to evaluate the latex agglutination (LA) and surface antigen detection assay as early diagnostic tests. 2. Measure cell-mediated immune response to human scrub typhus by migration inhibition and lymphocyte transformation (LT) assays. 3. Reinfect volunteers from previous doxycycline prophylaxis trial to determine immune status. 4. Analyze data stored in computer to learn more about epidemiology of scrub typhus. 5. Examine R.tsutsugamushi-infected endothelial cells by brightfield and electron microscopy to study pathogenesis of the disease. 6. Evaluate the isoenzyme patterns and karyotypes of several vector mite species to learn more about mite transmission and genetics.

25. (U) 81 10 - 82 09 1. Results of specificity and sensitivity testing of the LA test are very promising. 2. LTA results suggest that immunity to scrub typhus can be predicted. 3. Study was very successful, with 7 of 8 volunteers resisting the challenge by infected chiggers. 4. Three manuscripts have been submitted for consideration and two more are expected to be completed by December 1982. 5. Electron micrographs are being interpreted and hematoxylin and eosin-stained sections examined. 6. Electrophoretic studies of phosphoglucomutase are being analyzed and karyotyping has revealed that the diploid chromosome number for L. fletcheri is 14 and for L. arenicola is 28. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Applies 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 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 160 Field Studies of Rickettsioses and Other Tropical Diseases

Investigators:

Principals: LTC (P) Michael G. Groves, VC, USA; LTC George E. Lewis, Jr., VC, USA; LTC Robert L. Ridgway, VC, USA; Dr. Akira Shirai, Ph.D.; MAJ (P) Stanley C. Oaks, Jr., MS, USA; MAJ (P) John C. Twartz, RAAMC.

Assistants: Dr. Lim Thuang Seng, M.S., Ph.D.; Dr. Ng Kee Peng, Ph.D.

Associates: Miss Elsie Gan, B.S.

CLONING OF RICKETTSIA TSUTSUGAMUSHI AND ISOLATION OF AVIRULENT STRAINS

Background and Objectives: Antigenic heterogeneity is a well established characteristic of R. tsutsugamushi strains. Many isolates from humans, rodents, and chiggers have been shown by the direct fluorescent antibody (FA) test to be multiply-reactive to as many as six strains (10,35,38-42). The determination of whether this multiple reactivity is the result of a mixture of strains or of a single strain expressing a mosaic of antigens is important to the selection of potential vaccine strains.

The predominant antigens in R. tsutsugamushi isolates from countries in the endemic region are Karp or Karp-related (35-42). The Karp-related antigens are those expressed by the TA716, TA763 and TA686 strains, two of which (TA716 and TA686) are avirulent for mice. The majority of isolates that react with the Karp-related strain antigens by direct FA appear to be mixtures of two or more strains.

Characterization of scrub typhus rickettsial antigens in infected Leptotrombidium arenicola and L. fletcheri chiggers by direct FA reveals that there are shifts in the predominant antigen(s) from one generation to the next (39). Knowledge of the reason for this phenomenon could be important to vaccine development, particularly if the shifts are due to a variance in the expression of specific antigens by a single strain of R. tsutsugamushi.

The objectives of this project are: (i) to determine if certain isolates of R. tsutsugamushi are composed of single strains or are mixtures of two or more strains, (ii) to isolate clones of R. tsutsugamushi that are avirulent in mouse and monkey models, and (iii) to examine the shifts in predominant antigens that occur from one generation of R. tsutsugamushi-infected Trombiculid mites to the next generation.

The use of the plaquing technique (26) to clone isolates of R. tsutsugamushi will allow us to find the answer to the fundamental questions mentioned above. This technique allows us to separate out individual clones from a particular isolate, which can then be tested for reactivity to each of the eight prototype strain antigens by the direct FA test. If an isolate contains a mixture of several strains, we should be able to find clones that are reactive to only one strain each.

Cloning of isolates to satisfy our first and third objectives will be done in irradiated L-929 cells. However, for isolation of avirulent clones (objective ii), it will be necessary to establish a procedure for plaquing in a cell line suitable for vaccine work.

Progress: The plaquing and cloning procedures, using irradiated L-929 cells, have been successfully introduced into this laboratory. Unfortunately, the sole source of gamma radiation in the Kuala Lumpur area was unavailable for a 3½ month period (April-July), due to its being relocated. Work is now continuing on the cloning of local R. tsutsugamushi isolates. The first clones should be available late this year.

The MRC-5 diploid cell line has been acquired by the USAMRU-M from the American Type Culture Collection, is growing well, and stocks have been frozen away for back-up use. This cell line is acceptable for use in vaccine development and production. We are presently working out the parameters necessary for the plaquing of R. tsutsugamushi in monolayers of these cells. We anticipate this will require 8-10 months.

The plaquing of isolates from successive generations of chiggers is of a lower priority and has been delayed.

Recommendations and Future Objectives: This multiple objective project is so large and complex that we are proposing that it be split into individual projects during the 1983/1984 period. Due to the high priority we have placed on completing the first two objectives, and the labor intensive characteristic of each, we have deleted the third objective.

ANTIGENIC ANALYSIS OF RICKETTSIA TSUTSUGAMUSHI STRAINS FROM ENDEMIC AREAS

Background and Objectives: Information on the prevalence and distribution of R. tsutsugamushi strains is an essential part of a program to develop an effective scrub typhus vaccine. Thus far, research in this laboratory has shown that five of the eight prototype strains are predominant in isolates from Peninsular Malaysia, Thailand, Taiwan, the Philippines, Hong Kong, Australia and the islands of the Northern New Hebrides and Santa Cruz groups (35-42). While these areas represent a large segment of the endemic region, isolates from some of the countries on the periphery have not yet been obtained and characterized.

The objective of this project is to determine the geographic distribution of strains of R. tsutsugamushi within the endemic region.

The USAMRU-M has been collecting samples from various countries in the Asiatic-Pacific area for several years. These are being characterized using the direct fluorescent antibody (FA) test and a distribution pattern for each antigenic type is being developed. This will guide us in determining which strains must be included in a vaccine in order for it to be effective.

A recent technological advancement has allowed for the production of hybrid cell lines (hybridomas) which make monoclonal antibodies. These antibodies react with a single antigenic determinant on an organism, thus making it possible to discriminate between cross-reacting strains by isolating hybridomas that secrete antibody specific for an antigen exclusive to each strain of the organism. A battery of these monoclonal antibodies, used to prepare conjugates for direct FA, would allow for a very detailed antigenic analysis of new and existing R. tsutsugamushi isolates.

Progress: Isolates of R. tsutsugamushi from human, animal, and chigger sources have been obtained from Malaysia, Australia, Thailand, the Philippine Islands, Melanesia, the Pescadores Islands (Taiwan), and China. These have been partially characterized, using the direct FA test. The isolates from China were only recently received and are in the process of being propagated and characterized. Isolates from Japan, West Pakistan, and other countries are being actively sought in an effort to produce a more complete description of the distribution and prevalence of strains.

The hybridoma technology (21) has been introduced into the laboratory and several hybridomas producing antibodies specific for the TA716 strain have been isolated. However, upon recovery of these hybridomas from the frozen state (liquid N_2), we found that the antibody titer of ascitic fluid induced by the hybrid cells was substantially lower than prior to freezing (1:6,400 compared with 1:200). This was unexpected yet not without precedence. We are recloning these lines to determine if this was due to a non-producer which has overgrown the original line.

In attempting to develop hybridomas specific for other strains of R. tsutsugamushi, we have been experiencing very low yields of antibody producers, which on cloning, are being lost. The low yield appears to be due to the fact that antibody titers in the mice, used for obtaining spleen cells for the fusions, are low (1:50-1:200). It has been suggested that there is a direct relation between the yield of antibody producing hybrids and the antibody response of the donor animal. Modifications are being made to the mouse immunization procedure in order to stimulate a better response.

Recommendations and Future Objectives: We have developed a good perspective of the prevalence of the different strains in most of the scrub typhus endemic region. During the coming year, we will obtain and characterize isolates from those areas for which we have little or no reliable information. We have proposed the continuation of this vital project in the 1983/1984 grant proposal.

In regard to the hybridomas, we should have several new hybridomas available before the end of this grant period (31 Dec 82).

RICKETTSIA TSUTSUGAMUSHI INFECTION OF HUMAN ENDOTHELIAL CELLS IN VITRO

Background and Objectives: R. tsutsugamushi has been grown in primary cells and established cell lines, but a comparison of these in vitro studies to the in vivo pathogenesis of experimental infection or clinical disease is hampered by several factors. Established cell lines represent cells that have "dedifferentiated" both structurally and functionally from their tissue of origin. In addition, previous work has frequently been done using irradiation or chemical treatment of the cells to inhibit their multiplication. Endothelial cells,

however, maintain their differentiated structural and functional attributes when cultured in vitro (15,19,24). Since the endothelial cell appears to be a key "target cell" in the scrub typhus disease process (1,3,6,22,27), this model system is being explored as an ideal system for controlled studies of the growth and cytopathology of R. tsutsugamushi.

The objective of this project is to examine the growth characteristics and pathologic effects of R. tsutsugamushi in cultured human endothelial cells.

Cultured human endothelial cells, both R. tsutsugamushi infected and uninfected, are being examined by light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). In addition, the growth rate of the rickettsiae is being determined by titration of infected cell samples (taken at specific time intervals) in mice. These results will be compared with those from concurrent experiments using an established cell line.

Progress: The procedure for the isolation and subculturing of endothelial cells obtained from human umbilical cords (12) has been successfully introduced into this laboratory. R. tsutsugamushi-infected and uninfected endothelial and L-929 cells have been examined by SEM. The electron micrographs are presently being examined and will be compared with the TEM photos. Specimens are being prepared for ultra-thin sectioning, staining and subsequent examination by TEM. In addition, both infected and uninfected cells will be stained with hematoxylin and eosin, a standard pathologic stain, and will be examined by light microscopy. Pathologic examination of the cells will be done in collaboration with Dr. Michael Elwell, a veterinary pathologist located at the U.S. Army Laboratory (AFRIMS) in Bangkok, Thailand.

Titrations of rickettsiae in endothelial cells on days 0, 4, 8, 12, and 16 post-infection are presently being done.

Recommendations and Future Objectives: This project is progressing nicely. Due to technical problems with the TEM, we have been unable to proceed as rapidly with that portion as we have with the SEM.

We have proposed that this project be continued into the next grant period, so that we can complete it and also attempt to plaque R. tsutsugamushi in endothelial cell monolayers.

STUDIES IN CELL-MEDIATED IMMUNITY

Background and Objectives: Cell-mediated immunity (CMI) plays an important role in the protection of animals and man from intracellular infections (23,45). This has been substantiated in R. tsutsugamushi infections using the mouse model. Mice are protected from heterologous strain infections by the transfer of T, but not B, lymphocytes from immune mice (8,34).

In vitro correlates of CMI have been shown for many classes of rickettsiae: Coxiella burnetii - macrophage migration inhibition (16) and lymphocyte transformation (LT)(9,20); R. typhi - LT (5); R. tsutsugamushi - suppression of rickettsial growth in mouse peritoneal cells incubated with supernatants (containing lymphokines) from immune spleen cell cultures (25).

When immune lymphocytes are incubated with antigen, non-immunoglobulin substances (which mediate cellular responses) are released into the media. One of these substances is macrophage migration inhibition factor (MIF)(4). The indirect MIF assay used in our studies compares the migration of macrophages from an agarose micro-droplet when supernatants of test or control cultures are added (14).

The objectives of these studies are: (i) to develop one or more assays to measure the cell-mediated immune response after infection with R. tsutsugamushi; (ii) to study the onset and longevity of these responses in mice, monkeys, and humans; and (iii) to determine the relevance of the assays in predicting immunity to reinfection with homologous or heterologous strains of R. tsutsugamushi.

Progress: We have continued to use membrane and soluble fractions of French pressure cell treated, tissue culture grown R. tsutsugamushi (2).

Mouse Studies -- Optimal Karp and Gilliam strain antigen concentrations for differentiating immune and normal mice in LT and MIF assays were determined for BALB/c and C3H/He mouse strains. Gilliam antigen in C3H/He mice was found to be a poor discriminator of prior infection. Antigen concentrations for LT:

Mouse Strain	Infection Strain (10^3 MID_{50} S.C.)	Membrane Antigen conc. (μg/ml)	
		Karp	Gilliam
BALB/C	Karp	50	350
BALB/c	Gilliam	50	350
C3H/He	Karp	800	350
C3H/He	Gilliam	800	350

Soluble antigens (50 μg/ml) are used in the MIF assay.

To investigate the onset, longevity and correlates of scrub typhus immunity, a large group of BALB/c and C3H/He mice were infected subcutaneously with the Karp or Gilliam strain. At selected intervals, five mice from each of the four groups were tested for LT (2,28), MIF (2,14), and indirect fluorescent antibody (IFA)(33). At the same time, five mice from each group were tested for delayed-type hypersensitivity using the footpad swelling assay (11) with killed whole rickettsiae as antigen; several mice from each group were challenged with a "lethal" dose of rickettsiae to determine their immunity:

Mouse Strain	Infecting Strain	Challenge Strain
BALB/c	Karp	Karp
BALB/c	Gilliam	Karp
C3H/He	Karp	Karp, Gilliam
C3H/He	Gilliam	Karp, Gilliam

Although it was intended that each mouse be subcutaneously infected with 10^3 MID_{50}, the dilution used resulted in an inoculation of only $10^{1.3}$ MID_{50} Gilliam and $10^{0.8}$ MID_{50} Karp (titered intraperitoneally in ICR mice). IFA antibody was detected for the first time 21 days after infection. Peak titers (up to 1:400) occurred 35-56 days after infection. Only half of the Gilliam-inoculated C3H/He mice, and none of the Gilliam-inoculated BALB/c mice, developed antibody. This reflects the low and perhaps patchy inoculum that the mice received.

Back-challenge -- Karp-infected mice were solidly immune to back-challenge of Karp (in BALB/c) and Karp or Gilliam (in C3H/He) from day 14, the first day tested. Gilliam-infected C3H/He were partially immune to Karp or Gilliam challenge from day 21 but never achieved more than 60% protection (three mice out of five). No Gilliam inoculated BALB/c mice survived Karp challenge. This result is undoubtedly related to the low titer of the inoculum. The same level of immunity has persisted for 112 days post infection.

Lymphocyte Transformation (LT) -- Mitogen responses (to PHA) were suppressed in Karp-infected BALB/c and C3H/He mice from day 11 or 14 to day 28. Stimulation indices to homologous and/or heterologous antigens were significant for most Karp-infected mice on day 7, and then were suppressed until days 28 or 35. Responses are still significant at 112 days post-infection.

Migration Inhibition Factor (MIF) -- The majority of supernatants derived from Karp-infected mice tested from days 7-28 induced migration inhibition. At the time of peak infection (days 14-21) some supernatants from cells incubated without antigen contained significant MIF activity. This activity was not enhanced in cultures of cells with antigen, and the resultant migration index (= migration distance with supernatant)

$$\left(\frac{\text{from antigen + cells}}{\text{migration distance with supernatant}}\right)$$

$$\left(\frac{\text{from media + cells}}{\text{migration distance with supernatant}}\right)$$

was greater than 0.80, the arbitrary cut-off point.

Delayed-type Hypersensitivity (DTH) -- The antigen concentration (1.0 mg/ml) for this assay was derived using mice previously infected with 10^3 MID_{50}. None of the mice from the present study gave positive responses.

Human Studies -- The aim of these studies is to determine the CMI responses of human lymphocytes incubated with rickettsial antigens in order to develop a test which is capable of predicting immunity to reinfection. Members of the staff of the USAMRU-M have been used as donors. Their past histories include clinical scrub typhus (43), exposure to R. tsutsugamushi while taking doxycycline prophylaxis (43), or no history of scrub typhus exposure.

LT responses to the Karp, Gilliam and Kato antigens at concentrations of between five and 400 µg/ml were determined for donors with one of the three possible past histories.

Concentrations of 100 and 400 µg/ml were chosen for routine use, the higher concentration being necessary to obtain a response in some subjects with previously suppressed infections. Lymphocytes from normal volunteers never responded to rickettsial antigens, even at 400 µg/ml.

Lymphocytes were isolated every six days from the eight volunteers participating in the reinfection study who had successfully taken doxycycline prophylaxis in a study 21 months earlier (43). The results of the reinfection study appear elsewhere in this report. The only two volunteers who did not have positive LT responses to rickettsial antigens before their reinfection were the same two volunteers who subsequently had significant clinical disease. One was treated for scrub typhus; the other did not fulfil the criteria established for a diagnosis of scrub typhus, and after a week of low grade fever (≤36.4C) he recovered. LT in the other subjects was suppressed at some period between days 6 and 18 after challenge. Peak LT was seen at day 60. MIF assays are not complete at this time of writing but our initial impression is that the challenge infection did not cause a rise in MIF-producing lymphocytes, as measured in our assay.

Monkey Studies -- The onset of CMI responses in monkeys having their first exposure to R. tsutsugamushi, and the correlation of baseline responses in monkeys previously infected with R. tsutsugamushi to their clinical response after reinfection, is being studied.

Baseline LT and MIF assays have been performed, and the monkeys will be infected on 9 Oct 82.

Recommendations and Future Objectives: Mouse Studies -- Suppression of LT to unrelated antigens after rickettsial infection has already been reported for spotted fever group rickettsiae (29). Specific LT in the mouse persists after recovery from infection with R. tsutsugamushi, and thus-far correlates with back-challenge survival. MIF production was shown in our study to occur only briefly (days 7-28 after inoculation).

The experiment is being repeated with a higher titered inoculum.

Human Studies -- A larger scale study of the in vitro correlates of scrub typhus immunity is planned for 1983 using a rural Malaysian population which has heavy exposure to scrub typhus. The results of the small, laboratory-based study suggest that a

positive LT response is predictive of immunity. A negative LT response must be interpreted in light of the antigen concentration used in the assay, but the absence of LT at 400 μg/ml was associated with more evidence of disease after challenge.

Monkey Studies -- These must await the end of the study (December 1982).

STUDIES ON THE AVIRULENCE OF *RICKETTSIA TSUTSUGAMUSHI* IN THE MOUSE MODEL

Background and Objectives: Cross-protection studies in monkeys have shown that Karp-related strains of R. tsutsugamushi, avirulent for both monkeys and mice, protect in many instances as well or better than virulent strains (17). We believe this indicates that a living, avirulent scrub typhus vaccine is feasible. To be acceptable, however, an avirulent vaccine must be stable with regards to virulence.

One of the difficulties in developing an avirulent vaccine is the selection of candidate strains of R. tsutsugamushi. Following the isolation, cloning, and antigenic characterization, each clone must be tested for virulence. Those found to be avirulent must be further tested for the ability to induce cross-protection against specific heterologous strains.

Currently, there is no animal system that can be used to screen potential avirulent clones of R. tsutsugamushi as candidates for use in a vaccine to be given to humans.

The objectives of this project are twofold: (i) to devise a model mouse system for use in screening R. tsutsugamushi clones for avirulence and the ability to induce cross-protection against other cloned strains, and (ii) to test the stability of several avirulent strains of R. tsutsugamushi.

Progress: Prototype R. tsutsugamushi strains, known to be of reduced virulence for mice, will be used in developing the model. These strains will be injected subcutaneously into genetically susceptible C3H/He mice (13). Twenty-one days post-infection, these mice will be sacrificed and dilutions of their spleen cells will be prepared and injected into recipient C3H/He mice (34). Recipient animals will be challenged 24 hours after cell transfer with 10^3 MLD_{50} of either the Karp or Gilliam strain, and the results compared to a homologous system,

i.e. Gilliam challenge of mice receiving Gilliam immune spleen cells or Karp challenge of mice receiving Karp immune spleen cells. As a vaccination control, a portion of each group of donor mice will be challenged on day 22 with 10^3 MLD_{50} of the Karp or Gilliam strain.

One of the implied objectives of this project is to establish a rational method for selecting potential vaccine strains. By inoculating various dilutions of spleens cells into recipient mice, we propose to quantify the protection induced by avirulent strains against virulent infections.

Even though the virulence/avirulence characteristics of R. tsutsugamushi strains appear to be extremely stable, documentation of this is important if we are to successfully pursue development of an avirulent vaccine. Therefore, serial passages of the avirulent TA678, TA686, and TA716 strains of R. tsutsugamushi were made in mice and in Vero cell monolayers. Mouse passages were done by blind passage of spleens harvested 7 days post-infection. Vero cell monolayers were harvested after 7 days and new monolayers infected with the passage material. After every 5 passes the rickettsiae were titrated in mice to test for virulence.

Testing for stability of avirulence has been completed. The three avirulent strains of R. tsutsugamushi were each passed 25 times in ICR mice and, in a separate series, 25 passes in Vero cell monolayers. Testing for virulence was done, after every five passes, by the intraperitoneal injection of ICR mice and subsequent challenge (after 28 days) with the virulent Karp strain. A preliminary analysis of the data shows that the three strains used did not regain even partial virulence after continuous passage. Due to delays in the production of C3H/He mice and the increased mouse requirements for projects of a higher priority, it was necessary to delay the mouse model portion of this project.

Recommendations and Future Objectives: We anticipate that this project will be completed within the next few months.

ENTOMOLOGICAL STUDIES

Background and Objectives: Uninfected chiggers are capable of acquiring R. tsutsugamushi by feeding on infected mice (44). This infection is subsequently passed through the various developmental stages to the adult mite, but is not transmitted to succeeding generations. Using our infected and uninfected Leptotrombidium arenicola and L. fletcheri colony chiggers, previous studies of several successive generations have demonstrated very high filial and transovarial infection rates approaching 100% (31,32). In addition, these studies have also shown that the progeny of infected mites are almost exclusively female. Field studies have shown that multiple infections can occur in chiggers (36,38,39,41,42). There are several important questions regarding chigger transmission of rickettsiae: (i) how do chiggers become multiply-infected? (ii) does the rickettsial infection influence the sex ratio in the infected mites? (iii) can R. tsutsugamushi be transmitted by infected male mites or by cannibalism of infected mite eggs by uninfected mites? and (iv) what is the minimum attachment time required for an infected chigger to transmit scrub typhus?

Electrophoresis has become an important method for studying the genetic biology of a number of species. Isoenzyme studies in mosquitoes have focused on species relationships, formal genetics, surveys of natural populations for genetic variability, and reproductive biology and behaviour. The chigger colonies maintained by the USAMRU-M enable us to initiate similar studies on mites.

Most arthropod cytogenetic studies have been done with mosquitoes. Chromosome data are available on mite species in several families of the suborder Prostigmata; however, none of the species within the family Trombiculidae, which contains the vectors of scrub typhus, have been studied. Important questions to be answered are: (i) do any cytotaxonomic differences exist among these related species? and (ii) do any genetic mechanisms, such as sex determination, contribute to the various mite dynamics?

The objectives of this project are: (i) to examine the transmission of R. tsutsugamushi by Trombiculid mites with specific reference to the genetics of transovarial transmission and the influence of infection on the sex ratio, (ii) to develop an electrophoretic technique for use in differentiating the sex and species of mites, as well as their

infection status, and (iii) to perform chromosome studies on mites to see if there is a detectable karyotypic difference between infected and uninfected mites and between mite species and sexes.

Progress: The infected mites in our L. arenicola colony carry the Karp, TA686, TA716, TA763, and Kato strains, but not Gilliam (39). These and uninfected L. arenicola chiggers will be fed on mice experimentally-infected with the Gilliam strain. Their offspring will be followed for several successive generations to determine if the Gilliam strain can be acquired and subsequently transmitted both transtadially and transovarially.

In a separate set of experiments, four lines each of uninfected and infected L. arenicola and L. fletcheri will be identified and followed through all stadia. The number of dead and their sex will be recorded for each stage. The sex determination can be done morphologically in adults and by dissection and identification of reproductive organs in the nymphophanes, nymphs, and teliophanes. Unfortunately, the sex cannot be differentiated in the larval stage. These results will be analyzed to examine the influence of infection on the sex ratio.

Large numbers of mites in our infected colonies will be allowed to mature to the adult stage and a careful search made for male mites. If one or more are found, they will be mated with uninfected females. Subsequent generations will be followed to determine the infection rate, transmission rate, and sex ratio of the progeny. In addition, nymphs and adults from uninfected mite colonies will be fed infected mite eggs and subsequent generations will be examined for possible infection by R. tsutsugamushi.

To determine the minimum attachment time, large numbers of chiggers will be fed on mice and at predetermined intervals, a portion of the chiggers will be removed from the mice. Mice will then be processed for rickettsial isolation by standard procedures. This will be done using both L. arenicola and L. fletcheri chiggers.

The electrophoretic separation of isoenzymes using starch gel electrophoresis will be attempted using newly-emerged adult L. arenicola and L. fletcheri. Infected and uninfected female and uninfected male mites will be used. If available, infected male mites will also be used. We hope to be able to

differentiate sex, species, and/or infection status using isoenzyme characterizations.

Finally, using well-established karyotyping techniques, we will attempt to find species and/or sex differences among infected and uninfected L. arenicola and L. fletcheri mites.

Evidence accumulated during the present grant period suggests the following: (i) rickettsial infection does not appear to influence the sex ratio in infected mites, (ii) mites appear to be unable to acquire an R. tsutsugamushi infection by feeding on infected mite eggs, (iii) infected males are not produced by infected female mites, and (iv) the minimum attachment times required for L. fletcheri and L. arenicola chiggers to transmit scrub typhus are 3½ and 9 hours, respectively.

In addition, one of five infected L. arenicola lines tested was able to become infected with and transovarially transmit an additional strain of R. tsutsugamushi, the Gilliam strain. However, that strain was not recovered beyond the first generation.

The isoenzyme patterns of two enzymes, phosphohexoisomerase and phosphoglucomutase, were compared for each of the laboratory mite colonies available, as well as some field-collected L. fletcheri. The results are currently being analyzed.

Cytogenetic studies have been done as described above. We have found that the mitotic chromosomes of L. deliense and L. fletcheri show 2n = 14, while those of L. arenicola indicate 2n = 28.

Recommendations and Future Objectives: In view of the limited success of our attempt to infect L. arenicola chiggers with the Gilliam strain and establish transovarial transmission, we feel that a repeat experiment is warranted. This has been programmed into our 1983/1984 grant request. In addition, the inability of the mites to become infected by feeding on infected eggs brings up the possibility that this might be due to a gut barrier. We have also requested funding of this study in the 1983/1984 grant proposal.

Additional studies proposed deal with the electrophoretic separation of isoenzymes and the differentiation of karyotypes. Our results to date justify a continuance of these projects.

EARLY DIAGNOSIS OF *RICKETTSIA TSUTSUGAMUSHI* INFECTIONS

Background and Objectives: The conventional means of diagnosing scrub typhus relies mainly on clinical signs and symptoms, with laboratory confirmation by serological methods 2-3 weeks after onset of illness. Relevant epidemiological data are often also useful. Our experience, however, has shown that the recognition of clinical disease is often difficult in highly endemic areas, because classical signs of scrub typhus (like eschar and rash) are seldom observed (7). Fever and headache are of little help diagnostically since they are also observed in many other infectious diseases. Isolation of *R. tsutsugamushi* using mouse inoculation often requires two or more months before a definitive diagnosis is made. More sensitive and specific serological methods or a more rapid means of isolating and identifying the organisms are needed.

The objective of this project is to develop one or more laboratory tests for obtaining a more rapid definitive diagnosis of scrub typhus in humans.

Several approaches to early diagnosis will be used. One procedure, latex agglutination, focuses on antigen detection; two others, radioimmunoassay and reverse solid phase ELISA (enzyme linked immunosorbant assay) detect antibodies; and the last, surface antigen detection, will look for markers found on the surface of infected cells.

Progress: Preliminary investigations have shown that agglutination does occur between *R. tsutsugamushi* and latex particles coated with scrub typhus antibody. These investigations have dealt only with the specificity and sensitivity of the procedure, and results have been promising.

Preliminary work on the radioimmunoassay and reverse solid phase ELISA (IgM capture) procedures was not encouraging. Priorities were redirected to the more promising techniques, latex agglutination and surface antigen detection.

In earlier work we had an indication of the possible presence of rickettsial-associated antigen on the surface of *R. tsutsugamushi*-infected monocytes and lymphocytes. There is a precedent for this, as virus infected cells frequently express viral antigens on their cell membranes (18,30).

Antibody against reticuloendothelial cells (RBC), from C57Bl/6 mice infected with the Gilliam strain of R. tsutsugamushi, was prepared in CBA/Ca mice. This antibody, after absorption with normal C57Bl/6 REC, was conjugated with fluorescein isothiocyanate (FITC) and tested against spleen cells of normal and acutely-infected BALB/c mice. The results indicated that the FITC-conjugated anti-REC antibody bound only to the spleen cells from the infected mice. However, the titer of this antibody was low and dilution above 1:2 was ineffective in the assay.

Recommendations and Future Objectives: We are continuing to develop the latex agglutination test. We propose to test its ability to detect antigen in sera from experimentally-infected animals and eventually, from acutely-ill, human patients. This test is one that can easily be done in the field, using pre-prepared antibody-coated, latex beads.

We are presently working on obtaining a higher-titered anti-REC antiserum for use in further testing of the surface antigen test. Mice infected with the Karp, Gilliam, and Kato strains of R. tsutsugamushi will be tested at specific intervals post-infection to determine the potential of this antibody for use as an early diagnostic tool. Human peripheral blood lymphocytes from known scrub typhus cases, as well as from uninfected control individuals, will also be used in evaluating this procedure.

REINFECTION OF VOLUNTEERS PREVIOUSLY EXPOSED TO RICKETTSIA TSUTSUGAMUSHI WHILE ON DOXYCYCLINE PROPHYLAXIS

Background and Objectives: In a recently completed study (43), human volunteers receiving weekly doxycycline chemoprophylaxis and deliberately infected with R. tsutsugamushi via Leptotrombidium fletcheri chiggers were protected from acquiring scrub typhus. Although immunity lasting for two or more years is known to result from clinical scrub typhus, the immune status of persons receiving chemoprophylaxis at the time of infection is unknown.

The objective of this study was to determine if persons infected with R. tsutsugamushi while receiving weekly doxycycline, as a scrub typhus chemoprophylactic, developed immunity to the disease.

Progress: We proposed to reinfect the original volunteers from the previous study to examine their immune status after combined chemoprophylaxis and infection. The volunteers were reinfected using L. fletcheri chiggers from the same infected colony as in the original doxycycline study. Complete physical examinations were given prior to the challenge and volunteers were examined daily for signs and symptoms of scrub typhus. Blood samples, drawn at regular intervals, were tested for the level of rickettsemia by mouse inoculation and for antibody titer by the indirect fluorescent antibody test. Cell-mediated immunity studies were planned to determine whether or not we could accurately quantify the immune status prior to the challenge.

Eight volunteers were pre-exposed to infection with R. tsutsugamushi 21 months after successfully completing the doxycycline prophylaxis trial (37). Seven of the eight resisted the rickettsial challenge, showing that long-lasting immunity to scrub typhus does develop during a suppressed infection.

Recommendations and Future Objectives: We have submitted a proposal for the coming grant period, based on these highly encouraging results, to begin a multi-phase program that would culminate in the testing of an antibio-vaccine.

ANALYSIS OF DATA FROM MAJOR EPIDEMIOLOGICAL STUDIES

Background and Objectives: In the early to mid-1970's, epidemiological studies were initiated at the Mentekab Hospital, the Bukit Mendi Health Center, and the Jengka Triangle in central Peninsular Malaysia. While these focused primarily on collecting data relating to scrub typhus, information was also obtained regarding other febrile illnesses, such as leptospirosis, typhoid fever, malaria, melioidosis, and dengue fever. This data was introduced into our computer and stored until programs could be written to accomplish the required analyses.

The objective of this project is the analysis of epidemiological data collected during several years of work in central Peninsular Malaysia.

Progress: Computer programs will be prepared that are each capable of extracting specific types of data, analyzing it, and presenting the results in a format that is useable by USAMRU-M scientists in the preparation of manuscripts.

During the present grant period, much of the data has been analyzed and two manuscripts have been prepared for publication, with a third presently being completed. Another program has been written and will be entered in the computer during the next two months.

Recommendations and Future Objectives: We anticipate that the introduction of this latest program into our computer will allow us to prepare two additional manuscripts. The completion of these will complete this project.

Project 3M672770A803 TROPICAL MEDICINE

Work Unit 007 Field Studies of Rickettsioses and Other Tropical Diseases

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*Underlining indicates the individual who presented the paper.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
				DA 0B 6525	82 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. DIS'B'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AH	161
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2.2			

11. TITLE (Precede with Security Classification Code)*

(U) Anti-Schistosomal Drug Development and Malaria Vector Immunology and Studies

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012600 Pharmacology 002600 Biology 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 07	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	2.5	103
	CURRENT 83	2.5	108

19a. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Brazil; (U) Schistosomiasis; (U) Malaria; (U) Chemotherapy; (U) Immunology; (U) Epidemiology; (U) Drug Resistance; (U) Entomology

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

23. (U) Find new prophylactic and curative drugs for the prevention and cure of schistosomiasis infections and to study the clinical, epidemiologic, drug susceptibility and vector transmission patterns of falciparum malaria in the Amazon River basin of Brazil. Both are primary diseases which would be acquired by U.S. Military and DOD civilian personnel in the event of deployment to any of numerous tropical areas of the world.

24. (U) The WRAIR Anti-Schistosomal Drug Testing Program continues to submit candidate compounds for prophylactic (PMT) and curative (PCT) testing against schistosomiasis in mice. Compounds active in the primary screen are extensively reexamined for confirmation and dose response patterns. The malaria immunology studies include the testing of sera from endemic areas by the indirect fluorescent antibody test. Malaria vector transmission studies include field and laboratory analysis of morphological, behavioral physiological and DDT susceptibility patterns of Anopheles darlingi and other potential anophelene malaria vectors.

25. (U) 81 10 - 82 09. This research is complementary to studies being conducted under DA0B 6525, work Unit 086, entitled "Chemotherapeutic Studies on Schistosomiasis". During the reporting period, 457 compounds were screened in the PCT and PMT. Of these 5 were designated confirmed or unconfirmed active and 23 were toxic. Nine compounds were tested in the SCT. Upgrading of research mouse colony facilities was begun. Mark and release studies of Anopheles darlingi at the Ituxi River Study Area are being conducted to determine dispersal patterns from possible larval breeding sites. Construction of an insectary is nearing completion at the University of Brasilia. Preliminary studies to colonize An. darlingi have been initiated. For technical report see Walter Reed Army Institute of Research Annual Report, 1 Oct 81 - 30 Sep 82.

*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 65 (FOR ARMY USE) ARE OBSOLETE

PROJECT 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit: 161 Anti-Schistosomal Drug Development and Malaria Vector Immunology and Studies

Investigators: Dr. Aluizio Rosa Prata, MD; LTC Willis A. Reid, Jr.; MAJ Anthony B. Bosworth; and Mr. Norman Peterson.

PROBLEMS AND OBJECTIVES:

1. Schistosomiasis, malaria and leishmanisais continue to pose a threat to American military personnel who are or who might have to be stationed in the Middle East, Africa, the Far East, the Caribbean or Latin America. These diseases also inhibit development and cause great human misery, a potential source of political unrest, in many of the developing countries where they are found.

2. There currently is no single drug that is a totally satisfactory treatment for schistosomiasis. It also is highly desirable that, in addition to developing better therapeutic agents, we also develop prophylactic methods, either drugs, treatments for exposed skin or treatments for the uniform, which will reduce casualties resulting from exposure to schistosomiasis. The mode of transmission of this disease is such that troops moving through or stationed within an endemic area could be expected to experience a high level of exposure and infection. This would result in unaffordable and unnecessary loss of combat strength and would result in a burden on medical facilities. It should be possible, through sustained research effort, to avoid these problems. Research with the objective of identifying potential chemoprophylactic and chemotherapeutic agents for schistosomiasis is being conducted by the US Army Medical Research Unit/Brasilia (USAMRU/Brasilia), located at the Núcleo de Medicina Tropical (NMT) of the University of Brasilia (UnB). Standardized screening procedures in a mouse - Schistosoma mansoni - Biomphalaria glabrata system are being used.

3. Since 1975, reported cases of malaria in Brazil have quadrupled, according to Ministry of Health statistics. The vast

majority of these cases occur within that portion of the Amazon River basin lying within Brazil. Almost 200,000 cases were reported in 1981, and it is possible that several times that many occurred. In urban areas of the Amazon, malaria control is practiced and there is little malaria. However, in the rural areas where most of these cases originated, malaria control is inadequate and the system of reporting cases likely reflects a very conservative estimate. Drug resistance in falciparum malaria in the Amazon has necessitated a concentration on vector control by use of traditional residual spraying of houses with DDT in an attempt to ameliorate the serious effect malaria has on the rural population. However, residual insecticide usage has had a limited beneficial effect, because many houses are only partially enclosed and because DDT has been demonstrated to have a marked repellent effect on the primary malaria vector in the region, Anopheles darlingi. Vector biology studies by USAMRU/Brasilia have as their objective the development of sufficient knowledge about An. darlingi to permit the formulation of a practicable vector control strategy for this species under the above conditions.

4. Cutaneous and mucocutaneous leishmaniasis are zoonoses with many wild mammal reservoirs. It is widespread in Brazil. This vector borne disease is difficult to diagnose. Culture of some strains of the etiologic agent can not be done reliably. Treatment is extended, involves the use of toxic drugs and often has to be repeated because of ineffectiveness and/or relapse. It is difficult to confirm cure. The disease is potentially hideously disfiguring and may have a fatal outcome. The many days required for therapy and the detailed follow-up required to confirm cure would constitute an extreme burden on medical facilities. The grossly disfiguring effects of advanced mucocutaneous leishmaniasis would horrify and have a negative psychological affect on troops, unless they could be given genuine assurances. It is very important that we learn to prevent this disease. It is highly relevant to the development of locally effective control strategies that the animal reservoirs for the disease be identified. Leishmaniasis research at USAMRU/Brasilia has the objective of determining the reservoirs for leishmaniasis at a study site where the scope of transmission appears to have been extended to women and children, concomitant with habitat modification in government encouraged agricultural development.

PROGRESS:

1. Schistosomiasis: Following a period of interrupted testing activity due to inavailability of adequate numbers of mice of

desired quality, antischistosomiasis drug testing was resumed in October, 1981. In the Primary Mortality Test (PMT) system, designed to detect chemoprophylactic activity against Schistosoma mansoni, 604 compounds were given initial tests. Toxicity was noted in 161 of these compounds. Three compounds demonstrated significant antischistosomal activity. Retesting in the PMT was done on an additional 82 compounds. Of these, 21 were toxic. No significant antischistosomal activity was detected in retested compounds. In the Primary Curative Test (PCT) system, designed to detect therapeutic potential, 296 compounds were given initial testing. Toxicity was noted in 59 of these compounds. Sixteen compounds demonstrated significant antischistosomal activity. Retesting was done on 38 compounds in the PCT. Of these, 12 were toxic. Significant antischistosomal activity was detected in 3 of the retested compounds. Two compounds were tested in the Secondary Curative Test, designed to provide information on dose, time-to-action, and preferred route of administration of new compounds which demonstrate markedly significant antischistosomal activity. Many of the compounds tested are proprietary, and it is part of our agreement with the supplier that information regarding these specific compounds will not be generally distributed.

Reinitiation of testing was made possible by the establishment of a mouse colony in the Núcleo de Medicina Tropical, while the Central Bioterio was being partially renovated. This renovation is completed. Although it does not conform strictly to the high standard practiced in the United States for colonies producing animals for toxicological and drug testing, it is much improved. Breeding stock from the mouse colony at the Núcleo has been delivered to the Bioterio to reinitiate a colony there. We hope that mice of the quantity and quality needed can be produced there. Our snail colony remains capable of supporting our testing programs at full operational level.

2. Malaria: Successful mark, release and recapture studies of An. darlingi showed that this species could fly long distances (1 to 2 km) in less than a day and that it has strong human host seeking behavior. High percentages of marked mosquitoes (18.5%, 4.0%, 3.5% and 0.5%) from 4 release sites at about 1 km distances from the study area were recaptured at the study site within 8 days. Marked mosquitoes released from 4 additional release sites at 1.5-2 km distances from the study area were also recaptured at high percentages (6.0%, 1.0%, 0.5% and 0.5%). Larval surveys for Anopheles mosquitoes were made in conjunction with these studies in an effort to find breeding grounds. No An. darlingi immatures were found. Two separate tests for two different rates of fenitrothion application (1g/m^2 and 2g/m^2) showed that

the rates of application were not significantly different with regards to total numbers of mosquitoes which exited treated chambers. However, mosquito movement from chambers with treated paper was markedly higher than movement from the untreated control chambers. Mortality of mosquitoes was 72.6% and 78.8% of the total number tested in the chamber with $1g/m^2$ and $2g/m^2$, respectively. A field technician from Labrea was trained at UnB in Brasilia. During one month, he learned mosquito larval and adult surveillance techniques, data card records procedures, proper storage techniques and other entomological procedures. He was also trained to operate a radio station which is anticipated to be placed in the entomology laboratory in Labrea during the coming fiscal year. Over 500 female An. darlingi mosquitoes were collected near the city of Labrea and returned to UnB. These were used to produce mosquitoes for colonization attempts. This species has rarely been successfully colonized. Mating studies using 1,000 males and 1,000 females were conducted in a newly constructed insectary. Each day for 15 days, mating was checked by dissecting and examining 10 spermathecae; 30 mosquitoes were also blood fed and left for oviposition. The spermathecae were negative for spermatozoon, and no viable eggs were deposited. Force mating techniques were tried on over 100 females with over 300 males. These were also unsuccessful, even though force copulated pairs appeared to secure and clasp well. A 30 hour course entitled "Arthropods and Mollusks of Medical Importance" was given to 10 physicians at the Núcleo de Medicina Tropical. Introductory lectures were given in medical entomology, insect physiology and morphology, insect toxicology and biological control. Support for malaria serology studies was continued at only a low level because of the inavailability of an investigator to direct the effort. However, 858 examinations of sera by fluorescein labeled anti-humans IgG and anti-human IgM in indirect fluorescent antibody tests demonstrated the high level of malaria seropositivity we have previously seen in the Amazon River basin. Additionally, 40 sera from the Ituxi River area, examined at WRAIR to confirm locally acquired results, were 100% positive for the presence of IgG antimalaria antibodies.

3. Leishmaniasis: Arrangements were made for necessary space at the field laboratory maintained by the Núcleo de Medicina Tropical (NMT) in Três Braços for processing the potential mammalian reservoirs of leishmaniasis collected there and for storage of traps and other equipment. The hamster breeding and housing facilities were expanded to provide animals to use for inoculations in isolation, identification and diagnosis. A field vehicle has also been made available. Close coordination is being maintained between the epidemiological, clinical and parasitological studies being conducted by the NMT and the

present study to avoid duplication of effort and to reap the benefits of a multidisciplinary approach to research on this disease. From June through September, 1982, 24 nights of trapping produced 79 mammals. The secondary scrub habitat produced 76 mammals of 12 species (1 Metachirus nudicaudatus*, 1 Marmosa parvidens, 2 Marmosa murina*, 6 Didelphis albiventris, 9 Oryzomys capito*, 1 Oryzomys concolor*, 1 Oryzomys fulvescens, 2 Nectomys squamipes*, 39 Zygodontomys lasiurus, 8 Oxymycterus sp., 1 Holochilus brasiliensis and 5 Rattus rattus*), during 742 trap nights. Capture rate was one mammal per 9.8 trap nights. Two rodents (1 Zygodontomys lasiurus and 1 Oxymycterus sp.) were collected in the bananna and cacao plantation during 93 trap nights. One marsupial (Metachirus nudicatudatus) was captured in the tall humid forest during 141 trap nights. The species denoted by(*) have been identified as reservoir hosts for neotropical leishmaniasis elsewhere. Tissue specimens (skin, spleen and liver) from all mammals captured were inoculated intraperitoneally and into the feet of hamsters. These hamsters are being observed, but have not yet demonstrated signs of leishmaniasis infection. In only a small proportion of tests has sufficient time lapsed to suspect a negative determination. Domesticated dogs have previously been demonstrated to be infected in the study area.

RECOMMENDATIONS:

1. Increase emphasis on screening of compounds in the Primary Mortality Test, which provides an index of prophylactic potential.

2. Add to our screening system the anti-cercarial penetration screening protocol recently discontinued at WRAIR.

3. Continue studies of the dynamics of An. darlingi populations and movements in the study area on the Ituxi River.

4. Continue attempts to locate the larval breeding sites of An. darlingi in the study area.

5. Establish an Anopheles surveillance program in the town of Labrea near our study area on the Ituxi River.

6. Attempt to document natural malaria infections of An. darlingi and other species of Anopheles in our study area.

7. Continue efforts to colonize An. darlingi at the Núcleo de Medicina Tropical.

8. Continue the extensive program to capture mammals from all available habitats (e.g. tall forest, secondary forest, secondary scrub, cropland, pastureland and domiciliary) in the vicinity of Três Braços.

9. Conduct an intensive trapping program to capture mammals in and around houses where current leishmaniasis transmission in humans has been documented.

10. Study the ecology of the reservoir species and determine which control methods are practicable.

11. Collect ectoparasites and endoparasites, and preserve pathological specimens from the captured animals.

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This bibliography includes work done by the Transamazon Project previously stationed in Belém, Brazil.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OB 6530	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORG'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AH	162
b. [redacted]				
c. [redacted]	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: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	7.0	190
FISCAL YEAR CURRENT 83	7.0	191

19. RESPONSIBLE DOD ORGANIZATION

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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)Goat;(U)Immunity

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

23. (U) The objective of this program is to develop an effective, practical vaccine against African trypanosomiasis, useful to both military and civilian agencies. Related benefits include acquisition of knowledge pertaining to trypanosome immunity, host response and pathology of infection. There is a requirement for these studies which should provide a basis for rational development of a vaccine for this disease which would constitute a serious hazard for military personnel operating in the endemic area. 24. (U) Experiments conducted at WRAIR and in Kenya have demonstrated that experimental animals can be successfully immunized with irradiated trypanosomes. Rodents, cattle and monkeys can be rendered completely resistant to a challenging infection of T. rhodesiense. 25. (U) 81 10 - 8209 During this period the investigators continued to monitor the antigenic stability of parasites from western Kenya. Studies were conducted to assess the possible role of mechanical transmission in spread of T. rhodesiense. In a cow-cow study it appeared that this mode of transmission is unlikely to play a major role and would not therefore negate a metacyclic vaccine. It is not possible to totally rule out this form of transmission in cow-man or man-man epizootics but the negative data would not support a human volunteer study. In conjunction with local health authorities extensive patient follow up studies have been initiated to determine the extent of treatment failure/relapse and reinfection after a course of therapy recommended by WHO. An experimental model utilizing the goat is being evaluated since it appears that uniform central nervous system disease can be produced in a short period of time. This CNS disease is uniformly fatal if not successfully treated. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 October 1981 - 30 September 1982.

Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

PROJECT 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 162 Vaccine Development in Trypanosomiasis

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Introduction

Vaccine development remains a high priority in African trypanosomiasis research efforts. The Lambwe Valley, Kenya study area countinues to yield a stable serodeme of T.b. rhodesiense. The 1980-81 zoonotic in humans and domestic animals was brought under control in Feb-Apr 1981 largely as a result of an aerial application of insecticide. Monitoring of the tsetse population and epidemiologic surveys of human and cattle populations were initiated immediately after the spray campaigns and continue to the present. These studies will furnish new data on the effect of environmental pressures on tsetse and trypanosome populations.

Visceral leishmaniasis in East Africa, although subjected to much study, remains poorly understood. There is a paucity of data concerning host-parasite-drug interactions. Adequate second and third line drugs do not exist and the subject of parasite resistance vs patient non-responsiveness to therapy remains a subject of debate. Vector-reservoir relationships also are poorly understood. Objectives include better documentation of the action of available drugs both in vivo and in vitro, better definition of "resistance", biochemical typing of both parasites and vectors, and expansion of vector-reservoir field studies.

African Trypanosomiasis

Approximately 11,300 individuals from 1,300 households were either interviewed or accounted for by the head of the household. Figure 1 shows the survey areas and the location of cases. During the survey blood films were prepared for microscopic examination. Individuals with histories or clinical signs of trypanosomiasis were contacted later for more extensive laboratory workups. These examinations included the collection of blood for rodent subinoculation, complement fixation studies and serum protein electrophoresis. Analysis of these specimens is underway.

A limited survey of Kisegi (Fig 1) was performed in the vicinty of the home of a patient that had no contact with the Lambwe Valley. Two hundred fifty-two (252) humans were examined and 157 cattle. No further human cases were identified but 15 of 157 (10%) cattle were found to have Trypanosoma brucei type parasite by rodent subinoculation. G. pallidipes was not found in the area but G. fuscipes were collected in moderate numbers. G. fuscipes feeds predominately on cattle but will bite man. Studies are underway to characterize the parasites isolated from cattle and to compare these to those collected from humans and cattle in the Lambwe Valley.

New patients began to present in Oct 1981 but remained few in number until July 1982. From July to October 1982 approximately 20 cases presented to Homa Bay District Hospital. A corresponding increase in isolates of T.b. brucei from cattle and a return of tsetse flies in numbers equal to or greater than found in the Lambwe Valley during the 1980-81 outbreak strongly suggest the onset of a new transmission cycle which may become epidemic in scope.

The Kenya Trypanosomiasis Research Institute is in the process of establishing a central treatment facility for the care of sleeping sickness cases. This decision has been prompted by the sudden increase in patient load placed on the Homa Bay District Hospital, the need to have a more specialized facility and staff to adequatly evaluate and treat these patients and the need for a research-oriented patient care facility if clinical drug trials were to be performed. The Walter Reed Army Institute of Research has been requested to provide a clinician and to participate in this program.

Mechanical transmission studies utilizing a bovine-tsetse-bovine system in the laboratory indicate that this mode of transmission is probably not a significant factor in field transmission.

Interrupted feedings of teneral, laboratory-reared Glossina morsitans morsitans were used to study mechanical transmission of Trypanosoma brucei rhodesiense. Intervals between exposure of individual flies on parasitemic rats and refeedings on clean rats were varied from 5 min to 24 hr. Direct transmissions were demonstrated at each interval up to 160 min post-exposure. Proboscis dissections showed that active trypanosomes were present up to 320 min post exposure. No mechanical transmissions from bovine-to bovine occurred in 39 attempts, when groups of 20-120 flies exposed on parasitemic bovines were transferred immediately to uninfected cattle, but 2/40 individual flies exposed on parasitemic bovines mechanically transmitted trypanosomes to clean rats. Proboscis dissections done immediately after flies were exposed to a bovine with a parasitemia of 4.8×10^4 trypanosomes per mm^3 of blood showed that 11/20 (55%) had active trypanosomes in the food canal. The mean number of trypanosomes per proboscis (±1SD) was 29.4 (±20.5). Of 20 flies exposed on a bovine with a low parasitemia, however, only 1 trypanosome was seen in proboscis dissections. The parasitemia of the infected donor was an important factor in mechanical transmission. It appeared that an individual mechanically infected fly might not transmit a human-infective dose during refeeding. Previous work demonstrating transmission by probing and the more frequent feedings of infected flies more likely explain high transmission rates with a low percentage of infected flies.

Leishmaniasis

A comparison of three dosage regimens of sodium stibogluconate (Pentostam(R)) in the treatment of visceral leishmaniasis in Kenya was undertaken. Previously untreated patients were randomized to receive 31 doses of sodium stibogluconate, 10 mg Sb/kg per dose, administered once daily for 31 days (group A), every 12 hours for 15 days (group B) or every 8 hours for 10 days (group C). Of the 29 patients who completed treatment, 26 appeared cured 3 to 12 months later. Two patients in group B who initially responded to treatment relapsed 6 weeks after discharge but appear to have been cured by further treatment with sodium stibogluconate at 20 mg Sb/kg/day for 60 days. A third patient in group B failed to respond to initial treatment. None of the treatment regimens was toxic. Parasites disappeared from splenic aspirates more quickly and hemoglobin levels rose more rapidly in patients receiving sodium stibogluconate every 8 hours. Treatment of visceral leishmaniasis in Kenya with sodium stibogluconate at a dose of 10 mg Sb/kg every 8 hours for 10 days appears to be a safe and effective alternative to conventional treatment.

Quantitation of amastigotes of Leishmania donovani in smears of splenic aspirate from patients with visceral leishmaniasis. During a 19 month period, more than 500 splenic aspirations were performed in 79 patients with suspected or proven visceral leishmaniasis. The two complications which occurred (intra-abdominal bleeding and penetration of the intestine in one patient each) both resolved with conservative management. Parasite density in splenic aspirate smears was graded on a logarithmic scale from 0 (no parasites in 1,000 microscopic fields) to 6+ (> 100 parasites per microscopic field). Among 39 newly diagnosed and 17 relapsed or drug resistant patients with visceral leishmaniasis, the average initial parasite grade was 4.29 ± 0.97 (mean ± s.d.) and 4.15 ± 1.37, respectively. The grading system was useful in measuring the speed of response to treatment and in distinguishing slow responders from non-responders. This was especially valuable for managing patients with drug-resistant visceral leishmaniasis. The system also provided a means of comparing the efficacy of different tratment regimens and for calculating the optimum duration of treatment.

A comparison of microscopy and culture in the detection of Leishmania donovani from splenic aspirates. Three culture media were compared with Giemsa stained smears for the detection of Leishmania in splenic aspirates from Kenyan patients with visceral leishmaniasis. Ninety-nine splenic aspirates obtained from 26 patients at various times before, during and after treatment were cultured in Schneider's Drosophila medium and RPMI medium 1640, both supplemented with 20% fetal bovine serum, and McConnell's modification of Senekje's medium overlayed with 0.9% saline. From 13 splenic aspirates obtained before treatment, amastigotes were identified microscopically in all and promastigotes were cultured

in 12. During and after treatment. Schneider's medium was the most sensitive method for detecting parasites, followed by microscopic examination of stained smears which was more sensitive than either of the other two media tested.

Experimental East African cutaneous leishmaniasis. Eleven strains of cutaneous leishmania (8 East African, 2 Old World and 1 New World) were inoculated into BALB/c mice and lesion development and progression of infection were studied. BALB/c mice were susceptible at varying degrees to 8 of the 11 strains tested. In general, infections with Leishmania aethiopica were variable and inapparent. Parasites could be cultured from the noses of infected mice, however no swelling or lesions appeared. One strain of L. aethiopica produced lesions in 2 of 5 mice inoculated at 60 days post-inoculation. L. aethiopica infections did not visceralize in BALB/c mice. Inoculation with L. tropica minor also resulted in parasites in the nose without visible lesions. L. mexicana lesion development was slow and progressive with visible nose swelling beginning at 40 days PI. Visceralization did not occur with either L. tropica minor or L. mexicana in this strain of BALB/c mice. Inoculations with L. major produced fulminating, fatal infections in BALB/c mice. Visceralization and metastasis of lesions occurred in all animals. Albino WRAIR mice and golden hamsters were also susceptible to L. major.

A survey to examine small mammals for leishmanial parasites was initiated in the Perkerra Settlement Scheme, Baringo District, Rift Valley Province, Kenya. A total of 789 animals of 10 different species were trapped and examined. Leishmanial parasites were isolated from the spleens of 9 animals of 5 different species: 7 from Tatera robusta, 2 from Taterillus emini, 5 from Arvicanthis niloticus, 1 from Aethomys kaiseri and 2 from Mastomys natalensis. The isolations of Leishmania from Taterillus and Aethomys are the first recorded from these rodents in Africa.

Transmission of Leishmania donovani by experimentally infected phlebotomine sandflies. Evidence that the sandfly Phlebotomus martini Parrot is a vector of Leishmania donovani in Kenya includes its anthropophilic biting habits, its presence in areas where kala-azar is endemic or epidemic and the isolation, from this species, of leishmania parasites which are infective to man and indistinguishable, based on enzyme typing, from human-derived strains of L. donovani Additional confirmation of the vector status of this sandfly is provided in the present study. P. martini females, from a recently established laboratory colony, were experimentally infected with L. donovani and subsequently transmitted the parasite to hamsters while taking a blood meal.

Two problems arise in doing transmission work with P. martini. Hamsters, infected with L. donovani are not, in our experience, always infective to sandflies that feed on them; and female sandflies, which produce eggs following a single blood meal, usually die in the act of oviposition and are therefore not available to take a second 'transmission' blood meal. To circumvent these problems, fly infections in the present study were achieved by membrane feeding 2 day old females on cultures of L. donovani promastigotes (RPMI 1640 plus 20% FBS; 10^5 parasites/ml). All P. martini fed this way develop heavy midgut infections but do not produce eggs. Such flies continue to display normal biting behavior and can therefore be used in attempts to infect hamsters.

To assess the vector competence of P. martini with this system, 20 females were infected with L. donovani via membrane feeding, held for 7 days, then allowed to engorge on uninfected hamsters (5 females/hamster). The hamsters were held for 45 days at which time cultures (RPMI 1640 plus 20% FBS) were made to detect the presence or absence of parasites. Three out of 4 hamsters bitten by P. martini were culture positive for L. donovani. Larger scale experiments, using different concentrations of parasites for fly infection are currently underway.

Phlebotomus (Phlebotomus) duboscqi from Kenya: a new record. A group of light trap-captured sandflies collected in Baringo District, Rift Valley Province; Kenya (0° 30'N. Lat., 36°E long); included a single male which has been identified as Phlebotomus (Phlebotomus) duboscqi Neveu-Lemaire, 1906. This is a new record for Kenya and represents the first time any member of this medically important subgenus has been taken in the country.

In conjunction with this finding it is interesting to note that P. duboscqi is thought to be a vector of cutaneous leishmaniasis (Leishmania major) in a wide area of North Africa. While no cases of human disease due to L. major have been reported in Kenya, Leishmania from rodents, captured at Baringo, are biochemically and serologically identical to parasites isolated from human cases of cutaneous leishmaniasis in Senegal suggesting a reservoir of L. major in Baringo. The presence, here in Kenya, of an L. major-like parasite together with a putative vector of cutaneous leishmaniasis is an interesting situation that merits further investigation.

RECOMMENDATIONS

African trypanosomiasis

It is recommended that the Lambwe Valley study be continued with emphasis on case followup and evaluation of the demographic data. The typing studies using VAT, isoenzymes and neutralization techniques should continue and be coupled with attempts to identify immunologically important antigens. In the light of the reported relapse data, increased efforts should be made to reevaluate existing drugs and added emphasis should be placed on new drug development. The use of serological testing should be expanded.

Leishmaniasis

Drug efficacy and pharmacokinetic studies should continue on currently available compounds until such time as new compounds or new formulations are available for field trials. Vector-reservoir field studies should be expanded. Controlled biochemical typing, morphologic taxonomy and transmission studies should be implemented as colony raised sandfly become available.

Publications

1. Beach, R.F., Young, D.G. and Mutinga M.J.: Phlebotomus (Phlebotomus) duboscqi from Kenya: a new record. Trans. Roy. Soc. Trop. Med. Hyg. 76(5), 707.

2. Preston, J.M., Kovatch, R.M. and Wellde, B.T.: Trypanosoma congolense: Thrombocyte Survival in Infected Steers. Exp. Parasit. 54, 129-133.

3. Roberts, L.W., Boyce, W.L. and Lyerly, W.H.: Cordylobia anthropophagia (Diptera: Calliphoridae) Myiasis in an Infant and Dog and a Technique for Larval Rearing. J. Med. Ent. 19(3), 350-351.

Manuscripts In Press

1. Beach, R., Young. D.G. and Mutinga, M.J.: New Phlebotomine Sandfly Colonies: Rearing Phlebotomus martini, Sergentomyia schwetzi and Sergentomyia africanus (Diptera: Psychodidae). J. Med. Ent.

2. Chulay, J.D., Bhatt, S.M., Muigai, R., Ho, M., Gachihi, G., Were, J.B.O., Chunge, C. and Bryceson, A.D.M.: A Comparison of Three Dosage Regimens of Sodium Stibogluconate (Pentostam$^{(R)}$) in the Treatment of Visceral Leishmaniasis in Kenya. J. Inf. Dis.

3. Chulay, J.D. and Bryceson, A.D.M.: Quantitation of Leishmania donovani Amastigotes in Splenic Aspirate Smears. Am. J. Trop. Med. Hyg.

4. Chulay, J.D., Haynes, J.D. and Diggs, C.L.: Plasmodium falciparum: Assessment of in vitro Growth by [^{3}H] Hypoxanthine Incorporation. Exp. Parasit.

5. Lightner, L.K., Chulay, J.D. and Bryceson, A.D.M.: A Comparison of Microscopy and Culture in the Detection of Leishmania donovani from Splenic Aspirates. Am. J. Trop. Med. Hyg.

6. Wellde, B.T., Chumo, D.A., Adoyo, M., Kovatch, R.M., Mwongela, G.N. and Opiyo, E.A.: Haemorrhagic Syndrome in Cattle Associated with Trypanosoma vivax Infection. Trop. An. Hlth. Prod.

Manuscripts Submitted

1. Chulay, J.D., Spencer, H.C., Warshow, M.M., Mson, L., Saio, M.A., Musoke, S.S., Masembe, J.B., Rollins, A.J. and Churchill, F.C. II: Fatal Chloroquine Resistant Plasmodium falciparum Malaria from Tanzania.

2. Lightner, L.K.: In Vitro Cultivation of Kenya Leishmania

3. Lightner, L.K. and Reardon, M.J.: Dipetalonena dracunculoides in Dogs and Spotted Hyena (Crocuta crocuta) in the Turkana District of Kenya.

4. Lightner, L.K., Reardon, M.J. and Giture, J.I.: Isolation of Leishmanial Parasites from a Dog in the Turkana District of Kenya.

5. Roberts L.W., Wellde, B.T., Reardon, M.J. and Onyango, F.K.: Mechanical Transmission of Trypanosoma brucei rhodesiense by Glossina morsitans (Diptera: Glossinidae).

6. Spencer, H.C., Kipingor, T., Agere, R., Koech, D.K. and Chulay J.D.: Plasmodium falciparum in Kisumu, Kenya: differences in sensitivity to amodiaquine and chloroquine.

7. Spencer, H.C., Masaba, S.C., Chulay, J.D. and Nguyen-Dinh, P.: Field Evaluation in Kenya of the 48-Hour In Vitro Test for Plasmodium falciparum Sensitivity to Chloroquine.

8. Watkins, W.M., Sixsmith, D.G. and Chulay, J.D.: The Activity of Proguanil and Its Metabolites, Cycloguanil and p-Chlorophenylbiguanide, Against Plasmodium falciparum In Vitro.

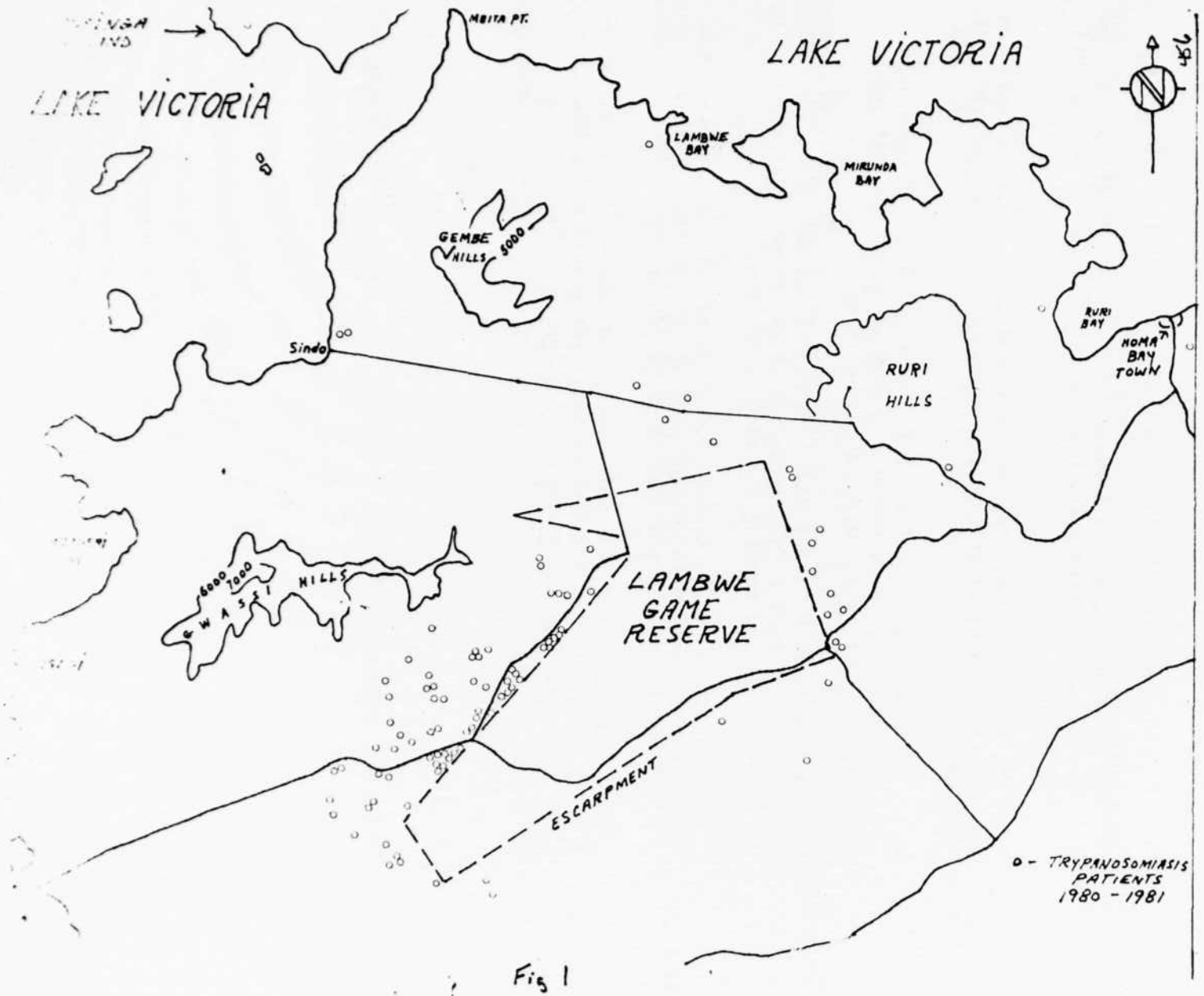
LAKE VICTORIA
MBITA PT.
LAKE VICTORIA
LAMBWE BAY
MIRUNDA BAY
GEMBE HILLS 5000
RURI BAY
HOMA BAY TOWN
Sindo
RURI HILLS
6000
7000
WASSI HILLS
LAMBWE GAME RESERVE
ESCARPMENT
o - TRYPANOSOMIASIS PATIENTS 1980 - 1981

Fig 1

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6500	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62770A	3M162770A871	AD	163 WWI5
b. CONTRIBUTING				
c. CONTRIBUTING	XXXXXXXX	STOG 80-7.2:2		

11. TITLE (Precede with Security Classification Code)

(U) Gastrointestinal Diseases of Military Importance

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology 008800 Life Support 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 07	CONT	DA	C, In House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: b. EXPIRATION:

c. NUMBER:

d. TYPE: e. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	8.0	697
	CURRENT 83	8.0	839

19a. RESPONSIBLE DOD ORGANIZATION

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NAME: CHENEY CPT, C.P.

NAME: REID LTC, R.A.

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede each with Security Classification Code)

(U) Bacterial Mucosal Adherence;(U) Pili;(U) Pathogenic E.coli;(U) Gut Associated Lymphoid Tissue;(U) Intestinal Epithelial Transport;(U) Myoelectric Activity

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

23 (U) Research efforts in this department continue to be directed toward Gastrointestinal diseases of military importance. Focus is on enteropathogenic bacterial diarrheal disease pathogenic E. coli, but also Salmonellosis, Shigellosis and Cholera. These have critical military relevance because of their influence on troop mobility, particularly following deployment of units to new areas.

24 (U) Studies of bacterial diarrhea are being conducted in 4 general areas 1) Mucosal Adherence as a determinant of bacterial colonization. 2) Intestinal immune response to bacterial infection. 3) Pharmacologic modification of effects of infections on intestinal transport and 4) Motility. Studies utilized preparations of intestinal membrane fraction and of bacterial adherence factors (pili), isolation and fractional characterization of intestinal mononuclear cells, in vivo volated intestinal loops, in vivo intestinal perfusions, Ussing chambers, voltage clamps, in vivo acute and chronic recording of intestinal activity.

25 (U) 81 10-82 09 Mucosal Adherence: Colonization factor antigen (CFA/II) adherence pili have been prepared in quantity for testing in humans as an oral vaccine against adherent, toxigenic E.coli which cause Traveler's diarrhea. Immunology: A synthetic octapeptide, the antigenic site for a larger immunogenic peptide, has been prepared and conformational analysis performed. Transport: Chloride secretion and D-glucose absorption stimulated by E.coli heat stable toxin was related to effects on the guanylate cyclase system in villous tip (but not crypt) cells. Motility: Induction of migrating action potential complexes stimulated by cholera toxin B subunit are identical to those induced by the whole toxin. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sept 82.

Available to contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1465A, 1 NOV 65 AND 1465B-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

Project: 3M162770A871 PREVENTION OF MILITARY DISEASE HAZARDS

Work Unit 163: Gastrointestinal Diseases of Military Importance

Investigators:

Principal: LTC Edgar C. Boedeker, MC
Associates: LTC Robert H. Reid, MC
LTC Robert W. Sjogren, MC
LTC Thomas Gage, MC
CPT Christopher P. Cheney, MSC
Dr. Yuan-Heng Tai, GS-13
Mr. William G. Marnane, GS-11

PROBLEMS AND OBJECTIVES

i. Role of Mucosal Adherence in Bacterial Colonization

Colonization of the small intestine is a prerequisite for the production of clinical diarrhea by many enteriopathogens, including the enteropathogenic (EPEC) and enterotoxigenic (EPEC) groups of 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? Can an adherence antigen be used as an effective oral vaccine against enteric infection with pathogenic E.coli?

ii. Role of Host Immune Mechanisms

The mechanisms of local immunization and the regulation of local immune responses at the level of the intestinal mucosa are being studied to develop better oral vaccines. Synthetic peptide immunogens are being developed as oral vaccines to give protective intestinal mucosal immunity. The goal is to have vaccines giving long term T lymphocyte immunity without interferring secretory antibody production thus allowing subsequent booster oral immunization for both specific T-lymphocyte and local secretory antibody immunity. Peptide antigenic site modification is the

approach being taken leading towards cross-reactive T-lymphocyte immunization without cross-reactive antibody formation as a prototype for initial intestinal immunization allowing for booster immunization with the modified peptide antigen. Lymphocytes from the gut associated lymphoid tissue (GALT) are being used for *in vitro* immunization and challenge with synthetic peptide antigens.

iii. Alterations of Intestinal Transport

The objective is to obtain a better understanding of certain functional properties of the intestine. We are particularly interested in the secretion of fluid and ions into the intestinal lumen. We hope to obtain further information on the nature of the secretory process (processes), including its anatomical location, mechanism of operation and its sensitivity to various reagents, both stimulatory and inhibitory. These secretory processes are of particular interest with respect to bacterial diarrhea because they may be involved in the relevant pathophysiology. Attempts to answer the following questions may aid the development of more effective or simpler therapies for bacterial diarrhea. What are the mechanisms for salt and water transport under normal conditions and in the secretory state induced by bacterial toxins and other secretory stimuli? Can pharmacologic agents reverse the salt and water secretion induced by bacterial toxins and other secretogogues?

iv. Alterations of Intestinal Motility

What are the mechanisms and neurohumoral pathways by which bacterial toxins change small bowel myoelectric patterns? Do luminal toxins produce similar changes in myoelectric patterns to native disease? Do toxins causing mucosal injury induce similar motility changes to those that do not cause mucosal damage? Are transport, myoelectric and microbiologic responses to toxins and infections related? How do myoelectric patterns correlate with radiographic peristalsis and transit time? Is colonic motility abnormal in response to fluid load, laxatives, antidiarrheals, antibiotics and infection of the small and large bowel?

PROGRESS

i. Role of Mucosal Adherence in Bacterial Colonization

Traveler's diarrhea (TD) is of great potential concern because of the high likelihood that it will affect troops, in a devilitating manner, shortly after their deployment to endemic areas. TD is most commonly caused by enterotoxigenic *E.coli*

(ETEC). ETEC strains from TD patients produce surface pili (CFAs) which promote association of organisms with the intestinal mucosa. Two antigenic type (CFA-I and CFA-II) are found in human ETEC isolates. Studies of analagous animal infections show that susceptible hosts can be protected from intestinal E.coli colonization by passive administration of IgA antipilus antibody. In theory, an effective vaccine against ETEC-induced TD should stimulate local secretion of IgA against CFA and oral administration of purified CFA should induce a maximal response. However, the antigenicity of purified CFA at the mucosal surface has remained undefined. To test whether intraluminal CFA can elicit a specific local IgA response, we purified CFA-II pili from an ETEC strain pathogenic for man; inoculated rabbit Thiry-Vella loops with the antigen and tested loop fluids for IgA and IgG antibody to CFA-II (as well as IgA antibody to CFA-I) using enzyme linked immunosorbent assays. CFA-II pili (pure by electron microscopic and subunit analysis) were prepared from our ETEC strain which was negative for CFA-I and Type I common pili. 0.1, 0.5, 1 or 2 mg doses of CFA-II were placed in the loops weekly for three weeks. Loop secretions were collected daily and sera weekly. The 0.1 and 0.5 mg doses gave no detectable IgA or IgG response to CFA-II, and no serum response. The 1 mg doses stimulated a loop fluid IgA response to CFA-II (titer 1:400) from day 14 through 28. The 2 mg doses gave a greater IgA response lasting from day 13 to 35. No loop fluids showed anti-CFA-II IgG despite a measurable serum IgG response. Nor was there loop IgA antibody to the antigenically distinct CFA-I pilus. In summary, mucosally applied CFA-II induces a dose-related, specific IgA response in intestinal secretions. This result supports the further study of purified CFAs as potential oral vaccines.

In collaboration with Dr. S. Berman at the Forest Glen facility we have prepared a lot of CFA/II pilus antigen which is sterile, immunogenic in animals, and safe for oral administration. An IND application is in progress to permit testing of the vaccine product for immunogenicity and efficacy in human volunteers under the supervision of Dr. M.M. Levine at the Center for Vaccine Development, University of Maryland.

A major focus of the department has been the elucidation of the mechanisms whereby a naturally occuring, enteroadherent 015:K-:NM E.coli strain induces diarrheal disease in rabbits with specific emphasis on defining the determinants of enteroadhernce both on the bacterial cell and the intestinal mucosa. Over the last year, this rabbit infection with E.coli strain RDEC-1 has emerged as a model highly analalgous to naturally occuring human disease caused by the enteropathogenic E.coli (EPEC) strains or serogroups. The relevance of RDEC-1 infection of rabbits as a

model for the pathogenesis of EPEC disease in human infants is suggested by: 1) close morphological similarity of the adherence process in the two diseases (close adherence with pedastal formation and loss of microvillar cytoskeletal architecture in areas of adherence) and 2) similar age of susceptibility (weaned infants in both instances). In investigating the determinants of RDEC-1 adherence to rabbit intestinal epithelial cell apical (brush border) membranes, we have developed evidence that the close enteroadherence of RDEC-1 to rabbit intestine is conferred by the expression of specific adherence pili which are distinct from the Type I or common pili but are expressed only under certain growth conditions. Three lines of evidence have been developed. First, when the expression of pili by RDEC-1 is phenotypically suppressed by growth in enriched (Brain Heart Infusion) media, the organisms lose their capacity for in vitro adherence to isolated rabbit brush borders. In contrast, when grown in Penassay broth, RDEC-1 organisms are densely piliated and adhere avidly to isolated rabbit brush borders. These pili are distinct from Type I pili by criteria of hemagglutination and immnoprecipitation. Second, the property of in vitro adherence to rabbit brush borders can be genetically transferred along with the transfer of RDEC-1 pili to other bacterial species. These properties appear to be transferred along with a plasmid of approximately 80,000 molecular weight. Third, isolated preparations of RDEC-1 pili adhere directly to the intestinal surface of the rabbit, but not of the rat, guinea pig or human ileum. The adherence of isolated pili to frozen sections of rabbit intestine is linear along the mucosal surface as demonstrated by immunofluorescence, and corresponds to the pattern of adherence seen with the intact, piliated, RDEC-1 organisms. Taken together, these result suggest that a unique type of pilus, expressed under certain growth conditions, is responsible for the species-specific adherence of RDEC-1 organisms to the rabbit intestine. If the analogy of RDEC-1 enteroadherence to EPEC enteroadherence is correct, EPEC strains may also elaborate specific adherence pili under appropriate growth conditions in vivo. This suggests an area for further investigations of EPEC strains.

Work on the elucidation of the intestinal mucosal receptors for RDEC-1 E.coli has progressed. Previous studies showed that receptors for the adherent pathogen, RDEC, on rabbit intestine are first detectable at the onset of weaning. Emergence of receptor activity also correlated with appearance of intestinal maltase (M) activity. Since (M) activity is localized to the sucrase/isomaltase (SIM) enzyme complex of the brush border membrane, we purified the SIM complex and determined whether or not SIM possessed RDEC receptor activity. SIM complex was released from the intestinal mucosa by papain digestion, precipitated by

ammonium sulphate and purified using affinity chromatography. Affinity column fractions were pooled by their enzymatic activity, concentrated and analyzed for RDEC receptor activity. Receptor activity was determined by the ability of a fraction to specifically agglutinate piliated RDEC but not nonpiliatd RDEC or type 1 piliated organisms as judged by phase microscopy. Of the 7 pooled fractions tested, only 1 had receptor activity. This fraction was enriched 15 fold in isomaltase but relatively devoid of sucrase activity. SDS PAGE showed one major protein band with a MW of 230,000 and another higher MW band that barely penetrated the gel. In summary, the intestinal brush border enzyme isomaltase appears to serve as the host receptor for RDEC.

Because of the observation that mucosal receptors for RDEC-1 E.coli appear at weaning, a systematic study was undertaken to define changes in available, reactive carbohydrate structures on the mucosal surface of the rabbit. The rabbit intestinal mucosal surface undergoes morphologic (glycocalyx emergence), enzymatic (increased sucrase, decreased lactase activities) and functional changes (new binding of pathogenic E.coli) with weaning (day 21). Such changes, and in particular the interaction of the surface with bacterial proteins, might correlate with the type and complexity of olgiosaccharide structures expressed at the surface. To investigate the development of intestinal surface properties, we examined the ability of a series of fluoresceinated lectins known to bind to specific sugars at certain locations in typical oligosaccharides from the amino acid linkage (L), to core (C) and peripheral (P) sites , to bind to ileum from rabbits aged 18, 21, 25, 28 days and adults. Lectins used (and sugar specificity) were Concanavalin A (Con A)-mannose (man); Ricinus Communis (RCA)-galactose (Gal); Wheat Germ Agglutinin (WGA)-Nacetylglucosamine (GlcNac); Soybean Agglutinin (SBA)-Nacetylgalactosamine (GalNac); and Ulex Europaeus (UEA)-fucose. Each lectin was incubated with ileal thin sections for 30 min. The apical surfaces of cells from crypt (C) to villus tip (V) were examined for the presence (+) or absence (-) of linear fluorescence. Results are tabulated:

			18 day	21 day	25 day	28 day	Adult
Lectin	Sugars	Site	C V	C V	C V	C V	C V
RCA	Gal	L/P	+ -	+ -	+ -	+ +	+ +
SBA	GalNac	L/P	+ -	+ -	+ -	+ +	+ +
WGA	GlcNac	L/P	- -	- -	+ -	+ +	+ +
Con A	Man	C	- -	- -	- +	+ +	+ +
UEA	Fuc	P	- -	- -	- -	+ +	- -

These results are consistent with the interpretation that 1) tip

cells minimally express reactive carbohydrates on their surface until weaning 2) crypt cells seem to express only rudimentary structures involving the linkage sugars before weaning 3) more complex carbohydrates are not found until day 28 on both cell populations.

Aeromonas hydrophila is frequently isolated from water sources throughout the world and is also isolated from patients with acute, diarrheal disease. Aeromonas strains have been demonstrated to produce an enterotoxin, fimbriae and a hemolysin (among other products) but the role of this organism in the pathogenesis of diarrheal disease remains undetermined. A prospective study was organized in collaboration with Dr. Peter Ecchevrria and the Dept. of Bacteriology at AFRIMS, Bangkok, Thailand to investigate the relation between Aeromonas isolation from the stool, severity of disease, and colonization, invasion or pathologic change in the small and large bowel mucosa of individuals presenting to a diarrhea center and from whom Aeromonas was isolated. A study has been set up and initiated at the diarrhea center of Bamrasnaradura Infectious Disease Hospital in Nontaburi, Thailand. The study is ongoing. To date 30 patients have been fully studied, but a lower than anticipated rate of isolation of Aeromonas precludes interpretation of results at the time of this report.

ii. Role of Host Immune Mechanisms

The rabbit ileal lamina propria mononuclear cells contain uncommitted lymphocytes and antigen presenting monocytes as reported in the 1981 Annual Progress Report. Any possible influence by coccidia occassionally found in the cell population has been ruled-out by repeating the experiments using coccidia free animals. The presence of T-lymphocytes in this cell population has been confirmed by using a monoclonal antibody specific for rabbit T-lymphocytes. Also, unusually small monocytes which may be the antigen presenting cells had been confirmed by an electron microscopic study. The T-lymphocyte/monocyte ratio may now be optimized by using the monoclonal antibody to remove the T-lymphocytes which can then be added back to adjust the T-lymphocyte/monocyte ratio for optimal responses. This system can be used to test synthetic peptide antigens for the development of better peptide immunogens to be used in oral vaccines.

The synthetic peptide (GLY-ASN-THR-ILE-VAL-ALA-VAL-GLU) is the 1-8 antigenic site on a 13 A.A. peptide found to be immunogenic for rabbit ileal lamina propria lymphocytes in vitro. Conformational restriction for this octapeptide in aqueous solutions has been found by an on-going 600 MH_2 high resolution proton NMR study as reported in the 1981 Annual Progress Report. The conformational

analysis has been further enhanced by taking the NMR results and applying computer programs for energy calculations and energy minimization. The resultant atomic X,Y,Z coordinates can now be used with additional computer programs and a color graphics computer terminal to display a filled-in molecule where all six sides views can be seen and photographied for comparision and 3 dimensional viewing. A ball and stick molecule can also be displayed and printed-out on an X-Y recorder for comparision and 3 dimensional viewing.

Initial peptide synthesis has resulted in the complete synthesis, including product purity confirmation, of the octapeptide GLY-ASN-THR-ILE-VAL-ALA-VAL-GLU. We employed the solid phase synthetic methodology developed by RB Merrifield Instrumentation including a prototype microprocessor controlled automated peptide synthesizer model 250 B from Vega Biochemicals. The prototype nature of the instrument necessitated several modifications, such as converting the constant pressure N_2 system to a closed system and tracing down an electronic problem. Vega Biochemicals supplied most of the reagents, and the starting material GLU, esterified to polystyrene resin.

Synthesis proceeded by an approach using 9-fluorenylmethoxycarbonyl chloride with promise for simplifying the final purification. This procedure terminates any unreacted peptide chains after the amnino acid coupling step, by acylation with acetic anhydride. Monitoring the synthesis progress by the Kaiser Ninhydrin Test gave an unsuspected negative indication after coupling the third amino acid. Applying the more analytical technique of amino acid analysis employing an analyzer established by R. Seid, Dept of Bacterial Diseases, revealed for each amino acid in the synthesized chain, a decrease of 20% from the preceeding amino acid. Restarting the synthesis and eliminating the acetic anhydride step solved the problem. Closer progress monitoring was accomplished by measuring the efficiency of each t-Boc deprotection step, and each amino acid dehydration coupling step, through amino acid analysis, and by developing a modified Dorman Chloride Determination method.

Initial removal of the synthesized peptide from a small resin sample with M Smith, NIH via reaction with H-F for 1/2 hour gave a product containing two peptides, according to C-18 HPLC. Composed of identical amino acids, one peptide demonstrated unsaturated character indicated by UV absorbance at 280 nm. One hour H-F cleavage followed by N O acyl shift reversal by base hydrolysis, reduced the unsaturated peak from 20% to 5%. HPLC analysis of the same octapeptide synthesized by Bachem gave the 20% double peak results. Final recovery of 800 mg of octapeptide at 80% yield gave

the desired amino acid sequence as analyzed with D. Corcoran, USUHS.

iii. Alterations of Intestinal Transport

Both cholera toxin and cAMP increased D-glucose and 3-O-methyl-glucose absorption, and net chloride secretion, and decreased net sodium absorption in the rat ileum. Phlorizin abolished the net absorption of D-glucose in both the control and cholera toxin-treated tissues. Removal of chloride from the bathing solutions decreased the basal D-glucose absorption by 50% and totally abolished the cAMP-stimulated increase in D-glucose absorption. The results are consistent with the concept that the cholera toxin- or cAMP-stimulated increase in D-glucose absorption is due to an increase in the D-glucose: Na^+ stoichiometry in the co-transport system in the brush border membrane.

The effects of the heat-stable toxin of E.coli (ECST) on glucose and chloride transport under short-circuit conditions and on the cyclic GMP (cGMP) concentrations in the isolated villous tip and crypt cells in the rat small intestine were studied. ECST increased glucose absorption and chloride secretion by 0.696 umol/h/cm^2 and 3.44 ueg/h/cm^2, respectively. ECST increased, in three minutes, the cGMP concentration in the villous tip cells from 0.24 $\pm$ 0.06 to 1.45 $\pm$ 0.50 pmol/mg prot. whereas the cGMP concentration in the crypt cells was unchanged throughout 20 min. after the addition of ECST. The results suggest that the intestinal response of chloride secretion and D-glucose absorption to ECST is predominantly due to the effect of ECST on the guanylate cyclase/cGMP system in the epithelial villous cells.

Acute elevation of the intraluminal hydrostatic pressure also caused intestinal secretion of water and electrolytes in the rabbit jejunum and ileum but did not alter the mucosal Na-K-ATPase and adenylate cyclase activities. It appeared that increased intraluminal hydrostatic pressure affected the hydrodynamics of the mucosal microcirculations to produce a driving force for passive filtration-secretion. A mathematical model for the dynamics of luminal fluid accumulation in intestinal obstruction was derived based on a luminal fluid material balance.

Growing new mucosa from remnants of small bowel remaining in patients with short-bowel syndrome might offer a strategy for solving this clinical problem. We have performed a series of experiments investigating the possibility of growing rabbit ileal mucosa on vascularized pedicle flaps of abdominal wall musculature based on the inferior epigastric artery. By patching a defect of distal ileum with a skeletal muscle flap, we were able to

demonstrate bowel augmentation by neomucosal ingrowth. Light and scanning electron microscopy confirmed the presence of essentially normal mucosa with well-developed villi atop the skeletal muscle pedicle flap. The mucosa was stripped from the skeletal muscle and compared with stripped mucosa from adjacent ileum in the Ussing chamber in 11 rabbits. The electrophysiologic studies showed no significant difference, or tissue conductance. The addition of 10 mM glucose resulted in similar unidirectional glucose flux and increase in Isc in both tissues. Bile salt absorption was also similar in both tissues. We conclude that neomucosa can be grown on flaps of skeletal muscle and is similar to normal mucosa by microscopic and eletrophysiologic evaluation.

Trifluoperazine (TFP) an inhibitor of the calcium dependent regulatory protein calmodulin (CDR), is an antisecretory agent effective against a range of secretagogues including 8-Br-cAMP, E.coli stable toxin and Ca-ionophor A23187. CDR, which exhibits a variety of regulatory activities in other systems including modulation of kinase activity and membrane phosphorylation, is abundant in the intestinal epithelial brush border (BB). The BB is also known to have an endogenous cyclic nucleotide dependent phosphorylation system. Since membrane phosphorylation might be a common mechanism whereby TFP exerts its antisecretory effects, we examined the relationships of TFP and CDR to BB phosphorylation. Total phosphorylation of rat small intestianl BBs was measured in the presence of 1mM Ca^{++} and $\gamma^{32}p$ -ATP. Addition of exogenous CDR (prepared by Teo's method) significantly increased the total phosphorylation of intestinal BBs (94.2 $\pm$ 3.7 vs 67.1 $\pm$ 6.9 fmoles ^{32}P incorporated/ug protein, $p<0.001$) and a consistent decrease without added CDR. Specific BB protein phosphorylation was examined by SDS-PAGE of BBs exposed to Ca^{++} and $\gamma^{32}P$ -ATP in the presence and absence of TFP. TFP decreased incorporation of ^{32}P into a single BB protein (m.w. 98,000). This protein differs from the previously demonstrated cyclic nucleotide dependent BB protein (m.w. 86,000) and TFP did not effect the cGMP-induced increase in the phosphorylation of the lower m.w. protein. These studies demonstrate that: 1) TFP inhibits the CDR stimulated increase in total BB phosphorylation; 2) TFP inhibits phosphorylation of a specific BB protein (m.w. 98,000), but does not influence the phosphorylation of the cyclic nucleotide dependent BB protein (m.w. 86,000). These results suggest that CDR mediates phosphorylation of a specific BB protein which can be inhibited by TFP. The relation of this phosphorylation to secretory events in the intestine remains to be determined.

iv. Alterations in Intestinal Motility

In rabbit ileal loops, bacterial toxins, specifically E.coli

heat labile toxin (LT) and cholera enterotoxin (CT), produce a diarrheogenic myoelectric pattern, the migrating action potential complex (MAPC). The MAPC is produced by the cholera B subunit, requires binding at the GM1 binding site and requires aggregation of B subunit components in a form more complex than the monomeric B subunit. This raises the possibility that potential B subunit vaccines may have untoward effects on intestinal motility. However, binding of the same receptor by TSH produces neither an electrical nor a fluid response. LT produces similar activity in similar concentrations as CT. The antigenic similarities between CT and LT are insufficient to block MAPC activity by preincubation of toxin with heterologous antiserum.

The lectins ricin and WGA produce MAPC responses similar to those of CT and LT in rabbit ileal loops but there are differences in fluid output. Unlike WGA, CT and LT, ricin causes mucosal destruction and induces, in addition to MAPC's a second myoelectric pattern, repetitive bursts of action potentials (RBAP). The lack of correlation between fluid output and motility in these responses raises the possibility that transport and motility may not be related.

Reproduction of MAPC and RBAP activity in denervated loops of bowel has been reported elsewhere. A prototype in vitro system for suspension of denervated loops of bowel in an oxygenated, nutrient bath has been constructed but problems in maintaining tissue viability are delaying further studies.

A non-peristaltic perfusion pump has been developed to enable in vivo perfusion of intestinal loops with C^{14} PEG to enable simultaneous measurement of fluid transport, myoelectric activity and intraluminal pressure change. Using this system, preliminary studies directly correlating transport with motility have commenced.

A non-human primate model for recording chronic intestinal myoelectric activity from unanesthesized, chair adapted monkeys has been developed. Our intial work has been in validation of the model by comparing fasting and fed changes in the migrating myoelectric complex (MMC) as monitored by computer analysis of spike burst activity It would appear that differences in these patterns may be partially mediated by blood glucose concentrations. Microorganisms, pathogenic for humans, have been used to produce infection in this model. Seven animals have developed clinical Shigella and five have developed Salmonella infection with diarrhea and dysentery. Motility patterns became clearly abnormal with clinical disease and resolved with antibiotic treatment. Furthermore, Shigella and Salmonella produce different

abnormal motility patterns. Common laxatives, antidiarrheals and antibiotics have also been tested in this primate model. Some result in enhanced motility responses (sorbital, MCT oil, caffeine), some in reduced motility (castor oil, PGI, PGE-2), some in unusual motility patterns (castor oil, lactose) and some were without effect on motility (ampicillin, saline, loperamide, lomotil, codeine). These studies will serve as control studies for tests of antibiotic/antidiarrheal medications during native infections described above.

A rabbit model for recording chronic intestinal myoelectric activity from unanesthesized, restrained rabbits has also been developed. Our initial work with this model has shown that unlike the primate model fasting and fed changes in the MMC occur irregularly. Present studies utilizing computer analysis of slow wave and of spike burst activity include testing common laxatives, antidiarrheals, antibiotics and toxins as well as RDEC and clindamycin-induced infectious diarrheas in a manner analogous to that used in the primate model. Modifications of electrode construction and placement in both rabbit and primate models may provide significant technical advances in improving the quality of electrical signals for analysis.

Computer programs have been developed to allow interpretation of MAPC's, RBAP's, MMC's, unpatterned electrical spike activity and slow wave frequency from analog digital recordings. This will allow greater precision and add the ability to appreciate electrical patterns previously unaccessable by visual record analysis. These developments should greatly extend the capabilities of future studies.

FUTURE PLANS AND RECOMMENDATIONS

i. Role of Mucosal Adherence in Bacterial Colonization

Development of an IND to permit testing of the CFA/II pilus preparation for immunogenicity following oral administration to humans, as determined by the production of specific local secretory immunoglobulin A in intestinal secretions, and for efficacy in protecting against diarrhal disease in challenge studies with a challenge strain of toxigenic E.coli producing the same colonization factor antigen will be pursued. Any additional studies required by the Bureau of Biologics will be pursued with highest priority. Attention is also being directed towards developing and testing methods for producing large lots of the pilus vaccine which are both sterile and antigenically intact. Currently the preparations are being sterilized by gamma irradiation which is efficacious at low dose in eliminating the

parent E.coli strains from which the fimbriae are isolated, but which requires high doses to remove possible airborn contaminants including bacillary spores. Alternative sterilization methods are being explored. In addition, amino acid composition and sequence data on the adherence pili is being obtained in collaboration with Dr. Robt. Seid of the Dept. of Bacterial Diseases. The efficacy of the peptide fragments of the pili as immunogens and as inhibitors of bacterial hemagglution are being investigated. The aim here is to determine specific binding and antigenic sites which might be produced by synthetic means, thus eliminating the need for isolation from potentially contaminating pathogens. In addition, studies on the plasmid DNA sequence required for pilus production are being undertaken in collaboration with Dr. Dennis Kopecko. The long range goals here include the production of pili in non-pathogens following plasmid transfer. Such organisms might be safely utilized as heat killed or live vaccine products.

Studies in the rabbit model of RDEC-1 infection in rabbits will be pursued with the aims of further purifying the epithelial receptor. Purified will be used to produce antibodies (including monoclonals) which will be tested for their efficacy in blocking enteroadherence. Emphasis will be placed on developing data on the optimum conditions for infectivity of rabbits with RDEC-1, including optimum age and size of rabbit which will permit consistent infection with the minimal dose of organisms, with a predictable time course. These conditions will then be utilized for studies of protection of rabbits against RDEC-1 infection using vaccines, including RDEC-1 pili, and substances which can interfere with pilus-receptor interactions such as hydrophobic gels. The relation of appearance of mucosal receptors to susceptibility to infection will be examined in detail.

Since, in classic studies of enteroadherence using the K88 positive E.coli strains, diarrheal disease was induced by organisms which possessed adherence pili but no recognizable enterotoxins; and since neither RDEC-1 nor EPEC E.coli strains produce classical enterotoxins, the relation of mucosal binding of E.coli adherence factors to the development of changes in intestinal fluid and electrolyte transport and permability will be investigated. We will use purified pili, isolated intestinal mucosa in Ussing chambers, whole intestinal cell suspensions and microvesicular microvillus membrane preparations.

ii. Role of Host Immune Mechanisms

The synthetic peptide (GLY-ASN-THR-ILE-VAL-ALA-VAL-GLU) is an antigenic site for rabbit ileal lamina propria T-lymphocytes and contains a smaller antigenic site for antibody binding. The larger

peptide antigenic site for the T-lymphocyte suggests that conformation of the antigenic site may be more important for the T-lymphocyte antigen receptor then for antibody binding. Since the conformation of the octapeptide is now known, the development of two analog series may be possible with the use of the already established computer assisted molecular modeling utilizing energy minimization strategies. One series would have basically very similiar over-all conformations but maximally different primary structures and the other series would have very dissimilar conformations with minimal changes in primary structure. These analogs would then be synthesized using the already established peptide synthesis capability. The peptides conformations would actually be determined using the 600 or 750 MH_2 high resolution proton NMR facility at Carniege-Mellon University. The analog peptides would be tested for the presence or lack of cross-antigenicity with the original octapeptide using rabbit ileal lamina propria T-lymphocytes in vitro and antibody binding. This study will be testing the hypothesis that the conformation of a peptide antigenic site is more critical then the amino acid sequence for recogniton by sensitized T-lymphocytes in contrast to antibody binding where the opposite is true. Several peptide analogs may be found which give good T-lymphocyte cross reactivity but no cross reactive antibody binding. These peptide analogs would then be prototypes for further development of oral vaccines. The analogs would be conjugated to synthetic polypeptides having specific receptors on enterocytes and possibly further conjugated to a synthetic adjuvant such as muramyl dipeptide. The conjugates will also be tested in vitro for maintenence of original analog antigenicity. The immunogenic conjugates will also be tested for mucosal absorption and ability to sensitize a chronic ileal looped rabbit. Ultimately it will be determined if rabbits can be protected from enteric pathogens following oral immunization with the apppropriate synthetic peptide vaccines.

iii. Alterations of Intestinal Transport

Substances to be absorbed or secreted by the intestine must cross at least two barriers in series, the membranes at the two sides of the epithelial cell layer. In order to have net active transport across this series array, the membranes at the two sides must have different functional properties. For the past several years, much of our research has been directed toward obtaining information on the transport properties across the whole intestine. Over the next year we intend to add to our research the experimental approach designed to obtain information on the transport properties of these individual barriers and to understand how the barriers contribute to the overall properties of the

intestine. Influx studies of ions and non-electrolytes across mucosal membrane using influx chambers and vesicle preparations are planned. The initial experiments will be to estimate the glucose: Na^+ coupling coefficient in the brush border membrane.

Further studies involving the anti-secretory agents berberine should include 1) the effect of berberine on the glucose-induced absorption of water and electrolytes and 2) establishing a correlation between berberine-induced changes in the phosphorylated protein intermediate and berberine-induced changes in cyclic nucleotide- induced electrolyte transport.

Drugs of potential use in diarrheal diseases including potential absorption stimulators, such as dopamine, bromocriptine etc. and potential secretion inhibitors, such as trifluoroperazine, chlorpromazine, fluphenazine etc., will be evaluated in terms of their effects on the basal and bacterial toxin-altered intestinal water and electrolyte transport. These drugs will also be evaluated on the basis of the alterations which they cause in cyclic nucleotide concentration and membrane phosphorylation levels; as well as the possible involvement of calcium and calcium binding proteins.

iv. Alterations in Intestinal Motility

Future investigation of bacterial toxins, infections and lectins in rabbit ileal loops will utilize two approaches: in vivo ileal loops continuously perfused with C^{14} PEG to allow simultaneous determination of myoelectric activity and fluid transport and in vitro denervated ileal loops in oxygenated nutrient bath. Utilizing these approaches the following questions will be investigated: is there a correlation between fluid transport and the motility response; what is the role of mucosal damage in fluid and motility responses; what are the neurohumoral pathways by which motility patterns are mediated and what is the effect of therapeutic agents on these pathways? Do chemical-biological agents interact with these pathways? If so, do these agents affect enteric microbial colonization, infection and host response to bacterial toxins?

Continuing development of computer software will follow recent hardware developments which have enabled continuous Fourier analysis of myoelectric signals. Continuous frequency analysis may provide an additional, simpler and more accurate means of assessing myoelectric frequency. These improvements in signal interpretation will allow analysis of motility patterns in the colon which are much more complex than those in the small bowel. Assessment of the effects of infection, toxins and pharmacologic agents on colonic

motility and transport is essential before definitive conclusions about clinical diarrheal states and responses to therapy can be addressed (ie. are observed small bowel events compensated or exaggerated by colonic responses).

Future investigation using the primate model will compare myoelectric effects induced by mucosal damage, radiation and by purified toxins to the native infection. The temporal relationships of motility, transport and microbiologic events in clinical diarrheal disease and their response to pharmacologic intervention will be determined. Continuation of ongoing studies using the rabbit model will allow comparison of data obtained from the rabbit ileal loop and in vitro bath to data obtained from unanesthesized rabbits with chronically implanted electrodes. Extension of the chronic rabbit model to incorporate electrodes sewn on to Thiry villa loops will enable direct instillation of toxins and microorganisms into the study loop and transport studies using C^{14}-PEG perfusion to be performed on unanesthesized animals.

Before these studies can be extended to patient populations, less invasive probes and more accurate computer programs for data interpretation must be available. Eventually studies correlating myoelectric, pressure, radiographic and transport should be performed. Oral pressure, myoelectric and radioelemetry probes need to be developed. Collaboration with development of similar probes required for intraintestinal pressure monitoring in "Blast Overpressure" studies may be rewarding.

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PROJECT 3S162771A874

METHODS AND TECHNIQUES FOR COMBAT CASUALTY MANAGEMENT

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 0G 6770	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	X YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AG	181
b. CONTRIBUTING				
c. XXXXXXXXXXX	STOG 80-7.2:5			

11. TITLE (Precede with Security Classification Code)

(U) Management of Military Blast Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 012900 Physiology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2.0	195
FISCAL YEAR CURRENT 83	2.0	169

18. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: COHEN, David J. MAJ

NAME:

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Blast injury; (U) Pulmonary dysfunction; (U) Gastrointestinal hemorrhaging; (U) Pulmonary hemorrhaging; (U) Medical/Surgical Treatment

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

23 (U) This project proposes to respond to the threat of potential blast related problems which may be experienced by the Army in the field. Our ultimate goal is to optimize the treatment of blast-injured casualties. Toward this goal we are evaluating techniques which will allow documentation of the natural history of gastrointestinal and pulmonary injuries. The threat of exposure of American soldiers to blast waves from enemy weapon systems which may exceed established thresholds is increasing.

24 (U) Fuel-air explosives will be used to create injuries in two different species to determine if the distribution of injuries are similar. The advantages and disadvantages of utilizing these two species of animals will be enumerated and evaluated. The rapid technique for the in vivo estimation of lung water content using a double-dilution technique will be assessed in the laboratory to determine the reliability, repeatability and accuracy of this technique so that the natural history of blast injuries can be followed.

25 (U) 81 10 - 82 09 A feasibility study was carried out using fuel-air explosives in San Diego, CA. We have demonstrated that it is possible to carry out the blast study under controlled conditions, and to carry out necropsies without leaving the blast facility. Following pulmonary injury with oleic acid, lung water measurements increased seven-fold. Lung water measurements have been found to be easily reproducible and to be a sensitive measure of pulmonary injury. This measurement is potentially useful for monitoring pulmonary injuries following exposure to blast. For technical reports see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

* Available to contractors upon originator's approval

Project 3S162772A874 METHODS AND TECHNIQUES FOR COMBAT CASUALTY MANAGEMENT

Work Unit 181 Management of Military Blast Injury

Investigator:
Principal: COL Arthur W. Fleming, MC
Co: MAJ David J. Cohen, MC

A. Determination of a Suitable Animal Model for Studying Blast Injuries

Background and Objectives:

Direct or primary blast injury is a term which has superseded the older designation of "air concussion" and has become accepted as an expression of pathophysiologic events resulting from exposure of an individual to a high compression, high velocity shock wave, usually emanating from a detonating explosion in the air. In its pure form, direct blast injury is characterized by lesions appearing in various internal organs, particularly the air-containing structures, without signs of external injury. In the usually catastrophic event, however, the clinical picture is often compounded by additional trauma caused by flying objects of varying size which have been energized by the blast (secondary effect); by rapid bodily displacement created by the force of the explosion causing an individual to strike stationary objects (tertiary effects); by explosive forces in water as may be experienced by troops landing on beachheads (underwater or immersion blast); by transmission of pressure waves through solid objects, such as the wall of a tank, sometimes without disruption of the solid object itself (solid blast); and/or the effects of fire, heat, gas, and dust (Emergency War Surgery, 1975).

Our primary concerns are twofold: (1) Determining the natural history of injuries to the lung and the GI tract secondary to blast; and (2) determining the most suitable model for studying blast injuries. Since the animal species chosen may influence the natural history to some degree, we will focus our attention initially on determining the most suitable model for our needs. Sheep have been used extensively for many years for blast research. The most significant advantages for using sheep from our viewpoint are: (1) a vast amount of information is available in the literature on the response to various overpressures; and (2) the sheep are economical to use and easy to handle. Two distinct disadvantages, however, come to mind: (1) the sheep have a greater anterior/posterior diameter to their lungs as opposed to man whose greatest diameter is from side to

side; and (2) man has no counterpart to the rumen which is present in the gastrointestinal tract of these animals. These differences in anatomy do not, however, necessarily negate their use for research purposes nor the extrapolation of data from these experiments.

We would like to compare the sheep, which may be the best model when all advantages and disadvantages are considered, with other species of animals. Any immediate thought of a potentially suitable animal model for studying blast injury would be the subhuman primate. Although the pulmonary and gastrointestinal anatomy of some subhuman primates more closely approximates that of humans, there may be significant disadvantages such as: (1) difficulty in handling; (2) the small blood volume available in small subhuman primates which would limit the amount of blood that could be drawn for specimens; (3) the increased cost of subhuman primates; (4) the potential that certain groups may lobby against this type of research being performed; and (5) the difficulty in obtaining some subhuman primates.

It is anticipated that a study such as this will validate the use of any of these species depending upon multiple factors. The most important factor, of course, is to determine whether or not the null hypothesis, that there is no difference in the lesions produced between the species of animals compared, is correct. If there is no difference, then one can always refer back to the study whenever they are using different species from other investigators. At the same time, it is anticipated that studies such as these, which are vital to understanding the therapy of blast injuries, may not be able to be carried out in future years because of the sensitivity of the study.

Progress:

After over four months of negotiating, we were unable to carry out a study comparing five species. We were, however, able to carry out a study comparing sheep and pigs. This study was carried out at S-Cubed, a civilian contractor for the Defense Nuclear Agency, in California. Likewise, because of increased sensitivity of doing the studies in California, the entire field study was carried out on the grounds of the Miramar Naval Air Station. All of the animals were housed within an isolated, guarded area. A fuel-air explosive was used as a source of the blast. Preliminary data from this study has demonstrated the following:

1. There were no distinct differences found in the distribution of the lung injuries between sheep and pigs.

2. The rumen of the sheep was found to be approximately five times larger than the stomach of the pig and was more frequently involved in abdominal injuries.

3. The overall mortality for sheep was 50% and the overall mortality for pigs was 70%.

4. There were more large intestinal injuries in the sheep; and more small intestinal injuries in the pig overall.

5. Histological data is pending.

Although this data is preliminary, it appears that the distribution of gastrointestinal injuries may be quite different in sheep and pigs, and the frequency of injury to the gastrointestinal tract is greater than previously seen in experiments of this nature. Most importantly, we have demonstrated the feasibility of carrying out a field study in a self-contained unit at the Miramar Naval Air Station.

Recommendations for the Future:

A new proposal has been submitted from S-Cubed to the Surgery Subcommittee meeting at LAIR and has been approved for funding. We hope to be able to carry out the study on the five species in a judicious manner in the very near future. We will continue to enumerate the advantages and disadvantages of the various species. Once a species has been selected, we will then turn our attention to assessing the natural history of both pulmonary and gastrointestinal injuries.

B. Appraisal of a Quantitative Technique for Measuring Extravascular Lung Water in Vivo

Background and Objectives:

Since World War II it has been recognized that organs containing air (the lungs and gastrointestinal tract) are vulnerable to blast waves from an air explosion. After blast injury to the lungs, the predominant lesion involves exudation of edema fluid and blood into the alveoli and the interstitial space.[1]

Lung damage can arise from the blast pressure wave itself (primary blast effect), from the patient being hit directly in the chest by missiles energized by the blast (secondary effect) and/or by the patient being thrown against an object by the force of the explosion (tertiary effect).[2] Over a period ranging from a few minutes to several days after the injuries, respiratory failure may develop either from a combination of blast effects, or by fluid overload during resuscitative efforts.

Pulmonary edema is a pathologic state in which there is an abnormal extravascular fluid accumulation in the lungs. Pulmonary edema may be induced by many conditions including thermal injury, hemorrhagic shock, sepsis head injury as well as the effects of blast.[3,4,5,6,7,8,9,10,11,12,13,14,15,16,24].

Accurate measurement of extravascular lung water in patients has been attempted by a variety of methods for several years with variable success.[17,18,19,20,21,25] Chest x-ray and arterial blood gas analysis are the most commonly used clinical tools for estimation of pulmonary edema, however both may be altered by other factors and do not give either specific or quantitative information about the amount of lung water present. An accurate and reproducible method of measuring extravascular lung water has been demonstrated by Lewis, Elings and Oppenheimer.[22,23] Their technique is more accurate than radioisotope methods, is a non-destructive technique, and has been utilized in the quantitation of extravascular lung water (EVLW) in both experimental animals and humans (Oppenheimer - 1979).[20] This technique employs the simultaneous bolus of two indicators, cold and indocyanine green dye. Cold diffuses into the pulmonary interstitial fluid while the green dye remains in the intravascular space. By subtracting the volume of distribution of the intravascular from the volume of distribution of the diffusible indicator, the extravascular lung water volume (EVLW) can be obtained. The use of cold as the diffusible indicator and an on-line microprocessor for computation allows for faster, easier and more reproducible measurements.

Our recent work has been an effort to demonstrate that extravascular lung water (EVLW) measurements made by the double indicator dilution technique are sensitive and accurately reflect wet to dry lung weights.

We have corrrelated extravascular lung water measurements obtained by the Edwards Lung Water Computer using the thermal green dye dilution technique with:

1. assessment of the chest x-ray
2. wet to dry lung weights
3. arterial blood gases
4. pulmonary capillary wedge presssure

Progress:

Experimental animals (dogs) were injected (IV) with varying doses of Olelic Acid followed one hour later by a 150 cc/kg saline challenge. Changes in values of cardiac output, pulmonary capillary wedge pressure or pulmonary artery diastolic pressure, Thermal-Green Dye EVLW and pO_2 are summarized in Table 1. In all cases the Thermal-Green Dye EVLW reflected the increasing pulmonary injury. Pulmonary Capillary Wedge Presssure did not increase significantly especially after the animal had time to spontaneously diurese. Pulmonary injury remained, however, as evidenced by Thermal-Green EVLVW, pO_2 and wet-dry weight of lungs after sacrifice.

Each animal was sacrificed at the conclusion of the procedure. Lungs were removed, homogenized and lung water determined gravimetrically. Gravimetric determination for lung water could be determined with and without a correction for blood in the specimen using hemoglobin. The final series of thermal green dye measurements in each animal was compared to the uncorrected gravimetric lung water and to the gravimetric weight corrected for hemoglobin. There was a much better linear fit with the uncorrected gravimetric lung water (correlation coefficient = +0.859) than with the corrected gravimetric lung water (correlation coefficient = +0.592). This can be explained by the pathologic sections showing hemorrhage of blood into lung parenchyma and alveoli with the larger does of Oleic acid. This is appropriately measured as lung water with the thermal-green dye method explaining the better linear fit with the uncorrected gravimetric technique.

Recommendations and Future Objectives:

The thermal-green dye technique for measuring lung water provides a good linear fit when compared to gravimetric techniques, at least in the range of cardiac output considered here (1.0 L/min - 9.0 L/min). It was a more sensitive indicator of pulmonary injury than either arterial pO_2, pulmonary capillary wedge pressure or chest x-ray. It would seem to be an easily reproducible, sensitive measure of pulmonary injury appropriate for use in the blast injured animal.

TABLE I

Oleic Acid Dose	0.0225 gm/kg	0.035 gm/kg	0.045 gm/kg	0.09 gm/kg
EVLW + SEM/kg Baseline	5.85 ± 0.88	3.21 ± 0.99	4.76 ± 1.17	3.77 ± 2.05
After Oleic Acid & Ringers Lactate	11.50 ± 2.96	24.37 ± 3.61	26.28 ± 5.74	31.35 ± 1.43
C.O. + SEM/kg Baseline	0.125 ± 0.02	0.135 ± 0.005	0.23 ± 0.02	0.08 ± 0.002
After Oleic Acid & Ringers Lactate	0.168 ± .002	0.125 ± 0.003	0.27 ± 0.08	0.10 ± 0.004
PCW + SEM Baseline	4.0 ± 0.7	19.0 ± 0	8.09 ± 2.41	6.0 ± 1.0
After Oleic Acid & Ringers Lactate	8.1 ± 3.61	15.00 ± 0.8	18.25 ± 3.01	14.5 ± 3.5
pO_2 + SEM Baseline	420 ± 12.7	395	419	---
After pO_2 & Ringers Lactate	199.7 ± 88.6	69.4	79.2	---

Project: 3S162772874 In House Laboratory Independent Research

Work Unit 181 Management of Military Blast Injury

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Publications: None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6769	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	BB	182
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2:6			

11. TITLE (Precede with Security Classification Code)

(U) Biomedical Aspects of Medical Material

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support 002400 Bioengineering

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	1.0	72
FISCAL YEAR CURRENT 83	1.0	97

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Laboratory models; (U) Medical Material Systems; (U) Biomedical support; (U) Life support systems

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

23 (U) The primary objective is to develop and provide laboratory models for biomedical assessment of medical material systems. Medical material systems currently being developed will continue to undergo operational testing to determine if such systems are usable and useful. Our objective will be to exploit these newly developed materials in the environment that they are designed to be used in. These studies will not only assure the military relevancy of such materials, but they will also assist in the intergration of such materials into the armamentarium of the Army Medical Corps.

24 (U) Appropriate animal models and bench models will be developed and utilized to accomplish our objectives. Each medical material system will have an individual evaluation to determine which method of assessment will be used. After completion of each assessment, the data will be analyzed statistically, where possible, and a summary statement issued.

25 (U) 81 10 - 82 09 Modern autologous transfusion devices developed specifically for military field use have been evaluated. A position paper on the applicability of salvage autologous blood transfusion techniques for combat casualty care has been forwarded to the Deputy Surgeon General of the Army for review. A report on the application of recent technical advances for intensive care treatment during wartime is being prepared. Cyanoacrylate tissue adhesives are being evaluated for their efficacy in obtaining hemostasis in massive bleeding. Long-term histotoxicity studies on cyanoacrylate tissue adhesives are also under evaluation. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

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

Project 3S162772A874 METHODS AND TECHNIQUES FOR COMBAT CASUALTY MANAGEMENT

Work Unit 182 Biomedical Aspects of Medical Materiel

Investigator:
Principal: COL Arthur W. Fleming, MC

Background and Objectives:

The primary objectives are to develop and provide laboratory models for biomedical assessment of medical materiel systems. Medical materiel systems currently being developed will continue to undergo operational testing to determine if such systems are usable and useful. Our approach will be to subsequently exploit these newly developed materiels in the environment that they are designed to be used in. Appropriate animal models and bench models will be developed and utilized to accomplish this goal. Each medical materiel system will have an individual evaluation to determine which method of assessment will be used. After completion of each assessment, the data will be analyzed statistically, where possible, and a summary statement issued. These studies will not only assure the military relevancy of such materiels, but they will also assist in the integration of such materiels into the armamentarium of the Army Medical Corps.

Progress:

Functional concepts of the working relationship between USAMBRDL and the Division of Surgery, WRAIR in regards to the biomedical aspects of medical materiels have been developed and are operative.

Interest in the use of a medical device, the cyanoacrylate tissue adhesive, was renewed and influenced by the news media. This renewed interest in the cyanoacrylate tissue adhesive prompted us to review the literature on the subject and to initiate a protocol on the long-term histotoxicity and carcinogenic potential of cyanoacrylates.

This study is being carried out according to guidelines of the one report which placed the stigma on cyanoacrylates (report by Page, Larson and Siegmund in 1966 in the Proceedings: Symposium on physiological adhesives, University of Texas Press 11-23). This study (by Page et al), however, reported on the histotoxicity of methyl-cyanoacrylate, whereas the present study involves the use of butyl-cyanoacrylate, a higher homologue with increased efficacy. Rats injected with either 0.1 ml or 0.4 ml of cyanoacrylate are being compared with normal saline controls

over a two-year period of time. There has been no evidence of carcinogenesis at eight months.

A protocol is also currently being carried out to evaluate the efficacy of cyanoacrylates in severe liver trauma.

Modern autologous transfusion devices developed specifically for military field use have been evaluated. A position paper on the applicability of salvage autologous blood transfusion techniques for combat casualty care has been forwarded to the Deputy Surgeon General of the Army for review. A report on the application of recent technical advances for intensive care treatment during wartime is being prepared.

Recommendations for the Future:

Full biomedical support for assessment of field medical materiels is planned for the next five (5) years. The scope and frequency of involvement, and the number of manhours expended will vary with the requirements of USAMBRDL.

Literature Cited: None

Publications: None

PROJECT 3S162772A875

MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6479	82 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	9. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A875	AA	161
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code)

(U) Development of Anti-Chemical Warfare Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology 012600 Pharmacology 012100 Organic Chemistry

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

17. CONTRACT/GRANT

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	2.0	356
FISCAL YEAR CURRENT 83	5.0	339

19. RESPONSIBLE DOD ORGANIZATION

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

20. PERFORMING ORGANIZATION

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: CANFIELD, Craig J., COL
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign Intelligence Considered.

22. KEYWORDS (Precede EACH with Security Classification Code) (U)Drug Development; (U)Chemical Defense; (U)Molecular Modeling; (U)Chemical Poisons; (U)Pharmacodynamics; (U)Nerve Agents; (U)Chemical Synthesis

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

23.(U) To develop new drugs with protective activity against injury to military personnel in the event of exposure to chemical poisons.
24.(U) Potentially active drugs will be identified and obtained by synthesis or purchase. Candidate drugs will be tested in laboratory model systems to establish protective efficacy, mechanisms of pharmacological effects, effects on physiological responses and pharmacokinetic characteristics. Results will be used as input to computer-assisted molecular modeling system for evaluation to guide design of new compounds. Information is used in selection of candidate drugs for clinical trials.
25.(U) 8110-8209 Installation of new laboratory facilities for primary screening of candidate drugs was completed. The new laboratory animal model system was established and has been used in screening of approximately 30 compounds for efficacy against toxic doses of cyanide. The chemicals tested to date are thiol-containing. Thirteen of the compounds were active with survival rates exceeding 60 percent. Two compounds, WR 2823 and WR 61643, had 100% survival rates in tests against double the 50 percent Effective Dose of cyanide. This Work Unit is changed by consolidation with Work Unit "Molecular Modeling Drug Design and Development of CW Antidotes/Prophylactics", with title change from "Chemoprophylaxis of Chemical and Ionizing Radiation Injury". For technical report, see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

* 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 65 (FOR ARMY USE) ARE OBSOLETE.

PROJECT 3S162772A875AA Medical Systems of Nonconventional Environment

WORK UNIT 161 Development of Anti-Chemical Warfare Drugs

INVESTIGATORS:

Principal: COL David E. Davidson, Jr., VC
Associate: Dr. David Davis
Ms. Marie M. Grenan

PROBLEM AND OBJECTIVES:

Antidotes currently available to protect or treat U.S. military personnel who may be attacked with chemical weapons are inadequate, and for some types of chemicals which could be used against us, antidotes are non-existent or unsuitable for mass administration. The development of effective defensive measures would deter use of chemical agents by an enemy, and would improve the ability of military units to perform effectively if chemical agents were used.

PROGRESS:

The laboratory model system for primary screening of candidate antidotes for protective activity against chemical injury was developed and has been successfully employed. The system has potassium cyanide as the standard chemical agent administered subcutaneously at twice its LD_{50}. Sodium thiosulfate is administered intraperitoneally at 2 g/kg as a positive control. The Chemical Defense Laboratory at Building 500 was completed and the screening is now being conducted there. Primary screening has been done with thiol-containing compounds; 31 compounds were screened in 51 individual experiments. A number of the compounds were found to have activity against cyanide challenge as evidenced by survival. Two compounds (WR 2823 and WR 61643) were completely effective by this criterion at tested doses.

Studies continued in the previously established system in which compounds are investigated for their protective capacity by measuring the increase in dosage of potassium cyanide required as LD_{50} in their presence. Eleven cobalt salts were studied, of which the most promising was sodium cobaltinitrite, which by intravenous administration had a Protective Index of 4.1, corresponding to an elevation of LD_{50} of KCN to 41 ± 2.82 mg/kg. A combination of cobaltous acetate and sodium nitrite (1:2 molar ratio) raised the KCN LD_{50} to 33 ± 3.29. The combination of

sodium thiosulfate and dimethylaminophenol (DMAP) in two intravenous administrations raised the KCN LD_{50} to 52 ± 4.48. In studies of duration of action, DMAP persisted for 10 minutes, sodium nitrite for 45 minutes, sodium thiosulfate for 60 minutes and the combination of nitrite and thiosulfate for 53 minutes.

FUTURE OBJECTIVES:

The primary screen animal model will be employed for further studies with thiols and other chemical classes and secondary studies of active compounds will be undertaken for characterization of combined efficacy. Further development of the model system by employment of other chemical agents as standard toxic challenges is planned. Collaborative pharmacological studies with pyridostigmine and atropine are being developed. Studies of mechanism of action, including production of methemoglobin, are to be continued.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6478	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'NT	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A875	AB	162
b. CONTRIBUTING				
XXXXXXXXX	STOG 80-7.2:1			

11. TITLE (Procede with Security Classification Code)

(U) Development of Antiradiation Drugs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012100 Organic Chemistry 021600 Pharmacology 001600 Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 82	4.0	457
FISCAL YEAR CURRENT 83	2.0	370

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

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TELEPHONE: (301) 427-5411

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Drug Development; (U) Antiradiation Drugs; (U) Radiation Protection; (U) Ionizing Radiation; (U) Pharmacodynamics; (U) Chemical Synthesis

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 new drugs with protective activity against injury to military personnel in the event of exposure to ionizing radiation.

24. (U) Potentially active drugs will be identified and obtained by synthesis or purchase. Candidate drugs will be tested in laboratory model systems to establish protective efficacy, mechanisms of pharmacological effects, effects on physiological responses and pharmacokinetic characteristics. Information is used in guiding new drug synthesis and in selecting candidate drugs for clinical trials.

25. (U) 8110-8209 The cyclophosphamides synthesized as latentiated WR 2721 and analogs proved to have very low efficacy as radioprotectors and efforts in this area have ceased. Five additional "no-nitrogen" type antiradiation compounds have been produced and are awaiting testing. Considerable progress has been made in the synthetic effort in adamantyl amidinium protectors, with 10 new samples submitted, of which 7 show protection of 50% or greater at the $LD_{99}/30$, one is inactive as tested and two are awaiting test. Efforts have begun in two other areas: synthesis of cyclic pyrimidine potential prodrugs of WR 2721 and synthesis of direct analogs of WR 2721. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82. The title of this Work Unit is changed from "The Synthesis of Antiradiation Drugs".

*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 3S162772A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

Work Unit 162 Development of Antiradiation Drugs

Investigators:

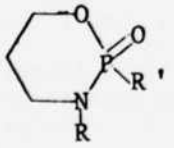
Principal: COL Craig J. Canfield, M.D.
Associates: LTC Robert O. Pick, Ph.D.; Daniel L. Klayman, Ph.D.; A.J. Lin, Ph.D.; William Y. Ellis, B.S.

The main thrust of this program is to design and synthesize effective antiradiation drugs that will be effective by oral administration and, at least, maintain the dose reduction factor obtained with WR 2721.

Efforts are continuing in the "no nitrogen" sulfide-sulfinate area, with five new samples submitted this year on which biology data are pending. The mercapto-amidinium adamantanes have significant activity in the primary test, but doses must be given which are near the toxic level. New efforts in the synthesis of analogs of WR 2721 have just begun.

Early attempts to produce hexahydropyrimidine "prodrug" forms of WR 2721 and analogs resulted in the formation of thiazolidines which have been sent for testing. Efforts are continuing in both areas, including attempts at protecting the phosphorothioate portion of the molecule.

The effort in latentiating WR 2721 has involved difficult problems in chemistry. The cyclophosphamide types could not be



R=H or CH_3

R'=antirad fragment such as WR 2721

prepared with R=H and, even with R=CH_3, the samples were hygroscopic and unstable. All of these types prepared have been considerably more toxic than WR 2721 and not radioprotective. Efforts have been discontinued in this area.

A method for the purification of a candidate antiradiation drug, WR 3689, was developed. Purity of the final product was confirmed by carbon -13 NMR spectroscopy.

The title of this work unit is changed from "The Synthesis of Antiradiation Drugs.")

Paper Published (See also work unit 156, DAOB6495 "Synthesis of of Antiparasitic Drugs."

1. Lin, A.J., Kelly, J.A., Breitman, T.R., Driscoll, J.S., Agents with Potential Specificity Against Melanotic Melanoma, J. Med. Chem., 25, 501 (1982).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6757	82 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 INSTN'S	9. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	H. Term	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A875	BD	163
XXXXXXXXX				
XXXXXXXXX	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code)

(U) Preclinical and Clinical Assessments of Antidotes

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 012600 Pharmacology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	4.0	462
	CURRENT 83	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: VON BREDOW, MAJ J.

NAME: PAMPLIN, LTC C. POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Pharmacology; (U) Antidotes; (U) Toxicity; (U) Pharmacokinetics; (U) Quantitation Methodology

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

23. (U) The technical objectives of this work unit are to obtain the necessary information to support a Notice of Claimed Investigational Exemption for a New Drug (IND) for candidate antidotes being developed for defense of military personnel in an integrated chemical/nuclear/conventional battlefield.

24. (U) A highly integrated, multidisciplinary effort is required to coordinate the extramural and intramural studies necessary to develop candidate antidotal agents. The actual studies performed are dictated by scientific rationale and existing federal regulations and include efficacy and toxicity studies, formulation development, pharmacokinetic and metabolic studies as well as clinical tolerance and efficacy studies.

25. (U) 81 10 - 82 09 Clinical safety and tolerance studies with the new formulation of 2 PAM Cl in autoinjectors were completed. Eighteen healthy subjects were divided into 3 dose groups and were given 1, 2, and 3 autoinjectors respectively. Minimal discomfort lasting 1-3 hours occured at the injection site(s). Protective plasma levels of 2 PAM Cl were maintained for approximately 1, 2 and 3 hours. No significant difference in bioavailability was observed in 2 subjects completing a crossover study comparing 600 mg 2 PAM Cl given IV with the same dose given by autoinjector. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81-30 Sep 82. This work unit is being terminated by consolidation with DA OH 0610 and DA OH 0609.

*Available to contractors upon originator's approval

DD FORM 1 MAR 86 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 85 (FOR ARMY USE) ARE OBSOLETE

Project No. 3S162771A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

Work Unit: 163 Preclinical and Clinical Assessments of Antidotes

This work unit is being terminated by consolidation with project number 3M463751D993, work units 061 and 063; assession numbers DA OH 0610 and 0609.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 8600	82 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
81 10 01	Change	U	U		NL	[X] YES [ ] NO	A. WORK UNIT

10. NO /CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A875	BC	164
b. CONTRIBUTING				
c. XXXXXXXXX	STOG 80-7.2;1			

11. TITLE (Precede with Security Classification Code)

(U) Behavioral Toxicology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

013400 Psychology 012900 Physiology 012600 Pharmacology 016800 Toxicology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 82	2.0	288
FISCAL YEAR CURRENT 83	2	320

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Hursh, S.R., MAJ

NAME: Kaufman, L.W., CPT

21. GENERAL USE

Foreign Inteiligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Chemical Defense; (U) Behavior; (U) Neuropsychiatry; (U) Toxicology; (U) Chronopharmacology

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

23. (U) Methods for assessing the impact of chemical defense-related compounds upon behavior will be evaluated. The overall objective will be development of testing protocols with maximum sensitivity to behavioral effects of chemical defense agents for use in the Army's effort to develop and field better antidotes to chemical agent exposure. There is military relevance in this research.

24. (U) The techniques of operant and respondent conditioning will be used to generate behavioral baselines which will be sensitive to the effects of chemical defense-related compounds. Dose-effect and time course functions will be determined in rodents and primates on procedures spanning a range of behavioral functions. Chronopharmacological effects will be evaluated. Methods of curve fitting and time series analysis will be used to evaluate drug effects.

25. (U) 81 10 - 82 09. Major findings: A sequence of operant conditioning tasks involving acquisition of new behavior, inhibition of behavior, discrimination of simple and more complex stimuli, and reversals of discrimination, was unaffected by prior exposure to a single, near-lethal dose of DFP, though performance in the radial arm maze is impaired at least 7 days post-DFP, and circadian feeding patterns are disrupted up to 21 days post-DFP. DFP toxicity is increased by high temperature, 27 hours of dehydration, but not by 24 hour dehydration or by subacute atropine pretreatment. Atropine was shown to degrade short-term memory, but not long-term memory of gerbils in a radial arm maze. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

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

Project: 3S162772A875 MEDICAL DEFENSE AGAINST CHEMICAL AGENTS

Work Unit: 164 Behavioral Toxicology

Investigators:

Principal:	Elsmore, Timothy F., Ph.D.
Associate:	Tyner, COL C.F.; Hursh, MAJ, S.R.; Raslear, CPT T.G.; Kaufman, CPT, L.W.

Objectives:

The overall objective of this work unit is the development of testing methods to evaluate behavioral effects of compounds used for pretreatment or therapy of chemical warfare agent exposure. Both the inherent toxicity of these compounds and their efficacy in preventing long-term behavioral and neurological damage will be evaluated. Thus, valid tests for detecting both acute and long-term behavioral deficits will be required. An additional goal is the definition of environmental variables affecting organophosphate toxicity.

Progress:

The finding that rats and other species of animals surviving exposure to the nerve agent "soman" may suffer diffuse neuronal degeneration throughout the brain (see WU 221, DAOG6753, Neuronal Mechanisms of Chemical Defense Agents and Antidotes), prompted a study of learning and memory in animals surviving poisoning with DFP, an organophosphate similar in action to the nerve agents. Rats were treated with approximately an LD20 dose of DFP, or saline, then exposed to a sequence of tasks involving acquisition of new behavior, extinction of that behavior, relearning, successive discriminations, simultaneous discriminations, and discrimination reversals. No differences were detected between DFP- and saline-treated animals. In a study in which rats lived in an environment permitting continuous monitoring of eating, drinking, and running in a running wheel, it was shown that DFP treatment produces changes in circadian rhythms of these activities including a decrease in activity, shifts in phase, and decreases in period. This study is being replicated. Other studies are in progress to evaluate the effects of DFP on sensory processes, perception, and startle reflexes. Preliminary results suggest that DFP treatment tends to speed up the perception of time.

A series of studies on the effects of nonpharmacologic manipulations on DFP toxicity are underway. These studies provide critical information on the way in which stressors that might be present in a chemical warfare environment will exacerbate the toxicity of the agents to which soldiers may be exposed. One hour exposure to high temperature (38 deg C.) was

shown to increase the lethality of a 1.75 mg/kg dose of DFP, compared with exposure to room temperature controls or rats exposed to cold (6 deg C.). Dehydration for 72 hours increased the lethality of DFP approximately 30% compared with 1 or 24 hour dehydration groups. Strenuous activity in the form of forced running in a running wheel or swimming to near exaustion was shown to have no effect on DFP toxicity.

The effects of atropine, which is currently the major component of antidotal therapy for nerve agent exposure, upon behavior are being examined in a study of short-term memory in gerbils. This study uses a radial maze with eight arms, four of which contain a food pellet. A well trained animal quickly finds the four pellets, and rarely enters arms of the maze which are never baited. Thus, both short term memory errors (entrance into previously entered arms) and long term memory errors (entrance into arms which are never baited) can be measured. Treatment with atropine sulfate in doses up to 10 mg/kg produced dose-related increases in time to complete the task, and in both types of errors, with the effect being greater upon short term memory. Injection with atropine methyl nitrate, which does not enter the brain, produced increases in time to completion, but did not increase errors.

Future objectives:

Long term effects of DFP exposure will continue to be investigated. The behavioral toxicity of atropine will be examined in a number of different procedures to develop an understanding of tests that are sensitive to manipulation of the cholinergic system. A rabbit model will be developed, to expand the species generality of our results. A primate display/response system based on a color video graphics and a touch sensitive screen will be developed to enable versatile programming of such tasks as visual tracking and various symbolic manipulation tasks.

Manuscript submitted

Raslear, T.G. and Kaufman, L. The effects of diisopropyl phosphorofluoridate (DFP) on circadian patterns of eating, running, and lever pressing. Submitted to Neurotoxicology.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OH 0608	82 09 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. NONK SECURITY	7. REGRADING	8a. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
82 02 01	Termination	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62734A	3M162734A875	AM	165
b. CONTRIBUTING				
c. [redacted]	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code)

(U) Molecular Modeling Drug Design and Development of CW Antidotes/Prophylactics

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 012600 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
82 02	82 09 30	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 81	0	0
	CURRENT 82	0.6	75

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Canfield, Craig J., COL MC

NAME: Tang, Lily C., M.D., Ph.D.

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U)Molecular Modeling;(U)Nerve Agents;(U)Antidotes;(U)Prophylaxis;(U)Drug Development

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 define, develop, maintain, and implement a rational approach to drug design as well as conduct both in-house and contract design, synthesis, and testing of potential candidate antidotes/prophylactics for military use.

24. (U) Computer-assisted molecular modeling system to evaluate physical-chemical parameters of active sites will be developed. Effect of candidate drugs will be biologically determined and results used as input to modeling system for design of antidotes/prophylactics. Appropriate molecular modifications will be synthesized and evaluated.

25. (U) 82 02-82 09 Laboratory requirements have been planned, equipment and supplies ordered, and a draft protocol written such that studies may be initiated when the laboratory is ready. Background information on modeling systems is being obtained, and an RFQ being written. This work unit is being terminated by consolidation with the old work unit #161, "Preclinical Studies of Anti-Chemical Warfare Drugs."

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1455A, 1 NOV 65 AND 1455-1, 1 MAR 65 (FOR ARMY USE) ARE OBSOLETE

Project 3M162734A875 MEDICAL SYSTEMS OF NONCONVENTIONAL ENVIRONMENT

Work Unit 165 Molecular Modeling Drug Design and Development of CW Antidotes/Prophylactics

Investigators:

Principal: LTC Robert O. Pick, Ph.D.
Associates: COL Craig J. Canfield, MC;
Lily C. Tang, M.D., Ph.D.

Laboratory requirements have been planned, equipment and supplies ordered, and a draft protocol written such that studies may be initiated when the laboratory is ready. Background information on modeling systems is being obtained, as well as information on the status of identification, isolation, production and purification of relevant components of the cholinergic system to be used in obtaining structural information for use in modeling and design of CW antidotes/prophylactics. This unit has only been staffed since February 1982 and is being terminated by consolidation with the old work unit "Preclinical Studies of Anti-Chemical Warfare Drugs," (DAOH 0609).

Publication

1. Tang, L.C.: Manganese and Levodopa, J. of Neurotoxicity, (in press).

Presentations

1. Tang, L.C. "Levodopa Enhances Brain Enzyme Activity and Immune Response in Aged Mice", Transactions of The American Society for Neurochemistry 13, 1982.

2. Tang, L.C. "Brain Enzyme and Tumor Incidence", Proceedings of 13th International Cancer Congress, 1982.

3. Tang, L.C. "Manganese and Levodopa (Relationship to Human Disease)", 1st International Neurotoxicity Symposium, 1982.

PROJECT 3E162777A878

HEALTH HAZARDS OF MILITARY MATERIEL

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OB 6484	2. DATE OF SUMMARY* 82 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 81 10 01	4. KIND OF SUMMARY D. Change	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING*	8A. DISP'N INSTR'N NL	8b. SPECIFIC DATA - CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A878	BB	041
b. CONTRIBUTING				
XXXXXXXXXX	STOG 80-7.2:4			

11. TITLE (Precede with Security Classification Code)*

(U) Biological Interactions with and Hazards of Microwave Radiation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

014100 Radiobiol 012900 Physiol 014000 Rad Chem 017000Wave Prog 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
71 07	CONT	DA			C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	3.0	752
	CURRENT 83	3.0	1,023

19a. 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

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:* L. E. Larsen

TELEPHONE: 202-576-3615

SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE

Foreign Intelligence Considered

ASSOCIATE INVESTIGATORS

NAME: J. H. Jacobi

NAME: E. L. Hunt POC: DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Microwave Hazards; (U) Bioeffects; (U) Dosimetry; (U) Biophysics; (U) Military Medicine; (U) Psychology

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

23. (U) To provide technical and medical information to the Surgeon General, system developers and agencies responsible for safety standards in order to protect the health and effectiveness of military units and affected civilian populations in microwave and RF environments. This requires analysis of the biophysics and bioeffects attributable to non-ionizing radiation under laboratory conditions which reasonably simulate and/or predict operational exposures.

24. (U) To perform basic and applied research on the problem of microwave and RF interactions with biosystems at all levels of analysis from the cellular and molecular to metazoan physiology, pathophysiology and behavior. This requires development of measurement systems for dosimetric analysis, in vitro and in situ; the evaluation of frequency, power level, polarization and modulation as important parameters of the radiation; and the use of low level energy to assess the functional state of cells and tissues.

25. (U) 81 10 - 82 09 The previous findings of increased ocular lens pathology in vitro with pulsed exposures as compared to continuous wave exposures of equal average power have been confirmed & extended. Furthermore, a distinctive energy-per-pulse dependance was shown in the damage threshold for pulse-modulated exposures. The dosimetric studies have continued with completion of a new three dimensional scanner and image display/processing systems. A new program for correction of refractive errors in dosimetric images has begun to compare ray based (linear as well as non linear) & diffraction methods. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sept. 82.

*Available to contractors upon originator's approval.

Project 3E162777A878 HEALTH HAZARDS OF MILITARY MATERIAL

Work Unit 041: Biological Interactions With and Hazards of Microwave Radiation

Investigators.
Principal: LTC(P) Lawrence E. Larsen, M.D.
Associate: John H. Jacobi, M.S.; Edward L. Hunt, B.A.; Peter V.K. Brown, M.S.; Charles N. Rafferty, Ph.D.

Objectives

The objectives of this research are to provide quantification, medical evaluation, prevention and treatment of the hazards of microwave energy. The mechanism of hazard production is used along with field and target characteristics to allow maximum generality and predictive value for the results so obtained. These objectives are reached through four program areas which are dosimetry, pulsed field effects, behavioral effects and dielectric relaxation.

Progress and Background

a. Dosimetry measures the spatial distribution of absorbed microwave energy in biological systems. Due to the non-uniformity of absorbed energy, the target organ concept is employed to allow generality of results. Progress includes development of a polarization diverse water coupled element, development of a rotary scanner to collect data on scattered fields at all angles and rebuilding of the water coupled arrays that were destroyed by corosion.

b. Thermoacoustic expansion is a hazard mechanism which adds a mechanical pressure wave to the heat value of the applied microwave energy. This addresses the importance of modulation in safety standards. Progress includes demonstration of qualitative and quantitative differences in cellular ultrastructure of the murine ocular lens under *in vitro* exposure for pulse and continuous exposure of the same average power. Further, an energy per pulse dependency in threshold for pulse related ultrastructural defects was demonstrated.

c. Behavioral studies are essentially suspended due to inoperable exposure facilities.

d. Dielectric relaxation studies relate to the constituitive properties of tissue as media for the propagation of microwave energy. These properties are predicted to behave differently under pulse or transient conditions than cw or steady state conditions. A new theory or dielectric relaxation has been developed which requires two relaxation times under transient conditions: one for a co-operative effect and a second one for individual dipole effects.

PROJECT 3E162777A878 HEALTH HAZARDS OF MILITARY MATERIAL

Work Unit 041: Biological Interactions With and Hazards of Microwave Radiation

Bibliography

1. Larsen, L.E., Jacobi, J.H.: Method of Microwave Imagery for Biological Applications, in Diagnostic Imagery in Medicine, Part II, Advances in Source and Detector Technology, Martinus Nijhoff, B.V., The Hague, 1982, in press.

2. Stewart-DeHaan, P.J., Creighton, M.O., Larsen, L.E., Jacobi, J.H., Ross, W.M., Sanwal, M., Trevithick, J.R.: In Vitro Studies of Microwave Induced Cataract: separation of heating and field effects, Exp. Eye Res., in press, 1982.

3. Guo, T.C., Guo, W.W.: A Transient-state Model of Dielectric Relaxation Accounting for Lag or Many-body Effect, J. Electrostatics, 12: 229, 1982.

4. Larsen, L.E., Jacobi, J.H.: The Use of Polarization Diversity in Microwave Transmission Imagery of Isolated Canine Kidney, Proc Symp Electromagnetic Dosimetric Imagery, L.E. Larsen, J.H. Jacobi, Eds, in press, 1982.

5. Jacobi, J.H., Larsen, L.E.: Linear FM Pulse Compression Radar Techniques Applied to Biological Imaging, op cit.

6. Fots, S.J., Flam, R., Aubin, J., Larsen, L.E., Jacobi, J.H.: Water Immersed Phased Array System for Biological Target Investigation op cit.

7. Guo, T.C., Guo, W.W.: A Transient-state Theory of Dielectrics Relaxation and the Curie-von Schweidler Law, Phys Rev Lett., in press, 1982.

8. Guo, T.C., Guo, W.W.: A Transient-state Theory of Dielectric Relaxation and Eupirical Laws, Proc Conf Electrical Insulation and Dielectric Phenomena, in press, 1982.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6472	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. RESEARCH	8a. RESP'N INSTN'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D Change	U	U		NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY				042
b. CONTRIBUTING	61102A	3E162777AB878	AB	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Non-auditory Effects of Blast Overpressure

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
017100 Weapons Effects 013300 Protective Equipment 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 03	CONT	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: 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 82	6.0	326
	CURRENT 83	6.0	422

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
Foreign Intelligence Considered

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TELEPHONE: (301) 427-5380
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME: Robert F. Hoyt, Jr., MAJ, VC
NAME: Andrew J. Young, CPT, MSC

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Human Volunteers; (U) Impulse Noise
(U) Blast Overpressure; (U) Pulmonary Physiology; (U) Gastrointestinal Physiology

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

23. (U) To define the physiologic effects of blast overpressure (BOP) exposure upon the human. To develop a laboratory model of blast injury. To assist in special studies of weapon specific BOP at the direction of HQ, USAMRDC.
24. (U) High velocity water jet of discrete impulse will model blast effects on large animals. Assays of lung water and parenchymal function will be used to assess injury. Pathologic comparison of water jet and blast-injured specimens will be done. Chronic effects of repeated BOP exposure will be assessed in man and animals. Pathophysiologic events of blast injury will be monitored by implantable transducers.
25. (U) Ten human volunteers were exposed to simulated M198/M203 blast waveforms in order to determine the effects of body orientation, arm position and clothing ensemble on intrathoracic pressure. Similar data was obtained from an anthropomorphic dummy. Provided non-auditory blast injury assessment for a double hearing protection study at Aberdeen Proving Ground. Performed initial validation study of water jet impactor. Designed additional test equipment needed for water jet validation studies. Completed iso-peak pressure/iso-impulse study and double peak study at Lovelace in order to develop a greater understanding of blast biophysics and resonance effects. Performed preliminary study of the effect of lung volume on injury potential. Developed tracheostomy prosthesis for chronic pulmonary function testing of unanesthetized sheep. Developed capability for a chronic thoracic lymph fistula preparation in sheep. Developed capability for chronic measurement of transthoracic electrical impedance in sheep using subcutaneous electrodes. Conducted a weapon-specific animal study at Lovelace demonstrating the relative safety of the 81mm mortar blast wave.
1 Oct 81-30 Sep 82.

*Available to contractors upon originator's approval

DD FORM 1498 1 MAR 88

Project 3E162777AB878: HEALTH HAZARDS OF MILITARY MATERIEL

Work Unit 042: Non-auditory Effects of Blast Overpressure

Principal Investigator: Yancy Y Phillips, M.D., MAJ, MC

Associate Investigators: James J. Jaeger, Ph.D., MAJ, MSC
Jeffrey L. Hess, D.V.M., MS, MAJ, VC
Robert F. Hoyt, Jr., D.V.M., MS, MAJ, VC
Andrew J. Young, Ph.D., CPT, MSC
Patrick E. Lorenz, Ph.D., CPT(P), MSC

Problem Statement and Objectives

Certain Army weapon systems, some currently in use, others still in development, produce levels of blast overpressure which exceed the limits defined in MIL STD 1474B. The research objective of the Department of Clinical Physiology is to define the risk of non-auditory injury to crewmembers from the blast overpressure produced by these weapon systems. Toward this end experiments are conducted to obtain various types of physiological and biophysical data which can be related to injury to the larynx, trachea, lungs or gastrointestinal tract.

Progress in FY 82

a. Human volunteer test subjects were exposed to a simulation of the blast wave observed in the operator's position of the M198 Howitzer firing the M203 charge. The purpose of the experiment was to determine the effects of body orientation, arm position, and clothing ensemble on the pressure recorded in the thorax. The statistical analysis of the data is nearly complete. A final report will be written in the first quarter of FY 83.

b. An anthropomorphic dummy on loan from Dr. Arne Jonsson, an investigator in the area of blast biology with the Swedish National Defense Research Institute, was exposed to the same levels of blast overpressure as described for the humans in (a.) above. When the analysis of the internal pressures recorded in the "thorax" of the dummy are complete, a comparison with the human data will be made. Data comparisons will be made in terms of resonant frequency as well as the effects of orientation, arm position and clothing.

c. Members of our research group provided substantial support for the human investigation conducted at Aberdeen Proving Ground in the first quarter of FY 82. The purpose of the study was to test the adequacy of single and double hearing protection in preventing hearing threshold shifts in operators of the M198/M203 weapon system. Our group provided the human risk assessment as well as help in recruiting, screening and evaluating test subjects for

possible non-auditory injury. Data from the study clearly indicate that single hearing protection (E-A-R® foam ear plugs) is sufficient to prevent significant threshold shifts in M198 crewmembers firing up to 25 rounds of M203 charge. There was no evidence of injury to the larynx or gastrointestinal tract of any of the test subjects as assessed by indirect laryngoscopy and stool testing for occult blood.

d. The water jet impactor, a device constructed to simulate air blast effects in animal investigations, was received from the contractor. The impactor was installed and after preliminary mechanical checks were made, three sheep were exposed to repeated blasts with peak pressure estimated to be 20 to 25 psi. The first animal received 20 consecutive blasts and the second two received 100 each. None of the animals exhibited injury to any air containing organ when examined at necropsy. Observations made during these firing series indicated that there were substantial timing errors in the arrival of the eight water jets. Further animal tests were postponed until the valve timing could be sychronized. Since the instrumentation needed to perform the procedure only became available in early October, the valve adjustment, mechanical testing and additional animal exposures will take place in the first quarter, FY 83.

e. The iso-peak pressure, iso-impulse animal exposures which were started in FY 81, were completed in FY 82. In these experiments, blast injury to the larynx, lungs and gastrointestinal tract were reported as a function of both the peak pressure and the impulse to which the animal was exposed. It was determined that as in the case of structures exposed to air blast, injuries to the air containing organs of sheep are a non-linear function of both peak pressure and impulse with a minimum peak pressure and a minimum impulse required.

f. An animal experiment designed to test the hypothesis that the resonant frequency of the thorax is an important factor influencing the degree of lung injury caused by air blast was conducted at the Lovelace research facility in Albuquerque, NM. A total of 25 sheep were exposed to blast waves with double peaks. The time interval between the peaks was systematically varied between 2 and 14 msec in 2 msec intervals. Since the resonant frequency of the sheep thorax is approximately 80 Hz, it was felt that between peak intervals of 8 to 10 msec should produce more severe lung injury than intervals of 2 to 4 msec. The relationship between wet lung weight (our index of injury) and peak-to-peak interval did not support the hypothesis. There was no apparent relationship between the level of lung injury and the peak-to-peak interval. These studies will have a significant impact on future studies on the effects of complex blast waves.

g. Preliminary animal experiments on 6 sheep were conducted at Lovelace to investigate the effects of the degree of lung inflation on pulmonary injury from blast. Sheep were exposed to injury producing levels of blast with airway pressure fixed momentarily at either +30 or -30 cm H_2O. Initial observations indicate that lung hemorrhage was more severe at low lung volume than at high, but due to technical problems and the large standard deviation of lung weight data, the study will need to be repeated with improved techniques and a larger set of animals. In future studies we plan to systematically vary airway pressure, and thus lung volume, over the entire physiologic range.

h. Several types of tracheostomy prostheses and implantation techniques have been tested in preparation for blast overpressure studies where physiologic measures of lung function are to be made in awake sheep. Devices were implanted in 13 animals which were then periodically examined for stability and function of the device. One of the devices, an original design of the investigator appears to have been quite successful, performing as expected over the first ten months of observation.

j. The major effort of the project's veterinary surgeon and staff has been the development of an acute and a chronic thoracic lymph fistula preparation. Twenty sheep have been utilized in the development and refinement of this preparation in our laboratory. The last three preparations have been successful in all aspects but for the length of time that the lymph cannula remained patent. It is expected that through consultation with other investigators using this preparation, final adjustments in technique will yield a working preparation for the desired 7 to 10 days. This thoracic lymph preparation will be the centerpiece for the FY 83 research effort providing valuable information on pulmonary vascular permeability changes following blast exposure as well as an ideal source of lymph from which a biochemical marker of lung injury may be isolated.

k. The measurement of transthoracic electrical impedance as an indicator of shifts in thoracic fluid volume has been established in a chronic animal preparation in our laboratory. To date, six animals have had circumferential wire electrodes implanted subcutaneously allowing repeated and long-term measurement of this physiological parameter. No problems have been encountered with animals over a 10 week measurement period. Data are being collected describing the within-day and day-to-day variability of transthoracic electrical impedance. In the first quarter of FY 83, chronically instrumented sheep will be exposed to injury producing levels of blast overpressure with the objective of describing the time course of thoracic fluid shifts following blast injury.

l. At the request of the USAMRDC Research Area III Manager and the Project Manager for the improved 81 mm mortar system, a weapon-specific animal study was conducted at the Lovelace research facility in the final quarter of FY 82. Thirty-six sheep were exposed to the worst case blast exposure condition for the improved 81 mm mortar in order to provide a statistically valid set of data upon which to base a human risk assessment for a walk-up study. Each animal was exposed to 300 consecutive shots with a peak pressure of approximately 5.7 psi and an A-impulse of 1.7 psi msec. No evidence of blast related pathology was detected at necropsy in any of the 36 sheep. This negative finding will form the basis of a human risk assessment allowing the exposure of human volunteers to mortar blast overpressure in a hearing protection study to be conducted in FY 83.

m. The project's laboratory data acquisition system was upgraded by the installation of the RT-11 operating system and the FORTRAN IV programing language. These added capabilities will aid the project in the development of fast fourier transform analysis for blast waves and in the development of more efficient programs for analysis of physioloigical data.

n. International contacts in the area of blast overpressure research were as follows:

MAJ Yancy Phillips and MAJ James Jaeger, U. S. Participants in the NATO AC/243 Panel 8, Research Study Group 6 on the Effects of Impulse Noise, Oslow, Norway.

MAJ Yancy Phillips and MAJ James Jaeger, site visits to the blast researach facility of the Institut Franco-Allemand, St. Louis, France and the Explosives Division National Defense Research Institute, Stockholm, Sweden.

Recommendations and Objectives FY 83

The major research efforts of FY 83 under this work unit will be:

a. The application of the measurement of transthoracic electrical impedance to blast injury studies.

b. The utilization of the chronic thoracic lymph fistula preparation in studies describing changes in pulmonary vascular permeability following blast exposure and blast exposure plus exercise.

c. The evaluation of the water jet impactor as a laboratory model of blast overpressure.

d. The accumulation of biophysical data from animals and humans to include chest wall motion parameters and intrathoracic pressures during blast exposure.

References Cited

None

Presentations

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Jaeger, J. J. et al. Low Level Blast Exposure in Humans. NATO AC/243 Panel 8, Research Study Group 6, Oslow Norway, May 1982.

Phillips, Y. Y et al. Biophysics of Injury from Repeated Blast. Triparte Technology Coordinating Program Panel W-2, Muzzle Blast Overpressure Workshop, Picatinny Arsenal, NJ, May 1982.

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Hoyt, R. F., Jr. Cricothyroidotomy, Tracheotomy and Tracheostomy. Emergency War Surgery Training Program, Symposium on Military Veterinary Medicine, WRAIR, Washington, D.C., March 1982.

Bibliography

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A. Cymerman, C. S. Fulco, A. J. Young and J. T. Maher. Acute Moutain Sickness: Relationship to Forearm Vascular Responses. Fed. Proc. 41:1616, 1982.

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Fulco, C. S., A. Cymerman, N. A. Pimental, A. J. Young and J. T. Maher. Anthropometric changes associated with weight loss at high altitude. Fed. Proc. 41:1617, 1982.

Jaeger, J. J. and Y. Y Phillips. A simplified technique for eucapneic hyperventilation. Fed. Proc. 41:1627, 1982.

PROJECT 3E162777A879

FACTORS LIMITING SOLDIERS EFFECTIVENESS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6453	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AA	041
b. [redacted]				
c. [redacted]	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: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	7.5	806
	CURRENT 83	7.5	746

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Harris, LTC J.

NAME: Van Vranken, LTC E. POC: DA

21. GENERAL USE

Foreign intelligence considered

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 minimization of psychiatric casualties.

24. (U) The methods of clinical psychiatry, social and clinical psychology, social anthropology and field epidemiology are used to identify factors that generate psychiatric casualties, behavior dysfunction and performance dysfunction and decrement in order to modify such factors or the relationship between them.

25. (U) 81 10-82-09 Studies of the effects of prolonged deployment on family stress and its interactive effects upon the active duty soldier utilizing the Battalion of the 82nd Airborne Div that deployed to the Sinai MFO were initiated and continue. Studies of the impact of NMS Cohort on family stress and the performance and sustainability of the active duty soldier are in the process of development as per tasking from TAGO. Studies of military communities at Ft Bragg, at the request of the 82nd ABN, and at Ft Hood, at the request of the 1st Cav Div, designed to look at the interaction between the military community, family and soldier stress and unit cohesion, were initiated and carried out during the past fiscal year. Further phases of these studies focused on the off-post dweller are projected for the coming fiscal year. Studies of the relationship of the health outcome and deployment in active duty members and military families continue at the 82nd ABN. Studies of generation of stress in Special Forces units and the interrelationship of A-team structure and high family stress and problems were initiated at the request of JFK Center, Ft Bragg and continue. Studies of the High Frequency User of the military medical care system are presently under way and will be completed in FY 83.

For technical report, see Walter Reed Army Inst of Research Annual Progress Report, 1 Oct 81-30 Sep 82.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 64 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1455A, 1 NOV 58 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 041 Military Preventive Psychiatry

Investigators.
Principal: David H. Marlowe, Ph.D.
Associate: COL Franklin D. Jones, MC; COL Norman M. Camp, MC; LTC(P) Jesse J. Harris, MS; LTC Edwin W. Van Vranken, MS; CPT Robert H. Stretch, MS; CPT Linda K. Jellen, MS; CPT Kathryn H. Knudson, MS; CPT Darleen M. Vernon, MS; CPT Terrence D. Fullerton, MS; CPT Ronald Smith, MS; William E. Datel, Ph.D.; Joseph M. Rothberg, Ph.D.; Peter K. Levison, Ph.D.; Richard Howard, M.A.; Richard Oldakowski; SFC Thaddeus M. Savage; SSG Edgar N. Marshall; SSG James E. Hall; SSG Richard W. Pickle; SSG Emily M. Kukura; SP5 Diana L. Smith; SP5 Calvin E. Cummings; SP5 Daniel J. Helm; SP5 William D. Rigney; SP5 Alvin B. Taylor; SP5 Veronica L. Davis; SP5 Dawn D. Caban; SP4 Donna M. Ross.

Description

Neuropsychiatric casualties have represented a major source of manpower loss in every armed conflict in which the United States Army has been involved. In times of peace the Army suffers significant personnel losses and costs as a function of behavioral dysfunctions, performance decrements, effectiveness deficits, psychosomatic illnesses, psychogenically based disorders and neuropsychiatric diseases. Many of these losses and costs appear to involve predisposing risk factors that are parts of the general and human ecology of the Army. Unique aspects and demands of military life engender both strains and stresses that further the risk of the individual and the group for dysfunctional and ineffective behavior. The symptomatic and often costly responses to stressful events and factors in the military are in part determined by the health status and coping styles of the individual and in part by the social milieu in which stressful events are experienced. This unit examines the interaction of the individual and group within this special set of ecological settings, ranging from the intense, life-threatening multiple stresses of combat to the daily stresses and strains of garrison and training, examines the dynamics of those specific factors within the military organizations and environments that conduce to psychiatric illness, operate to produce psychiatric casualties, and lead to ineffectiveness, the generation of dysfunctional behaviors, and decrements in military performance.

Progress

Field studies with the 82nd Airborne Division continue. Data collection and analysis continue in the health problems of deployment study initiated in FY81 at Ft Bragg. Preliminary data indicate that minor health and family problems have little or no effect on the serviceman and his deployment. The pre-deployment period seems most stressful for the wives. Such stresses include depression, breakdown, family problems and tensions, and husbands' absence. The most pertinent problems for most wives during the deployment period were problems with child care and child behavior and child rearing. In this sample there was no increase in requests for attention for minor ailments for either spouses or children during the deployments.

During this FY, studies were commenced, an an extension of health problems of deployment, of both the active duty members and families of the battalion which deployed to the Sinai as part of the MFO. Two observers accompanied the battalion to the Sinai and remained with it for its full tour in Egypt. A large data base consisting of questionnaires, interviews, and observational materials was acquired and is presently being arrayed and prepared for analysis. Studies were and continue to be carried out on the impact of deployment on families, family stress, and family structure, as well as on the mitigating effects of both interpersonal supports and the family support system offered by the 82nd Airborne Division. In passing, it should be noted that the wives of officers and senior NCOs in this sample were the best supported and those of lower ranking enlisted at greatest risk. The primary locus of support within the military community was the rear detachment of the battalion.

Studies have commenced at the request of the John F. Kennedy Center of problems of stress in Special Forces A teams and in the families of members of Special Forces. Initial interviewing and observation is presently under way and data will be collected for the next three years.

At the request of the 82nd Airborne Division, a study of an enlisted housing community at Ft Bragg was carried out. An interview and observational study was completed and data are presently under analysis.

At the request of the 1st Cavalry Division, Ft Hood, Texas, a survey of three on-post housing areas was carried out during the past FY. Questionnaire materials were gathered on 325 households. Materials are presently being arrayed and analyzed.

A study of patterns of stress experienced by drill sergeants in the training base commenced during FY82. Data has been gathered on a sample of 45% of the drill sergeants in CONUS and an equivalent number of controls (NCOs also in the training base). The data has been coded and arrayed and is presently undergoing analysis.

The training program in combat psychiatry and combat stress for foreign visiting military psychiatrists was developed under the auspices of the Combat Stress Working Group. In addition, a comprehensive bibliography on combat stress, etiology, prevention and treatment have been developed. An outline for a handbook of combat psychiatry has been developed and is now in draft form.

Data analysis has continued examining the impact of drug and alcohol use on cohesion in tank crews. Preliminary results indicate that perceptions soldiers have of one another are influenced by drug use. These results indicate that as the level of drug use increases (beyond occasional or recreational use) evaluations are degraded. Social relationships among soldiers appear to be influenced more by drug and alcohol use than job evaluations. Final results are expected next fiscal year.

Future Recommendations and Objectives

Anticipated progress through FY83 will emphasize further extensions of early stage projects as well as completion of end-stage projects in progress. Expected results include further elucidation in questions regarding the relationship between stress in special military occupations such as that of drill instructor or in Special Forces troops and social/family support factors; clarifications regarding structure and unique social dynamics of the military family and community as soldier support network, both under conventional circumstances and following introduction of the New Manning System cohort units; as well as elaborations from the data collected both from families remaining in CONUS and from deployed units in Egypt regarding patterns of stress and adjustment.

TAGO has tasked the Department of Military Psychiatry with family research in support of the New Manning System. Protocols have been developed utilizing research on the relationship between the military family and New Manning System Cohort Unit that will get under way in FY83. This research will take place at an Army posts in CONUS as well as OCONUS in collaboration with USAMRU-E and ARI.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 041 Military Preventive Psychiatry

Papers Presented at Scientific Meetings

1. Harris, J.J., LTC(P). The Health of Women in the Army. Paper for Panel on Women in Military and Paramilitary Organizations. American Psychological Association meetings, Washington, DC, August 1982.

2. Harris, J.J., LTC(P) & Knudson, Kathryn H.M., CPT. Women in the Army: a study. Michigan Women's Studies Association, April 3, 1982.

3. Jellen, Linda K., CPT. Army Social Work Reflections on the Past & Challenges for the Future, Social Work Practice Course, 1982.

4. Jones, F.D., COL. Division Psychiatry: Role of the Physician (with M.G. Deekin, G.L. Belinky, P.A. Newhouse, S.D. Eshelman, M.T. Parker), Annual American Psychiatric Association Meeting, 15-21 May 1982.

5. Jones, F.D., COL. Training Military Psychiatrists for Their Future Roles (with R.E. Hales), Annual American Psychiatric Association Meeting, Toronto, 15-21 May 1982.

6. Jones, F.D., COL. Applications of military psychiatry to terrorism, hostage negotiation, disasters, and refugees (with P. Harris and Y.H. Fong), AMEDD Division and Combat Psychiatry Conference, Denver, CO., 13-16 April 1982.

7. Jones, F.D., COL. The role of the psychiatric consultant in future combat and comments on combat use of psychotropics. AMEDD Military Psychiatry Conference, Uniformed Services University of Health Sciences, Bethesda, MD., 25-28 May 1982.

8. Jones, F.D., COL. Neuropsychiatric casualties of NBC warfare. XXXVI Congress of the Interallied Confederation of Medical Reserve Officers (CIOMR), Washington, DC, 8-15 August 1982.

9. Knudson, Kathryn H.M., CPT; Jellen, Linda K., CPT; Harris, Jesse J., LTC(P); Schneider, Robert J., MAJ; Oldakowski, R. Health and family problems related to short-term Army deployments. Paper for the meetings of the American Psychological Association, Washington, DC, 1982.

10. Marlowe, D.H. Women in the Armed Forces. XXIV International Congress of Military Medicine and Pharmacy, Athens, Greece, April 1982.

11. Marlowe, D.H. Health Problems of Women in the Army. USAREUR Medical Service Corps, Annual Meeting, Garmisch, Germany, May 1982.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 041 Military Preventive Psychiatry

Publications

1. Jones, F.D. An evolutionary learning perspective on pain. Chapter 12 in The Treatment of Pain, H. Wain and D. Devaris (Eds), Jason Aronson Publisher, Chicago, IL, 1982.

2. Jones, F.D. (with G.L. Belenky). The evacuation syndrome in military exercises: A model of the psychiatric casualties of combat. Proceedings, Users' Workshop on Combat Stress. A.D. Mangelsdorff and P.T. Furukawa (Eds), Academy of Health Sciences, 2-4 Sep 1981, published Nov 1981.

3. Jones, F.D. (with R.E. Hales). Teaching the principles of combat psychiatry to Army psychiatry residents. Military Medicine, in press.

4. Knudson, Kathryn H.M. & Kagan, Spencer. Differential development of empathy and prosocial behavior. Journal of Genetic Psychology, 1982, 140, 249-251.

5. Marlowe, D.H. The AVF and the Draft. In Robert K. Fullenwider, ed., The Morality of Military Service. Maryland Studies in Public Philosophy. Totowa, N.J.: Rowman and Littlefield, 1983, forthcoming.

6. Marlowe, D.H. Women, Men, and the Draft. In Robert K. Fullenwider, ed., The Morality of Military Service. Maryland Studies in Public Philosophy. Totowa, N.J.: Rowman and Littlefield, 1983, forthcoming.

7. Marlowe, D.H. The Utilization of Women in the Armed Forces, Proceedings, XXIV International Congress of Military Medicine and Pharmacy, Athens, April 1982.

8. Segal, M. The Argument for Female Combatants. In Nancy L. Goldman, ed., Female Soldiers - Combatants or Noncombatants: Historical and Contemporary Perspectives. Westport, Conn.: Greenwood Press, 1982, pp 267-290.

9. Segal, M. Social Change and the Participation of Women in the American Military (with David R. Segal). In Louis Kriesberg, ed., Research in Social Movements, Conflicts and Change, Vol 5. Greenwich: JAI Press, 1982, forthcoming.

10. Segal, M. Women's Roles in the U.S. Armed Forces: An evaluation of Evidence and Arguments for Policy Decisions. In Robert K. Fullenwider, ed., The Morality of Military Service. Maryland Studies in Public Philosophy. Totowa, N.J.: Rowman and Littlefield, 1983, forthcoming.

11. Segal, M. Scientific Knowledge Applicable to Decisions Regarding Women in the Military (with Kathryn Knudson). First draft of the report presented at the 1982 meeting of the Committee on Women in the N.A.T.O. Forces, Oslo, Norway, June 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	3. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6454	82 10 01	DD-DR&E(AR)636

2. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISS'R INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AA	042
b. [redacted]				
c. [redacted]	STOG 80-7.2.4			

11. TITLE (Precede with Security Classification Code)

(U) Military Psychiatric Epidemiology

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 013400 Psychology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	5.5	404
	CURRENT 83	5.5	498

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Camp, COL, N.

NAME: Rothberg, J. M., Ph.D. POC: DA

21. GENERAL USE

Foreign intelligence considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Military Adjustment; (U) Psychiatric Illness; (U) Epidemiology; (U) Behavioral Dysfunction; (U) Psychosocial 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)81 10-82 09 Collection and analysis of outpatient data concerning troops and families of the 82nd ABN continue. Data collection is being shifted to 2nd Bde in support of NMS Cohort studies. A wide-scale prevalence study of stress and perceived stress among drill sergeants is presently under way. Materials have been gathered on several thousand drill sergeants and controls and are presently under analysis. A study of patterns of psychiatric care giving and use of psychotropic medication during the Vietnam conflict is presently under way involving a survey of all psychiatrists who served in RVN. Data gathering phase of a study of the careers and incidents of delayed stress syndrome among Vietnam veterans who remained on active duty has been completed. Data gathering from controls is still under way and preliminary analyses are being undertaken. Work continues on studies of cohorts of female personnel and their psychosocial and career health outcomes. Analysis of past and present patterns of psychiatric, psychosomatic, and stress-related diseases as well as behavioral dysfunctions continues as do special epidemiological studies. For technical report, see WRAIR Annual Progress Report, 1 Oct 81-30 Sep 82.

*Available to contractors upon originator's approval

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 042 Military Psychiatric Epidemiology

Investigators.
Principal: David H. Marlowe, Ph.D.
Associate: COL Franklin D. Jones, MC; COL Norman M. Camp, MC; LTC(P) Jesse J. Harris, MS; LTC Edwin W. Van Vranken, MS; CPT Robert H. Stretch, MS; CPT Linda K. Jellen, MS; CPT Kathryn H. Knudson, MS; CPT Darleen M. Vernon, MS; CPT Terrence D. Fullerton, MS; CPT Ronald Smith, MS; William E. Datel, Ph.D.; Joseph M. Rothberg, Ph.D.; Peter K. Levison, Ph.D.; Richard Howard, M.A.; Richard Oldakowski; SFC Thaddeus M. Savage; SSG Edgar N. Marshall; SSG James E. Hall; SSG Richard W. Pickle; SSG Emily M. Kukura; SP5 Diana L. Smith; SP5 Calvin E. Cummings; SP5 Daniel J. Helm; SP5 William D. Rigney; SP5 Alvin B. Taylor; SP5 Veronica L. Davis; SP5 Dawn D. Caban; SP4 Donna M. Ross.

Description

The military environment places demands and strains upon its population that are markedly different from those of civilian environments. The demands and differences in terms of individual and unit effectiveness and performance, mental and physical health, and behavioral disruption and dysfunction have chronic effects in peacetime. In periods of deployment and combat, such stresses may have acute effects on the capability of units and individuals to perform their missions. This unit examines military organizational, social psychological, and environmental factors that create risk for and militate against psychiatric disease, psychosomatic and physical illness, behavioral dysfunction and disruption of performance as they affect Army personnel and impact on care giving agencies. The methods of epidemiology, including records surveillance, population and demographic cohort studies and methods of the psychological and social sciences are used to delineate factors conducing to risk as well as mitigation for such illnesses, disruptions and dysfunctions.

Progress

During the past year the department continued to gather health data relevant to the relationship of presenting symptoms and troop deployment at Ft Bragg. Analysis of the data continues.

It has been observed that the initiative training which proceeds deployment elevates the outpatient sick call rates for deployable combat troops as compared to non-deployable support troops. The categories of visits in which the rates increased (blisters, injuries, psychological symptoms) are consistent with the increased physical and psychosocial stress experienced by the soldiers.

A set of questionnaires designed to inventory those aspects of health, self-perception, and well-being that bear on the interaction of psychosocial and health factors with the soldiers' career was completed and assembled during the year. The comprehensive health hazards and protectors inventory is now available for use as a whole or utilization of its sub-modules.

A research protocol designed to delineate psychiatric treatment patterns and the behavior of Army mental health professionals during the Vietnam conflict was completed. This protocol focuses as well on the use and utilities of psychotropic medication in combat.

A pilot study of the data gathering questionnaire utilizing a small panel of psychiatrists who served in the Republic of Vietnam has been successfully completed and the full-scale study has begun.

Research continues on studies of Vietnam and Vietnam-era veterans still on active duty and the prevalence of delayed stress response syndrome. Comparable data has been collected from 324 active duty Vietnam and Vietnam-era veterans as well as from 925 Vietnam and Vietnam-era reservists. Coding on the samples has been completed and data analysis will shortly be initiated. Contact with a third control group, veterans who remained in neither the active Army or reserve awaits approval from OMB which is expected shortly.

Studies of suicides among active Army personnel continued.

Studies of high frequency users of medical care within the active duty military commenced during this fiscal year. A random sample (20%) of the health records at an east coast military post were surveyed and the results generally replicated those of the previous survey. Once again, a small proportion of younger active duty personnel were responsible for a disproportionate number of visits to outpatient medical services. A questionnaire and interview study of both health frequency users and controls was commenced and is presently under way.

A wide-scale study of general well-being of Army women is presently under way at a number of posts in CONUS. This study involves the collection of General Well-being Scales, the Cornell Medical Index, the Zung Depression Scale, and background information on a large sample of Army men and women.

Work continues on the analysis of past and present patterns of psychiatric diseases, psychosomatic illnesses, physical illnesses and behavioral dysfunctions among Army personnel.

A study of death rate for soldiers in peacetime 1980 showed a death rate about half that for a comparable civilian population. Disease deaths were a trivial fraction of the mortalities. Injury was responsible for elevated mortality rates in a number of duty MOSs; i.e., Cavalry Scout, Combat Engineer, Motor Transport Operator, and Armor Crewman. These occupations share exposure to heavy moving machinery and it is possible that psychophysiological factors contribute to these rates. This may be a topic for future study.

Future Recommendations and Objectives

Basic epidemiological analyses presently under way will be continued into the future with the immediate goal of developing sets of indicators relevant to troop readiness status, and to individual and unit abilities to perform optimally on the battlefield of the present and the future.

Developments during the course of the next FY will concentrate on the continuation and completion of studies presently under way. Further work is anticipated on the relationship between family health status and stress, unit cohesion and health and performance outcome for the active duty soldier. Work on social supports and other protectors against stress consequences, ill-health and breakdown will continue.

Monitoring of other data will continue in order to develop medical early warning indicators of unit status and potential patterns of disruption. Further work will be developed in the study of the military unit as a social support system protecting against or conducing towards illness and performance disruption and maintenance.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 042 Military Psychiatric Epidemiology

Papers Presented at Scientific Meetings

1. Camp, N.M. Dysfunction and Demoralization in U.S. Forces in the Phasedown Period of Vietnam. AMEDD Course on Combat and Military Psychiatry, Ft Carson, CO, April 1982.

2. Jones, F.D. & Marlowe, D.H. Shell Shock and Battle Fatigue: Importance of a Name (with G.L. Belenky). Presented at World Psychiatric Association, American Psychiatric Association Regional Meeting entitled Critical Issues in Psychiatry for the 80's, New York City, NY, 30 Oct - 3 Nov 81.

3. Jones, F.D. Stress in the Military Family (with G.L. Belenky, N.L. Rock, S.N. Xenakis, R.H. Bridenbaugh, & S.L. Baker). Annual American Psychiatric Association Meeting, Toronto, 15-21 May 1982.

4. Jones, F.D. Contemporary Studies in Military Psychiatry: A Review (with G.L. Belenky). Annual American Psychiatric Association Meeting, Toronto, 15-21 May 1982.

5. Jones, F.D. Division Psychiatry: Demography of Patients (with P.A. Newhouse, G.L. Belenky, M.G. Keekin, S.D. Eshelman, M.T. Parker). Annual American Psychiatric Association Meeting, 15-21 May 1982.

6. Jones, F.D. & Camp, N.M. Vietnam Psychiatry Revisited (with H.C. Holloway, H.D. Silsby, H.S. Rumbaugh, and W.W. Johnson). Annual American Psychiatric Association Meeting, Toronto, 15-21 May 1982.

7. Jones, F.D. Division Psychiatry: Role of the physician and demography of patients (with G.L. Belenky, M. Deekin, P.A. Newhouse, S. Eshelman, M. Parker). AMEDD Division and Combat Psychiatry Conference, Denver, CO, 13-16 April 1982.

8. Jones, F.D. Neuropsychiatric Casualties of NBC Warfare (with J. Stokes, P.A. Newhouse, & G.L. Belenky). AMEDD Division and Combat Psychiatry Conference, Denver, CO, 13-16 April 1982.

Project 3E162777A879 Factors Limiting Soldiers' Effectiveness

Work Unit 042 Military Psychiatric Epidemiology

Publications

1. Datel, W.E. and Jones, F.D. Suicide in United States Army Personnel, 1979-1980. Military Medicine, 147(10):843-847, Oct 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6457	82 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
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AC	043
b. CONTRIBUTING				
c. XXXXXXXX	STOG 80-7.2;4			

11. TITLE (Procede with Security Classification Code)

(U) Military Stress: Circadian and Ultradian Factors

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016200 Stress Physiology 013400 Psychology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	5.0	537
	CURRENT 83	5.0	601

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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ASSOCIATE INVESTIGATORS

NAME: Redmond, LTC D.

NAME: Thorne, D. Ph.D. POC: DA

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Stress; (U) Biological Rhythms; (U) Chronobiology; (U) Electrophysiology; (U) Psychophysiology; (U) Human Volunteer

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede 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 enviroments. 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) 81 10 - 82 09 Sleep deprivation is confirmed in a series of observed continuous operations field maneuvers (as Reforger exercises) to be a primary correlate of disruptions in cognitive performance in such diverse activities as Command (Tactical Operations), Communications and Fire Direction Center Operations. These disruptions have similarly been observed to be associated with problems in the regulation of attention, arousal and affect that contribute to the interpersonal strains which accompany decrements in military performance. Thus sleep deprivation (up to 72 hours) and recovery following a four hour sleep period were studied in a group of twelve subjects (mixed military/civilian, male/female).

Preliminary data analysis of eight cognitive PAB tasks reveals a consistent pattern of decrement and recovery consistent with a strong fixed linear component of cognitive performance decrease upon which are superimposed circadian (24-hour) rhythmic components. These performance change curves represent combined speed and accuracy decrements and are submitted to Training and Doctrine Command for incorporation into models of battlefield scenarios that give Commanders better estimates of the effectiveness of their human resources to use in tactical planning. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

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 3E162771A879 FACTORS LIMITING SOLDIERS' EFFECTIVENESS

Work Unit 043: Military Stress: Circadian and Ultradian Factors

Investigators:

Principal: Frederick W. Hegge, Ph.D.

Associates: LTC Sander G. Genser, MC; LTC Daniel P. Redmond, MC; David Thorne, Ph.D.; Harvey Babkoff, Ph.D. (Visiting Scientist); Helen Sing, M.S.

Problems and Objectives

The temporal organization of physiologic function and performance in military environments is studied in laboratory and field settings. Investigations seek to determine the magnitude and time-course of stress-induced performance degradations and the progressive psychophysiologic adaptation to stressors such as sleep deprivation, continuous combat operations, temporal desynchronization, and life threat. Current efforts focus on the impact of rapid troop deployment over long distances by air, the characterization of the stress related effects of combining occupational life threat with shiftwork, and the relationship between cerebral hemispheric laterality and circadian variations in military performance.

Progress

Participant observation in the Reforger '82 deployment of the 82nd Airborne Division covered (1) pre-deployment activity and trans-Atlantic flight, (2) the Battalion Aid Station, (3) a combat infantry platoon and (4) the Tactical Operations Center (TOC) of the Battalion during two weeks of covert, nocturnal and continuous performance maneuvers. Disruptions in cognitive performance in such activities as TOC communications, e.g., transposed digits, verbal pronunciation problems, as well as significant incidence of illness and injury were observed in association with time zone shift, altered sleep structure, sleep deprivation and affective lability. Conclusions include (1) Sleep is the key element (outside of hostile fire, disease, water supply and weather) which will determine the sustainability of military performance -- especially that dependent upon cognitive functions, group control, coordination and communications, (2) Massive sleep deprivation (less than 3 hours per day for at least 10-14 days, often accompanied by transient illusions or hallucinations) occurs in those soldiers whose activities are predominantly cognitive, while for others irregular sleep timing (with adequate amounts) and pulsatile patterns of activity are more typical, (3) Personal, ideosyncratic and volitional strategies for dealing with sleep deprivation and choosing the timing and duration of sleep form a significant component of individual soldiers' behavior patterns, (4) Circadian and ultradian patterns emerge in mood, sleep, rest, activity and performance. These patterns are modulated by individual differences (such as morning/evening preferences), affect the subjective perception of time, often occur outside the conscious awareness of the individual and determine the relationship between quality of sleep and time of day.

(5) Time zone change results in a relentless phase shift of physiological and social rhythms. Thus countermeasures should be considered only within the contexts of the operational scenario in the new time zone (nocturnal, continuous or daytime) and the broader issues of the paucity, irregularity and timing of sleep, (6) There is very little organized understanding of the profound effects of the aforementioned factors on operational performance, so that, for example, the sleep patterns of the Commander may filter down the hierarchy to create additional loads on subordinates.

Sleep deprivation (up to 72 hours) and recovery following a four hour sleep period were studied in a group of twelve subjects (mixed military/civilian, male/female). Eight separate cognitive tasks previously shown to be sensitive to fatigue and tapping a variety of cognitive operations such as short term memory, logical reasoning, pattern detection, spatial memory, and arithmetic calculation were administered as a Performance Assessment Battery (PAB) using Apple Computers on an every two-hour schedule. Additional tasks were directed towards assessment of verbal behavior (reading), subjective affect and activation, vigilance/discrimination (using auditory and/or visual stimuli) and specific cerebral hemispheric abilities (using a lexical decision task presented tachistoscopically to each visual hemifield). Concurrent physiological data collected include twenty-four hour urines for analysis of electrolytes and cortisol, oral body temperature, wrist motor activity and heart rate. Preliminary data analysis of PAB tasks reveals a consistent pattern of decrement and recovery consistent with a strong fixed linear component of cognitive performance decrease upon which are superimposed circadian (24 hour) rhythmic components. These performance change curves represent combined speed and accuracy decrements and are submitted to Training and Doctrine Command for incorporation into models of battlefield scenarios that give Commanders better estimates of the effectiveness of their human resources to use in tactical planning.

A library of Fortran IV programs realizing multiple complex demodulation as an analytical tool for such time series data is made exportable to others using magnetic tape and is being used to quantify circadian rhythmicity disruption in the aforementioned studies.

Future Objectives:

Immediate goals include the continuation of the analysis of the data from the sleep deprivation studies and Reforger deployment. Joint consideration of the field observational and laboratory data will feed back into efforts to construct a battery of field deployable sleep assessment instruments using physiological (Actigraph and EKG) and psychometric (mood/activation assessment, morningness/eveningness questionnaire) techniques for quantitative field studies of fragmented sleep deprivation and rest/activity rhythm effects on military performance. Such a battery aimed towards the development of a taxonomy and ecology of sleep in the field should facilitate the design of laboratory studies of fragmented sleep more precisely directed towards the development of countermeasures for field problems.

Presentations and Publications

1. Redmond, D., Sing, H., and Hegge, F.W. Biological Time Series Analysis Using Complex Demodulation, Rhythmic Aspects of Behavior. F. Brown and R. C. Graeber (Eds.), Hillsdale, N.J., Lawrence Erlbaum Assoc., 1982.

2. Sing, H.C., Hegge, F.W., and Redmond, D.P. Complex Demodulation. Proceedings of the XV International Conference of the International Society for Chronobiology, in press.

3. Redmond, D. Trip Report: Reforger '82. Division of Neuropsychiatry, Walter Reed Army Institute of Research, October 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OC 6452	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMRARY	5. SUMRARY SCTY	6. WORK SECURITY	7. REGRADING	8. [illegible] INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AC	044
b. CONTRIBUTING				
XXXXXXXXXXX	STOG 80-7.2:4			

11. TITLE (Procode with Security Classification Code): (U) Neuroendocrine Response to Military Stress

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 012600 Pharmacology 002300 Biochemistry 013400 Psychology 016200 Stress Physiology 003500 Clinical Medicine

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

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	3.0	460
	CURRENT 83	3.0	417

19. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: (202) 576-3559
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Belenky, G.L., MD, LTC, MC
NAME: Mougey, E.H.

21. GENERAL USE
Foreign Intelligence Considered.

22. KEYWORDS (Procode EACH with Security Classification Code): (U) Stress; (U) Hormones; (U) Neuropeptides; (U) Combat psychiatry; (U) Human volunteers

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

23. (U) To examine neuroendocrine correlates of stressors specific to the military environment. Types of stress to be studied will include physical and psychological stressors, including continuous performance and stressful social interaction.

24. (U) In both laboratory and field studies, we will attempt to correlate endocrine and performance data. This will provide a basis for optimization of work/rest schedules and stress management, and consideration of medical prevention/treatment regimens.

25. (U) 81 10 - 82 09. Psychological stress can produce large increases in the physiologically potent peptide, beta-endorphin, in both male and female rats. Studies in rats indicate that thyrotropin-releasing hormone may be clinically useful as a stimulant drug which does not produce the disruption of performance seen with amphetamines. We found that nicotine increases plasma beta-endorphin in a dose-related manner. We began studies of the behavioral and psychoendocrine effects of replacing a trained member of a working group with an untrained newcomer. Initial studies show that stressful exercise increases both plasma catecholamines and cyclic AMP. We have collected data from a foreign country that 10% of total casualties in a recent conflict were due to battle stress. Of those psychiatric casualties given rest and treatment near the front, 80% could be returned to combat duty. For technical report see Walter Reed Army Institute of Research Annual Progress Report 1 Oct 81 - 30 Sep 82.

*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 3E162777A879 MEDICAL FACTORS LIMITING SOLDIER EFFECTIVENESS

Work Unit 044 Neuroendocrine Response to Military stress

Investigators:
Principal: Meyerhoff, J.L., M.D.
Associate: Belenky, G.L., M.D., LTC, MC; Kant, G.J., Ph.D.; Mougey, E.H., M.S.; Pennington, L.L., B.S.

Objectives:

The study of neuroendocrine responses to stressors typical of combat and the military environment in order to identify conditions and processes leading to physical and/or psychiatric breakdown in combat. Types of stressors studied will include physical and pharmacological as well as psychological stressors.In both laboratory and field studies, we will attempt to correlate endocrine and performance data. This will provide a basis for optimization of work/rest schedules and stress management, and consideration of medical prevention/treatment regimens.

Progress:

It is established that physical stress can increase plasma levels of the peptide hormone beta-endorphinin the rat. We have demonstrated that exposure to a psychological stress elevates plasma beta-endorphin in male rats, and have recently extended this finding to female rats, as well. We have also shown that this response may be linked to highly specific stimuli (such as a tone).

Work continued on studying the effects of acetylcholinesterase inhibitors and cholinomimetic agents on the beta-endorphin system in the rat. Nicotine, when injected at a dose of 0.8 mg/kg produced a maximal increase of plasma beta-endorphin at between 5 and 10 minutes with levels returning to baseline after 120 min. The injection of increasing doses of nicotine from 0.1 mg/kg to 1.6 mg/kg produced increasing levels of plasma endorphins.

An experiment was performed to study the effects of dexamethasone on the beta-endorphin response to stress or cholinergic agents in the rat. It was found that dexamethasone injected at 24 hrs prior to and again at 2 hrs prior to testing did result in a partial decrease in plasma endorphin levels to about half the control levels. Dexamethasone pre-treatment suppressed the increase in plasma beta-endorphin following 0.5 mg/kg of the cholinesterase inhibitor, neostigmine, but only minimally suppressed the eighteen-fold increase in response to footshock.

Methods have been estblished for assaying beta-endorphin in human serum and cerebrospinal fluid, using an antibody and extraction techniques developed in this laboratory. The assay for plasma beta-endorphin will be employed in clinical and field studies.

In eollaboration with the Neuropharmaeology Braneh, we have eompared the behavioral effeets of amphetamine with those of thyrotropin releasing hormone (TRH). Both TRH and amphetamine are known to stimulate inereased motor aetivity in the rat. We have found that amphetamine but not TRH disrupts the performanee of rats in an eight arm maze. These data suggest that TRH may have potential utility as a stimulant drug to extend performanee without impairing judgement or cognition. We plan to extend this work by sereening additional drugs.

We have eolleeted data from a foreign eountry that 10% of total easualties in a recent eonfliet were due to battle stress. Of those psyehiatrie easualties given rest and treatment near the front, 80% eould be returned to eombat duty.

In eollaboration with investigators at Johns Hopkins University, we have begun to examine the effeets on team performance of the soeial stress of replaeing an established group member with an untrained neweomer. We have measured ehanges in urinary testosterone in response to these interventions.

We have sueeessfully modified plasma eolleetion techniques to permit assay of eyelie AMP and eyelie GMP in plasma of human volunteers. Stimulation of peripheral beta-adrenergie reeeptors elevates plasma eyelie AMP while stimulation of alpha-adrenergie reeeptors elevates eyelie GMP. Stress is known to elevate eirculating eateeholamines. Measurement of plasma eyelie nueleotides may permit inferenees regarding the state of peripheral adrenergie reeeptors in individuals exposed to stress, and may be predietive of individuals predisposed to panie disorder.

Future Objeetives:

Plans inelude measurement of plasma eyelie nueleotides, eortisol, prolaetin and beta-endorphin in soldiers during promotion board interviews, in subjeets during publie speaking and stress interviews and in patients with mitral valve prolapse, a eondition assoeiated with poor psychologieal toleranee of stress. We also hope to study these variables in patients with panie disorder. In eonjunetion with the Department of Military Psyehophysiology, we plan to study eortisol as a eorrelate of performanee deerement induced by sleep deprivation.

Our earlier studies of eonditioned beta-endorphin response to psychological stress in rats will be extended to directly eompare the dynamies of responses in males and females. We plan to extend studies of effects of cholinergic drugs on plasma beta-endorphin to inelude effeets of physostigmine, a carbamate type of eholinesterase inhibitor. In collaboration with investigators at the Institute for Chemical Defense, we hope to study the effects of Soman on plasma beta-endorphin levels in rats.

Presentations

1. Mougey, E.H. and Meyerhoff, J.L. Effect of cholinomimetics and cholinesterase inhibitors on plasma beta endorphin. Society for Neuroscience, 11th Annual Meeting, Los Angeles, CA, Oct 1981.

2. Meyerhoff, J.L.,Mougey, E.M. and Bunnell, B.N. Plasma beta-endorphin in rats is increased by psychological stress. Neuroscience Abstracts 7(57.6) 1981.

3. Belenky, G.L. Combat psychiatry in future wars. Army Medical Department Symposium: Division and Combat Psychiatry, Colorado Springs, Colorado, 12-16 April 1982.

4. Belenky, G.L. Contemporary studies in combat psychiatry. American Psychiatric Association Annual Meeting, Toronto, Canada, 15-21 May 1982.

5. Belenky, G.L. The role of endorphin systems in the effects of single and repeated electroconvulsive shock. 14th Collegium Internationale Neuropsychopharmacologicum, Jerusalem, Israel, 20-25 June 1982.

6. Belenky, G.L. Battle stress casualties in the war in Lebanon. Presentation to Army Reserve Unit, Ohio State University School of Medicine, Columbus, Ohio, 10 July 1982.

7. Belenky, G.L. Battle stress casualties in the war in Lebanon. Department of Psychiatry Grand Rounds, Walter Reed Army Medical Center, Washington, D.C., 29 July 1982.

8. Belenky, G.L. Battle stress casualties in the war in Lebanon. Department of Psychiatry Grand Rounds, Uniformed Services University of the Health Sciences, Bethesda, Maryland, 11 August 1982.

9. Belenky, G.L. Battle stress casualties in the war in Lebanon. Department of Psychiatry Grand Rounds, Madigan Army Medical Center, Fort Lewis, Washington, 28 September 1982.

10. Emurian, H.H., Brady, J.V., Meyerhoff, J.L., and Mougey, E.H. Testosterone responses to a change in group composition and size. Presented at the Annual Convention of the Eastern Psychological Assn., Baltimore, MD, Apr 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OC 6470	82 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. ISSU'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	XX YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AA	046
b. CONTRIBUTING				
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 Psychology 013400 Psychology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 82	3.0	55
	CURRENT 83	2.5	55

19. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: ROCK, CPT S.

NAME:

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21. GENERAL USE

Foreign Intelligence 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, questionnaires, field and cohort studies, and various observation methods are employed to develop requisite data.

25. (U) 81 10-82 09 A study of soldiers' knowledge about attitudes toward battle stress casualties (psychiatric battle casualties) has been completed. Major findings: None of the groups surveyed (officers, medics, and other enlisted soldiers) was able to specify adequately how stress casualties could be recognized or treated. Most respondents also indicated a negative attitude toward this important source of recoverable manpower. A study of battle stress casualties in the Wehrmacht has been substantially completed. Major findings: Even the best leadership will not necessarily preclude battle stress casualties from occurring. Study of the assimilation of families into a military community. Results: This is designed to learn how families are assimilated into military communities as a function of housing area and to determine the long term implications (health and general well-being) of this assimilation. This research is in progress. Study of the socialization and assimilation of new officers into USAREUR units. This work is in progress. For technical report see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*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 MAR 68 (FOR ARMY USE) ARE OBSOLETE

Project 3E162777A879 FACTORS LIMITING SOLDIER EFFECTIVENESS

Work Unit 046 Medical Factors Limiting Soldier Effectiveness

Investigators

Principal: Schneider, MAJ R.J.
Associate: Rock, CPT S.K., Jr.

Description

This field unit, stationed in West Germany with the US Army Europe and Seventh Army, identifies and investigates physical, psychological, social, and organizational factors bearing on the individual, unit performance, and battle readiness. Initial efforts have focused on four areas identified by commanders as important concerns within the European theater.

BATTLE STRESS AND READINESS

Battle stress reaction (BSR) will be a major problem to the commander in any future war. Medical data from Korea and the World Wars indicate that between one out of two and one out of eight (average one out of four) casualties resulted from battle stress. Future war is likely to be waged at unprecedented levels of intensity and mobility in a sustained (round-the-clock) mode. A high stress casualty rate can be expected within the first 24 hours. The absolute proportions of the total force likely to be temporarily incapacitated due to BSR, the proportions of casualties likely to be stress-related, and the rapidity with which they will occur will be unacceptable to the commander.

We do not believe that BSR should be viewed strictly as a medical problem. Medical resources will not be able to adequately cope with the probable numbers of BSR in future war. Rather, concerted efforts by individuals at all levels will be required. Stress management is battle-proofing. It comprises steps taken to recognize stress and deal with it before the individual becomes dysfunctional in combat. Battle-proofing can not only help maintain combat effectiveness, but can contribute to increased levels of garrison functioning. This requires knowledge of how soldiers act and respond to stress of and how the negative impact of such stress can be reduced. Stress

casualties are a rapidly recoverable source of manpower. Leaders must know the correct treatment doctrine to ensure that it is followed, since improper treatment can lead to slowed recovery or even chronic disability. Leaders must also be aware of how their soldiers will react to those who are or who have been battle ineffective due to a temporary stress reaction. Attitudes or behaviors which detract from their reintegration and functioning as soldiers must be prevented.

This research assessed soldiers' knowledge about BSR. It included a study of beliefs concerning recognition and treatment, and assessment of attitudes toward BSR. Only a small proportion of the total sample recalled having had classes or seeing simulations of BSR. Few soldiers had an accurate idea of how to recognize or treat it. The small numbers of correct descriptors and correct treatment procedures recalled or recognized indicated an unacceptably low overall level of knowledge. We grouped our respondents into enlisted medics, enlisted non-medics, and officers. Although there were some differences among the three groups, generally favoring the officers and medics, the absolute numbers of correct responses were uniformally small for each group. It is clear that there has been little effective dissemination of information to those who could most benefit: (1) the soldier who will usually be the first man available to recognize BSR and provide buddy aid, (2) the medic who provides first-line combat medical support, and (3) leaders who must conserve manpower.

Most respondents reported that they would not trust a returned BSR casualty. It seems likely that this could contribute to inappropriate treatment (e.g., restraining him, immediately evacuating or hospitalizing him, or reassigning him to different jobs upon his return). Based on past experience, with proper treatment we can expect to return about 80 percent to full duty within three days. All soldiers must realize that the alternative to accepting back a seasoned, battle-ready soldier might be to receive "no one".

As the Army moves to increased dispersion on the integrated battlefield, division level medical support will become ever more difficult. Small unit cadre and medical personnel will have a corresponding greater responsibility for the recognition and treatment of BSR. The combat soldier should be included in any training program emphasizing recognition and treatment. He is likely to be the first person in contact with an individual in the initial stages of BSR. To augment limited medical resources on the battlefield, the service member should be taught minimal skills in recognition and how to apply the simple doctrinal treatment principles. Such training in garrison could

assist preventive medicine efforts by providing early identification of stress-related disorders, and in many cases, provide adequate ameliorative support. Stress management can profitably be viewed as a unit and buddy function. The psychological literature clearly shows the importance of having someone with whom to talk to reduce the negative impact of transient stress problems. Only if personnel at company level fail to mitigate or control dysfunctional stress should higher level assistance be necessary. To the extent that this obviates the "need" for a clinic visit, it would provide the most cost effective use of garrison soldier and medical resources. To the extent that it compliments medical efforts on the battlefield, it will also help conserve manpower in battle.

Any training program should emphasize relatively simple principles. It should help soldiers recognize that there might be a need for special short term considerations for their battle buddy. Such considerations could include giving him temporary rest, getting him a hot meal, and the importance of having his battle buddy sit down and talk about his problems. In a garrison environment, these are the kinds of actions which foster loyalty, trust, and commitment, the building blocks of unit cohesion. This in turn is part of battle-proofing — and increasing readiness -- through reducing the probability that stress problems will occur.

ASSIMILATION OF FAMILIES INTO A MILITARY COMMUNITY

An observational and interview study of a cohort of 100 families is being conducted. The purpose of this is to assess how cohesion (or social supports) is developed in military communities as a function of housing types. Four types of housing areas are being compared: on base housing, military sub-community housing, off base (economy) housing with availability of US Army support, and off base (economy) housing without US Army support. These housing types were selected based on earlier work suggesting that they relate to a number of important family issues, including differences in adjustment.

Each family (active duty soldier and his spouse) was interviewed within 90 days of its arrival and two additional times at six month intervals. Final interviews were completed in October 1982. Data are currently being arrayed for analysis.

Preliminary results indicate that families' perceptions of their introduction and assimilation are fairly poor, although they generally find assignment to Germany better than expected.

There are differences between what the military organizations attempt to do for residents, and the perceptions of the residents as to what is actually done for them. Housing type also appears to relate to these perceptions.

Analysis and write up is expected to be completed in early 1983.

CRITERION TESTING ON THE DRAGON ANTI-TANK MISSILE

European war scenarios presume high speed armor thrusts designed to penetrate NATO defenses. Our ability to effectively kill tanks will determine whether we survive such a war. The DRAGON anti-tank missile is an integral part of our anti-armor defense, and we must maintain the highest possible level of training for its use.

The purpose of this research was to provide the unit trainer with our observations concerning training during DRAGON missile firing. We emphasize the importance of two principles in such training: 1) whenever possible, group actions should be used to reinforce desired individual behavior, and 2) often it might be better to compromise the opportunity to evaluate for the opportunity to train. These are to help the commander further increase training time and decrease non-productive or counter-productive waiting time.

Our data show that less than 20 percent of soldiers had ever before fired a DRAGON, and because only about 25 percent of the soldiers had ever stood next to a DRAGON being fired, the smoke and magnitude of the report were generally unanticipated. When a missile was lost (went far off target or crashed), it was lost in the first two seconds after firing. Within these two seconds, it was clear that large flight corrections would be required, because the missile was already far off the gun-target line. The corrections required at this point are either technically impossible (the guide charges are used up) or behaviorally unlikely (due to a tendency to quickly jerk the sight onto the target and frequently overcorrecting past the target).

Soldiers waiting in a group behind the firing line (usually about 50 meters away) gain no experience or information which could help them achieve a target hit. They do not gain knowledge of the magnitude of the report, the amount of smoke generated, the common errors made in live fire, the probability of a particular kind of error, the problems reported by the gunner, or the corrections or critique of the attendant

spotters. In fact, it is probable that the waiting soldiers exert a counter-productive effect. The recriminations for failure to hit a target, and especially for a lost missile, are minimal for the gunner as an individual soldier. However, these recriminations are considerable for the gunner as a peer group member. As he assumes his firing position, the soldier can be confident that if he fails, he will be publicly booed and derided by his group. It is likely this increases anxiety, tension, and the inability to perform the deliberate and fine muscle movement required for a hit.

Criterion testing should be viewed only as a tool to assess the level of training. It should not be considered an end in itself. Criterion testing with emphasis on fairness and "scientific" concern for comparable conditions for all leads trainers to preclude the use of live fire as a training experience. In an effort to be fair and to obtain valid measures of performance, they do not let later gunners learn from the mistakes of the earlier gunners. This occurs in spite of the fact that firing one live round from a sitting position might be the single most critical step in training. In this case, it is likely that the soldier who does make a hit has a large training advantage over one who does not. He knows what to do right. The goal of testing in this kind of situation should not be to make test results fair, or to make them look good. The goal must be to ensure that each individual gains the maximum amount of experience and training in a situation which is extremely expensive to reproduce.

During criterion testing the individuals waiting to fire could be utilized in two ways. First, a small group (about four) could be on the firing line with the gunner. They could replace the two spotters, under the supervision of the NCO. Following the launch, they would provide a debrief and critique. The remaining individuals would observe the gunner, also looking for inappropriate body movement or tube movement. The NCO would emphasize their role in helping achieve a target hit. Because these group members would then have a vested interest in the outcome, derision for poor performance is likely to decline dramatically. All would experience the report, smoke, and associated conditions directly during firing. Further, all would experience the information provided in the debrief and critique. To further emphasize the importance of the individual's work group, the percentage of hits should not be reported as individual or battalion scores (the aggregate of individuals), but should focus instead on whether the company performed adequately. In field training exercises in which one gunner is selected to fire, another gunner should be located with him or as close to him as possible to gain additional

experience with the sensory conditions associated with firing.

Second, during our observations of criterion testing, and without exception, after the gunner stated he was "ready", a slight nudge to the tube or the gunner's body led to a large deflection (30mm) of the tube. After about four such nudges, a push considerably harder than the recoil of a shotgun would not dislodge the gunner or displace the tube. The next soldier in line could actually set up his tube with two additional spotters, one or two meters from the gunner. An audible sound is emitted one-half second prior to when the missile leaves the tube. One of these additional spotters could jar the tube of this soldier in a manner approximating the weapon's recoil. Regardless of the role they played, all soldiers would have emphasized the importance of maintaining a correct firing position, and recognize how deviations in body and tube movement occur. This would take place under conditions of live fire, including the temporary loss of vision due to smoke during the crucial first seconds of flight.

Preliminary Report on the Assimilation of New Lieutenants into USAREUR

This summarizes the preliminary results of a study of lieutenants newly assigned to the 3d Armored Division (3AD) in Europe. The study was initiated by USAMRU-E at the request of the CG, 3AD, and is an effort to delineate how newly assigned second lieutenants are integrated into their assigned units and positions. Informal interviews were conducted with each recently assigned lieutenant and with their platoon sergeants and immediate commanders. Questions concerning the sponsor program, relations with each other, and what things contributed to or interfered with the integration of a new lieutenant in the unit were asked.

This project is currently in progress. However, preliminary results indicate that:

1. The lieutenants are highly motivated and want to do a superior job.

2. The lieutenants, their commanders, and their sergeants perceive the lieutenants' role and his performance differently.

3. The lieutenants are unsure how to develop a working relationship with their sergeants.

4. The lieutenants are hesitant to take their problems and questions to their commanders, are afraid of their commanders and higher ranking officers, and seem to feel that they are relatively unimportant in the functioning of the unit.

5. The training system is frustrating to the lieutenants; they believe that it does not work as intended and does not support the needs of their soldiers.

6. The sponsor program is not working the way commanders believe it works, or as it was designed to work.

We have completed all lieutenant interviews, including six month followup interviews. Additional interviews of captains (mostly company commands) will be completed by Dec 82. A final report will be prepared within 90 days of that date.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 300237	82 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62777A	3E162777A879	AB	047 WW.TM
b. CONTRIBUSING				
XXXXXXXX	STOG 80-7.2:4			

11. TITLE (Precede with Security Classification Code)

(U) Neuropharmacological Management of Military Performance and Casualties

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
81 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 82	2.0	165
	CURRENT 83	4.0	327

19. RESPONSIBLE DOD ORGANIZATION

NAME: Walter Reed Army Institute of Research

ADDRESS: Washington, D.C. 20012

RESPONSIBLE INDIVIDUAL

NAME: Russell, Philip K., COL

TELEPHONE: (202) 576-3551

20. PERFORMING ORGANIZATION

NAME: Walter Reed Army Institute of Research

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

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

NAME: Holaday, J.W., GM 14

TELEPHONE: (202) 576-3028

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Long, Joseph B., CPT

NAME: Mobley, William C., MAJ POC: DA

21. GENERAL USE

Foreign Intelligence Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Shock; (U) Stress; (U) Pharmacology; (U) Trauma; (U) Stimulants; (U) Nervous System; (U) Behavior

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

23. (U) The objectives are to develop research designed to evaluate the therapeutic utility of neuropharmacologic agents in experimental models of shock and trauma, to evaluate the role of endogenous substances in arousal and depressed states to include both pharmacological and physiological models, and to provide pharmacological insights into the mechanisms of adaptation and habituation to environmental and behavioral stressors. Investigations into the mechanisms of action of neuropharmacologic agents serve to relate these research areas to existing information on autonomic and endocrine function. There is military relevance in this research.

24. (U) Using established methods of animal pharmacological, physiological, and behavioral measurements, attempts will be made to define initially the alteration of normal function by various physiological and environmental stressors. Having developed an understanding of the resulting pathophysiology and abnormal behaviors, a variety of neuropharmacological agents will be injected to determine their ability to restore normal function in an othewise dyshomeostatic situation. In conditions where available methodology is insufficient or lacking, new models appropriate to the experimental objectives will be developed and standardized.

25. (U) 81 10 - 82 09. A novel delta opioid receptor antagonist (M 154,129) was shown to successfully reverse endotoxemia without antagonizing morphine induced analgesia. This substance provides direction to future development of more selective delta opiate antagonists for the management of battlefield shock while relieving pain with morphine. TRH was further shown to be better than naloxone in reversing shock and preventing paralysis following spinal injury. Monkeys were shown to respond with improved hemodynamics and survival following TRH in experimental endotoxic and hemorrhagic shock. Atropine, but not naloxone or TRH, improved toxic autonomic responses to diisopropyl fluorophosphate (DFP). For technical report, see Walter Reed Army Institute of Research Annual Progress Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval

Project 3E162777A879 MEDICAL FACTORS LIMITING SOLDIER EFFECTIVENESS

Work Unit ~~010~~ 047 Neuropharmacological Management of Military Performance and Casualties

Investigators:
Principal: Holaday, J.W., Ph.D.
Associate: Mobley, W.C., MAJ, MC; Long, J. CPT, MS; Tortella, F.C., Ph.D.

Objectives:

To develop research designed to evaluate the therapeutic utility of neuropharmacologic agents in experimental models of shock and trauma; to evaluate the role of endogenous substances in arousal and depressed states using pharmacological, behavioral and physiological models; and to provide pharmacological insights into the mechanisms of adaptation and habituation to environmental and behavioral stressors. Investigations into the mechanisms of action of neuropharmacological agents allow for a correlation of these research areas to existing information on autonomic and endocrine function. Basic science approaches are used to provide potential insights into the etiology and treatment of these various issues pertaining to both military and clinical applications.

Progress:

Work in our laboratories at WRAIR has confirmed that TRH appears to have greater efficacy than naloxone in reversing shock due to endotoxemia and hemorrhage. Collaboratively with Drs. Reynolds, Gurll, and Vargish at the University of Iowa, we have investigated the effects of TRH in endotoxic and hemorrhagic shock in cynomulgus monkeys. Those results indicated that TRH improved cardiovascular function in both models of circulatory shock. In hemorrhagic shock models, survival was significantly improved by TRH. However, in monkeys subjected to endotoxic shock, hemodynamic improvement following TRH did not result in greater survival in this model of endotoxemia.

Continuation of conjoint studies conducted with Dr. Faden at the Uniformed Services University of Health Sciences provided direct comparison among naloxone, TRH, and dexamethasone in preventing neurological deficits following experimental spinal injury in anesthetized cats. Of these three pharmacological approaches, TRH was shown to be more efficacious than naloxone; however, dexamethasone (currently used in the treatment of spinal injuries) was without significant therapeutic effect. Thus, TRH may be established through future clinical trials to be the drug of choice in the treatment of spinal cord injuries such as those which are frequently encountered on the battlefield.

Through the use of newly available, specific opiate-receptor antagonists, it was demonstrated in our laboratories at WRAIR (collaboratively with R. D'Amato, B. Ruvio, L. Robles, and C. Johnson) that the "opiate" component of shock pathophysiology is mediated by endogenous opiates acting at "delta" receptors, whereas the analgesic properties of injected morphine are mediated at "mu" receptors. These results have provided the first demonstration of a selectivity of action for physiologically activated endogenous opiates at specific receptor subpopulations. Moreover, this selectivity of action at distinct subpopulations of opioid receptors allows for the potential use of delta opiate antagonists to reverse shock while morphine could be co-administered for the relief of pain associated with traumatic injuries.

The pharmacological properties of opiates such as morphine allow for pain relief; however, adverse side effects such as respiratory depression and orthostatic hypotension limit its usefulness in the acute treatment of pain. In collaboration with Dr. Ward from the University of Minnesota, research conducted in our laboratories at WRAIR revealed that these various desirable and undesirable properties of morphine can be partially disassociated. Specifically, through the use of selective "delta" and "mu" receptor antagonists, morphine was shown to act upon both receptor subtypes to produce respiratory depression, "mu" receptors mediated analgesia and bradycardia, however the hypotensive effects of morphine were difficult to demonstrate in conscious rats, thus opioid receptor subtype involement could not be shown.

In a series of studies using gerbil models of cerebral ischemia and stroke, it was demonstrated in our laboratories (collaboratively with R. D'Amato) that neither naloxone nor TRH provided any functional improvement in the neurological sequelae which these procedures produce. These findings refuted a published report from another laboratory which used naloxone in such models, and the reasons for the discrepancies in these studies were established by our investigations.

A novel technique for evaluating respiratory rate and depth which utilized existing cannulation procedures was devised. This afforded the opportunity to conduct many of the studies described above, as well as experiments which evaluated the cardiorespiratory effects of organophosphate toxicity as described below.

Research using the organophosphate DFP (collaboratively with Dr. Meyerhoff, Sp 5 Ruvio, and J. Kenner) established for the first time in unanesthetized, low-stress rats that DFP produces pronounced hypertension (greater than 50% increase in mean arterial pressure), slight tachycardia, and respiratory depression. During the pre-terminal phase, respiratory depression usually preceded cardiovascular collapse, indicating its principal role in the lethal effects of DFP. Naloxone or TRH were without effect upon cardiorespiratory measures or survival, whereas atropine successfully reversed the pathophysiological responses to DFP.

Prior work by us demonstrated that electroconvulsive shock (ECS) in rats activates opioid systems, and that cross sensitization between chronic ECS and chronic morphine exposure occurs. Both of these procedures were recently shown (in collaboration with Dr. Belenky, Neuroendo. Neurochem. Br. and Dr. Hitzemann at the Univ. of Cincinnati) to result in an apparent increase in the number of available opioid receptors, thus linking these pharmacological and biochemical observations for the first time.

During the last quarter of the Fiscal Year '82, three new senior investigators joined the Neuropharmacology Branch. Due to the newness of their arrival and the lack of available laboratory space, little progress of note has been made other than the procurement of equipment needed by these researchers. It is hoped that laboratory space will be made available to accomodate these researchers and allow their implementation of proposed research in immunohistochemical morphology, seizure disorders, and regulation of blood brain barrier permeability.

Future Directions:

Plans for 1983 include the priority of obtaining laboratory and office space to afford the new investigators the opportunity to pursue research objectives outlined in approved and planned research proposals. These studies within the Neuropharmacology Branch will integrate available talent to provide novel insights into the neuropharmacology of the blood brain barrier and its alteration during stress and toxic substance exposure. Morphological studies will be conducted which will afford the opportunity to correlate the localization of various neuroactive transmitters with their receptors and, of greatest importance, function as measured by glucose utilization and subcortical electroencephalographic monitoring. Prior work with selective opiate antagonists provided new ideas regarding pharmacological approaches to seizure induction and treatment. These insights will be evaluated using fluorothyl and electroshock seizure models. Further work on shock and trauma will emphasize multiple opiate receptor approaches in order to distinguish between shock and pain mechanisms.

Plans for 1984 - unknown.

Presentations made, JWH:

1. Merck Institute, West Point, PA, invitational lecture on "Neuropeptide involvement in shock and spinal injury", Dec 1981.

2. U.S. Food and Drug Administration Special Lecture, "The role of neuropeptides in shock and spinal cord injuries", Rockville, MD., Jan 1982.

3. Autonomic Research Study Group, Uniformed Services University of the Health Sciences, lecture on "Differential cardiorespiratory effects of 'mu' and 'delta' opiate agonists following 3rd and 4th ventricular injections" Jan 1982.

4. Department of Psychiatry, Walter Reed Army Hospital, lecture on "Neuropsychiatric implications of endorphins", Feb 1982.

5. Paralysis Cure Research - American Paralysis Association, invited participant and lecturer on "TRH for early treatment of spinal cord injuries", Airlie, Virginia, Mar 1982.

6. Chairman, Federation of American Societies for Experimental Biology, session on "Enkephalins and Endorphins", New Orleans, LA, May 1982.

7. Department of Pharmacology, University of California, San Francisco, Invited lecture on "Endorphins in shock and spinal injuries: Update on autonomic mechanisms", May 1982.

8. Symposium on Recent Advances in the Pathogenesis & Treatment of Septic Shock, invited lectureship, "Role of endorphin and endorphin antagonists in the pathogenesis and treatment of septic shock", Portland, OR, May 1982.

9. Spinal Cord Society, meeting on "Recent Research Aimed at the Cure and Treatment of Spinal Cord Injuries", invited lectureship, Seattle, WA, May 1982.

10. Shock Society Annual Meeting, symposium chairman, "The role of endogenous opiates in shock", Smuggler's Notch, VT, June 1982.

11. Molecular and Cellular Aspects of Shock and Trauma, invitational symposium participant, lecture on "Endorphins in Shock and Spinal Injury: Therapeutic Effects of Naloxone and Thyrotropin Releasing Hormone", Kona, Hawaii, June 1982.

12. Basic Mechanisms and Clinical Management of Shock, invitational symposium participant, lecture on "Endorphins in Shock", Merrillville, Indiana, Sept 1982.

13. Pharmacological Basis of Anaesthesiology, invitational symposium participant, lecture on "Clinical Implications of Endorphin Research", Milan, Italy, Nov 1982.

14. Session Chairman, "Opiate Receptors" Society for Neurosciences Annual Meeting, Minneapolis, Minnesota, 31 Oct. - 5 Nov. 1982.

15. Sixth Annual Pain Symposium, invited faculty to present a lecture on "Endorphins: An Update", Department of Psychiatry, Walter Reed Army Medical Center, Washington, DC, 17 Sept. 1982.

Publications:

1. Holaday, J.W., Ruvio, B.A., and Faden, A.I. Thyrotropin releasing hormone improves blood pressure and survival in endotoxic shock. Eur. J. Pharmacol. 74:101-105 (1981).

2. Holaday, J.W. and Faden, A.I. Endorphins and thyrotropin releasing hormone in shock and trauma. Advances in Pharmacology and Therapeutics II, Vol. 1, H. Yoshida, Y. Hagihara, and S. Ebashi, (eds.) Pergamon Press, Oxford, pp. 45-55, (1982).

3. Tortella, F.C., Cowan, A., Belenky, G.L. and Holaday, J.W. Opiate-like electroencephalographic and behavioral effects of electroconvulsive shock in rats. Eur. J. Pharmacol. 76:121-128 (1982).

4. Holaday, J.W. and Faden, A.I. Naloxone and thyrotropin releasing hormone have additive effects in reversing endotoxic shock. Advances in Endogenous and Exogenous Opioids, H. Takagi et al., (eds.) Kodansha, Tokyo, pp.367-370, (1981).

5. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Comparison of early and late naloxone treatment in experimental spinal injury. Neurology 32: 677-681 (1982).

6. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Thyrotropin releasing hormone improves functional neurological recovery following experimental spinal injury. New England J. Med. 305:1063-1067 (1981).

7. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Neuropeptides and spinal cord injury. Regulatory Peptides: Functional and Pharmacological Aspects, E. Costa and M. Trabucchi, eds. Raven Press, New York (Advances in Biochemical Psychopharmacology 33: 131-138, 1982).

8. Holaday, J.W., D'Amato, R.J., Ruvio, B.A., and Faden, A.I. Action of naloxone and TRH on the autonomic regulation of circulation. Regulatory Peptides: Functional and Pharmacological Aspects, E. Costa and M. Trabucchi, eds. Raven Press, New York (Advances in Biochemical Psychopharmacology 33: 353-361, 1982).

9. Faden, A.I., Jacobs, T.P., and Holaday, J.W. Thyrotropin releasing hormone for spinal trauma (letter: Hall, E.D. and Braughler, J.M.). New Engl. J. Med. 306: 429-430 (1982).

10. Holaday, J.W. and D'Amato, R.J. Naloxone or TRH Fails to Improve Neurologic Deficits in Gerbil Models of "Stroke". Life Sci., 31: 385-392 (1982).

11. Holaday, J.W. and D'Amato, R.J. Naloxone in Cerebral Ischaemia. Lancet i: 1238 (1982).

12. Holaday, J.W., Ruvio, B.A., Robles, L.E., Johnson,C.E., and D'Amato, R.J. ICI M 154,129, A Putative Delta Antagonist, Reverses Endotoxic Shock Without Altering Morphine Analgesia. Life Sci., 31: 2209-2212, 1982.

13. Holaday, J.W., Hitzemann, R.J., Curell, J., Tortella, F.C., and Belenky, G.L. Repeated Electroconvulsive Shock or Chronic Morphine Treatment Increases The Number of ^{3}H-D-Ala^{2}, D-Leu^{5}-Enkephalin Binding Sites in Rat Brain Membranes. Life Sci., 31: 2359-2362, 1982.

Manuscripts in press:

1. Holaday, J.W. and D'Amato, R.J. Naloxone is Without Effect Upon Ischemic Neurologic Deficits in the Gerbil. Science, (in press).

2. Faden, A.I., Jacobs, T.P., Smith, M.T., and Holaday, J.W. Comparison of Thyrotropin Releasing Hormone (TRH), Naloxone, and Dexamethasone Treatments in Experimental Spinal Injury. Neurology (in press).

3. Holaday, J.W. Cardiovascular Consequences of Endogenous Opiate Antagonism. Invited commentary, Biochem. Pharmacol. (in press).

4. Holaday, J.W. Endorphins in Shock and Spinal Injury; Therapeutic Effects of Naloxone and Thyrotropin Releasing Hormone. in Molecular and Cellular Aspects of Shock and Trauma (in press).

5. Holaday, J. W. Cardiovascular Effects of the Endogenous Opiate System. in Annual Review of Pharmacology (in press).

6. Holaday, J. W. Endorphins in Current Concepts (in press).

7. Holaday, J.W. and Reynolds, D.G. The Role of Endogenous Opiates in Shock: Introductory Comments. in Advances in Shock Research S. Reichard and D. Reynolds, eds. (in press).

8. Holaday, J.W. and Faden, A.I. Spinal Shock and Injury: Experimental Therapeutic Approaches. in Advances in Shock Research S. Reichard and D. Reynolds, eds. (in press).

Manuscripts submitted:

1. Tapp, W.N., Holaday, J.W., and Natelson, B.H. Ultradian Glucocorticoid Rhythms in Monkeys and Rats Continue During Stress. (submitted).

2. Holaday, J.W. Cardiorespiratory effects of mu and delta opiate agonists following 3rd or 4th ventricular injections. Peptides (submitted).

3. Holaday, J.W., Pasternak, G.W., and Faden, A.I. Naloxazone pretreatment modifies cardiorespiratory and behavioral effects of morphine. Brain Res. (submitted).

4. Holaday, J.W., Pasternak, G.W., D'Amato, R.J., Ruvio, B.A., and Faden, A.I. Naloxazone lacks therapeutic effects in endotoxic shock yet blocks the effects of naloxone. Eur. J. Pharmacol. (submitted).

Patents pending;

1. "Narcotic antagonists in the therapy of shock" Continuation in part, Serial # 248,622, re: neurogenic shock and spinal injury.

2. "Thyrotropin releasing hormone in therapy of shock and as a central nervous system stimulant" Patent application serial number 252,443.

Abstracts:

1. Holaday, J.W., and Faden, A.I. Selective Cardiorespiratory Differences Between Third and Fourth Ventricular Injections of "Mu" and "Delta" Opiate Agonists. Fed. Proc. 41:1468 (1982).

2. Holaday, J.W., Ruvio, B.A., and Sickel, J. Morphine Exacerbates the Cardiovascular Pathophysiology of Endotoxic Shock in Rats. Circ. Shock 9:169 (1982).

3. D'Amato, R.J. and Holaday, J.W. Thyrotropin Releasing Hormone Reverses Endotoxic Shock Through Autonomic Effects at Central and Peripheral Sites. Circ. Shock 9:202 (1982).

4. Holaday, J.W., Ruvio, B.A., Robles, L.E., and Johnson, C.E. ICI M 154,129, A Putative Delta Antagonist, Reverses Endotoxic Shock Without Altering Morphine Analgesia. Proceedings of the Int. Narc. Res. Conf., Sea Crest, North Falmouth, MA June, 1982.

5. Holaday, J.W., Hitzemann, R.J., Curell, J., Tortella, F.C., and Belenky, G.L. Repeated Electroconvulsive Shock or Chronic Morphine Treatment Increases The Number of 3H-D-Ala2, D-Leu5-Enkephalin Binding Sites in Rat Brain Membranes. Proceedings of the Int. Narc. Res. Conf., Sea Crest, North Falmouth, MA June, 1982.

6. Reynolds, D.G., Gurll, N.J., Holaday, J.W., and Ganes, E. Thyrotropin Releasing Hormone (TRH) in Primate Endotoxic Shock. Physiologist (Proceedings of the American Physiological Society, Fall Meeting) in press, 1982.

7. Gurll, N.J., Reynolds, D.G., Holaday, J.W., and Ganes, E. Improved Cardiovascular Function and Survival Using Thyrotropin Releasing Hormone (TRH) in Primate Hemorrhagic Shock. Physiologist (Proceedings of the American Physiological Society, Fall Meeting) in press, 1982.

8. Holaday, J.W. and Ward, S.J. Morphine-Induced Bradycardia is Predominantly Mediated at Mu Sites, Whereas Morphine-Induced Hypotension May Involve Both Mu and Delta Opioid Receptors. Soc. Neurosci. Abstr. 8: (in press) 1982.

9. Ward, S.J. and Holaday, J.W. Relative Involvement of Mu and Delta Opioid Mechanisms in Morphine-Induced Depression of Respiration in Rats. Soc. Neurosci. Abstr. 8: (in press) 1982.

10. Holaday, J.W. Endorphins in Shock and Spinal Injury: Therapeutic Effects of Naloxone and Thyrotropin Releasing Hormone. Molecular and Cellular Aspects of Shock and Trauma: U.S.A. - Japan binational conference, Kona, Hawaii, 21-24 June, 1982.

PROJECT 3M463751D993

MEDICAL DEFENSE AGAINST CHEMICAL WARFARE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OH 0609	82 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
81 10 01	H. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63751A	3M463751D993	AB	061
b. ~~SECONDARY~~				
c. ~~SECONDARY~~	STOG 80-7.2.1			

11. TITLE (Procode with Security Classification Code)

(U) Preclinical Studies of Anti-Chemical Warfare Drug Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 012600 Pharmacology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING	82	2.0	257
FISCAL YEAR CURRENT			

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:

21. GENERAL USE

Foreign Intelligence Not Considered

ASSOCIATE INVESTIGATORS

NAME: VON BREDOW, MAJ. J.

NAME: PAMPLIN, LTC C.

22. KEYWORDS (Procode EACH with Security Classification Code) (U) Pharmacology; (U) Antidotes; (U) Toxicity; (U) Pharmacokinetics; (U) Quantitation Methodology

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

23. (U) The technical objectives of this work unit are to obtain the necessary information for chemical warfare agent antidotes to support a Notice of Claimed Investigational Exemption for a New Drug (IND). The antidotes will be developed for the defense of military personnel in an integrated chemical/nuclear/conventional battlefield.

24. (U) A highly integrated, multidisciplinary effort is required to coordinate the extramural and intramural studies necessary to develop candidate chemical warfare agent antidotes. the actual studies performed are dictated by scientific rationale and existing federal regulations to include the completion of efficacy and toxicity studies, formulation development, pharmacokinetic and metabolism studies.

25. (U) 81 10 - 82 09 A high pressure liquid chromatography method for the assay of 2-Praldoxine Chloride (2-PAM) in solutions has been developed. This method has been used to study the compatability and stability of solutions containing 2-PAM and atropine. The efficacy of pyridostigmine for prophylactic therapy of nerve agent poisoning in the rhesus monkey model has been thoroughly re-examined. This data will support an IND submission on pyridostigmine. For technical report see Walter Reed Army Institute of Research Technical Annual Report, 1 Oct 81 - 30 Sep 82.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

Project 3S623751993 MEDICAL DEFENSE AGAINST CHEMICAL WARFARE

Work Unit 061 Preclinical Studies of Anti-Chemical Warfare Drug Development

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
Associate: MAJ J. von Bredow, CPT D. Korte, Jr., LTC C. Pamplin, Dr. L. Fleckenstein, Dr. H. Lowensohn, J. DiGiovanni, SP6 Norman Wright

1. Description.

The development of antidotes against various chemical warfare agents requires a highly integrated, multidisciplinary approach spanning a broad spectrum of preclinical and clinical pharmacological studies. The ultimate goal of these studies is to obtain the necessary information to support granting of a Notice of Claimed Investigational Exemption for a New Drug (IND) by the Food and Drug Administration (FDA) for each candidate antidote.

2. Progress.

The analysis of six 8 year old samples of PAM-Cl was completed. The samples were originally formulated to contain 300 mg/ml. Analysis of the six samples resulted in an average value of 270.4 ± 16.9 mg/ml. Therefore, these unbuffered PAM-Cl solutions in six autoinjectors maintained at room temperature for 8 years indicated a 10% degradation of PAM-Cl with a concomitant reduction in pH from 3.5 to 0.9.

An accelerated stability study is in progress to compare the degradation of PAM-Cl in buffered and unbuffered solutions. Combinations of PAM-Cl and atropine in buffered and unbuffered solutions are also in progress.

The development of a HPLC (high pressure liquid chromatography) technique for the analysis of PAM-Cl and its degradation products as well as atropine sulfate and its degradation products in the presence of each other is in progress and will be used to analyze these samples. The results of this accelerated stability study will be completed in FY 83.

A new laboratory has been developed for the use of dilute chemical agents. The laboratory will be operational for the in vitro and in vivo assessment of the effectiveness of new antidotal combinations.

3. Future Work.

Work under this project has been terminated and will continue.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	3. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DAOH 0610	82 09 30	DD-DR&E(AR)636

2. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA* CONTRACTOR ACCESS	9. LEVEL OF SUM
81 10 01	H. Term	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63751A	3M463751D993	AC	062
b. CONTRIBUTING				
c. CONTRIBUTING	STOG 80-7.2:1			

11. TITLE (Precede with Security Classification Code)*

(U) Preclinical Studies of Antiradiation Drug Development

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine 012600 Pharmacology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING: 82	2.5	218
FISCAL YEAR CURRENT:		

19. RESPONSIBLE DOD ORGANIZATION

NAME:* Walter Reed Army Institute of Research

ADDRESS:* Washington, DC 20012

RESPONSIBLE INDIVIDUAL

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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.

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: FLECKENSTEIN, Dr. L.

NAME: PAMPLIN, LTC C.

21. GENERAL USE

Foreign intelligence not considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Pharmacology; (U) Antidotes; (U) Toxicity; (U) Pharmacokinetics; (U) Quantitation Methodology

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

23. (U) The technical objectives of this work unit are to obtain the necessary information to support a Notice of Claimed Investigational Exemption for a new Drug (IND) for antiradiation agents being developed for defense of military personnel in an integrated chemical/nuclear/conventional battlefield.

24. (U) A highly integrated, multidisciplinary effort is required to coordinate the extramural and intramural stuides necessary to develop candidate antiradiation agents. The actual studies performed are dictated by scientific rationale and existing federal regulations to include the completion of efficacy and toxicity studies, formulation development, pharmacokinetic and metabolic studies.

25. (U) 81 10 - 82 09 The radioprotective compound WR 2721 was microencapsulated into 3 formulations. All three are resistant to acid hydrolysis of pH 1 and are readily released in simulated intestinal solution. A high pressure liquid chromatography method for the assay of WR 2721 has been developed. Chromatographic conditions have been established to separate metabolite and interfering substances. For technical report see Walter Reed Army Institute of Research Technical Annual Report, 1 Oct 81 - 30 Sep 82.

*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 3S623751993 MEDICAL DEFENSE AGAINST CHEMICAL WARFARE

Work Unit 062 Preclinical Studies of Antiradiation Drug Development

Investigators:
Principal: Melvin H. Heiffer, Ph.D.
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1. Description.

The development of agents which protect against the effects of radiation injury requires a highly integrated, multidisciplinary effort to undertake a broad range of preclinical and clinical pharmacological studies. The ultimate goal of this work is to obtain the necessary information to support granting of a Notice of Claimed Investigational Exemption for a New Drug (IND) by the Food and Drug Administration (FDA) for each candidate radioprotectant.

2. Progress.

WR 2721 is an aminopropyl aminoethyl phosphorothioate compound currently being developed by the department for submission to the FDA as an antiradiation drug. The drug shows good radioprotectant activity after intavenous administration. The drug undergoes rapid hydrolysis under acidic conditions and thus would not be expected to survive the acidic conditions of the stomach without significant degradation. As expected, the drug shows little radioprotection in animal studies following oral administration. To be useful in military settings, under field conditions, it is necessary to develop a dosage form which can be taken orally. To this end, drug microencapsulation has been undertaken to protect the drug from acid hydrolysis and deliver the parent drug intact to a favorable absorption site in the intestine. Microencapsulation of WR 2721 in various fatty materials has led to three experimental formulations which demonstrate promising *in vitro* characteristics. All 3 formulations show good protection against acid hydrolysis in a pH 1 test medium. Further, it has been demonstrated that the drug is readily released from the dosage form *in vitro* under conditions designed to simulate the intestinal environment.

Development of a specific assay for WR 2721 will allow bioavailability testing of the experimental encapsulated formulations. Development of a high pressure liquid chromatographic assay has proceeded far enough to demonstrate the

feasibility of this technique. Chromatographic conditions have been worked out to separate WR 2721 from metabolites and endogenous interfering substances in plasma. Detection methods, internal standards and stability have been investigated. the method will be finalized once reproducibility and accuracy experiments currently underway are completed.

3. Future Work.

The pharmacokinetic profile of WR 2721 will be investigated in the dog model. Bioavailability studies will be undertaken in animals to test the oral absorption of experimental microencapsulated formulations. Formulation work will continue to optimize microencapsulation formulations.

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