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ANNUAL RESEARCH PROGRESS REPORT

RCS-MEDDH-288(R1)

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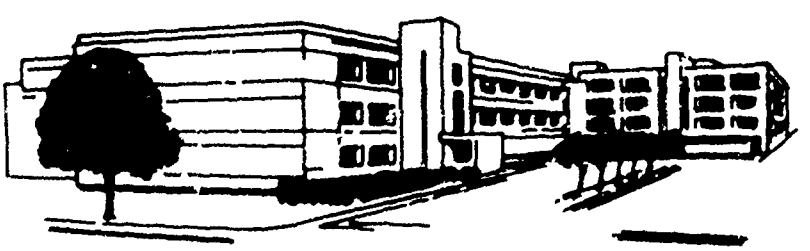

LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

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READ INSTRUCTIONS BEFORE COMPLETING FORM

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3A161101A91C
3M161102BS02

404912

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

During Fiscal Year 1978 progress was attained at the Letterman Army Institute of Research in the following research areas: basic and applied research in skin diseases of military importance; the effects of hemorrhagic shock on the heart and brain; basic nutritional biochemistry; basic biochemical processes of metabolism; basic and applied nutrition; clinical nutrition; the metabolism of normal man and as altered by disease; the evaluation of insect repellent; serodiagnosis of leishmaniasis; the determination of exposure thresholds of coherent (see reverse)

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Block No. 20 (Cont)

radiation producing damage to the eye and skin; basic and applied studies on blood and blood products storage; work performance on man and military dogs; and research computer science. The progress made in this fiscal year is described in the reports of the work units presented.

FOREWORD

The research conducted at the Letterman Army Institute of Research, Presidio of San Francisco, California, was accomplished in Fiscal Year 1978 under the following Department of the Army projects:

3A161101A91C - In House Laboratory Independent Research

3M161102BS02 - Basic Mechanisms of Recovery from Injury

3M162772A810 - Military Skin Disease

3M162772A811 - Military Nutrition and Food Hygiene

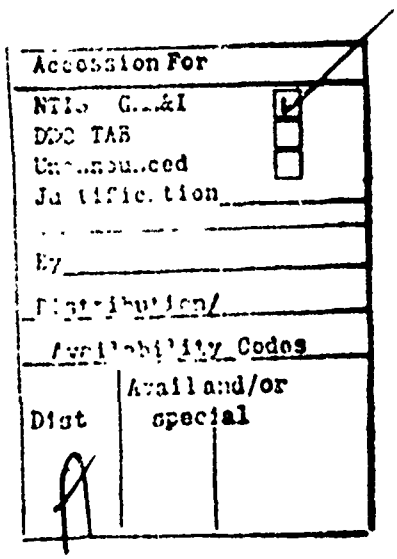
3M162772A812 - Military Research Animal Resources

3E162772A813 - Health Effects of Military Lasers

3S162662A814 - Military Trauma and Resuscitation

Projects are subdivided into work units and studies, as appropriate, to accomplish project objectives.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.



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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OB 6300	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
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(U) The Molecular Basis of Vitamin A Activity (06)

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002300 Biochemistry

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24. TECHNICAL OBJECTIVE: 25. APPROACH: 26. PROGRESS (Procede with Security Classification Code)

23. (U) The participation of vitamin A isomers in the visual process has been known for some time, while other functions of the vitamin which became evident in different animals remain obscure. Of obvious military interest is its apparent participation in resistance to infection and stress, its involvement in dark adaptation, and its requirement for wound healing. Vitamin A is also responsible for normal differentiation of epithelial tissues such as in the respiratory tract. In order to provide a better rationale for the use of vitamin A and its requirements, investigations will be conducted on vitamin A requirements at the molecular level. These studies will be designed to determine the specific tissue functions of vitamin A and its active form.

24. (U) Studies should be conducted on the involvement of vitamin A in glycoprotein biosynthesis, on sulfur amino acid metabolism and sulfation, on cell and tissue membrane synthesis, on wound healing and infection, and on DNA and RNA metabolism. Studies will use tissue and organ culture techniques, cellular blocking agents, labeled substrates, electron microscopy, and other methods. Particular attention should be focused on the effects of vitamin A on the maintenance of different organ cultures of the respiratory tract since these tissues are particularly susceptible to injury from airborne chemical and biological exposure.

25. (U) 77 10 - 78 09 Human plasma has been fractionated by ion-exchange chromatography to obtain fractions which are enriched with Prealbumin-Retinol Binding Protein (PA-RBP; Fraction IV) and another fraction (Fraction III) which contains no vitamin A but which contains a protein with a fluoresence excitation spectrum almost identical to that of PA-RBP. Fractions III and IV are being used to prepare pure PA-RBP, PA, RBP and to purify and characterize the protein in Fraction III with the fluorescent characteristics of whole RBP. This work unit is terminated due to directed realignment of the Institute research program.

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.

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 040 — The Molecular Basis of Vitamin Activity

The following investigation has been conducted under this work unit:

STUDY NO. 2 — Development of a microfluorometric assay for vitamin A

The most widely accepted method for extracting serum and plasma vitamin A is a colorimetric method which measures the intensity of the color produced when trifluoracetic acid reacts with the vitamin A (retinol plus carotene) obtained in a hexane extract of the plasma or serum samples. Previous studies in this laboratory have required plasma samples of 2 ml to perform the analyses. A micromethod has now been established which requires only 0.5 ml. Computer programs have been written to perform calculations and comparisons of standards and samples to insure the procedure conforms to the new DHEW Good Laboratory Practices regulations. The disadvantages of the assay have been discussed in a previous report from this laboratory and a study was initiated to compare the results obtained with colorimetric assay with those using a direct fluorometric method. A good correlation was found between the colorimetric and fluorometric assays, as well as an immunological method for determining plasma retinol binding protein (RBP). During the course of these studies evidence was obtained which suggested that a second plasma protein, not containing vitamin A (retinol) and chromatographically distinct from retinol binding protein, had a fluorescence excitation and emission spectrum which was quite similar to that of retinol binding protein. Since this protein could potentially interfere with the vitamin A analyses using the direct fluorometric method, studies were initiated to separate RBP from the interfering protein conveniently. This was accomplished by ion-exchange chromatography or DEAE-Sephadex. The contaminating protein has been tentatively identified as the blue copper-containing protein ceruloplasmin.

BODY OF REPORT

WORK UNIT NO. 040 — The Molecular Basis of Vitamin A Activity

STUDY NO. 2 — Development of a microfluorometric assay for vitamin A

PROBLEM

Vitamin A is essential for maintaining vision, skeletal development, reproductive capacity, and normal differentiation of many epithelial tissues (skin, trachea, etc.). With the exception of the role of vitamin A as the light-sensitive prosthetic group of the visual pigments, the mechanism(s) of action is (are) not yet known. This lack of basic knowledge has undoubtedly hindered the development of new therapies involving vitamin A. Recent progress in the vitamin A field has led to the use of derivatives of the vitamin for the effective treatment of cancer in tissues of epithelial origin. The importance of vitamin A nutrition in wound healing, resistance to infection, coping with stress, and vision makes this an area of research obviously important to the military forces. The development and evaluation of a rapid sensitive assay for vitamin A are necessary for both practical applied studies as well as for experimental studies of the mechanism(s) of action of vitamin A.

RESULTS AND DISCUSSION OF RESULTS

The most widely accepted method for measuring vitamin A is a colorimetric procedure in which the sample is reacted with trifluoracetic acid (TFA) to produce a blue color, the intensity of which is proportional to the amount of vitamin A in the sample. Such an assay has been used in this laboratory since the assay was developed, but the procedure established required relatively large samples, e.g., 2 ml of plasma. The assay has now been modified to a semi-micro level, permitting detection of retinol in the range of 5 to 100 µg of retinol per deciliter of plasma with a 0.5 ml sample. The method is reproducible. Quality control procedures have been established to insure compliance with the 1978 Good Laboratory Practice regulations. While the method is acceptable, there are disadvantages which have been discussed in some detail in a previous report. Many of the objections to the TFA methodology were avoided in a newer method involving the direct fluorometric analysis of retinol in dilute samples of human plasma. Samples as small as 25 µl are required as opposed to 500 µl for the TFA methodology, and the assays could be performed in a fraction of the time. During the course of these studies, evidence was obtained which suggested that another substance was present in human plasma which was distinct from the retinol-binding protein-retinol (RBP-R) complex but which had fluorescence properties quite similar to those of RBP-R. When human plasma was fractionated by ion-exchange chromatography on DEAE-Sephadex, four

fractions could be obtained. Fractions I, II, and III contained no vitamin A, which was found exclusively in Fraction IV. Nevertheless, both fractions III and IV had almost identical fluorescence spectra with excitation peaks at both 292 nm and 335-340 nm. Such results would be expected for Fraction IV which contains RBP-R, but not for Fraction III which does not contain RBP-R. Subsequently, it was determined that Fraction III contains a copper-containing protein, ceruloplasmin, which has an absorbance maximum at 325 nm (as well as other ultraviolet and visible maxima) which could account for the observed fluorescence spectrum. Obviously, it would be possible for two samples of plasma, having identical RBP-R levels, to give different fluorescence readings if the sample contained different amounts of ceruloplasmin. It has not yet been determined, quantitatively, what the contribution of ceruloplasmin is to the total fluorescence of plasma in the microfluorometric method.

CONCLUSION

A direct microfluorometric assay for retinol in plasma and serum samples may require correction for ceruloplasmin levels.

RECOMMENDATIONS

1. Work should be continued with microfluorometric retinol assay to evaluate the contribution to the total measured fluorescence due to ceruloplasmin and retinol-binding protein-retinol.

2. Methods should be sought which would minimize, eliminate, or permit quantification of the fluorescence of ceruloplasmin in samples which contain both ceruloplasmin and retinol-binding protein-retinol.

3. Tissue/organ culture studies should be initiated in support of work to be done on the effects of retinol-binding protein-retinol on wound healing.

PUBLICATIONS

1. PLOPPER, C.G., D.L. WALLACE, T.J. BUCCI, and H.E. SAUBERLICH. Autoradiographic localization of vitamin A in the kidney of rats. Proc Soc Exptl Biol Med 155:124, 1977

2. BASHOR, M.M. Dispersion and disruption of tissue. In: Methods in Enzymology. Cell Culture. 58, Academic Press, Inc., New York, 1978 (In press)

3. BRINK, E.W., W.D.A. PERERA, S.P. BROSKE, R.A. CASH, J.L. SMITH, H.E. SAUBERLICH, and M.M. BASHOR. Vitamin A status of children in Sri Lanka. Amer J Clin Nutr, 1978 (In press)

4. BASHOR, M.M. and J.A. WILLOTSON. Isolation of a riboflavin-binding apoprotein from chicken egg white and its use in a radioassay for urinary riboflavin. Fed Proc 37:672, 1978 (Abstract)

Note: Due to directed realignment, this work unit is being terminated.

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 042 — Long-Term Cryopreservation of Platelets for Immediate Field Use

The following investigations are being conducted under this work unit:

STUDY NO. 1 Cryopreservation strategies

STUDY NO. 2 In vitro viability/function testing

STUDY NO. 3 Nonhuman in vitro and function testing

STUDY NO. 4 Maximization of platelet harvest

STUDY NOS. 1, 2, 3, and 4 Massive transfusion of stored blood or blood substitutes following severe combat injuries leads to dilutional thrombocytopenia and increased bleeding secondary to the lack of platelets. Platelet transfusions can correct this defect, but conventional liquid storage of platelets is impractical for field use since platelets are only viable when stored for less than 72 hours. Studies under this work unit explore methods for freezing platelet concentrates so that they may be preserved inuefinitely and infused without time-consuming washing procedures. Metabolic integrity of platelets is being evaluated with a serotonin uptake test. The procedure for this has been standardized and the data subjected to extensive mathematical analysis. Kinetic models have been tested and are providing valuable insights into overall platelet metabolic integrity. Promising cryoprotective agents have been evaluated using serotonin uptake and morphology tests. Six combinations have shown good results after freezing at a rate of 2 degrees per minute.

BODY OF REPORT

WORK UNIT NO. 042 Long-Term Cryopreservation of Platelets for Immediate Field Use

STUDY NO. 1 Cryopreservation strategies

PROBLEM

Following massive transfusions of blood products or blood substitutes for severe combat injuries, thrombocytopenia is the most common cause of generalized bleeding. While this bleeding may be prevented by platelet transfusions, conventional liquid storage of platelet concentrates is limited to 72 hr at room temperature or 4 C. For field use outside of CONUS, these time limits are too restrictive to provide effective support for combat forces. The objective of this work unit is to develop strategies for preserving platelets indefinitely in the frozen state so that they may be infused immediately after thawing. Current methods of platelet cryopreservation employ cryoprotective agents that are toxic in the concentrations used and hence require extensive post-thaw manipulation and washing. This study seeks to exploit the synergistic effects of different admixed cryoprotective agents so that nontoxic concentrations will cryoprotect platelets while eliminating post-thaw washing. All of the studies of this work unit relate to this objective.

RESULTS AND DISCUSSION OF RESULTS

We have continued to evaluate different cryoprotective agents. These agents have undergone in vitro testing for effect of injury by the cryoprotective agent in the non-frozen state as well as for the effect of the agent with the freezing. The various combinations that have been shown to be promising under the in vitro tests performed last year were revaluated with the modified serotonin uptake test (see Study No. 2). Of the 23 combinations in which the cryoprotectants did not appear harmful, 6 combinants successfully withstood freezing-thawing. The following combinations tolerated a 2 degree/min freezing down to -80 C: 2% dimethyl sulfoxide (DMSO)/2.5% glycerol/2% dextrose; 1% DMSO/ 5% hydroxyethyl starch (HES)/5% polyvinylpyrrolidone (PVP); 1% DMSO/1% glycerol/2.5% HES; 1% DMSO/1% glycerol/5% HES; 1% DMSO/10% HES and 5% HES/5% DHA.

CONCLUSIONS

There are several combinations of cryoprotective agents which show promise. HES and DMSO-containing preservatives appear to maintain platelet metabolic integrity best. Since these agents have different colligative actions, a synergism between a penetrating agent and a nonpenetrating agent appears possible.

RECOMMENDATIONS

The freezing condition and cryopreservatives, indicated in this study, should be studied further to achieve optimal cryopreservation. Experimental plans designed to find combinations of cryopreservation conditions and concentrations of cryopreservative agents which result in optimization of in vitro platelet tests should be implemented to refine the system further. Optimal cryopreservatives should be tested in an animal model and eventually in man.

PUBLICATIONS

None

STUDY NO. 2 In vitro viability/function testing

PROBLEM

The objective of this study is to develop an in vitro system for assessing the metabolic integrity of cryopreserved platelets.

RESULTS AND DISCUSSION OF RESULTS

The major thrust of this year's research has been directed toward establishing a reliable, in vitro assay for platelet metabolic integrity. We have worked extensively with serotonin uptake kinetics since serotonin uptake is a process which involves a multitude of metabolic functions (membrane, cytosol, intracellular binding and metabolism), the sum total of which manifests in platelet survival and function. In a series of experiments, we have established the optimal conditions for kinetic study of serotonin transport and binding by platelets: starting concentration of serotonin (250 ng per 10^8 platelets), pH 6.0 to 6.5, temperature 37 C, platelet count approximately 300,000/µl. Additionally, after experiments demonstrated a pH dependent maximal uptake rate, we evaluated different systems for maintaining the pH constant during the entire experiment. A "closed" system was chosen. By maintaining the platelets in a capped syringe, pH changes due to carbon dioxide loss were minimized. The data from these uptake experiments have been fitted by several mathematical models in an effort to characterize the kinetics.

CONCLUSIONS

Serotonin uptake is a valuable method for understanding platelet physiology and study of its kinetics may yield information relevent to platelet viability. It is reproducible and amenable to kinetic analysis. The conditions of the assay are now

characterized and, pending development of a final mathematical model, should allow for comparison with in vivo function as well as insight into platelet physiology.

RECOMMENDATIONS

Efforts should be made to test various mathematical models and select the appropriate one. The serotonin uptake test should then be correlated with an in vivo animal model to provide a simple procedure to evaluate and screen various cryopreservation systems.

PUBLICATIONS

None

STUDY NO. 3 Nonhuman in vivo viability and function testing

PROBLEM

Conventional tests of platelet metabolic integrity and function correlate only roughly with clinical effectiveness. Animal models must be developed to verify the usefulness of cryopreserved platelets prior to any trials in man. The animal model proposed employs a rabbit first rendered thrombocytopenic and then exposed to a small wound in the jugular vein. Correction of the prolonged bleeding time and measurement of normal life span of transfused human platelets are the desired end points.

RESULTS AND DISCUSSION OF RESULTS

No further work has been done on the animal model during this year.

CONCLUSIONS

None

RECOMMENDATIONS

This study has been temporarily curtailed pending results from Studies No. 1 and 2. In vitro tests as well as basic cryobiological properties of the test system are being evaluated. Following this, an animal model should be used to correlate in vitro tests and in vivo function of frozen-thawed platelets. The rabbit model can be used for this purpose but does have a potential drawback in that reticuloendothelial blockage has been difficult to accomplish. A safe and effective system for blocking the reticuloendothelial system should be developed or modification of the model should be explored to exclude the reticuloendothelial system (ex vivo model).

PUBLICATIONS

None

STUDY NO. 4 Maximization of platelet harvest

PROBLEM

Large platelets are heavier and more active metabolically and functionally than small platelets. The preparation of platelet concentrates involves differential centrifugation which may remove the large heavy platelets. To insure optimal platelet concentrates, specific preparatory methods must avoid the loss of large platelets and maintain platelet size distribution (PSD).

RESULTS AND DISCUSSION OF RESULTS

Various procedures for separating platelets from whole blood have been attempted to obtain the largest yields of platelets with minimal damage. We tried using 10 cc polypropylene tubes with 1 cc of balanced citrate and 9 cc of whole blood, at various centrifugation speeds, centrifugation times, and the addition of buffered saline-glucose diluent. These variables were manipulated by using a simplex technique to determine optimal yields. By this technique 85 to 100% yields could be obtained in two centrifuge spins with the addition of 3 cc of diluent.

CONCLUSIONS

These platelet yields represent a two-fold improvement over current procedures with a little additional manipulation. More sophisticated techniques are now available to obtain 85 to 100% yields but require complex manipulation and are impractical for blood banks. Our results are encouraging in light of our efforts to reach a goal in which virtually all platelets can be harvested with simple practical procedures. High yield platelet harvest will provide a greater proportion of dense, and probably younger, platelets that will improve their probability of surviving freezing injury.

RECOMMENDATIONS

High yield procedures should be evaluated further. Blood collected in 450 cc bags should be evaluated by simplex optimization to improve yields by practical procedures that will afford a large scale applicability.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6106	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SECTY	6. WORK SECURITY	7. REGRADING	8a. GROUP MARKS	8b. SPECIFIC DATA RESTRICTION ADDED	9. LEVEL OF SUM
77 10 01	H. Termination	U	U	NA	NL	YES ☒ NO ☐	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	043
b. CONTRIBUTING				
c.	None			

11. TITLE (Include Security Classification Code)

(U) The Effect of Gastrointestinal Hormones on Gastrointestinal Function

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry, 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMING ORGANIZATION
76 03	77 09	DA	

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: Not Applicable — EXPIRATION:
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 78	.5	34
FISCAL YEAR 79	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: Letterman Army Institute of Research
b. ADDRESS: Presidio of San Francisco, CA 94129

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d. GENERAL USE

Foreign Intelligence Not Applicable

20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
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NAME: Herman, R. H., COL, MC POC: DA

21. KEYWORDS (Precede Each with Significant Letter) (U) Combat Wounds, (U) Gastrointestinal Function, (U) Gastrointestinal Surgery, (U) Gastrin, (U) G.I. Enzymes, (U) Abdominal Injuries

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Furnish individual paragraphs identified by number. Precede text with date period covered.)

23.(U) Dietary substances cause changes in enzyme activities in the small intestine. Dietary substances cause the release of numerous hormones from the gastrointestinal (GI) tract. The GI hormone, gastrin, has a trophic action on the small intestine. In the absence of gastrin, the small intestine atrophies and disaccharidases decrease in activity. It is possible that dietary substances affect small intestinal enzymes via the stimulation of gastrin. Healing of combat-incurred GI tract injury may be facilitated by the administration of gastrin.

24.(U) The effect of gastrin on small intestinal enzymes of the partially gastrectomized dog has been studied. Analyses were done to ensure that the animal was gastrin deficient. Various chemical forms of gastrin were used to determine if small intestinal enzyme activity was increased. The response to dietary substances with and without gastrin were tested. If gastrin is effective, other hormones of the GI tract should be tested.

25.(U) 77 10 - 78 09 Studies to date demonstrate the feasibility of maintaining partially gastrectomized dogs in sufficiently good health so that small intestinal tissue can be obtained on a periodic basis for analysis. The basic studies have been carried out, however, not all the analytical procedures have been completed. Those which have been completed demonstrate that pentagastrin causes a 100% increase in protein synthesis. This project is being terminated due to realignment of the Institute.

26. PUBLICATIONS (References)

DD FORM 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

ABSTRACT

PROJECT NO.	3A161101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	043	The Effects of Gastrointestinal Hormones on Gastrointestinal Function

The following investigation has been conducted under this work unit:

STUDY NO. 1 In vivo study of the role of gastrin in the control of small intestinal mucosal enzymes on the dog

Gastrin, a polypeptide hormone, synthesized in the gastric antrum, has trophic effects on the small intestine in rats. Gastrin deficiency due to absence of enteral food or antral tissue leads to gastrointestinal atrophy. If the trophic effect of gastrin is required for normal intestinal protein synthesis, then gastrin deficiency (resulting from gastric or intestinal resection) will lead to abnormal protein and enzyme synthesis and subsequent abnormal gastrointestinal function. The acutely injured soldier with abdominal injuries requiring gastric or intestinal resection may be gastrin deficient and consequently postoperative gastrointestinal function may be favorably influenced by gastrin therapy. Studies of the effects of gastrin on intestinal glycolytic enzymes in antrectomized (gastrin deficient) dogs are underway. The technical problems of partial antrectomy and serial small-intestinal biopsies have been resolved. The specific, basic studies have been performed, and all the necessary tissue obtained, however, not all the analytical procedures have been completed. Those which have been completed demonstrate that pentagastrin causes a 100% increase in protein synthesis.

BODY OF REPORT

WORK UNIT NO. 043 — The Effects of Gastrointestinal Hormones on Gastrointestinal Function

STUDY NO. 1 — In vivo study of the role of gastrin in the control of small intestinal mucosal enzymes on the dog

PROBLEM

An important aspect of acute gastrointestinal disease involves combat-related abdominal injury and its sequelae. Abdominal injuries occur frequently in any military operation and develop serious complications. In World War II, in one field hospital, wounds of the stomach comprised 416 of 3,154 cases (13%) of abdominal injuries. The fatality rate was 40%. Approximately 30% of the abdominal injuries consisted of wounds of the small intestine. Approximately 20% of the total number of injuries required partial resection of the gastrointestinal tract. Many patients with abdominal injuries will have altered gastrointestinal function secondary to resection of portions of the intestinal tract. With improved techniques of first aid, evacuation, blood replacement, surgery, and prophylaxis and treatment of infection, we can expect an increased number of combat-wounded soldiers to reach the postoperative period. At this point only general supportive measures are available and no specific therapy is known which can hasten healing and restore function of the gastrointestinal tract. Food intake, intestinal hormones and intestinal adaptation all make considerable contributions to the recovery process after intestinal resection. Several observations suggest that the antral hormone, gastrin, has trophic effects on the gastrointestinal tract. In rats, gastrin has increased ^{14}C-leucine incorporation into protein, ^{14}C-orotic acid incorporation into RNA, and ^{14}C-thymidine incorporation into DNA. Gastrin trophic effects have been demonstrated in in vitro tissue cultures of rat gastric and duodenal mucosa. Pentagastrin (PG) stimulated epithelial cell growth, decreased cell doubling time, and decreased cell contact inhibition.

Two different laboratories have demonstrated the importance of food intake in regulating small intestinal enzymes. In rat, intravenous hyperalimentation decreased intestinal maltase and sucrase activities. Tissue gastrin fell concomitantly. The disaccharidases were restored to control levels by PG which suggests that gastrin may control intestinal disaccharidases. Both tissue gastrin and intestinal disaccharidases returned to normal after oral feeding. Previous studies in this laboratory have demonstrated increased activity of jejunal glycolytic enzymes in response to carbohydrate meals. Specific sugars caused adaptive changes in the enzyme most concerned with the metabolism of the specific substrate. In addition there was a generalized increase in enzyme activity attributed to calories alone. Since food intake influences gastrin and intestinal enzymes, and

since gastrin has documented trophic effects in the gut it is conceivable that gastrin has a generalized effect on protein synthesis in the gut. If the trophic effect of gastrin is required for normal intestinal protein synthesis, then gastrin deficiency states occurring as a consequence of gastric or intestinal resection could result in abnormal protein synthesis and subsequent maladaptation of intestinal enzymes. There is ample in vivo and in vitro support for a gastrin trophic effect. There is also evidence to suggest that food intake is important in determining the level of intestinal enzymes and the amount of tissue gastrin. The acutely injured soldier who has lost variable amounts of stomach and small intestine has reduced intestinal function by virtue of the surgical resection. The enzyme activities in the remaining gut are responsive to food intake and gastrin, both of which have been reduced by the surgical procedure. It is reasonable to believe that replacement of gastrin will restore intestinal enzymes to normal and hasten restoration of gastrointestinal function.

RESULTS AND DISCUSSION OF RESULTS

We have studied the role of gastrin in the control of selected intestinal enzyme activities in 4 dogs made gastrin deficient by antrectomy. The various surgical problems related to antrectomy, long-term maintenance of the animals, and repeated small intestinal biopsies have been resolved satisfactorily. In these 4 animals the basic studies have been finished, and a large amount of biopsy tissue was obtained for analysis. Much of this analytical work is pending. Analyses which have been completed demonstrate that pentagastrin causes 100% increase in protein synthesis as measured by ^{14}C leucine incorporation.

CONCLUSIONS

The various surgical and technical problems related to long-term maintenance of antrectomized dogs and repeated small bowel biopsies have been resolved. Initial results indicate that antrectomy results in diminished activity of selected intestinal enzymes. Protein synthesis as measured by ^{14}C leucine incorporation is stimulated 100% by the infusion of pentagastrin. Additional conclusions cannot be drawn until the remainder of the tissue has been analyzed.

RECOMMENDATIONS

The analytical procedures should be computed on the tissue samples which have been obtained and are being stored. This project is being terminated due to realignment of the institute.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6109	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY REV: U	6. WORK SECURITY: U	7. REGRADING: NA	8a. ENTER PROFILE: 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	044
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

10. TITLE: (U) Influence of Stress and Environment on the Nutrition and Metabolism of Military Personnel

11. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002300 Biochemistry; 012900 Physiology

12. START DATE	13. ESTIMATED COMPLETION DATE	14. FUNDING AGENCY	15. PERFORMING ORGANIZATION
76 10	CONT	DA	C. In-House

16. CONTRACT/GRANT

a. DATE EFFECTIVE: Not Applicable
b. NUMBER:
c. TYPE:
d. AMOUNT:
e. KIND OF AGRMT:
f. CUM AMT:

17. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
78	1.8	105
79	1.5	60

18. PERFORMING ORGANIZATION

Name: Letterman Army Institute of Research
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Responsible Individual
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19. GENERAL USE: Foreign Intelligence Not Applicable

20. PERFORMING ORGANIZATION

Name: Letterman Army Institute of Research
Division of Nutrition Technology
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Principal Investigator
Name: Klain, G.J., DAC
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Social Security Account Number:

Associate Investigators
Name: Sauberlich, H.E., DAC

POC:DA

21. (U) Military Nutrition; (U) Military Medicine; (U) Metabolism; (U) Nutritional Alterations; (U) Environment; (U) Laboratory Animals; (U) Adaptation; (U) Endocrine

22. TECHNICAL OBJECTIVE, APPROACH, PROGRESS (Furnish individual paragraphs identified by number. Precede each with date of effort and security classification code.)

23. (U) Various stress factors and environmental exposure markedly alter the nutritional and metabolic patterns of mammalian species. Military personnel likewise are exposed to stressful states and environments in the performance of military duties. Fundamental studies will be conducted with the use of laboratory animals to identify the metabolic reactions and nutritional alterations that occur in response to a given stress or environment. The initial objectives include: (a) evaluate the biochemical and endocrine responses that occur with a stressful environment such as cold, calorie restriction, and adverse diets; (b) determine the degree of adaptation induced by dietary changes and environmental exposures; (c) investigate adverse effects of stress; (d) determine deleterious effects and interactions of various stresses such as dietary excesses, restricted nutrient intakes, cold, heat, etc., on physical and mental performance.

24. (U) The studies require suitable laboratory animal models and the development of novel biochemical techniques. Various metabolic pathways and hormonal systems were followed in response to nutritional alterations and stress guidance for applied investigations on the influence of stress and environmental factors on the nutrition and metabolism of military personnel.

25. (U) 77 10 - 78 09 Growth hormone stimulates in vitro oxidation of hepatic glucose in fed rats and reduces CO_2 production in adipose tissue from refed animals. The hormone also reduces lipogenesis in adipose tissue from refed animals. Growth hormone stimulates in vivo protein synthesis and increases leucine oxidation. Glucagon enhances the activity of specific enzymes involved in the biosynthesis of adrenal corticosteroids. In contrast, the hormone reduces the activity of the enzyme complex controlling the synthesis of progesterone.

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

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 044 — Influence of Stress and Environment on the Nutrition and Metabolism of Military Personnel

The following investigation has been conducted under this work unit during the past year:

STUDY NO. 3 Nutritional state and metabolic effects of growth hormone

STUDY No. 4 Glucagon and enzyme activity in the adrenal gland

STUDY NO. 3 In vivo and in vitro effects of growth hormone on leucine and glucose metabolism were studied in fed, fasted, or refed rats. In vivo, growth hormone enhanced leucine oxidation in fed and refed rats and had no effect in fasted rats. The hormone also stimulated leucine incorporation into tissue proteins in fed or refed animals. In vivo synthesis of hepatic and adipose tissue fatty acids was depressed by the hormone. Growth hormone reduced the activity of several hepatic enzymes involved in protein degradation.

In vitro, CO_2 production from glucose was stimulated by growth hormone in liver from fed rats. In contrast, the hormone depressed CO_2 production in adipose tissue from refed rats. In vitro hepatic and adipose tissue fatty acids synthesis was reduced in refed rats. No effect was observed in fed or fasted rats. In general, the effects of the hormone were more pronounced in refed than in fed rats.

STUDY NO. 4 Intravenous administration of glucagon reduces the activity of the enzyme complex controlling the conversion of cholesterol to progesterone in the adrenal gland. In contrast, the hormone stimulates the enzyme system regulating the formation of corticosterones. These findings are in accord with an increase in the synthesis of several adrenal hormones induced by glucagon administration.

BODY OF REPORT

WORK UNIT NO. 044 — Influence of Stress and Environment on the Nutrition and Metabolism of Military Personnel

STUDY NO. 3 — Nutrition state and metabolic effects of growth hormone

PROBLEM

Available evidence indicates that the secretion of growth hormone (GH) is markedly affected by nutritional, metabolic, or physical stress. In this respect, a decrease in food intake reduces plasma levels of GH. Changes in diet composition also affect GH secretion. A high-carbohydrate diet suppresses GH secretion and this effect may be altered by changing the protein content of the diet. GH secretion is stimulated by some amino acids, notably by lysine and arginine. Thus, it appears that the protein effect may be related to the effect of specific amino acids. Physical exercise also increases plasma levels of GH. Elevated plasma levels of GH have been observed in diabetic patients. Several observations suggest that GH plays a role in glucagon secretion.

Beyond these observations, little is known about the impact of stress on metabolic effects of GH in specific target tissues. Metabolic effects of several other hormones regulating body metabolism are considerably affected by the prior dietary state. Consequently, a pronounced effect of the nutritional state on metabolic responses to GH can be anticipated.

GH has been implicated in the regulation of body fat metabolism, blood glucose homeostasis, and in conserving body proteins during periods of food deprivation. The role of GH in mitigating harmful effects of stress as may be encountered in military combat is thus apparent.

RESULTS AND DISCUSSION OF RESULTS

Male rats weighing about 250 g were fed a complete casein-sucrose diet for 7 days. After this period of dietary adjustment, the rats were divided randomly into 3 treatment groups. The first group continued on the ad libitum feeding schedule, while the second and the third group of animals were fasted for 3 days. The third group of rats was subsequently refed for 3 days. Thereafter, 6 rats from each treatment group were injected subcutaneously with a saline solution of GH (0.4 mg/100 g body weight). Four hours later the rats were sacrificed and glucose-U-^{14}C incorporation into hepatic, kidney, and adipose tissue metabolites was determined.

In another experiment, fed, fasted, or refed rats were injected twice daily for 3 days with GH. On the fourth day, the rats were intraperitoneally given an injection of L-leucine-U-^{14}C dissolved in saline

(6 μCi/100 g body weight). Expired $^{14}CO_2$ was absorbed in a solution of NaOH and radioactivity was determined periodically during the 3-hour collection period. At the end of the collection period, the rats were sacrificed and leucine incorporation into tissue components and the activity of several hepatic enzymes were determined.

The data indicate that GH stimulated hepatic glucose oxidation only in fed rats, with no effect in fasted or refed animals. In contrast, GH reduced glucose oxidation in adipose tissue from refed rats, with no effect in fed or fasted rats. Furthermore, GH decreased hepatic and adipose tissue synthesis of cholesterol in fed and refed rats. No effect was observed in fasting animals. Glycerol synthesis was stimulated by GH only in adipose tissue from fed rats. GH markedly reduced fatty acids synthesis in adipose tissue from fed and refed rats. Fatty acids synthesis in fed animals was not affected by the hormone.

GH enhanced in vivo leucine oxidation in fed and refed rats, and stimulated leucine incorporation into hepatic, heart, kidney, and muscle proteins. The pool size of free leucine in these tissues was slightly increased. In contrant, the hormone markedly reduced leucine conversion into fatty acids in refed rats. In addition, the hormone decreased the activity of leucine transaminase, hepatic arginase, and arginine synthetase in fed and refed rats. GH did not affect enzyme activity in fasted rats. In general, metabolic effects of GH were more pronounced in refed rats than in fed animals.

CONCLUSIONS

The present study demonstrates pronounced effects of altered nutritional states on metabolic actions of GH. The hormone plays an important part in the continuous regulation of protein and energy metabolism by stimulating protein synthesis, lipolysis, and glucose conservation both in fed and refed animals. Thus, it may be possible to attribute to GH in protein synthesis a role analogous to that which it performs in energy metabolism, namely, protection of the organism against the adverse effects of a short-term fast.

RECOMMENDATIONS

Further investigations of the basic interrelationships between GH and energy-protein metabolism, in particular in fasting states, are needed. These should include studies concerned with interactions between GH and insulin.

STUDY NO. 4 Glucagon and enzyme activity in the adrenal gland

PROBLEM

The role of adrenals in adaptive phenomena and the role of glucocorticoids in protective reactions to stress have been recognized.

The factors which regulate the synthesis of the adrenal hormones should, therefore, be clearly defined. Our previous studies demonstrated that glucagon stimulated in vivo conversion of progesterone into deoxycorticosterone, corticosterone, 17-OH-progesterone, and dehydroepiandrosterone. Since the rate of synthesis of these hormones is dependent on the activity of a number of complex enzyme systems, it can be postulated that glucagon affects the activity of specific enzymes in such systems. Accordingly, in the following experiments the effect of glucagon on the activity of several enzymes involved in steroidogenesis was studied.

RESULTS AND DISCUSSION OF RESULTS

Male rats weighing about 300 g were fed a complete casein-sucrose diet for 7 days. After this period of dietary adjustment, the rats were administered via the tail vein a solution of glucagon (100 µg/100 g body weight). Control rats were injected with the corresponding volume of the diluent. Twenty minutes later the rats were sacrificed, the adrenal glands were quickly removed and chilled. The mitochondrial and microsomal fractions were prepared by differential centrifugation. Appropriate fractions were incubated with specific radioactive precursors, the products were extracted and separated by thin layer chromatography. Radioactive components were subsequently located by radioautography and identified by co-chromatography with authentic reference compounds and by color or fluorescence reactions.

The results indicate that glucagon reduced the activity of Δ^5-3-hydroxysteroid dehydrogenase and Δ^5-3-ketosteriod isomerase, the enzyme complex regulating the final step in the conversion of cholesterol to progesterone. In contrast, glucagon enhanced the activity of C-18-hydroxylase activity and had no effect on the activity of C-11β-hydroxylase, the enzyme system involved in the synthesis of corticosterone and OH-corticosterone.

CONCLUSIONS

Intravenous administration of glucagon decreases the activity of the enzyme system regulating the formation of progesterone. The hormone enhances the reaction sequence leading to the formation of corticosterone. The results suggest that this mechanism channels pregnenolene to the endoplasmic reticulum for more efficient utilization during adrenocortical hyperactivity. The results further indicate that glucagon may have several effects in the adrenal gland all of which may serve to provide a fine control over corticosteroidogenesis under widely varying conditions of stimulation.

RECOMMENDATIONS

Further studies concerned with interactions of glucagon and physical stress and their effect of corticosteroidogenesis are recommended.

PUBLICATIONS

1. KLAIN, G.J. Growth hormone and in vivo leucine metabolism in the rat. (Abstract) Fed Proc 37:537, 1978

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

				DA OE 6101	78 10 01		DD-DR&E(AR)636	
77 10 01	D. Change	U	U	NA	NI			
	6 11 01 A	3A161101A91C		00	045			
	none							

(U) Development of a Model Host System of American Cutaneous Leishmaniasis

75 08	CONT	DA		C. In-House	
			78	2	57
Not Applicable			79	2	40

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Foreign Intelligence Not Applicable

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Wilson, H. R., DAC
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Mellick, P. W., MAJ

POC: DA

(U) Protective immunity; (U) Infectivity; (U) Animal model; (U) Leishmania braziliensis; (U) Leishmania mexicana; (U) Pathology

23. (U) The objective is to develop a model host system of American cutaneous leishmaniasis for better understanding of the pathogenesis, immunity, and transmission of the disease. Knowledge gained from these studies is essential to the support of investigations leading to the development of improved measures for the protection and treatment of military personnel serving in endemic areas.

24. (U) An animal model of human *Leishmania braziliensis* infection is being developed in which resolution of lesions, resistance to challenge infection, and delayed-type hypersensitivity occur. Emphasis is being placed on use of inbred mice and evaluation of the immunogenic potential of different *L. braziliensis* strains. Chemical and biologic agents will be used to effect and modify these responses. Infections are being produced and monitored by standardized quantitative methods established in this laboratory.

25. (U) 77 10 - 78 09. Primary *L. braziliensis* lesions of inbred CBA mice ceased growing by 15 weeks following infection but did not regress or resolve during an additional 9 weeks of observation. However, acquired resistance in these mice was demonstrated by the lack of development of lesions from challenge inoculation at 16 weeks after primary inoculation. Routine serial passage of two additional *L. braziliensis* strains was achieved in hamsters. Lesions, produced by one of these two strains, ceased growing at least 3 weeks before the lesions produced by our standard *L. braziliensis* strain or the other strain. An improved cryopreservation procedure was developed which permits efficient and consistent recovery of *Leishmania* amastigotes or promastigotes from liquid nitrogen.

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

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 045 — Development of a Model Host System of American Cutaneous Leishmaniasis

Acquired resistance to *L. braziliensis* infections was demonstrated in inbred CBA mice with primary lesions when challenge infections failed to produce secondary lesions; uninfected control mice developed lesions. Routine serial passage of two additional *L. braziliensis* strains has been achieved in hamsters. Sustained slow growth of lesions of one newly passage strain (HSJD-11) and of our standard strain (M-1287) was still evident at 8 weeks following inoculation. Growth of lesions of the other newly passaged strain (HSJD-1) was entirely comparable to those of HSFD-11 and M-1287 for the first 5 weeks. However, the lack of significant growth of HSFD-1 lesions after 5 weeks indicates a more effective host response and potentially greater immunogenicity of this strain. An improved cryopreservation procedure has been developed for *Leishmania* which permits consistent recovery of cultures of this parasite from liquid nitrogen.

BODY OF REPORT

WORK UNIT NO. 045

Development of a Model Host System of American Cutaneous Leishmaniasis

PROBLEM

The American cutaneous leishmaniases are sand fly-borne parasitic diseases encountered by U.S. military personnel stationed in endemic areas. Due to the potential life-threatening nature of *L. braziliensis* it is imperative to develop adequate diagnostic and preventive measures to insure the protection of personnel susceptible to infection. However, achieving these goals is dependent upon establishing appropriate animal models to support these investigations.

Specific models are needed to evaluate the capacity of leishmanial antigens to elicit protective immunity, to elucidate mechanisms of protective immunity, and to study biologic transmission and its interruption. In studies of protective immunity, an ideal animal model should be capable of effecting protection. This requirement will be fulfilled by the demonstration of resistance to homologous challenge infection following induced or spontaneous resolution of the primary lesion. An economical animal model displaying these characteristics has not been described.

RESULTS AND DISCUSSION OF RESULTS

Inbred CBA mice are able to control and resolve *L. tropica* and *L. donovani* infections and are resistant to subsequent homologous challenge infections. Similar behavior of CBA mice infected with *L. braziliensis* will allow the development of this mouse strain as a practical and economical model. Groups of 10 CBA mice were inoculated in the forepaw with 10^6, 10^4 and 10^2 *L. braziliensis* amastigotes. All mice inoculated with 10^6 and 10^4 amastigotes developed lesions within 3 weeks; 1 mouse inoculated with 10^2 amastigotes developed a lesion which was detected at 6 weeks. Although lesions induced in mice by 10^6 amastigotes ceased growing by 15 weeks, no regression or resolution was noted. Secondary lesions did not result from challenge with 10^4 amastigotes 16 weeks after primary infection in any mouse in which a primary lesion had developed; 8 of 10 untreated control mice developed lesions from the challenge inoculum. The failure of lesions to develop implies the presence of sufficient acquired resistance to protect against homologous challenge even though resolution of primary lesions had not occurred. Such results are promising and indicate the need for additional studies.

It is an established principle of research in infectious diseases that different strains of the same organism can differ markedly in terms of infectivity, pathogenicity, and immunogenicity. Such differences

can be exploited in the development of animal models. We have been able to effect routine hamster-to-hamster passage of two strains of L. braziliensis (HSFD-1, HSJD-11) in addition to our standard strain (M-1287), with which they are being compared. Onset of lesions of *L. braziliensis* HSJD-1 and HSJD-11 occurred in 4-6 days at a dose of 10^6 amastigotes, equivalent to our standard strain. Growth of lesions of *L. braziliensis* HSJD-1, HSJD-11 and M-1287 was entirely comparable during the first 5 weeks following infection. Measurable growth of lesions of *L. braziliensis* HSJD-1 did not occur after this time; however, lesions of *L. braziliensis* HSJD-11 and of our standard M-1287 strain continued to grow for longer than 8 weeks. The comparable time of onset and initial growth of lesions suggest a similar degree of pathogenicity of the 3 *L. braziliensis* strains. However, the early cessation of growth of HSJD-1 lesions suggests that this *L. braziliensis* strain elicits a stronger and more effective host immune response. This characteristic would make strain HSJD-1 more suitable for use in studies of immunity than the strain presently used.

A standing operating procedure (SOP) for preparing *Leishmania* amastigotes and promastigotes for cryopreservation in liquid nitrogen has not been available for use in our laboratory. We modified and developed one procedure recommended for the cryopreservation of mammalian cells as an SOP for freezing *Leishmania*. Features of this modified procedure include: the use of *Leishmania* maintained in optimal physiologic condition prior to freezing, the use of Lebovitz (L-15) medium with 20% fetal calf serum, and the adding of an equal part of L-15 medium with 15% dimethylsulfoxide (DMSO) to the culture. This last step avoids toxic concentrations of DMSO which may occur when undiluted DMSO is added to a culture. In routine use, this cryopreservation procedure is uncomplicated and has consistently provided recovery of viable *Leishmania*.

CONCLUSIONS

Acquired resistance to *L. braziliensis* was demonstrated in CBA mice when challenge inoculation failed to produce lesions. Thus, one of the criteria of an animal model of immunity to *L. braziliensis* infection, resistance to challenge infection, was fulfilled in the CBA mouse. However, primary lesions had not resolved at the time of challenge, and resolution of lesions must be accomplished before the CBA mouse can be fully utilized as a model. Growth of lesions of a newly passaged *L. braziliensis* strain, HSJD-1, ceased before that of two other strains. This finding suggests a more pronounced host response to this *L. braziliensis* strain and, consequently, its greater potential to evoke host immune responses, including resolution of infection. This characteristic would make this strain more suitable to studies of immunity than the strain presently used. Consistent recovery of viable *Leishmania* after freezing in liquid nitrogen has been achieved by use of a modified cryopreservation procedure, which appears suitable for adoption as an SOP in our laboratory.

RECOMMENDATIONS

The CBA mouse should be further investigated and developed as a potential animal model of *L. braziliensis* infection with emphasis upon achieving resolution of lesions. As indicated by the differential host response to *L. braziliensis* HSJD-1 lesions, additional strains of *L. braziliensis* should be investigated for their potential to evoke host immune responses, including resolution of lesions and resistance to challenge infection. The cryopreservation procedure should be continued as the procedure for preservation of *Leishmania* in liquid nitrogen.

PUBLICATIONS

WILSON, H.R., B.S. DIECKMANN and G.E. CHILDS. *Leishmania braziliensis* and *Leishmania mexicana:* experimental cutaneous infections in golden hamsters. (Cleared for publication)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6310		2. DATE OF SUMMARY: 78 10 01		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. GRP'G INSTR'N: NL	9a. SPECIFIC DATA - CONTRACTOR ACCESS: ☒ YES ☐ NO		9b. LEVEL OF SUM: A. WORK UNIT
10 NO /CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER		
a. PRIMARY	61101A	3A161101A91C		00		046		
b. CONTRIBUTING								
c. CONTRIB-ING	10JE							

11. TITLE (Precede with security classification code)

(U) Biochemical Adaptations and Dietary Interactions of Exercise Training

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 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. DATE/EFFECTIVE: — EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: — d. AMOUNT:

e. KIND OF AWARD: — f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR: PREVIOUS 78	2.0	64
FISCAL YEAR: CURRENT 79	1.8	45

19. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
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20. GENERAL USE

Foreign Intelligence Not Applicable

21. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Green, M.D., CPT, MS
NAME:

POC:DA

22. KEYWORDS (Provide KEYWORDS for the highest classification code): (U) Diet; (U) Adaptations; (U) Military Performance; (U) Laboratory Animals; (U) Exercise; (U) Biochemistry; (U) Nutrition; (U) Enzymes

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs indexed to number provide list of keys and security classification code.)

23. (U) The objective is to determine the critical pathways of energy utilization during long-term exercise training. The feasibility of utilizing dietary modifications to enhance physical performance and increase resistance to stress of prolonged chronic physical exertion will be tested. Such studies may identify dietary modifications that can enhance physical performance of military personnel in combat assignments and stressful environments.

24. (U) Metabolic adaptations occurring in response to exercise training were investigated using the treadmill trained laboratory rat as an experimental model. Identification of adaptive enzyme changes were used as a starting point in formulating experimental diets designed to amplify exercise induced adaptations. The initial focus was upon energy generation from fat, carbohydrate, and protein metabolism. Subsequent studies should investigate the interactions of specific minerals or vitamins.

25. (U) 77 10 - 78 09 Diets previously proven to be effective in lessening the severity of starvation-induced hypoglycemia were tested for gluconeogenic properties in support of exercise performance. Supplemental dietary linoleate or triundecanoin fed to exercising rats did not enhance gluconeogenesis or physical performance. Exercise training did not increase the capacity rat brain ketone body metabolizing enzymes to oxidize ketones, but did alter the response of phosphofructokinase to citrate inhibition. Exercise training increased the turnover rate of fatty acids in adipose depots with the greatest increase occurring in the mesenteric depot and the smallest increase in the subcutaneous depot.

* Provided in machine-produced copy and camera-ready copy.

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

ABSTRACT

PROJECT NO. 3A161101A91C — In-House Laboratory Independent Research

WORK UNIT NO. 046 — Biochemical Adaptations and Dietary Interactions of Exercise Training

Four experiments dealing with critical aspects of energy metabolism following physical training have been conducted under this study: Experiment 1 - Efficacy of dietary linoleate and undecanoate as gluconeogenic energy sources for exercising rats; Experiment 2 - Effect of exercise on brain enzymes; Experiment 3 - Effect of anatomical site and physical training on the turnover rate of adipose tissue; and Experiment 4 - Effect of physical training on muscle protein metabolism. The results of these investigations indicate that neither supplemental dietary linoleate nor triundecanoin enhance the formation and deposition of glucose from non-glucose substances under the conditions of chronic exercise. These dietary additives appear to be more useful in maintaining carbohydrate reserves under the conditions of sedentary fasting than exercise. Unlike skeletal muscle, the brain does not appear to adapt by increasing its capacity to oxidize ketone bodies following excercise conditioning. This further emphasizes the importance of maintaining an adequate level of blood glucose during rigorous exercise for brain energy demands. The exercising rat does not draw upon all adipose depots equally for fatty acids to be oxidized as energy sources. Results indicate that the mesenteric fat depot has a significantly greater turnover of fatty acids than that of the subcutaneous fat depot. These differences should be considered when selecting an adipose depot to study during exercise adaptation studies. The mobilization, deamination, and oxidation of certain amino acids during exercise are currently under investigation to determine the contribution of protein to energy generation during exercise.

BODY OF REPORT

WORK UNIT NO. 046 — Biochemical Adaptations and Dietary Interactions of Exercise Training

STUDY NO. 1 — Dietary control of energy metabolism

PROBLEM

It is well documented that sub-optimal nutrition can have a deleterious effect on physical performance. On the other hand, comparatively few dietary modifications have been demonstrated to enhance physical performance in well-nourished individuals. The objective of this study is to obtain information on the potential of dietary modifications to enhance physical performance. Diets that elevate muscle and liver glycogen stores have proven to be the most reliable methods of prolonging work time to exhaustion. The timing of delivery of these high carbohydrate diets is critical, making such diets impractical under combat conditions where activity bursts cannot be predicted. The research conducted under this study is designed to test the overall hypothesis that diets designed to enhance the utilization of body fat depots or increase the conversion of non-carbohydrate precursors to carbohydrate will be beneficial to physical performance.

RESULTS AND DISCUSSION OF RESULTS

Results published in the scientific literature suggest that certain unsaturated fatty acids and certain odd-carbon chain length fatty acids fed to animals maintain their blood glucose at a higher level during starvation than control diets. The feeding of diets supplemented with either linoleate or triundecanoin (a synthetic 11-carbon fatty acid triglyceride) was not effective in increasing muscle or liver glycogen, blood glucose, or other predictors of gluconeogenesis in exercising rats. We conclude that the metabolic stresses of chronic exercise training must be of a different nature than that of fasting.

Exercise training failed to increase the activity of 3-oxoacid CoA transferase in rat brain but did increase enzyme activity in skeletal muscle. Brain tissue appears to regulate its metabolic activity in response to substrate and metabolite concentrations in the blood during exercise but does not necessarily undergo adaptive enzyme changes similar to that of skeletal muscle. Phosphofructokinase activity in homogenates of brain obtained from trained rats was less sensitive to ATP-citrate inhibition than brain homogenates obtained from untrained rats. The basis for this altered regulatory mechanism is currently under investigation.

The turnover rate of adipose tissue fatty acids was measured in subcutaneous, perirenal, epididymal, and mesenteric adipose depots in both trained and sedentary rats. This experiment was conducted to

identify the adipose depot most active in providing fatty acids oxidized during exercise. Chronic exercise increased the turnover of fatty acids in all depots; however, the increase was greatest in the mesenteric depot and least in the subcutaneous site. Mesenteric adipose tissue, by virtue of its rapid turnover rate, appears to be the most appropriate depot to sample for metabolic studies.

A final experiment was conducted to determine if skeletal muscle amino acid oxidation undergoes an adaptive increase in response to exercise training similar to that observed for skeletal muscle fatty acid oxidation. This study involved the measurement of leucine aand glutamate oxidation, myofibrillar protease and glutaminase activity, and 3-methyl histidine excretion. The results of this experiment are currently being analyzed. Preliminary results indicate that training causes an adaptive increase in leucine oxidation but has no effect on myofibrillar protease activity.

CONCLUSIONS

Diets that lessen the severity of hypoglycemia in starvation did not have a similar gluconeogenic effect during exhaustive exercise. Diets containing supplemental levels of linoleate or triundecanoin may have an application under conditions of reduced food intake, but do not appear to have ergogenic merit. Exercise did not influence ketone body oxidation by the same mechanism in the brain as in skeletal muscle. Enzyme induction did not occur in the brain, although brain metabolism did appear to be influenced by exercise conditioning, possibly by a modulation of enzyme activity in response to metabolite concentrations. Adipose tissue adapted to exercise training by increasing the turnover rate of its fatty acids. The increase in turnover rate was not equal in all depots. The most active provider of fatty acids to be oxidized during exercise was the mesenteric depot.

RECOMMENDATIONS

None at this time due to the uncertain status of nutritional research at LAIR.

PUBLICATIONS

1. ASKEW, E.W., A.L. HECKER, V.G. COPPES, and F.B. STIFEL. Cyclic AMP metabolism in adipose tissue of exercise-trained rats. J Lipid Res 19:729, 1978.

2. ASKEW, E.W., G.L. DOHM, P.C. WEISER, R.L. HUSTON, and W.H. DOUB, JR. Supplemental dietary carnitine and lipid metabolism in exercising rats. Nutr Metab, 19788 (in press)

3. ASKEW, E.W. Effect of nutritional factors on lipid metabolism. In: Handbook of Nutrition and Food, edited by M. Rechcigl, Jr. (in press) Florida: CRC Press, Inc., 1978

4. ASKEW, W.E., S.S. KULINSKI, J.R. LOWDER, and W.P. WISE, JR. Comparison of turnover rates of four adipose tissue depots as influenced by exercise. (Abstract) Fed Proc 37:428, 1978

5. GREEN, M.D., and E.W. ASKEW. Brain ketone metabolizing enzymes in exercise trained rats. (Abstract) Fed Proc 37:447, 1978

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | DAOA 6377 | 78 10 01

1. DATE PREV SUMRY	2. KIND OF SUMMARY	3. SUMMARY SEC	4. WORK SECURITY	5. DOD/AGENCY	6a. GRADE REQ	6b. DOCUMENT DATA-CONTRACTOR ACCESS	7. LEVEL OF SUM
77 10 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A WORK UNIT

8a. PRIMARY	61102A	3M161102BS02	00	055
8b. CONTRIBUTING				
8c. CONTRIBUTING	CARDS 116f			

9. TITLE (U) Design and Support of Military Biomedical Research Information Systems (06)

10. DESCRIPTIVE AND TECHNOLOGICAL AREAS
00420 Computers; 009700 Mathematics and Statistics

11. START DATE	12. ESTIMATED COMPLETION DATE	13. FUNDING AGENCY	14. PERFORMANCE METHOD
71 07	CONT	DA	C, In-House

15. CONTRACT/GRANT
a. DATE/EFFECTIVE — EXPIRATION
b. NUMBER Not applicable
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM. AMT.

16. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
78 (INCURRED)	6.8	239
79 (PLANNED)	2.2	95

17. RESPONSIBLE DOD ORGANIZATION
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19. GENERAL USE
Foreign Intelligence not applicable

20. KEYWORDS (U) Digital Computers; (U) Data Base Management; (U) Data Files; (U) Biomedical Research Information; (U) Statistics

21. TECHNICAL OBJECTIVE, 22. APPROACH, 23. PROGRESS (Precede with number of publications reported in Block 24)

23. (U) The objective is to design, implement, and document computer programs and program systems for the management of LAIR research data. These programs will (a) process the results of clinical and laboratory studies to derive their conclusions and to test applicability to specific military situations, (b) maintain and utilize effectively a repository of past research data for direct application to military problems and correlation with future approved military research, (c) evaluate data reported in the open literature to determine its applicability and to apply it to the military environment by correlation and transformation techniques, (d) support the requirements of privacy and the freedom of information acts, (e) provide data base and rapid analysis for information within the mission areas of LAIR in the event of mobilization.

24. (U) General purpose computer programs will be used to the greatest extend possible. Where the unique information processing requirements of a specific research protocol cannot be met by available general purpose computer programs, special purpose programs will be developed.

25. (U) 77 10 - 78 09. Special purpose programs were produced to support military biomedical research projects such as antimetabolite studies of irradiated foods, graphic recording on microfilm of reduced nutrition survey data, owl monkey behavior analysis, and petri plate counting studies. A generalized bar chart, histogram and linear plotting package has been designed and developed to fill the growing requirement for microfilm graphics output of publication quality. Network links among several of the presently independent computer systems are now under development. Several new statistical packages have been implemented for general use. A discrete-event simulation model is being developed for assessing the impact of potential R&D improvements within the blood research area. Data acquisition software has been developed for stress and strain analysis for ligament repair studies.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND USED UNTIL EXHAUSTED. AR 70-10 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	055	Design of Support of Military Biomedical Research Information Systems

The following studies have been conducted under this work unit:

STUDY NO. 1	General mathematical and statistical systems development
STUDY NO. 2	Direct mathematical, statistical and data processing support of military biomedical research
STUDY NO. 3	Design of distributed information processing faciiities
STUDY NO. 4	Data management systems development
STUDY NO. 5	Biomedical engineering and data acquisition

STUDY NOS. 1,2,3,4, and 5. As the use of quantitative techniques in biomedical research continues to expand, the need for better, more efficient and more sophisticated data and information handling techniques has become more vital in the areas of data acquisition, mathematical modelling, graphics, data base management and statistical analysis. This work unit involves the design and development of tools and techniques for the acquisition and management of LAIR research data. These systems are necessary to (a) process the results of laboratory and clinical studies to derive their conclusions and to test their applicability to specific military situations, (b) maintain and utilize effectively a repository of past research data for direct application to military problems and correlation with future approved military research, (c) evaluate data reported in the open literature to determine its applicability and to apply it to the military environment by correlation and transformation techniques, (d) support the requirements of the privacy and freedom of information acts, (e) provide data base and rapid analysis of information within the mission area of LAIR in the event of mobilization.

BODY OF REPORT

WORK UNIT NO. 055 — Mathematical and Computer Support of Military Biomedical Research

STUDY NO. 1 — General mathematical and statistical systems development

PROBLEMS

Statistics is a collection of methods which allows one to make objective inferences about uncertain data. The term data analysis, however, is used to describe techniques more recently developed in the computer environment. The objectives of computer data analysis have been described as (1) achievement of a more specific description of what is loosely known or suspected; (2) finding unanticipated aspects of the data, and suggesting unthought-of-models for the data's summarization and exposure; (3) employing of data to assess the adequacy of a contemplated model; (4) attempting to provide incentives and guidance for further analysis of the data, and (5) keeping the investigator fully stimulated while he absorbs the feeling of the data and considers what to do next. The objective of this study is to provide the mathematical and statistical computer software necessary for comprehensive data analysis in support of the medical military research conducted at LAIR.

Although most data analysis procedures needed by LAIR investigators can be performed by using stand-alone prepackaged programs. Such generalized programs do not readily lend themselves to implementation on minicomputers such as the ECLIPSE C/300 because of size constraints. Indeed, running large packaged programs on the ECLIPSE would significantly reduce response time to interactive users. Therefore, large packaged programs are maintained at Lawrence Berkeley Laboratory (LBL).

RESULTS AND DISCUSSION OF RESULTS

LAIR maintains three statistical packages at Lawrence Berkeley Laboratory. The Generalized Research Analysis Statistical System (GRASS) is a package developed by the Department of Information Sciences and designed to be easy to use for the researcher relatively unfamiliar with computers. GRASS has the capability to produce a variety of descriptive statistics, plots, histograms, and non-parametric tests. GRASS also has data transformation and data manipulation capabilities.

In addition to GRASS, GENSTAT, a general statistical program developed at Rothamsted Experimental Station, England, was installed by LAIR this year on the LBL CDC 7600 GENSTAT has a powerful statistical syntax which allows programs for complex statistical analyses to be written quickly and debugged. GENSTAT is particularly strong in the areas of generalized linear models and the analysis of designed experiments giving LAIR capabilities not previously available. GENSTAT also has many pre-programmed procedures including the ability to store and retrieve structured data sets.

The BMDP Biomedical Computer Programs developed at the UCLA Health Sciences Computing Facility are also maintained by LAIR at LBL. The BMDP package is a series of twenty-nine stand alone programs for statistical analysis. The programs share a free format English-based control language. BMDP has abilities in the area of data screening, analysis of qualitative data, analysis of variance and covariance, regression, multivariate analysis, and non-parametric statistics.

In addition to the packages maintained by LAIR, LBL supports the Statistical Package for the Social Sciences (SPSS), an integrated package for data manipulation and analysis. This year SPSS was updated to contain new capabilities in spectral and survival analysis. LBL also maintains a variety of FORTRAN subroutine libraries which may be used to build special purpose programs.

Limited statistical software is also available to researchers on the in-house ECLIPSE C/300 computer. MINITAB, a statistical computing system developed at Penn State University, provides data analytic capabilities to researchers who are analyzing small to medium sized data sets. MINITAB is easy to learn, well-documented and runs interactively on the ECLIPSE computer. MINITAB capabilities include plotting, data manipulation and transformations, inferential and descriptive statistics, and matrix operations.

It is acknowledged that packaged programs will not solve all statistical computing problems at LAIR. Often special programs must be written to implement special techniques or data analytic methodologies. The Department of Information Sciences believes that such programs are most easily developed on the ECLIPSE C/300. To facilitate building such programs, the International Mathematical and Statistical Library (IMSL) is maintained on the ECLIPSE C/300. IMSL contains approximately 200 mathematical and statistical FORTRAN subroutines. Several special purpose programs have been written with the use of IMSL building blocks.

CONCLUSION

A variety of statistical software are used to perform data analysis at LAIR. Program packages allow an investigator to select which statistical routines are most appropriate without having to master the computational techniques. Lawrence Berkeley Laboratory only supports one statistical

package SPSS. A good remote computing site should provide several such packages, as well as a data base management system which is able to interface directly with statistical packages. The burden to maintain general software should be on the remote computing facility, not on users such as LAIR.

RECOMMENDATIONS

It is recommended that the feasibility of obtaining remote batch processing services at another site which better supports general statistical software be investigated. Effort to develop specialized statistical software on the ECLIPSE as applications arise should continue. BMDP, GENSTAT, MINITAB and IMSL should be updated as new revisions become available from the respective distributors.

PUBLICATIONS

None

STUDY NO. 2 — Direct mathematical, statistical and data processing support of military biomedical research

PROBLEM

Unique characteristics of modern military biomedical research require support to design and conduct experiments and to analyze experimental results. Technical support provided by this study is required to assure efficiency in data collection and to identify appropriate statistical analysis principles and techniques. Likewise, an objective of this study is to provide direct support to LAIR investigators and to aid data acquisition, management and analysis functions integral to experimental processes.

RESULTS AND DISCUSSION OF RESULTS

Consultation has been available on an as required basis in experimental design, data analysis and the use of mathematical and statistical computer software. Objectives sought in formulating good experimental designs are (1) a formal definition of the primary goals of the experiment, (2) employment of a statistical model appropriate to the experimental material which provides unambiguous results, and (3) a design which is feasible within the working conditions of the investigator. After the data have been collected from the designed experiment, support is available to reduce and interpret the data statistically.

Computing resources for institute-wide use are available in two data processing environments, the CDC 7600/6600 digital computers which are accessed remotely at the Lawrence Berkeley Laboratory (LBL) and the Data General ECLIPSE C/300 central minicomputer maintained at LAIR. Software and auxilliary storage limitations on the C/300 require the management and analysis of larger data files in the LBL batch processing environment. The use of time-shared processing at LBL cannot be evaluated as non-ERDA users of the LBL facility are significantly limited in time-shared access during prime time. User orientation to timesharing on the C/300 is widely accepted and is preferred over batch processing methods.

More recently the acquisition of data by using peripherals controlled by a desk top programmable calculator has been designed and programmed. The availability and use of local processors with a data communications capability to the institute central minicomputer are now being tested, the outlook for implementation of significant experimental data acquisition and control functions by using dedicated processors seems favorable.

Department of Information Sciences is one of the participating departments in the nutrition studies in support of the DoD Food Program. The computer programming, statistical, data analysis, and data banking support provided by this department are described under Work Unit 086.

A. Department of Comparative Medicine

Animal Resources Division. Data processing support was requested to aid the study of owl monkey behavior. Activity profiles were established on two groups of animals: a paired group (adults) and a group of juvenile monkeys. Behavior patterns were classified for approximately 19000 behavior observations among both groups. Specifically, a system was designed and programmed which facilitated the on-line entry, validation, reporting, recoding, cumulative analysis, and plotting of behavior observations and animal demographic data.

B. Department of Dermatology

Cutaneous Infection Division. Dermatology clinic outpatient records (134289 count) collected over 3 years at Brooke, Fitzsimons, Letterman, and Walter Reed Army Medical Centers were categoriced by diagnosis code. For the 54 most frequently occurring diagnoses, outpatient records were cross-tabulated by hospital, and collectively for all hospitals by age, race, and sex groupings. A request from LTC Charles Lewis, HSC, BAMC to cross-tabulate dermatology outpatient records with a recorded diagnosis of perioral dermatitis was also processed.

Cutaneous Protection Division. The design of an ECLIPSE C/300 based system to manage mosquito repellent effectiveness testing data was completed. With the recent implementation of the MINITAB interactive general statistical program on the LAIR central minicomputer, local processing was preferred to allow greater user control over data entry, maintenance, and analysis functions. The task of unloading the existing repellent effectiveness testing data base management under the Remote File Management System (RFMS) to SPSS for statistical analysis was completed. Processing at LBL no longer exists, rather, programs are being designed to unload the data base for transport to the LAIR ECLIPSE C/300 minicomputer. A program to report a repellent code-formulation dictionary has been designed and programmed.

C. Department of Nutrition

Bioenergetics Division. Specialized programs were developed at LBL to generate publication quality microfilm recordings of bar charts and histograms summarizing nutrient intake data previously reduced from data collected in the Twentynine Palms and USS Saratoga ship afloat nutrition surveys. Data were recorded by using an SC4060 microfilm recorder available at the LBL facility.

Food Hygiene Division. On-going maintenance of the dictionary of standardized food class, food item, and organism designations is being supported. Reporting by laboratory of all data collected in 1977 was completed and selective retrievals of food class, food item, organism and bacteria count data are in progress. A program was developed locally to aid interactive entry of Petri plate count data in preparation for transfer of data to LBL and subsequent statistical analysis.

A system to support the collection of animal and food weights for anti-metabolite studies of irradiation sterilized tests foods was developed. An HP9815A programmable calculator controlling precision balance and bar code reader peripherals is being used for weight collection. Programs to control weight collection, data unloading to the ECLIPSE C/300 minicomputer and reporting of daily animal weight change and food intake have been implemented. Design and programming to develop data maintenance programs and an animal group definition program are in progress.

Radioisotope Division. On-going maintenance to programs continued which calculate beta counter calibration curve coefficients (program CRV) and disintegration rates (program DPM). Reference to a validated orthogonal polynomial regression function satisfied from a standardized statistical analysis library, IMSL, was integrated into program CRV. Decay correction, used when calculating disintegration rates for more active isotopes, was integrated into program DPM along with functions to control the archival of disintegration rate results for future reference and analysis. A requirement exists to design programs to

display and integrate peaks in disintegration rate counts and to calculate disintegration rates of spiked isotope solutions. The requirement for data processing support to reduce and analyze radioimmunoassay data acquired from institute gamma counters is carried over from last year.

CONCLUSIONS AND RECOMMENDATIONS

Appropriate use of statistical methods is essential to accomplish the scientific research mission at LAIR. Continued development of new techniques and implementation of accepted techniques for data analysis is essential to effective evaluation of research data. To reduce system development time and overall software maintenance requirements, the use of generalized software packages is preferred over reprogramming of existing functional software.

PUBLICATIONS

FRUIN, J.T., J.F. FOSTER, D.L. STUTZMAN, W.H. LANGLEY, J.L. FOWLER and K.E. TREFZ: Report of 1976 Microbiological Data Collection Program. Report No. 55. San Francisco, California: Letterman Army Institute of Research, July 1978.

SPENCER, T.S., K.L. ZELLER, W.A. AKERS, and W.H. LANGLEY: A Data Storage and Retrieval System for a Mosquito Repellent Test Program. (Submitted to LAIR Publications Review Committee).

STUDY NO. 3 — Design of distributed information processing facilities

PROBLEMS

Constantly changing information science technology and an increasing need for greater computing power in biomedical research require distributed computing systems which cannot be purchased as off-the-shelf items. The objective of this study is to develop this facility, specifically the hardware level of interfacing and the computer programs necessary to integrate many independent components into a coordinated facility.

RESULTS AND DISCUSSION OF RESULTS

Development of link between in-house computing facility and Lawrence Berkeley Laboratory (LBL). The link from the in-house ECLIPSE machine to LBL has been developed and is now being tested and improved. It should be fully functional in approximately 3 months. The link permits bidirectional transfer to research data between the two facilities in order to take full advantage of the complementary capabilities of these facilities. The data transfer process is fully protected for data integrity by an error detection with automatic retransmission capability. From the LBL facility it is possible to transfer directly into the DoD Advanced Research Projects Agency Network (ARPANET).

Development of the in-house network. The laboratory computer supporting the Treadmill Automated System (TAS) has been linked to the central in-house computer. In the next several years, it is planned that several other small laboratory machines will be interconnected by using the same procedures. These links offer fully protected error detection with automatic retransmission capability to insure the integrity of the research data. This system will offer to the laboratory computers a greatly expanded capability for data storage and retrieval as well as the ability to execute more powerful computational processes which may directly interact with the small laboratory machine.

On a smaller scale, we have been evaluating microprocessor systems for inclusion in the LAIR NETWORK (LAIRNET). Many laboratory automation tasks could be most effectively handled by dedicating a small processor to the task with linkage to the network. This cannot be effectively done, however, by randomly selecting individual microprocessors with different characteristics and installing them on a one-by-one basis. The individual development, installation, and maintenance costs of such a conglomeration are prohibitive. It is necessary to use a single design so that development costs can be distributed over many copies of the same device and so that linkage of the device into the network can be standardized. Candidates for this development are currently being studied.

CONCLUSIONS AND RECOMMENDATIONS

More effective information processing systems have become essential. The work described above has resulted in the development of more effectively distributed information processing facilities for LAIR. Continued development is essential in the area of network protocols between dissimilar machines, data acquisition equipment, and microcomputer systems in order to make acquisition and processing of laboratory data more timely, more efficient, and more effective.

PUBLICATIONS

None

STUDY NO. 4 — Data Management Systems Development

PROBLEM

The scientific researcher faces special problems in research data base management. Most generalizd data base systems (such as System 2000) are not designed specifically with research data in mind. Although such systems will handle complex data structures adequately, they usually require high level programming ability and do not provide smooth interfacing with statistical analysis packages. Although some statistical packages have data management capabilities, they often lack adequate variable documenting capability, updating options,

security protection, and the ability to define complex data structures conveniently.

RESULTS AND DISCUSSION OF RESULTS

In an effort to provide the LAIR research community with a data management system specifically designed for research data analysis, the Lawrence Berkeley Laboratory is installing a data base management system called Scientific Information Retrieval (SIR) on the CDC 7600 at the request of the Department of Information Sciences. SIR is designed specifically for the scientific environment. The system is based upon the syntax used in the Statistical Package for the Social Sciences (SPSS) and therefore, the researcher familiar with SPSS is provided with an easy transition into data management. SIR contains the following capabilities: hierarchical file structure, data editing and updating, data security options, data retrieval language, report generator, and interfaces with BMDP and SPSS statistical packages.

Remote users of the LBL facility have been informed of the implementation of Systems 2000 and the planned discontinued support of Remote File Management System (RFMS). At present, two data bases are managed under the RFMS data base management: data collected in the DoD Food Wholesomeness Study and Mosquito Repellent Effectiveness Testing Data. The limited use of RFMS in the past can be attributed to several limiting features of the system. Users are constrained by the number of attributes that can be defined in a data base. A maximum of 128 elements are definable in RFMS. Data managed under RFMS are not easily interfaced to statistical analysis systems and that it is recognized that ideal generalized data management features are programmed in RFMS which support the definition, loading, retrieval, reporting, update, and saving of a data base.

Requirements for data management resources on LAIR's central minicomputor are presently being assembled. As the number of applications being developed on that machine increases, the need for data management features at several levels above existing programming language I/O processors is apparent. Desirable features of a minicomputer-based data management system coincide with the ideal features of similar type packages discussed above. Emphasis is also placed on features which support a data archival capability and uniform internal identification of file contents and structure.

CONCLUSIONS AND RECOMMENDATIONS

SIR provides a powerful, easy to use data base management system designed for research scientists. The usefulness of the system to LAIR researchers should be evaluated in the coming year to determine if the Department of Information Sciences should support and/or extend the package.

For continued support of data bases presently managed in RFMS, the more feasible method of conversion to System 2000 and conversion programming requirements should be identified, designed, and programmed. In conversion an LBL consultant indicated that users must bear the responsibility of conversion programming. A generalized RFMS to System 2000 conversion program will not be supplied by LBL. Enhanced features of System 2000 should be tested and reported for general information. RFMS vs System 2000 cost comparison should be documented. Additionally, maintenance required to existing unique application programs which pre- and post-process data managed by RFMS should be identified and completed.

PUBLICATIONS

None

STUDY NO. 5 — Biomedical engineering and data acquisition

PROBLEM

Rising sophistication of biomedical research activities at LAIR has increased the requirements for biomedical engineering support. The objective of this study is to provide such support as needed by investigators at LAIR, to maintain responsive, state-of-the-art techniques sufficient for future needs, and to collaborate with institute investigators when appropriate.

RESULTS AND DISCUSSION OF RESULTS

A. Statistical Library and Computer Graphics. A general purpose statistical library has been created for use on the Data General ECLIPSE C/300. Among the capabilities of the library are calculation of basic descriptive statistics, correlation coefficients, histograms, principal component analysis, T-test analysis, and polynomial regression analysis. Capability has also been provided for plotting data and results of certain analyses on the CRT terminal screen, on the line printer, and on the Textronix plotter.

B. Stress-Strain Data Acquisition of Ligament Repair Study. This project involves measuring the force maintained by ligaments and their resultant elongation as a function of time as they are mechanically pulled to rupture. Special purpose, automatic on-line data acquisition software has been created for this purpose and installed on the Data General NOVA 3/12. The obtained data are stored on disk in a form compatible with previously written off-line analysis programs.

C. Cardiopulmonary Analysis. This project involves on-line data acquisition of cardiopulmonary variables from anesthetized dogs, on-line analysis providing real-time feedback to the investigator in the form

of analog graphic displays, and off-line analysis of the stored data toward the end of describing the cardiopulmonary effects of various kinds of anesthesia.

The major part of the on-line data acquisition aspect of this project was initiated in 1976-77 and completed this year. Off-line analysis programs have been implemented which provide capability for data editing and extensive analysis of respiratory and cardiovascular function, including an integrated analysis of blood gas and expired air content.

D. On-Line Outlier Detection. The problem is to devise a way to detect the occurrence of outlying (or obviously erroneous) data points or waveforms as they are being acquired on-line by computer without any a priori knowledge of the statistics or other characteristics of the data. Mathematically, the problem is defined as deriving an algorithm which selectively rejects sequential data in a manner such that the mean of the accepted data converges faster than the mean of the total (accepted plus rejected) data, the variance of the accepted data is less than that of the total data and, under certain limiting assumptions, the error due to outliers in the mean calculated from the accepted data is smaller than the error in the mean of the total data. In statistical terms, such an algorithm produces, in the limit, a more robust estimate of the mean of the sequentially arriving data.

Progress has been made toward identifying the necessary trade-offs involved in approaching such a problem and in developing the necessary mathematical framework for deriving a solution.

E. Base Excess Nomogram. A system of programs has been created and implemented on the Data General ECLIPSE C/300 which (1) accepts data concerning PCO_2 content, pH hemoglobin level, and measured base of excess of blood samples, (2) uses statistical curve fitting routines to calculate predicted values of pH as a function of base excess and PCO_2, (3) automatically modifies the fitted curves (and therefore the prediction of pH) to be consistent with both known and accepted hypothetical relationships between pH, PCO_2, and base excess, and (4) uses the resultant functions to produce computer plotted nomograms of PCO_2, pH hemoglobin, and base excess.

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA OE 6112	78 10 01		REPORT CONTROL SYMBOL DD-DR&E(AR)636
77 10 01	N. Termination	U	U	NA	NL	YES NO	
10 NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61102A	3M161102BS02		00	056		
b. [redacted]							
c. [redacted]	CARDS 114f						

(U) Biochemical Basis of Nutrition, Disease and Recovery in Military Personnel

002300 Biochemistry; 003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMING METHOD
76 10	78 09	DA			C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: [redacted] EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR 78	2.0	86
FISCAL YEAR 79	0.0	00

19. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

ADDRESS:

RESPONSIBLE INDIVIDUAL
NAME: Marshall, J.D.,COL, MS
TELEPHONE: 415-561-3600

20. GENERAL USE

Foreign Intelligence Not Applicable

21. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Biochemistry Division
ADDRESS: Department of Nutrition
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Provide name and telephone number)
NAME: Milne, D.B., DAC
TELEPHONE: 415-561-5872
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Turnbull, J.D., CPT, MS
NAME: Askew, E.W., MAJ, MS POC:DA

22. KEYWORDS: (U) Nutrition; (U) Metabolism; (U) Military Nutrition; (U) Military Medicine; (U) Laboratory Animals; (U) Nutritional Disorders

23. TECHNICAL OBJECTIVE: (U) Various nutrients have been implicated in wound healing, bone repair and integrity, prevention of various cardiovascular and degenerative diseases, adaptation to environment and stresses, muscle functions and energy metabolism. Objective is to conduct fundamental research directed toward present or potential military problems concerning nutrition and metabolism, and the role of diet in health, disease, infection, immune response, and recovery from injuries.

24. APPROACH: (U) Through use of laboratory animals and tissue culture systems, the biochemical basis of the role of nutrients and related compounds will be investigated as they may relate to the prevention and treatment of injury and disease, and to the functioning of military personnel under various conditions. Included for study are interactions of nutrition in toxicology and parenteral nutrition, aspects of medicinal nutrition, and pharmacological effects of vitamins and other nutrients. Techniques will be developed and investigations conducted that will provide knowledge as to the requirements, metabolism, interactions, utilization, and functions of nutrients that may be utilized in applied studies in military medicine and ration development.

25. PROGRESS: (U) 77 10 - 78 09 A study on copper, ascorbic acid interaction in monkeys is currently in progress. High levels of ascorbate tend to lower ceruloplasmin and serum copper levels in monkeys fed diets containing marginal levels of copper. This work unit is terminated due to directed realignment of the LAIR research program.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3M61102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	056	Biochemical Basis of Nutrition, Disease, and Recovery in Military Personnel

The following investigation has been conducted under this work unit:

STUDY NO. 2 Trace elements in wound healing, collagen metabolism, and bone metabolism

STUDY NO. 2 The effects of high levels of ascorbic acid (vitamin C) and marginal levels of dietary copper have been studied in cynomolgus monkeys. Data from this study are still being analyzed. However, preliminary observations indicate that high levels of dietary ascorbate may have adverse effects on copper utilization and metabolism.

BODY OF REPORT

WORK UNIT NO.	056	Biochemical Basis of Nutrition, Disease, and Recovery in Military Personnel
STUDY NO.	2	Trace elements in wound healing, collagen metabolism, and bone metabolism

PROBLEM

Many trace elements are involved in bone calcification and collagen or elastin metabolism and thus may be important in recovery from injury. Copper, an essential trace element, is required in elastin and collagen metabolism. It is also necessary for normal iron utilization and energy metabolism. A recent survey of a military population at Alameda Naval Air Station indicated that intakes of copper may be marginal and below National Research Council Recommendations.

There has also been a recent surge of interest in vitamin C and also an increase of intakes of vitamin C by the general population because of claims regarding its efficacy in optimizing health in general. However, studies in rats and chickens have shown that large amounts of vitamin C are detrimental to copper absorption and metabolism.

The initial experiment in this study was designed to study the impact of high and moderate intakes of vitamin C on marginal copper intakes in cynomolgus monkeys, a species with similar requirements as man.

RESULTS AND DISCUSSION OF RESULTS

Eight cynomolgus monkeys were maintained on a liquid diet containing marginal levels of copper for 29 weeks. During the first 4 weeks, no vitamin C was added to the diet. After 4 weeks, the monkeys were divided into 2 groups. One group was fed the recommended level (1 mg/kg BW/day) of vitamin C for adult monkeys. The other 4 were fed 100 times that amount. After 209 weeks, copper was added back to the diet (50 mg/kg BW/day) at 1½ times the estimated requirement. After another 4 weeks, the animals were returned to normal chow diets. Blood samples were taken at weekly intervals and the following parameters were determined: hemoglobin, hematocrit, cell counts, serum ferritin, serum iron, serum total iron binding capacity, serum copper, ceruloplasmin (a copper containing enzyme responsible for normal iron mobilization) and serum and whole blood ascorbate levels.

Preliminary observations indicate that added ascorbate, in higher amounts, adversely affects the serum copper and ceruloplasmin levels. Some increases in serum iron were noted. Many of the samples are currently being analyzed and the data will be evaluated upon completion.

CONCLUSION

Preliminary observations indicate that high levels of ascorbate (vitamin C) may adversely affect copper utilization in monkeys and may present a problem in military nutrition.

RECOMMENDATIONS

1. Interactions between different nutrients present in military diets should be carefully considered.

2. Studies on the effects of other trace elements on wound healing, connective tissue metabolism, and bone metabolism are necessary.

3. Studies on the roles of essential trace elements on performance and muscle dysfunction should be initiated.

PUBLICATIONS

ZIPORIN, Z.Z., P.P. WARING, R.L. MORRISSEY, M.E. LYSNE. Effect of Vitamin D and Dietary Content of Calcium and Phosphorus on Protein Synthesis in Rat Duodenal Mucosa. Report No. 50. San Francisco, California: Letterman Army Institute of Research, March 1978.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6113	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DRE&E(AR)636
3. DATE PREV SUM'RY: 77 10 01	4. KIND OF SUMMARY: H. Termination	5. SUMMARY SCTY: U	6. CODE SECURITY: U	7. GRADING: NA	8. CRDA MARKS: NL	9. SPECIFIC DATA CONTRACTOR ACCESS: [X] YES [ ] NO
10. SMI CODES: a. PRIMARY	PROGRAM ELEMENT: 61102A	PROJECT NUMBER: 3M161102BS02		TASK AREA NUMBER: 00	WORK UNIT NUMBER: 057	
b. [redacted]						
c. [redacted]	CARDS 114f					

11. TITLE: (U) Nutritional Physiology in Health and Prevention of Injury in the Military

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine; 012900 Physiology; 006500 Food; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	78 09	DA	C. In-House

17. CONTRACT/GRANT: a. DATE/EFFECTIVE: b. NUMBER: Not Applicable; c. TYPE: d. KIND OF AWARD: e. AMOUNT: f. CUM. AMT:

18. RESOURCES ESTIMATE:

PERFORMANCE	FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
PREVIOUS	78	4.0	160
CURRENT	79	0.0	00

19a. RESPONSIBLE DOD ORGANIZATION: NAME: Letterman Army Institute of Research; ADDRESS: Precidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL: NAME: Marshall, J.D., COL, MS; TELEPHONE: 415-561-3600

20. GENERAL USE: Foreign Intelligence Not Applicable

19b. PERFORMING ORGANIZATION: NAME: Letterman Army Institute of Research; ADDRESS: Bioenergetics Division, Department of Nutrition, Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR: NAME: Johnson, H.L., DAC; TELEPHONE: 415-561-5092; SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS: NAME: Schnakenberg, D.D., MAJ, MS; NAME: Fults, R.D., DAC; NAME: Kretsch, M.J., DAC

21. RESEARCH AREAS: (U) Nutrition and Environment; (U) Body Composition; (U) Nutrient Requirements; (U) Nutrition and Performance; (U) Human Volunteers

22. TECHNICAL OBJECTIVE:

23. (U) Attainment and maintenance of optimum metabolism is of prime import to the Armed Forces and requires a constant search for new methods for improving and evaluating physiological status. Status reflects nutriture and body hydration, and may be affected by age, sex, state of health, dietary constituents, vitamins, hormones, therapeutic agents, and varied stresses, all of which should be reflected in body composition changes and related physiological parameters. General objective is to conduct fundamental research that will provide guidance about dietary alterations and nutritional practices can be applied for increased efficiency of military personnel operating under various combat and environmental stresses.

24. (U) Approaches will be to (a) investigate the physiological basis of nutrition, pulmonary function, and physical status, utilizing human subjects and experimental animals; (b) study the physiologic and metabolic adaptations to nutritional and environmental stresses, including research on the relationship of nutrient requirements; and (c) develop techniques and biomedical instrumentation to measure body composition, caloric requirements, and other nutrient requirements; and (d) investigate through the use of animal models the metabolic and/or neurophysiological basis of stress and diet-induced changes in voluntary food and water consumption.

25. (U) 77 10 - 78 09 An interface providing computer control of two treadmills simultaneously has been developed. The final version of the Treadmill Automation System (TAS) has been delivered by Lawrence Berkeley Laboratories. The respiratory mass spectrometer has been received and is being implemented under TAS control. Protocols utilizing this new equipment are being drafted. One protocol for using this system to evaluate the energy expenditure in a group of Marines has been developed. Analysis of data from two studies of protein requirements in humans has been completed and a report is being drafted.

DD FORM 1490 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 MAY BE USED FOR ARMY USE UNTIL OBSOLETE

ABSTRACT

PROJECT NO. 3M61102BS02 Basic Mechanisms of Recovery from Injury

WORK UNIT NO. 057 Nutritional Physiology in Health and Prevention of Injury in the Military

The following investigations have been conducted under this work unit:

STUDY NO. 6 Evaluation of protein requirements during heavy physical activity

STUDY NO. 7 Development of instrumentation and methodology for evaluating physiological status

STUDY NO. 6 Analyses of samples and processing of data collected from two studies on the adequacy of protein allowances during heavy physical training (e.g., basic training of military inductees) have been ongoing. All data have been processed except for the results of the endurance walk and ^{40}K whole body counting which will be processed after completion of necessary computer software. Analyses of variance or covariance have been completed on all of the processed data. Post hoc multiple comparison tests are presently being prepared. Preliminary evaluation of the results indicates that dietary protein levels had minimal effects on work performance and body composition. Both the NRC (0.6g/kg of body weight) and the military (1.49g/kg of body weight) allowances for protein appear to have been adequate for maintenance of work capacity and body composition. Final conclusions will be drawn after processing endurance and ^{40}K data and with further statistical analyses including the post hoc multiple comparisons and possibly multivariate analysis of variance.

STUDY NO. 7 The complete and final version of the Treadmill Automation System (TAS) software has been received from the vendor (Lawrence Berkeley Laboratories). The Respiratory Mass Spectrometer (RMS) was delivered by the University of Colorado and has been interfaced with the Data General Nova 1220 minicomputer. This system is now operational. Interfaces among the Modcomp II minicomputer and the treadmills, ^{40}K counter and Data General Eclipse C/300 (located in the Department of Information Sciences) are all under final test or near completion. Software for treadmill control and computer-to-computer communication is under final testing. However, much software remains to be written in order to utilize fully the capabilities of this laboratory system. Preparations are underway to perform graded exercise testing in the field early in FY 79. Protocols for further studies in which this equipment is used and for expanding present experimental methodology are being developed.

BODY OF REPORT

WORK UNIT NO.	057	Nutritional Physiology in Health and Prevention of Injury
STUDY NO.	6	Evaluation of protein requirements during physical training and heavy physical activity

PROBLEM

Basic training for military inductees, advanced and specialized training for most combat arms personnel, and the refresher training upon activation of the National Guard or Reserve personnel have a large portion of time dedicated to physical training and conditioning. Any factors that would contribute to the efficiency and effectiveness of these programs would benefit all services and the personnel undergoing training. The amounts of dietary protein required during physical conditioning have been a long-term controversy (for at least 120 years) and have recently reappeared in the literature. This study was designed to evaluate the adequacy of the National Academy of Sciences--National Research Council's Recommended Allowances (NRC) versus those of the military (AR 40-25) during periods of physical conditioning and heavy physical activity.

RESULTS AND DISCUSSION OF RESULTS

The first two phases of this study were conducted during January through April 1976, and the experimental details were presented in the Annual Reports FY 76. Mineral and nitrogen catabolite concentrations were determined in samples of blood, urine, feces, and sweat collected during the study. These data have been processed and examined via analysis of variance. Appropriate post hoc multiple comparison tests are being prepared. Preliminary information from nitrogen balance data indicated that balances could be attained at the NRC allowance of protein (0.6 g/kg of body weight) during physical conditioning after a 12-day sedentary period of adaptation to low dietary levels of protein, and that the military allowances (100 g/day or 1.4 g/kg of body weight) were adequate for nitrogen retention (which is indicative of increased muscle mass or body protein stores). Positive potassium balances were consistent with these results. These studies were conducted with well-defined, highly digestible liquid diets. Processing of exercise physiology data is complete through analysis of variance for variables obtained from graded exercise tests, pulmonary function analysis, and body composition by anthropometry and water displacement. Post hoc multiple comparison tests are being prepared for these data. The data from the treadmill endurance tests and ^{40}K shadow shield counting will be processed after specialized computer software has been developed. This software is roughly 75% completed.

CONCLUSIONS

Initial indications were that the NRC allowances for protein would provide for nitrogen balances during physical training after adapting to these diets and that military allowances would provide sufficient proteins for increasing muscle mass. At this point, it appears that only minimal changes were observed in work capacity, pulmonary function, and body composition. Both diets appear to have been adequate for maintenance of these parameters. Multivariate analysis of variance is being considered as a means of discriminating the small differences that may exist between diet groups and from day to day.

RECOMMENDATIONS

Data analyses should be completed and the final phase of the study should be conducted, probably with the use of normal military diets. The design for the final phase of the study will depend upon the complete evaluation of the first two phases.

STUDY NO. 7 — Development of instrumentation and methodology for evaluating physiological status

PROBLEM

The effects of nutritional and environmental stresses are reflected by physiological status, including cardiopulmonary function, body composition, and physical work capacity. Highly sophisticated instrumentation and methodology are required to investigate adequately the effects of stress on these parameters. In addition, many military situations require the soldier to perform strenuous physical activities while under nutritional and environmental stress. To ensure that the soldier is able to perform adequately under such conditions, heavy physical activity must be incorporated into experimental protocols. Since physical activity increases the difficulty of measuring physiological parameters, more complex instrumentation is required for studies involving activity than for studies under static conditions. Complex instrumentation and methodology also allow investigation of the dynamics of the responses to stress, an important factor in understanding the response. The development of a laboratory incorporating high-speed instrumentation, automated data acquisition, complex computerized analysis porcedures, and suitable methodology will facilitate extensive evaluation of the effects of nutritional and environmental stress.

RESULTS AND DISCUSSION OF RESULTS

All development work aimed at automating data acquisition under control of the Modcomp II minicomputer (MCII) and providing data processing of the MCII and the C/300, via a protocol similar to Digital Equipment Corporation Network (DECNET), has been completed. An

information exchange system between the Departments of Nutrition and Information Sciences is being prepared. The Respiratory Mass Spectrometer (RMS) has been received and is being interfaced in the MCII under Treadmill Automation System (TAS) control. The RMS has been used as a stand-alone device and has demonstrated excellent performance. Early stages of integration under TAS have met with success.

A study of energy expenditure, to be performed during the nutrition study at Twentynine Palms, California, early in FY 79, is planned. Preparations are being made for the MCII to control a treadmill according to the subject heart rate and to acquire data from the RMS, process the data for immediate feedback during the experiment, store the data on disc for future evaluation, and transmit the data to the C/300 for back-up archival.

CONCLUSIONS

The acquisition of a minicomputer for on-line data processing and experimental control during testing, and the delivery of the RMS have greatly enhanced our capabilities for determining nutrient requirements for personnel undergoing stresses commonly encountered in military situations, and for the evaluation of physical and physiological status of military personnel. Most of the instrument interfacing and computer programming required for this system has been completed. Protocols are being developed to utilize this equipment and techniques for evaluation of nutrient requirements of personnel performing military duties in various environments.

RECOMMENDATIONS

1. The present system should be completed and tested under field conditions during investigations of nutritional status of military personnel.

2. Instrumentation and software will need to be modified to remain current with state of the art and, as required, to accomplish expeditiously objectives of studies.

3. Protocols should be developed to respond to military needs for evaluation of current problems and to maintain optimal combat readiness for the future utilization of these techniques.

PUBLICATIONS

1. CONSOLAZIO, C.F., J.A. TILLOTSON, and T.A. DAWS. Riboflavin Depletion and Work Capacity in Humans. Report No. 47. San Francisco, California: Letterman Army Institute of Research, January 1978

2. JOHNSON, H.L., C.F. CONSOLAZIO, R.F. BURK, T.A. DAWS, and R.G. LUFKIN. The Effects of Abrupt Altitude Exposure (4300M) upon the Metabolism of Glucose-^{14}C-UL in Man. Report No. 44. San Francisco, California: Letterman Army Institute of Research, January 1978

3. KRZYWICKI, H.J., C.F. CONSOLAZIO, H.L. JOHNSON, and N.F. WITT. Metabolic Aspects of Caloric Restriction (500 Calories) Body Composition Changes. Institute Report. San Francisco, California: Letterman Army Institute of Research, (submitted for review and clearance)

4. KRZYWICKI, H.J., C.R. CONSOLAZIO, H.L. JOHNSON, and N.F. WITT. Effects of Exercise and Dietary Protein Levels on Body Composition in Humans. Institute Report. San Francisco, California: Letterman Army Institute of Research, (submitted for review and clearance)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6114	2. DATE OF SUMMARY: 78 10 01		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. CHANGE	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. GROUP MARK: NL	8a. SPECIFIC DATE CONTRACTOR ACCESS: [X] YES [ ] NO	9. LEVEL OF SUM: A. WORK UNIT
10. MC CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	62772A	3M162772A812		00		005	
b. SECONDARY	61102A	3M161102BS02		00		061	
c. TERTIARY	CARDS 114						

11. TITLE (Enter title and security classification code): (U) Disease Mechanisms at the Cellular Level

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002600 Biology; 010100 Microbiology; 002300 Biochemistry; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
76 10	CONT	DA			C. In-house

17. CONTRACT/GRANT
a. DATE EFFECTIVE: — EXPIRATION: —
b. NUMBER: Not Applicable
c. TYPE: — d. AMOUNT: —
e. KIND OF AWARD: — f. CUM. AMT.: —

18. RESOURCES ESTIMATE

	PROFESSIONAL MAN YRS	FUNDS ($ Thousands)
FISCAL YEAR 78	1.7	119
FISCAL YEAR 79	0.8	50

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129
b. ADDRESS:
c. RESPONSIBLE INDIVIDUAL
NAME: Marshall, J.D., COL, MC
TELEPHONE: (415) 561-3600
d. GENERAL USE: Foreign Intelligence Not Applicable

20. PERFORMING ORGANIZATION
a. NAME: Letterman Army Institute of Research
Pathology Services Group
b. ADDRESS: Division of Research Support
Presidio of San Francisco, CA 94129
c. PRINCIPAL INVESTIGATOR
NAME: Mellick, P.W., LTC, VC
TELEPHONE: (415) 561-3855
SOCIAL SECURITY ACCOUNT NUMBER:
d. ASSOCIATE INVESTIGATORS
NAME:
NAME:
POC:DA

21. KEYWORDS: (U) Histochemistry; (U) Electron Microscopy; (U) Diagnosis: (U) Infection; (U) Laboratory Animal; (U) Metabolic Disease

22. TECHNICAL OBJECTIVE, APPROACH, AND PROGRESS:

23. (U) Prevention or control of disease depends on complete understanding of the abnormal processes involved, from initial injury of the cell to repair. These experiments will encompass diseases of military importance such as skeletal injury of soldiers and economically important diseases of laboratory animals. They will provide information on cellular response to specific injury and will form a basis for studies of a more clinical nature.

24. (U) Diseases of particular importance to the Army which are poorly understood will be studied at the cellular level in appropriate human or animal cells. Electron microscopy, histochemistry, and quantitative analytical methods will be used. Basic differences between normal and affected cells will be sought and hypotheses regarding the disease mechanism will be proposed and tested. Initial studies will involve control of calcium metabolism and early detection of diseases of laboratory animals. A new enzyme histological technique, peroxidase-antiperoxidase localization of biologically important proteins, will be standardized and implemented.

25. (U) 7710-7809 Progress was hampered by the departure of the principal investigator and requirements of assigned investigators and technicians to support other LAIR projects. Rabbit anti-owl monkey IgG was prepared, purified, and partially tested. Initial attempts to demonstrate IgG in plasma cells in owl monkey lymph nodes failed. Because of the reorganization of LAIR and unavailability of professional personnel, experimental investigations under this work unit will be terminated. Instead, new studies to develop and adapt improved histological, histochemical, and ultrastructural techniques to support LAIR investigators will be initiated under this work unit during FY 79.

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

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	061	Disease Mechanisms at the Cellular Level

The following investigations have been conducted under this work unit:

STUDY NO. 1	Feasibility of the use of immunoenzyme-labeled antibody in the histopathological diagnosis of viral laboratory animal diseases
Unnumbered Studies	(Reported under work units of other LAIR investigators)

Work performed under this work unit included animal necropsy, histopathology, clinical pathology (hematology, clincal chemistry, microbiology), scanning and transmission electron microscopy, and special morphometric procedures. These techniques were applied to one study in the Department of Comparative Medicine addressing cellular responses in disease, and to 30 additional studies in collaboration with or in support of principal investigators from other departments in LAIR and LAMC as required. The specific work units and programs requiring complete veterinary pathology support are tabulated in the text of this report and all resulting publications are listed.

STUDY NO. 1 Initial attempts to use the peroxidase labeled-antibody technique to demonstrate feline syncytial forming virus (FeSFV) in cell cultures were unsuccessful. Rabbit anti-owl monkey IgG was prepared and purified. This antibody solution was used, along with the immunoperoxidase procedure, in attempts to demonstrate IgG in plasma cells in sections of lymph node. The presence of background staining and other technical difficulties led to negative results.

BODY OF REPORT

WORK UNIT NO. 061 Disease Mechanisms at the Cellular Level

STUDY NO. 1 Feasibility of the use of immunoenzyme-labeled antibody in the histopathological diagnosis of viral laboratory animal diseases

PROBLEM

Healthy laboratory animals are a vital resource for Army medical research. Diseased animals are wasteful of this resource and the loss is compounded when experiments cannot be evaluated properly because of intercurrent disease in the research animals. Accurate and expeditious means to detect and diagnose diseases, particularly viral diseases, are therefore highly desirable. It is usually impractical to use standard procedures to culture, isolate, and identify the virus(es) that cause disease in laboratory animals. Techniques using enzyme-labeled specific antibody have been used for rapid detection and identification of viral antigens in human tissue. The advantage of these techniques lies in their rapidity, sensitivity, and reliability. This method will be adapted to detect and identify viral antigens in fixed and frozen tissue as a rapid and economical means to diagnose specific viral diseases of laboratory animals. When the technique is established for a model system, it will be adapted by using appropriate antisera for quick identification of other specific viral antigens which may be present in the tissues of both sick and apparently healthy laboratory animals.

Candidate diseases for which a rapid specific detection method is needed include herpes and syncytial viral (FeSFV) infection in cats, adenoviral infection in owl monkeys, and Sendai viral infection in rodents. Surveillance for presence of viral agents is conducted under Project No. 3M762772A812, Work Unit No. 002, Study No. 3; viral isolates and known infected tissue from that work are effectively used in this study. If this study is successful, this relatively inexpensive technique will preclude future need for expensive culture, isolation, and identification of these and similar viruses.

RESULTS AND DISCUSSION OF RESULTS

Since many of the cats used as experimental animals at LAIR carry FeSFV, we attempted to use this system as a model to test the feasibility of the enzyme-labeled antibody technique for immunochemical diagnosis. Cell cultures known to contain FeSFV were stained by the indirect method using rabbit anti-FeSFV antibodies and goat anti-rabbit IgG labeled with the peroxidase enzyme. Results were disappointing because of nonspecific staining, poor fixation of material, and other technical difficulties.

Rabbit anti-owl monkey IgG was prepared for use in immunohistochemical procedures as follows. Owl monkey IgG was precipitated from whole serum by addition of saturated ammonium sulfate. After purification with the superfine grade of Sephadex G-200, the IgG with Freund's complete adjuvant was injected 3 times into rabbits. Gamma globulin from the resulting hyperimmune rabbit anti-owl monkey serum was precipitated with ammonium sulfate 3 times, redissolved and stored in vials at -40°C. Initial attempts were made to use this material to demonstrate IgG in plasma cells of owl monkey lymph node. The peroxidase-antiperoxidase technique was used. Intense background staining, poorly preserved lymph node tissue, and technical difficulties led to negative results. Work on this study had to be curtailed in order to provide support to other studies with higher priority.

CONCLUSIONS

The development of a peroxidase-labeled antibody method to diagnose viral diseases in laboratory animals may be feasible. If an accurate, reliable, and sensitive technique were available, it would be extremely valuable in animal colony disease surveillance and control. Development and testing of the technique will be difficult and time-consuming and should be undertaken by an investigator who can devote considerable time to the study.

RECOMMENDATIONS

This study will be terminated because of the departure of the principal investigator and co-investigator, and reorganization of LAIR. New studies to develop and adapt improved histological, histochemical, and ultrastructural techniques to support LAIR investigators should be initiated following reorganization.

PUBLICATIONS

None

UNNUMBERED STUDIES

In addition to the cell-level studies of disease mechanisms, this work unit provided support in pathology and electron microscopy for all investigators of LAIR and Clinical Investigation Service (CIS), Letterman Army Medical Center. Specific veterinary pathologic services, i.e., animal necropsy, microbiology, histopathology, and clinical pathology; and special techniques, i.e., histochemistry, morphometry, and electron microscopic autoradiography, were performed. Based on careful records for FY 1978, approximately $28,000 was spent from this work unit for pathology services to investigators in other work units. The sum includes civilian technicians' pay and benefits, expendable supplies, and prorated overhead for civilian and military technicians. Officer (investigator) salaries and overhead, equipment, and costs attributable to

administrative functions and leave are not included in this figure.

Support and collaboration by veterinary pathologists were provided for the LAIR studies listed below. Outcome of those studies is reported elsewhere in this annual report. Publications resulting from collaborative efforts are listed at the end of this section.

LAIR STUDIES PROVIDED WITH COLLABORATION/SUPPORT

BY VETERINARY PATHOLOGISTS - FY 1978

Project No.	Work Unit	Project No.	Work Unit
3A161101A91C	040	3M762772A812	001
	042		002
	043		003
	045		024
	046	3E762772A813	025
3M161102BS02	056	3S76277A2814	004
	065		008
	067		009
	070		011
	071		013
3M62772A810	001		016
	004		019
	010		
3M762772A811	001		
	002		
	004		
	007		
	010		

Support and collaboration were also provided by veterinary pathologists to several Clinical Investigation Service, Letterman Army Medical Center studies, the majority of which are still incomplete. The services included animal tissue evaluations in studies of synthetic prostheses for large veins, treatment of experimental spinal cord trauma, induced pregnancy toxemia, and osteomyelitis.

PUBLICATIONS

1. CHANDLER, J.S. and T.J. BUCCI. The ontogenesis of calcium-binding protein in fetal rat kidney. (submitted for publication)

2. FRIEDMAN, H.I., F. DeVENUTO, L. LOLLINI, P. MELLICK, and T. ZUCK. Morphological effects following massive exchange transfusions with a stroma free hemoglobin solution. I. Liver. Lab Invest 39:167-177, 1978

3. FRIEDMAN, H.I., F. DeVENUTO, L. LOLLINI, P. MELLICK, and T.F. ZUCK. Morphological effects following massive exchange transfusions with a stroma free hemoglobin solution. II. Kidney & brain. (submitted for publication)

4. KELLEY, S.T., T.J. BUCCI, and S. SILVERMAN. Physical growth and skeletal maturation in laboratory born owl monkeys (Aotus sp.). (submitted for publication)

5. MORRISSEY, R.L., D.T. ZOLOCK, T.J. BUCCI, and D.D. BIKLE. Immunoperoxidase localization of vitamin D dependent calcium-binding protein. J Histochem Cytochem (in press)

6. MORRISSEY, R.L., D.T. ZOLOCK, D.D. BIKLE, R.N. EMPSON, JR. and T.J. BUCCI. Intestinal response to a 25-dihydroxy cholecalciferol. I. RNA polymerase, alkaline, phosphatase, calcium, and phosphorous in vitro and in vivo calcium transport and accumulation. Biochim Biophys Acta 538:23-33, 1978

7. MORRISSEY, R.L., R.N. EMPSON, JR., D.T. ZOLOCK, D.D. BIKLE, and T.J. BUCCI. Intestinal response to a 25-dihydroxy cholecalciferol. II. Cellular localization of calcium-binding protein. Biochim Biophys Acta 538:34-41, 1978

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6115	2. DATE OF SUMMARY: 78 10 01		REPORT CONTROL SYMBOL: SD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: B.TERMINATION	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. GROUP MEMB'S: NL	8A. SPECIFIC DATA: CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT
10. NO./CODES: PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3M161102BS02		00		062	
b. [illegible]							
c. [illegible]	CARDS 114 f						

11. TITLE: (U) Response of the Respiratory System to Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002600 Biology; 012900 Physiology; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
76 10	78 09	DA			C. In-house

17. CONTRACT/GRANT: a. DATE/EFFECTIVE: — b. NUMBER: Not Applicable — c. TYPE: — d. AMOUNT: — e. KIND OF AWARD: — f. CUM. AMT.: — g. CONCURRENCE:

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS ($ thousands)
FISCAL YEAR: PREVIOUS 78	0.7	70
FISCAL YEAR: CURRENT 79	0.0	00

19a. RESPONSIBLE DOD ORGANIZATION
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19b. PERFORMING ORGANIZATION
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Pathology & Comparative Studies Div
ADDRESS: Department of Comparative Medicine
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PRINCIPAL INVESTIGATOR (Person responsible for performing work unit)
NAME: Mellick, P.W., LTC, VC
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SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME:
NAME:

20. GENERAL USE: Foreign Intelligence Not Applicable

POC:DA

21. KEYWORDS: (U) Respiratory Injury; (U) Cell Kinetics; (U) Wound Repair; (U) Electron Microscopy; (U) Airway; (U) Chemical Irritants; (U) Smokes

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

23. (U) Upper respiratory infection is the most common illness that affects military personnel. Soldiers are frequently exposed occupationally to airborne chemical agents that damage respiratory tissues. Knowledge of basic mechanisms by which respiratory epithelium is repaired following injury is inadequate and is required so that clinical management of personnel with acute respiratory injury can be improved. An animal model to study basic repair mechanisms in the respiratory tract will be developed.

24. (U) The initial experiment in this work will be done in rats. Animals will be exposed to sublethal concentrations of a corrosive gas to produce respiratory injury. The duration of cell cycle phases in exposed and control animals will be determined at three levels of the respiratory tract by autoradiographic techniques. Epithelial proliferation and differentiation will be studied by light microscopy, scanning and transmission electron microscopy, and electron microscopic autoradiography. Semiquantitative techniques to assess the regenerative capacity of respiratory epithelium will be developed and standardized. Smokes and gases noxious to troops can then be compared.

25. (U) 7710-7809 Lung tissues of all control and ozone-exposed rats that were sacrificed at intervals from 30 minutes through 48 hours after injection with tritiated thymidine have been prepared for light microscopic autoradiography. Preliminary examination of lungs from dogs, cats, pigs, rhesus monkeys, and owl monkeys suggests that the primate lung more closely resembles the human lung morphologically and would thus provide the most suitable animal model for future pulmonary studies. Progress was curtailed greatly because of requirements to provide electron microscopy support to other LAIR projects with higher priority. This work unit will be terminated because of reorganization of LAIR.

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

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	062	Response of the Respiratory System to Injury

The following investigations have been conducted under this work unit:

STUDY NO. 1	Epithelial regeneration in the upper respiratory tract following acute oxidant injury
Unnumbered Studies	(Reported under work units of other LAIR investigators)

Work performed under this work unit included transmission and scanning electron microscopy, special morphometric procedures, and autoradiography (both light microscopy and transmission electron microscopy). These techniques were applied to one study in the Department of Comparative Medicine addressing the regenerative response of the lung following acute oxidant injury and to 12 additional studies in collaboration with or in support of other principal investigators. The latter effort represented complete support in ultrastructural evaluation of tissue for LAIR investigators who required it. The specific work units and programs supported are tabulated in the text of this report and all resulting publications are listed.

STUDY NO. 1 Fifty-two sections of lung and fifty-two sections of trachea from twenty-six ozone-exposed and twenty-six control rats killed at intervals following tritiated thymidine injection were processed for light microscopic autoradiography. Much of this work had to be repeated because of technical difficulties associated with development of the photographic emulsion and subsequent staining of tissue sections. Examination of these preparations indicates that they are of sufficient quality and labeling intensity for analysis. Preliminary examination of autoradiographs of tracheas from control animals revealed only a few labeled cells, a situation which poses major difficulties in determination of component phases of the cell cycle. Normal lungs of three dogs, three cats, one pig, two rhesus monkeys, and one owl monkey were examined by light microscopy and scanning electron microscopy in an effort to determine which of these species had distal pulmonary airway structure (respiratory bronchioles) most closely resembling that of human lungs. Sections of distal airway from one animal of each of these species was examined by transmission electron microscopy. Of the distal airways examined, those of the rhesus monkey most closely resembled those of human lungs suggesting that this species might provide

the most useful model for studies designed to study the response of distal airways to inhaled materials.

BODY OF REPORT

WORK UNIT NO.	062	Response of the Respiratory System to Injury
STUDY NO.	1	Epithelial regeneration in the upper respiratory tract following acute oxidant injury

PROBLEM

Upper respiratory infection is the most common illness that effects military personnel both in training and in combat situations. Soldiers are frequently exposed occupationally to airborne chemical agents that damage respiratory tissues. Of the 91 chemical compounds that are components, by-products, or residues of pyrotechnic devices in use or under development by DOD agencies, 25 have been shown to be moderately or severely toxic to humans or animals when the fumes are inhaled. Explosive and incendiary munitions have similar chemical composition and, therefore, constitute an additional toxic hazard, especially to troops exposed in confined spaces such as bunkers, entrenchments, and armored vehicles. Epidemiological evidence and clinical experience indicate that chemically induced injury to respiratory tissues can predispose a person to upper respiratory infection and other non-infectious but equally debilitating pulmonary disease.

Knowledge of basic mechanisms by which upper respiratory epithelium heals is inadequate and is required so that treatment and clinical management of patients with upper respiratory injury can be improved. This study was initiated to develop a laboratory animal model for study of basic repair mechanisms in the respiratory tract.

RESULTS AND DISCUSSION OF RESULTS

As indicated in the Annual Research Progress Report for FY 77, upper respiratory epithelial injury was produced in rats by subjecting them to non-lethal concentration (0.8 ppm) of a highly corrosive gas (ozone) for 8 h. Exposed and control rats were given 500μ CI ^{3}H thymidine by intraperitoneal injection immediately following ozone exposure. They were killed at intervals following injection of the isotope. Trachea and lungs from each animal were fixed by airway perfusion with Karnovsky's fixative for subsequent examination by light microscopy (LM), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and LM and TEM autoradiography.

No lesions were detected in tissues of control animals. Examination of tissues from selected exposed rats by LM and SEM indicated that the degree of epithelial injury produced by ozone exposure should be

sufficient to stimulate epithelial regeneration in terminal bronchioles and alveolar ducts.

During the reporting period, cross sections of mid-cervical trachea and longitudinally bisected sections of terminal bronchioles from the 52 rats killed during the first 48 h after injection were coated with photographic emulsion for LM autoradiography. LM autoradiographic preparation of many tissue specimens had to be repeated because of technical difficulties encountered in the development of photographic emulsion and subsequent staining of tissue sections.

Examination of autoradiographs indicates that the quality of preparation and intensity of nuclear labeling is sufficient to identify post mitotic cells. However, the number of cells labeled, especially in sections of trachea from control animals, may not be sufficient to determine cell cycle times.

In human lungs the typical distal airway consists of well-developed respiratory bronchioles. These small airways are prime targets for damage by inhaled noxious agents. The pathogenesis of chronic obstructive pulmonary disease has been linked to damage of these airways. Rats lack well-developed respiratory bronchioles and are, therefore, not an ideal animal model for pulmonary studies designed to evaluate the effect of inhaled materials on distal airways. During this reporting period, normal lungs from 3 dogs, 2 cats, 1 pig, 2 rhesus monkeys, and 1 owl monkey became available for study. These animals were involved in other LAIR studies which had no effect on pulmonary structure. Lungs from these animals were examined grossly, histologically, and by scanning electron microscopy. Distal airways from one animal of each species were examined by transmission electron microscopy. Although there are many variations in ultrastructure of bronchiolar epithelium in the species examined, results indicate that the dog, cat, rhesus monkey, and owl monkey have respiratory bronchioles more closely resembling those in human lungs (although there are significant differences).

CONCLUSIONS

Exposure of rats to 0.8 ppm ozone for 8 h produces sufficient damage in terminal bronchioles to stimulate epithelial regeneration; it is doubtful that such exposure significantly increases the labeling index in the trachea. Increase in the duration of exposure or the concentration of ozone would probably increase the value of the model for study of epithelial repair in the trachea. The rhesus monkey has distal airway structure similar to that of man, and, therefore, would be a more suitable animal model for definitive pulmonary studies than would either the rat, pig, dog, cat, or owl monkey.

Progress on this investigation was sharply curtailed because of requirements to support other LAIR work units.

RECOMMENDATIONS

Although it has multiple potential application in military medicine, the electron microscopy facilities will be required for support of other projects after the reorganization of the Institute. Since there will be insufficient technical assistance and investigator input available to continue this investigation, this study is being terminated.

PUBLICATIONS

None

UNNUMBERED STUDIES

In addition to pulmonary studies, this work unit provided support in electron microscopy, morphometry, and autoradiography for other LAIR projects. Approximately $23,000 was spent from this work unit for services to investigators in other work units. The sum includes civilian technicians' pay and benefits, expendable supplies, and pro-rated overhead for civilian and military technicians. Officer (investigator) salaries and overhead, equipment and costs attributable to leave and administrative functions are not included in this figure. Support and collaboration by officers and technicians on studies requiring electron microscopy are indicated below. Results of those studies are reported elsewhere in the Annual Report.

LAIR Studies Provided With Support

in Electron Microscopy, Morphometry, or Autoradiography

Project No.	Work Unit
3A161101A91C	046
	042
3M161102BS02	056
	061
3M762772A810	001
3M762772A811	007
3M762772A812	001
3E762772A813	025
3S762772A814	004
	011
	016
	019

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | DA 0E6102 | 78 10 01 | DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY LEVEL	6. DOD SECURITY	7. GRADING	8. CONTR NUMBER	9b. SPECIFIC DATA CONTRACTOR ACCESS	10. LEVEL OF SUM
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

11. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102B50I	00	203
b. [redacted]	61102A	3M161102B50Z	00	065
c. [redacted]	CARDS 1141			

12. TITLE (Security Classification Code)

(U) Military Stress and Combat Effectiveness

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

013400 Psychology; 005900 Environmental Biology; 016200 Stress Physiology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
75 08	Cont	DA	C. In-House

18. CONTRACT/GRANT

a. DATE EFFECTIVE: Not Applicable — EXPIRATION:
b. NUMBER:
c. TYPE: — d. AMOUNT:
e. KIND OF AWARD: — f. CUM. AMT.

19. RESOURCES ESTIMATE

FISCAL YEAR	a. PROGRAMS	b. PROFESSIONAL MAN YRS	c. FUNDS ($ Thousands)
	78	2.5	132
	79	2.5	92

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
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21. GENERAL USE

Foreign Intelligence Not Applicable

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATOR(S)
NAME: Stamper, D. A. DAC
NAME: POC:DA

22. KEYWORDS (Provide at least one Security Classification Code)

(U) Military Performance; (U) Human Performance; (U) Visual Tracking; (U) Psychological Stress

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide brief but precise statements identified to number correspond with those in block 23. Security classification codes must be used for each security classification item.)

23. (U) The severe stress encountered in warfare may influence the soldier's ability to perform combat essential activities with maximum efficiency. The objectives of this research are to study 1) weapons effects on the performance of military tasks, 2) weapons systems environments and combat effectiveness, and 3) biomedical factors limiting soldier effectiveness. Research is conducted under field conditions and in the laboratory.
24. (U) Animals or human subjects are subjected to conditions which produce stress of varying intensity and durations. The effects of stressors are confirmed biochemically and through observation of physiological and psychological indices. Experimental stress is then related to the ability of subjects to perform various tasks. For human subjects, target acquisition and tracking, communications, endurance, and vigilance tasks are employed. Operant techniques are used with animalsubjects.
25. (U) (7710-7809). A field simulation laboratory has been constructed which will facilitate the controlled investigation of biomedical factors which may influence laser designator operations through their effects on the individual. The laboratory includes a sand bag bunker housing a laser designator, a terrain model, and scale model remote contr targets. Automatic data acquisition and computer control of experimental conditions are employed. Baseline designator operator performance data are being collected. These data will be used to validate the simulation against comparable data collected under field conditions.

* Available in summary upon subsequent approval

DD FORM 1498 — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498 - NOV 71 AND 1498-1 1 MAR 69 FOR ARMY USE ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3E161102BS02 — Basic Mechanism of Recovery from Injury

WORK UNIT NO. 065 — Military Stress and Combat Effectiveness

The following investigation has been conducted under this work unit:

STUDY NO. 6 Biomedical factors affecting laser designator operator performances.

The increasing use of laser devices in training, as well as their proposed use in combat operations, has led to the study of biomedical factors related to laser operations. A laboratory has been established which will provide essential information concerning the effects on laser designator operators of psychological stress environmental factors, and anti-personnel countermeasures. Preliminary data are available from volunteers who used a laser designator to track scale model remote controlled targets under simulated field conditions. These data will be used to validate the simulation through comparison of the laboratory data with published data obtained using laser designators under field conditions.

BODY OF REPORT

WORK UNIT NO. 065 — Military Stress and Combat Effectiveness

STUDY NO. 6 — Biomedical factors affecting laser designator operator performance

PROBLEM

Laser rangefinder-designator devices and laser weapon systems will play an important role in future military operations. The increasing use of laser devices in training, as well as their proposed use in combat operations, has led to the study of biomedical factors related to laser operations. As one part of this effort, a laboratory has been established in which those biological and behavioral variables which could influence laser rangefinder-designator operator performance will be studied. This laboratory will provide essential information concerning the effects on laser designator operators of psychological stress, environmental factors and anti-personnel countermeasures.

RESULTS AND DISCUSSION OF THE RESULTS

The field simulation which has been developed consists of a sand-bag bunker, terrain model, a laser rangefinder-designator, remotely controlled targets, and a control room. The bunker houses the laser designator. It is also used to control the soldier's working environment and to provide a degree of isolation from distractions occurring elsewhere in the laboratory. The terrain model represents a desert environment, but may be readily modified to simulate other operational environments. The laser rangefinder designator was designed to incorporate features of similar devices which are being considered for field deployment. Thus, the operator performance data obtained with the use of this device will be applicable to existing and proposed laser designator systems. The target vehicles are capable of variable speed remote controlled movement anywhere on the terrain model. Each vehicle is equipped with an electronic locating device which is used to monitor target movements during the execution of manual or computer controlled target maneuvers. The target vehicles are also equipped with laser sensors which transmit coded radio frequency signals whenever laser irradiation from the designator contacts critical target zones on the vehicle. These signals are processed to provide one source of information concerning the aiming and tracking abilities of the designator operators.

Preliminary data are available from volunteers who used the laser designator to track targets during simple left-to-right and right-to-left target maneuvers. These data will be used to validate the simulation through comparison of the laboratory data with published

data obtained when laser designators are used under field conditions. Statistical analyses of the preliminary data are in progress.

CONCLUSIONS

None

RECOMMENDATIONS

Data reduction and analyses should be completed.

PUBLICATIONS

None

OTHER PUBLICATIONS PREPARED BY GROUP

1. STAMPER, D. A. Physiological, Psychological, and Symptomatic Factors Affecting Prolonged Physical Performance. Report No. 56. San Francisco, California: Letterman Army Institute of Research, July 1978

2. LEIBRECHT, B. C., A. LLOYD, and P. A. O'MARA. Field Study of Stress: Psychophysiological Measures during Project SUPER. LAIR Report No. 59. San Francisco, California: Letterman Army Institute of Research, July 1978

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

				DA OB 6913	78 10 01			
77 10 01	D. Change	U	U	NA	NL	YES		
	61102A	3M161102BS02		00	066			
	CARDS 1147							

(U) Physical, Chemical Characteristics of Human Stratum Corneum

003500 - Clinical Medicine

67 07	CONT	DA		C. In-House

Not Applicable

FISCAL YEAR 78	78	2.6	48
FISCAL YEAR 79	79	1.6	15

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Foreign Intelligence Not Applicable

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Eisenberg, George, H.G., Jr., MAJ,MSC
POC: DA

(U) Stratum Corneum; (U) Absorption; (U) Permeability; (U) Water; (U) Water Vapor; (U) Chemicals; (U) Persistence; (U) Skin; (U)Human Volunteer

23. (U) The objective is to define the physical-chemical characteristics of the stratum corneum and its interaction with water, chemicals, ultraviolet radiation, and environment. These characteristics are fundamental to the etiology of several epidemic dermatological disorders caused by exposure of the soldier's skin to the environment and are also applicable to the behavior of topical preparations in human skin.

24. (U) Measurements of skin permeability and evaporation rates of insect repellents in vitro and in animals continue to provide guidelines for formulating compounds and vehicles to extend the duration on the skin and thereby increasing protection. These measurements also provide toxicological data for estimation of chemical exposure following topical application in man.

25. (U) 77 10 - 78 09. The percutaneous penetration of three radiolabeled insect repellents was determined in hairless dogs. Following both intraveneous and topical administration, urine, feces and blood samples were assayed for radioactivity by scintillation counting. The evaporation/penetration characteristics of three radiolabeled insect repellents were determined in vitro permeability chambers; methodology developed can be used to determine repellent evaporation in man for non-radiolabeled repellents.

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

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	066	Physical, Chemical Characteristics of Human Stratum Corneum

The following investigations have been conducted under this work unit:

STUDY NO. 1 Percutaneous absorption of C-14 labeled insect repellents in hairless dogs

STUDY NO. 2 Penetration/evaporation of C-14 labeled insect repellents in vitro

STUDY NO. 3 Chemical assay of insect repellents

STUDY NO. 1 The percutaneous penetration of three insect repellents, m-deet, p-deet, and ethylhexanediol were determined in the hairless dog. The results compared favorably to available human data.

STUDY NO. 2 The in vitro evaporation-penetration characteristics of m-deet, p-deet, and ethylhexanediol have been studied on excised skin. The technique developed can be extended to measurement of evaporation rates with non-labeled repellents.

STUDY No. 3 Chemical assays have been developed and determinations have been made of physical characteristics of 3 insect repellents (m-deet, p-deet, and ethylhexanediol). These procedures provide necessary support for forthcoming formulation studies.

BODY OF REPORT

WORK UNIT NO. 066 — Physical, Chemical Characteristics of Human Stratum Corneum

STUDY NO. 1 — Percutaneous absorption of C-14 labeled insect repellents in hairless dogs

PROBLEMS

The hairless dog is a promising animal model for screening insect repellents. The dog compares favorably to man in respect to the duration of time that repellents remain effective. For the most commonly used insect repellents, N,N-diethyl-m-toluamide (m-deet), N,N-diethyl-p-toluamide (p-deet), and 2-ethyl-1,3-hexanediol, the duration of protection time in man versus dog is 5.7 vs 7.2 h, 4.8 vs 5.2 h and 3.9 vs 3.3 h, respectively, when all were tested at a dose of 0.22 mg/cm^2 (Study No. 2, Work Unit 003). It was therefore important to determine the permeability characteristics of the skin on the dog's back (where insect repellents are tested) and to compare this to available human data.

The study was divided into two phases for each compound: intravenous administration of approximately 2 µCi to 3 dogs and then topical application of approximately 5 µCi to 3 dogs. The intravenous phase permitted correction of incompleteness of excretion during the topical phase. During both phases, the hairless dogs were housed individually in metabolic cages for urine and feces collection.

RESULTS AND DISCUSSION OF RESULTS

Urinary excretion was the major route of elimination of the Carbon-14 labeled insect repellents m-deet, p-deet, and 2-ethyl-1,3-hexanediol in the hairless dog after intravenous and topical application. Insignificant amounts were found in the feces from both administration routes. After intravenous administration, average urine recoveries were 90.6% for m-deet, 87.5% for p-deet, and 86.1% of the applied dose for 2-ethyl-1,3-hexanediol; the bulk of the radioactivity was recovered within the first 24 h. Blood level radioactivity fell to near background after 4 to 8 h with all of the compounds after intravenous administration.

After topical administration, average urinary recoveries (corrected for incompleteness of excretion as determined by the intravenous administration) were 7.9% for m-deet, 7.6% for p-deet, and 10.3 % for 2-ethyl-1,3-hexanediol. Excretion was near complete after 4 to 5 days.

Radioactivity levels in the blood samples after topical administration were too low to be determined accurately.

Substantial amounts of radioactivity were recovered from the peripheral protective patches used in the topical experiments with average recoveries of 75.3% for m-deet, 73.6% for p-deet, and 52.8% for 2-ethyl-1,3-hexanediol. Since the patches did not physically touch the application areas, these levels probably arose from evaporation of repellent from the skin surface, and from naturally shed stratum corneum that was trapped in the patch. Total recoveries for the topical phase were 83.7% for m-deet, 82.0% for p-deet and 63.0% for 2-ethyl-1,3-hexanediol. Only small amounts (approximately 1% or less of the applied dose) of radioactivity were recovered from the skin surface at 48 and 96 h after application. This was expected, since the compounds studied provide only 3 to 7 h of protection against mosquitoes at the doses used (0.32 mg/cm^2) (Study No. 2, Work Unit 003).

In our study, recovery of radioactivity in the dog urine after intravenous administration was considerably higher than has been reported by others in man (90.6% vs 52.3%), although the rate of urinary excretion in the dog following intravenous administration was comparable to that in man. Although differences in technique make it difficult to compare the results obtained from the dogs with those in man with regard to percutaneous penetration of m-deet, we conclude that the percutaneous penetration of m-deet does not differ markedly from that found in man. No reports of the percutaneous penetration of p-deet or 2-ethyl-1,3-hexanediol in man have appeared, although it can be assumed that the percutaneous penetration of p-deet would resemble its isomer, m-deet, as found in the dog.

CONCLUSIONS

The permeability characteristics of the hairless dog's skin do not differ markedly from human skin. More carefully controlled studies will need to be done to establish the value of the hairless dog as an animal model for other percutaneous penetration studies.

RECOMMENDATIONS

The lack of marked difference in permeability of mosquito repellents in man and dog suggests the use of the hairless dog as an animal model for repellent duration studies. From a toxicological viewpoint, the effect of changing dose/unit area versus percent penetration should be investigated. Chemical dose/area magnitudes lower than actual use dosage may lead to misleadingly high percent penetration.

PUBLICATIONS

1. PEIFENRATH, W.G., J.A. HILL, P.B. ROBINSON, D.L. MCVEY, W.A. AKERS, M. ANGO and H.I. MAIBACH. Percutaneous absorption of C-14 labeled insect repellents in hairless dogs (Submitted for publication)

STUDY NO. 2 — Penetration/evaporation of C-14 labeled insect repellents in vitro

PROBLEM

The development of improved mosquito repellent foundations requires that excessive evaporation from the surface of the skin and penetration into the skin be minimized. Development of in vitro techniques to measure these modes of loss is required. From a toxicological standpoint, estimates of relative in vivo penetration of insect repellents could then be made from the in vitro data.

RESULTS AND DISCUSSION OF RESULTS

The in vitro evaporation-penetration characteristics of three labeled insect repellents, m-deet, p-deet and ethylhexanediol on excised skin were studied. The average percent of applied dose to evaporate (corrected for efficiency of recovery was 17.5 $\pm$ 3.5 for m-deet, 11.4 $\pm$ 4.9 for p-deet, and 45.6 $\pm$ 5.3 for ethylhexanediol). The average percent of applied dose found to penetrate (as determined by the amount of radioactivity entering the lower Ringer's lactate chamber during a 12 h period) was 3.4 $\pm$ 3.2 for m-deet, 11.8 $\pm$ 9.4 for p-deet, and 15.4 $\pm$ 5.5 for ethylhexanediol. Total recoveries in these experiments were 68 $\pm$ 8 for m-deet, 83 $\pm$ 10 for p-deet, and 85 $\pm$ 4% for ethylhexanediol.

As expected, the more volatile ethylhexanediol has a higher percent evaporation than either of the two deet isomers. The evaporation levels ($\mu g/cm^2/h$), versus time for the repellents were 6.5 (1 h), 6.8 (3 h), 3.8 (5 h) and 3.4 (10 h) for m-deet; 7.1 (1 h), 5.7 (3 h), 2.9 (5 h), and 2.7 (10 h) for p-deet; and 30.2 (1 h), 18.9 (3 h), 10.1 (5 h), and 5.0 (10 h) for ethylhexznediol. Since the average duration of protection in volunteers for m-deet is 5.0 h, for p-deet is 4.8 h, and for ethylhexanediol is 3.3 h, it is expected that the minimum effective evaporation levels for the deet isomers would be near the 5th hour evaporation levels, while that for ethylhexanediol would be near the 3rd hour evaporation level. Measurements of the evaporation level at the minimum effective dose (as measured in volunteers with non-labeled repellent) for these repellents are in progress.

CONCLUSIONS

The in vitro measurements of repellents on excised skin provide estimates for two important modes of loss of repellents from the surface of the skin, evaporation, and penetration. The variability in the data reflects that which exists for human skin, as different skin samples were used for replication. The evaporation levels can be used to estimate the minimum effective evaporation rate (MEER) necessary to repel mosquitoes. Formulations of these mosquito repellents designed to reduce loss by excessive evaporation and penetration will have to maintain evaporation rates above the MEER.

RECOMMENDATIONS

These in vitro studies should be extended to two other available labeled repellents, carbamide and sulfonamide. The techniques developed in this study for vapor collection above skin can and should be used for similar measurements with non-labeled repellents in volunteers and for screening repellent formulations in vitro.

PUBLICATIONS

1. **MUELLER, H.M., T.S. SPENCER, and W.A. AKERS. Effect of harvest methods on hydration properties of stratum corneum. (Submitted for publication)**

STUDY NO. 3 Chemical assay of insect repellents

PROBLEM

Qualitative assay of mosquito repellent chemicals for purity and reproducibility from sample to sample is required. Quantitative assay is necessary for assessment of lifetime of repellents on the skin. Various physical chemical measurements of repellent properties are needed for formulation work.

RESULTS AND DISCUSSION OF RESULTS

Gas chromatography is a method employed to determine the purity of insect repellent chemicals, and to assay them quantitatively by the internal standard method. Gas chromatographic conditions have been determined for the quantitative determination of a variety of insect repellents in dichloromethane or carbon disulfide solution with diethylphthalate as an internal standard. Solid state adsorption techniques for sampling repellent above the skin have been developed. In collaboration with Patrick Jones, Ph.D., Department of Chemistry, University of the Pacific, work is in

progress for gas chromatography/mass spectrometer analysis of trapped repellent vapor. Thin layer chromatographic conditions for qualitative assay (stability studies) of various repellents in ethanol solution are ongoing. Procedures for measurement of viscosity (Thomas Stormer) surface tension (hanging drop method) and density have been developed and the measurement for a variety of established and candidate repellents are in progress. These data are necessary for formulation work. Data from physical measurements are input into computer data management systems (Study No. 1, Work Unit 003)

CONCLUSIONS

A variety of physical-chemical measurement techniques have been developed for qualitative and quantitative assay of mosquito repellents.

RECOMMENDATIONS

The procedures outlined in this part of the work unit provide necessary support for formulation studies, and measurement of repellent lifetime on the skin, and should continue

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA OC 6783	78 10 01	
77 10 01	D. Change	U	U	NA	NL	
	61102A	3M161102B502		00	067	
	CARDS 1147					

(U) Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections

002300 - Biochemistry; 010100 - Microbiology; 003500 - Clinical Medicine

73 09	CONT	DA		C. In-House

	78	3	99
Not Applicable	79	3	21

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Jaeger, June, DAC
Eisenberg, G.H.G., Jr., MAJ, MSC POC: DA

(U) Skin; (U) Pathogenicity; (U) Iron; (U) Exocellular products

23. (U) Under unfavorable conditions, epidermis of fungal infections can incapacitate 38 percent of combat soldiers within 16 days of first exposure, with the average period of incapacitation 7.3 days. The biochemical pathways fungi use to attack skin will be investigated. Mechanisms of iron acquisition by dermatophyte will be investigated. Mechanisms of cell wall synthesis and production of toxic or irritant products by dermatophytes will be investigated.

24. (U) The approach will be to 1) determine the iron requirements, the mechanisms of iron acquisition, the relation of iron acquisition capacity to pathogenicity of the dermatophytes, 2) determine conditions under which dermatophytes produce toxic or irritant products, 3) determine if the toxic or irritant products are significant factors in the pathogenesis of dermatophytosis.

25. (U) 77 10 - 78 09. A purified antigen has been prepared from *Trichophyton mentagrophytes*. The antigen has been evaluated for reactivity and has been designated as standard antigen for future use in our department. Iron requirements of 5 strains of dermatophytes have been determined. Production of exocellular iron chelators in response to low iron environment has been measured. A medium has been developed which promotes arthrospore formation in all dermatophytes tested to date. Exocellular products isolated from fungi grown on this medium have been observed to elicit highly inflammatory cutaneous reactions in guinea pigs.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498-1 WHICH IS FOR ARMY USE ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M161102BS02 Other Tropical Medicine

WORK UNIT NO. 067 Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections

The following investigations have been conducted under this work unit:

STUDY NO. 1 Preparation of purified skin test antigens of the dermatophytes

STUDY NO. 2 Morphological and chemostructural characterization of ontogenetic development of Trichophyton mentagrophytes.

STUDY NO. 3 Determination of role of iron acquisition on growth of dermatophytes.

The studies are designed to determine mechanisms of pathogenesis of dermatophytes.

STUDY NO. 1 The first study concentrates on development of antigens useful in studies of immune response to dermatophyte infection. A large quantity of antigens has been produced for use as standard antigen and a defined medium has been formulated which provides adequate yield of antigen.

STUDY NO. 2 The study seeks to determine the relationship of morphologically distinct fungal forms to the events of fungal infections. Nutrient requirements and environmental conditions required for arthrospore production are being determined. A fluorescent staining procedure has been developed. The procedure is capable of staining surface carbohydrates of fungi and is based upon specificity of lectins for individual carbohydrates.

STUDY NO. 3 The third study is designed to investigate the role of iron acquisition of dermatophytes in establishment of infection. Iron requirements for five strains of dermatophytes were determined. In addition, the five strains were evaluated for their capacity to obtain iron by measuring their production of iron chelating compounds and their sensitivity to normal human serum.

BODY OF REPORT

WORK UNIT NO. 067 — Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections

STUDY NO. 1 — Preparation of purified skin test antigens of the dermatophytes

PROBLEM

Dermatophyte infections are prominent producers of medically debilitating skin lesions in soldiers. The predominant etiologic agents for such infections in U.S. Army personnel in the Republic of Vietnam has been identified as Trichophyton rentagrophytes var. granulare. Although there is a voluminous amount of literature written about these fungal infections, a paucity of information remains regarding the specific mechanisms of pathogenesis. Research from this study has been centered upon preparation and characterization of trichophytin antigens. Preparation of adequate quantities of antigen will allow further investigation into the mechanism of pathogenesis due to the host immune response.

RESULTS AND DISCUSSION OF RESULTS

We have developed a defined medium which supports growth of dermatophytes and which provides adequate yields of trichophytin antigen. The medium was required to avoid a problem of sensitizations with media used previously. The new medium is based upon typical lot analysis of a cottonseed flower product used as medium for production of antibiotics. Since the medium is completely synthetic, it precludes sensitization of individuals to meat proteins found in other media, and also lends itself for use in other projects. Antigens prepared in this medium appear to be immunologically indistinguishable from those produced in complex ox liver medium when tested against rabbit antisera specific for purified trichophytin.

CONCLUSIONS

Trichophytin prepared in large quantities in a highly purified state is presently available in the Department of Dermatology Research, however, we have found no advantage of the purified antigen when compared to commercially available trichophytin. The cost of preparation is high in terms of time, personnel, and supplies. The trichophytin antigen does not contribute to immunity in dermatophytosis, rather its contribution appears to be that of a major sensitizing antigen which is responsible for hypersensitivity and probably many of the observed pathologic effects.

RECOMMENDATIONS

Prophylaxis or therapy of dermatophytosis based upon immunization or immune system stimulation appears to hold little promise. Efforts should be focused upon determining specific mechanisms of pathogenesis with the goal of interfering with one of these processes.

PUBLICATIONS

None

STUDY NO. 2 — Morphological and chemostructural characterization of the events of ontogenetic development of Trichophyton mentagrophytes

PROBLEM

Determining how saprophytic septate branching mycelium changes to the pathogenic hypersegmented mycelium or arthrospore form will lead to the identification of the conditions causing this alteration and its accompanying enzyme systems. As they become known, this will permit altering the physical conditions of the host or blocking some of the enzyme systems by chemotherapy which may be prophylactic or therapeutic measures against cutaneous fungal infections which molest our combat troops.

RESULTS AND DISCUSSIONS OF RESULTS

An artificial medium has been developed which apparently provides the required growth conditions for formulations of arthrospores in a wide variety of dermatophytes. Trichophyton mentagrophytes (2 strains), Trichophyton rubrum (3 strains), Trichophyton verrucosum (one strain), Trichophyton schonleinii (one strain), Trichophyton tonsorans (one strain), Microsporum canis (one strain), Microsporum gypseum (one strain), and one strain of the dematiscieous fungus Phialophora verrucosa. All produce abundant arthrospores on this medium. Arthrosporulation on this medium occurs at 28, 32, or 37 C in either liquid medium or on solid medium and is independent of CO_2 concentration. T. mentagrophytes produces a keratinase as measured with keratin azure. Isolated arthrospores are found to germinate spontaneously when stored in sterile water at room temperature overnight.

A fluorescent staining method has recently been developed which selectively stains polymers of N-acetyl-glucosamine (chitin, chitosan). The method stains only polymers exposed or near the surface of fungal elements. Some progress has been made in delineating the events of cell wall production of the dermatophytes with this stain. The method is capable of detecting dermatophytes in frozen sections of skin.

CONCLUSIONS

A method which provides morphologic forms similar to those observed in infected skin has been developed. This method coupled with the fluorescent stain method may lead to identification and production of units of dermatophytes which maintain pathogenic determinants while growing on artificial media. As a result, well defined studies to determine those factors which promote dermatophyte growth in skin may be delineated.

RECOMMENDATIONS

A major step required at this time is to equate the morphologic forms observed on the special medium described with those forms observed in infected skin and in the chorioallantoic membrane model described in FY 77 annual report. Determination of the structure of the dermatophyte cell wall and comparison of that structure to the structure observed in vitro may represent a valid method to equate the two morphologic forms. The fluorescent staining method, when combined with selective chemical/enzymatic digestion, should provide those comparisons.

PUBLICATIONS

None

STUDY NO. 3 — Role of microbial iron acquisition in dermatophyte infections

PROBLEM

Dermatophytes have long been known to be sensitive to iron deprivation by normal human serum. The competition between a host and an infectious microorganism for available iron stores may well provide a dividing factor in the eventual outcome of the interaction. Since microbial iron acquisition proceeds by a different mechanism than mammalian iron acquisition, it is believed that the system is one which is suitable for investigations leading to interference with iron uptake resulting in growth inhibition of the dermatophytes.

RESULTS AND DISCUSSION OF RESULTS

The study has been approached by (1) measuring the susceptibility of various strains of Trichophyton mentagrophytes to iron deprivation by normal human serum, and (2) the response of these strains to iron deprivation in synthetic media. Measurements of growth inhibition by normal human serum have shown a correlation between serum sensitivity and virulence. The less virulent strains (interdigitale types) are

more sensitive than granular types. When subjected to varying concentrations of iron in synthetic media, all strains were limited in growth by less than 0.1 mg iron/ml medium. In addition, all strains produced exocellular iron chelators in response to the low iron conditions.

CONCLUSIONS

Trichophyton rentagrophytes have a limited capacity to acquire iron, as evidenced by the ability of normal human serum to restrict its growth. However, under limiting iron concentrations, the organism can respond with high levels of exocellular iron chelators, capable of acquiring iron from a low iron environment. The ability of the dermatophytes to acquire iron may be a significant pathogenic factor.

RECOMMENDATIONS

The studies should continue by investigating directly the role of iron on infection in animals. We must determine if exocellular iron chelators are produced in vivo and if restriction of iron in the skin can significantly decrease the time course of dermatophyte infection.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

			DA 0E 6301	78 10 01		DD-DRAE(AR)636		
77 10 01	Termination	U	U	NA	NL	YES	NO	

	611102A	3M161102B52	00	070
	CARDS 1147			

(U) Influence of Nutrients, Hormones and Related Substances on the Recovery from Injury

002300 Biochemistry; 012900 Physiology; 002600 Biology

76 10	78 09	DA	C. In-House

Not Applicable

FISCAL YEAR	78	2.0	148
	79	0.0	00

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POC: DA

(U) Fracture of bone; (U) Stress fracture; (U) March fracture; (U) Non-union of bone; (U) Mal-union of bone; (U) Vitamin D metabolism; (U) Small intestine.

23.(U) Military personnel in training and combat incur a high incidence of march (stress) and traumatic fractures which cause much morbidity and disability. March fractures often heal poorly. About 30% of patients never return to duty. A large percentage of traumatic fractures heal more slowly than normal or not at all. We will determine if the metabolism of vitamin D is abnormal in these patients and responsible for the poor healing of bone.

24.(U) The metabolism of vitamin D will be studied in patients of military age with march fractures and in animals. Small intestinal mucosa obtained via peroral biopsy in the patients will be incubated in vitro and the uptake of calcium and phosphate, the activity of alkaline phosphatase, and ribonucleic acid (RNA) polymerase, and the concentration of calcium binding protein will be determined. These parameters will be measured while the patients are on low and high calcium diets. The status of bone will be determined by x-rays, osteodensitometry, bone biopsy, and ultra-sound measurements. The levels of 25-hydroxy vitamin D_3, 1,25-dihydroxy vitamin D_3, and parathormone will be determined. Parallel studies will be done in rachitic chicks.

25.(U) 77 09 - 78 09. Calcium uptake in chick mitochondria was no different in rachitic and non-rachitic animals. Calcium uptake in chick mitochondria was enhanced by 1,25-dihydroxy vitamin D_3 added in vitro. Chicks treated with vitamin D_3 accumulated 1,24,25-trihydroxyvitamin D_3 in the gut mucosa rather than 1,25-dihydroxyvitamin D_3. The trihydroxy compound was decreased when the animals were fed a high Ca and P diet. In chick renal mitochondria the enzyme 24-hydroxylase was stimulated by Ca, K, P, and bicarbonate. In patients with hyperparathyroidism, calcium enhanced the effect of parathormone on the renal production of cyclic AMP and the excretion of phosphate. These same patients fail to decrease their intestinal absorption of calcium when placed on a high calcium diet. This work unit is being terminated.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. PAGE NO. 83

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	070	Influence of Nutrients, Hormones and Related Substances on the Recovery

The following investigations have been conducted under this work unit:

STUDY NO. 1 The effect of 1,25-dihydroxyvitamin D_3 and 1α-hydroxyvitamin D_3 on calcium activated ATPase, calcium binding protein, and calcium and phosphate uptake in the chick intestine

STUDY NO. 9 The role of vitamin D in the etiology and treatment of fractures

STUDY NO. 1 In the course of exploring the mechanism by which 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) mediates calcium transport across the intestine, we more fully explored mitochondrial calcium uptake. We developed an efficient means of measuring this process in vitro and characterized it biochemically. We observed that little difference could be observed between mitochondria from vitamin D-deficient and vitamin D-replete animals. However, $1,25(OH)_2D_3$ added in vitro in physiologic concentrations enhanced calcium uptake.

The influence of dietary concentrations of calcium (Ca) and phosphate (Pi) on vitamin D metabolism and actions in chicks maintained on a vitamin D replete diet was evaluated. We found that $1,24,25(OH)_3D_3$, not $1,25(OH)_2D_3$, was the principal metabolite in the gut under these conditions. This trihydroxy vitamin D metabolite, like $1,25(OH)_2D_3$, was regulated both by dietary Ca and Pi. Like calcium binding protein (CaBP) concentrations and Ca transport, these metabolites were reduced in chicks eating diets with high Ca and Pi.

The above observations suggested exploration of the ionic control of the 24-hydroxylase, the renal mitochondrial enzyme which produces $24,25(OH)_2D_3$ from $25OHD_3$ and, presumably, $1,24,25(OH)_3D_3$ from $1,25(OH)D_3$. Similar to their effects on the $25OHD_3$ 1α-hydroxylase, the permeant cations calcium and potassium acetate and the permeant anions phosphate and bicarbonate stimulated the 24-hydroxylase. It seems apparent that both 1-hydroxylation and 24-hydroxylation of vitamin D play a role in regulation of vitamin D action in the body.

STUDY NO. 9 Clinical studies in patients with a variety of bone diseases have been performed since receiving approval of the protocol in March 1978. Failure to adapt properly to changes in dietary

calcium has been demonstrated in some but not all patients with hyperparathyroidism. Calcium has been found to enhance the effect of parathormone on renal production of cyclic AMP and excretion of phosphate. Development of the assays for the vitamin D metabolites required for this protocol is nearing completion, but routine application of these assays suffers from lack of equipment and personnel.

BODY OF REPORT

WORK UNIT NO. 070 — Influence of Nutrients, Hormones and Related Substances on the Recovery from Injury

STUDY NO. 1 — The effect of 1,25-dihydroxyvitamin D_3 and 1α-hydroxyvitamin D_3 on calcium and phosphate uptake in the chick intestine

PROBLEM

Stress fractures occur with high frequency (up to 10%) in troops in training. We have evaluated in animals the impact that manipulations of the essential components of bone, namely calcium and phosphate, and one of the principal hormones controlling bone mineral homeostasis, namely vitamin D, have on bone mineral transport across the intestine. It is expected that such investigations will facilitate understanding, diagnosis, and treatment of stress fractures.

RESULTS AND DISCUSSION OF RESULTS

Because of our recent observations that 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) the biologically active metabolite of vitamin D_3, stimulated calcium transport across the intestine without the need for de novo protein synthesis, we were encouraged to examine the effects of $1,25(OH)_2D_3$ directly on intestinal mitochondria, the subcellular organelle most likely to be involved in calcium (Ca) movement through the intestinal epithelial cell. Although no substantial differences in mitochondria from rachitic as opposed to vitamin D-replete animals could be demonstrated, we were able to stimulate calcium uptake by mitochondria with the addition of as little as 25 pmoles/ml of $1,25(OH)_2D_3$ in vitro. Vitamin D also stimulated calcium uptake, but at higher concentrations. Mitochondrial calcium uptake was inhibited by ruthenium red, required energy provided by ATP and/or oxidizable substrate, and was detected only with a concentration greater than 3×10^{-6} M. These results indicate a direct effect of $1,25(OH)_2D_3$ on one means by which the intestine moves calcium across its epithelium, and provide further evidence that $1,25(OH)_2D_3$ need not exert its effect of calcium transport by way of protein synthesis.

To characterize the means by which dietary calcium (Ca) and phosphate (Pi) regulate vitamin D action, we designed a study in which chicks were fed one of 9 diets varying in Ca and Pi but supplemented with 20 μg vitamin D/kg of diet. We found that the metabolite found in highest concentrations in the duodenal mucosa of these animals was not $1,25(OH)_2D_3$ but $1,24,25(OH)_3D_3$, a metabolite with substantial but lower

biological activity than $1,25(OH)_2D_3$. Like $1,25(OH)_2D_3$, which was also found in the intestine in substantial amounts, this trihydroxy vitamin D metabolite was regulated over a 1000-fold range by the dietary content of Ca and Pi. The levels of calcium binding protein and calcium transport correlated with the intestinal concentrations of these metabolites. An optimal diet was noted which permitted maximum growth increase and bone ash. Clearly, dietary concentrations of Ca and Pi profoundly affect the general well-being of the organism as well as the hormonal milieu which regulates bone mineral metabolism.

Since the production of $1,24,25(OH)_3D$ requires hydroxylation of $25OHD_3$ (the metabolite of vitamin D formed in the liver) by two kidney mitochondrial enzymes, the 1α-hydroxylase and 24-hydroxylase, and since control of vitmain D metabolism and its subsequent action is centered on regulation of these two hydroxylases, the ionic control of the 24-hydroxylase was evaluated. As for the control of the 1α-hydroxylase, the permeant cations calcium and potassium, and the permeant anions, acetate, phosphate, and bicarbonate, exert profound effects on the renal mitochondrial 24-hydroxylase. These studies indicate that, at least, the acute controls of 1- and 24-hydroxylation of vitamin D metabolites share remarkable similarities.

CONCLUSIONS

1. $1,25(OH)_2D_3$ directly modulates the ability of intestinal mitochondria to transport calcium.

2. $1,24,25(OH)_3D_3$ and not $1,25(OH)_2D_3$ may be the principal active metabolite of vitamin D_3 in the gut of animals raised on normal amounts of vitamin D.

3. Dietary calcium and phosphate control the amount of $1,24,25(OH)_3D_3$ as well as $1,25(OH)_2D_3$ in the gut in addition to controlling CaBP production and calcium transport.

4. Calcium and phosphate acutely control 1α- and 24-hydroxylase, the renal mitochondrial enzymes which produce $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, and $1,24,25(OH)_3D_3$.

RECOMMENDATIONS

The biologic actions of $1,24,25(OH)_3D_3$ as well as $1,25(OH)_2D_3$ should be explored with continued emphasis on determining their molecular mechanisms of action and control of these mechanism by environmental and physiologic factors. Due to lack of funding the project is being terminated.

PUBLICATIONS

1. MORRISSEY, R. L., R. N. EMPSON, JR., D. T. ZOLOCK, D. D. BIKLE, and T. J. BUCCI. Intestinal response to 1α, 25-dihydroxycholecalciferol. II. Optimal study of the intracellular localization of calcium binding proteins. Biochim Biophys Acta 538: 34-41, 1978

2. MORRISSEY, R. L., D. T. ZOLOCK, T. J. BUCCI, and D. D. BIKLE. Immunoperoxidase localization of vitamin D dependent calcium binding protein. J Histochem Cytochem (in press)

3. BIKLE D. D., L. HAGLER, L. O. LOLLINI, S. F. HULL, and R. H. HERMAN. Work induced muscle hypertrophy in vitamin D deficient rats. Am J Clin Nutr (in press)

4. BIKLE, D. D., R. L. MORRISSEY, D. T. ZOLOCK, and R. H. HERMAN. Dietary regulation of $1,25(OH)_2D_3$ stimulated calcium binding protein, alkaline phosphatase and calcium transport. (Abstract) Clin Res 26: 580A, 1978

5. MORRISSEY, R. L., D. T. ZOLOCK, and D. D. BIKLE. Influence of dietary calcium and phosphorus on the induction of calcium binding protein (CaBP) in response to 62.5 pM of 1,25 dihydroxy vitamin D_3. (Abstract) Fed Proc 37: 408, 1978

6. BIKLE, D. D., E. W. ASHEW, D. T. ZOLOCK, R. L. MORRISSEY, and R. H. HERMAN. Calcium accumulation by intestinal mitochondria from rachitic and $1,25(OH)_2D_3$ treated chicks. (Abstract) Fed Proc 37: 1300, 1978

7. ZOLOCK, D. T., R. L. MORRISSEY, and D. D. BIKLE. 1,25-dihydroxy-cholecalciferol ($1,25(OH)_2D_3$) mediated bone mineral uptake and its blockage by cycloheximide (cyclo). (Abstract) Fed Proc 37: 1316, 1978

8. BIKLE, D. D., C. C. PECK, R. L. MORRISSEY, D. T. ZOLOCK, and R. H. HERMAN. Pharmacokinetics of 1,25 diphydroxyvitamin D_3 in plasma and gut. (Abstract) Endocrinology (suppl) 102: 316, 1978

STUDY NO. 9 — The role of vitamin D in the etiology and treatment of fractures

PROBLEM

In an effort to understand the reasons why some military personnel seem predisposed to stress fractures, we have tested vitamin D metabolism in human subjects and patients with bone disease. We evaluated the physiologic adjustments of these patients during periods of low and high calcium intake and the response of target tissues to the principal hormones controlling bone mineral homeostasis: parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$).

RESULTS AND DISCUSSION OF RESULTS

The study has only recently begun, but several observations have been made. Certain patients with primary hyperparathyroidism demonstrate a failure to adapt to a high calcium diet by reducing their intestinal absorption of calcium, their renal excretion of phosphate, and their renal production of cyclic-AMP.

The appropriate binding proteins, and tracer metabolites to measure $25OHD_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ in the blood of patients have all been prepared and reliable standard curves for each assay have been performed. However, sample preparation requires equipment which we have been unable to obtain because of budgetary limitations.

CONCLUSIONS

1. Abnormalities in vitamin D and mineral metabolism can be detected in patients with known metabolic bone disease by using an approach that can be applied to individuals with stress fractures.

2. Dietary calcium influences not only hormone production (i.e., PTH and $1,25(OH)_2D_3$) but also hormone action on target tissues.

RECOMMENDATIONS

The study shows promise and should be supported. Due to lack of funding, this project is being terminated.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6302	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. MATH MATRIX	9A. DOD SPECIFIC DATA: CONTRACTOR ACCESS	9B. LEVEL OF SUM
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	020
b. [redacted]	61102A	3M161102BS02	00	071
c. [redacted]	CARDS 114(			

11. TITLE (Enter title and security classification code)*

(U) The Response of Muscle to Injury

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
76 10	CONT	DA			C. In-House

17. CONTRACT GRANT

a. DATE EFFECTIVE — EXPIRATION

b. NUMBER* Not Applicable

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
	PREVIOUS		
FISCAL YEAR 78		3.4	89
FISCAL YEAR 79		0.5	50

19. RESPONSIBLE DOD ORGANIZATION

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20. GENERAL USE

Foreign Intelligence Not Applicable

21. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME Scott, Rhonda L., 1LT, MSC

NAME POC: DA

22. KEYWORDS (Enter descriptive terms selected to identify project and security classification codes)

(U) Skeletal Muscle; (U) Myoglobin; (U) Metmyoglobin Reductase; (U) Heatstroke; (U) Oxygen Utilization-by Muscle

23. TECHNICAL OBJECTIVE* 24. APPROACH. 25. PROGRESS (Provide brief chronological description of work performed during previous year and planned work for current year. Security classification codes required.)

23. (U) The acutely injured soldier develops negative nitrogen balance and loses muscle mass through mechanisms which are unknown. One of the factors which may be involved is myoglobin, a heme-protein which transports oxygen within muscle cells. Myoglobin and its overall metabolic relationships within the muscle cell serve as useful markers in the study of muscle injury. Injured muscle loses myoglobin into the peripheral circulation where it may cause secondary renal damage for unknown reasons. Failure of myoglobin to maintain sufficient intracellular oxygen supply may lead to decreased energy production, weakness, and failure of mechanisms upon which recovery from injury depend.
24. (U) Selected aspects of the effects of injury on muscle will be evaluated. Strategies designed to minimize and/or reverse the detrimental effects of injury on muscle will be determined. The effects of muscle injury on other body systems, including the kidney, will be studied. The relationship between myoglobin (and its associated reactions in the muscle cell) and immobilization-induced muscle atrophy, exercised-induced muscle hypertrophy, and recovery from injury will be studied.
25. (U) 77 09 - 78 10 Metmyoglobin reductase has been repeatedly purified to a maximum of 2000-fold, to specific activities in excess of 150,000 U/mg protein. The enzyme has a pH optimum about 6.5, a Km of 5.0×10^{-5}M, and is unaffected by the absence of O_2. The molecular weight is about 30,000. Studies comparing the enzyme with commercial diaphorase, and with partially purified bovine methemoglobin reductase, demonstrate differences leading us to conclude that the muscle enzyme is a distinct entity. This work unit was transferred from the former Department of Medicine at the time of the reorganization.

* A change in previously reported data is required.

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

ABSTRACT

PROJECT NO.	3M161102BS02	Basic Mechanisms of Recovery from Injury
WORK UNIT NO.	071	The Metabolic Response of Muscle to Injury, Exercise, and Diet in Health and Disease

The following investigation has been conducted under this work unit:

STUDY NO. 1 Studies concerning the mechanism which controls the redox state of myoglobin

Previous studies clearly demonstrated the presence of metmyoglobin reducing activity in the supernatant of crude muscle homogenate. This metmyoglobin reducing activity has been repeatedly purified maximally to as much as 2000-fold, and has yielded specific activities in excess of 150,000 U/mg protein. The addition of bovine serum albumin or myoglobin to the purified enzyme protects the activity of material when stored at -70C. The enzyme has a pH optimum about 6.5, a Km of 5.0×10^{-5}M, and is unaffected by the absence of O_2. Sodium dodecyl sulfate gel electrophoresis revealed a molecular weight around 30,000. The reactivity of the enzyme is markedly influenced by the composition of the buffering milieu. Activity is inhibited by sulfhydryl reagents and quinacrine hydrochloride and stimulated by flavin mononucleotide. Purified enzyme did not react with lipoamide. The properties and characteristics of the enzyme were different than those of diaphorase, but appeared to be similar to those of partially purified methemoglobin reductase.

BODY OF REPORT

WORK UNIT NO. 071 — The Metabolic Response of Muscle to Injury, Exercise, and Diet in Health and Disease

STUDY NO. 1 — Studies concerning the mechanism which controls the redox state of myoglobin.

PROBLEM

Hemoglobin (Hb) of red blood cells and myoglobin (Mb) of red muscle share a number of properties which include reversible oxygenation to form oxyhemoglobin (HbO_2) or oxymyoglobin (MbO_2); or irreversible oxidation to methemoglobin (MetHb) or metmyoglobin (MetMb), respectively. Whether these heme proteins undergo oxygenation or oxidation depends on a number of factors which are complex and not completely understood. Under physiological conditions in vivo, only 2 to 3% of hemoglobin in red blood cells is in the met-form. Several efficient enzymatic systems have been described which continually reduce MetHb, thereby preventing its accumulation to any appreciable extent. The enzymes responsible for this reduction utilize NADH or NADPH, and in some cases require an electron carrier such as methylene blue for in vitro study. By far the most active system requires ferrocyanide ion activation.

Much less attention has been given to the possible existence of similar systems which reduce MetMb. MetMb normally is not thought to be present in muscle in any appreciable quantity despite the greater susceptibility of Mb to oxidation than Hb. It is reasonable to assume that muscle must contain a highly active mechanism for MetMb reduction; otherwise, the continued formation of MetMb would go unopposed. The presence of diaphorases in muscle is well known. However, the existence of a specific MetMb reductase, analogous to MetHb reductase activity in red blood cells, has not been convincingly demonstrated heretofore.

Enzymatic reduction of MetMb by NADH and NADPH dependent mechanisms has been shown by other investigators. However, a specific MetMb reductase activity was not found. An enzyme which will reduce metmyoglobin has been described in dolphin muscle. Enzyme activity was demonstrable with either NADH or NADPH at pH 7.0 and required the presence of methylene blue. How this enzyme differs from diaphorase is not clear. Presumed enzymatic reducing activity has also been demonstrated in both intact and ground meat, but without clarification of the mechanism. Furthermore, it has been shown that efficient non-enzymatic MetMb reduction can occur in vitro under suitable circumstances. Moreover, immunologic and electrophoretic studies have shown that ferrocyanide-activated MetHb reductase activity was detectable in several tissues including muscle.

Despite the failure of past investigators to demonstrate convincingly specific enzymatic MetMb reduction, it seemed logical to conclude that if MetMb reductase exists in red blood cells, an analogous enzyme for MetMb reduction should exist in muscle. Initial studies (discussed in the 1975 Annual Report pp 101-105) demonstrated the presence of a specific NADH-dependent metmyoglobin reductase in the soluble supernatant fraction of homogenized beef heart. The optimum assay conditions and some of the properties of the enzyme (in the crude system) were established. The next series of studies and experiments were aimed at purifying the enzymatic reducing activity in the supernatant fraction of homogenized beef heart.

Muscle function is impaired in wounded soldiers by direct injury (trauma, muscle wounds, excessive exercise) and/or immobilization of limbs and/or bed rest. In order to facilitate healing and to reverse atrophy of muscle it is important to understand the mechanism involved in exercise-induced hypertrophy and the immobilization-induced atrophy of muscle. It is postulated that myoglobin is involved in these exercise-dependent responses of muscle via its function as an intracellular carrier of oxygen. Since oxygen also oxidizes myoglobin to the met-form it can be logically argued that there must exist a mechanism for the reduction of metmyoglobin. If so, one might expect also that defects in the metmyoglobin reduction system can lead to exercise-induced injury, diminished hypertrophy of muscle during exercise, accelerated atrophy during immobilization, and prolonged recovery after injury.

RESULTS AND DISCUSSION OF RESULTS

Metmyoglobin reducing activity has been repeatedly purified maximally to as much as 2000-fold, and yielded specific activities in excess of 150,000 U/mg protein. The addition of bovine serum albumin or myoglobin to the purified enzyme protected the activity of material stored at -20C. The enzyme has a pH optimum about 6.5, a Km of 5.0×10^{-5}M, and is unaffected by the absence of O_2. Sodium dodecyl sulfate gel electrophoresis revealed a molecular weight around 30,000. The reactivity of the enzyme was markedly influenced by the composition of the buffering milieu. Activity was dependent on the presence of $K_4Fe(CN)_6$ and NADH in the reaction mixture. Activity was inhibited by p-chloromercuriphenylsulfonic acid and N-ethylmaleimide. Quinacrine hydrochloride inhibited activity, whereas flavin mononucleotide increased activity almost 2-fold. The temperature optimum was 37C, and activity was abolished by heating to 50C. Purified enzyme did not react with lipoamide and demonstrated characteristics which clearly distinguish it from diaphorase. Bovine erythrocyte methemoglobin reductase was purified by similar techniques and compared to the purified muscle enzyme. These studies demonstrated that the muscle and red cell enzyme were similar. On acrylamide gel electrophoresis, however, the two enzymes produced different patterns when stained for activity which speaks against their being the same.

CONCLUSIONS

These studies have demonstrated some of the characteristics of bovine metmyoglobin reductase which has been purified as much as 2000-fold. The enzyme represents a distinct entity in muscle which is not diaphorase or contaminating methemoglobin reductase. Its properties are in keeping with the conditions of pH and temperature which are known to exist in exercising muscle. The discovery of this enzyme and its characterization add a new dimension to the consideration of the role of myoglobin in muscle function.

RECOMMENDATIONS

This is important work and should be continued. However, due to loss of funding and personnel this work unit is being terminated.

PUBLICATIONS

HAGLER, L., R. I. COPPES, Jr., and R. H. HERMAN. Metmyoglobin reductase: Identification of a reduced nicotinamideadeninedinucleotide-dependent enzyme from bovine heart which reduces metmyoglobin. Submitted for publication.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6303	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. GEOGRAPHY	8. GEOPOLITICAL	9. SPECIFIC DATA: CONTRACTOR ACCESS	10. LEVEL OF EFFORT
77 10 01	H.Termination	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

11. MD /CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A810	00	001
b. CONTRIBUTING				
c. [redacted]	CARDS 114f			

12. TITLE (Procede with Security Classification Code)

(U) Prevention of Fungal Infections of the Soldiers Skin

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY			17. PERFORMANCE METHOD
76 10	78 10	DA			C. In-House

18. CONTRACT GRANT

a. DATE EFFECTIVE — EXPIRATION

b. NUMBER

c. TYPE Not Applicable — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

19. RESOURCES ESTIMATE

	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR — PREVIOUS	78 — 3	120
FISCAL YEAR — CURRENT	79 — 0.0	00

20. RESPONSIBLE DOD ORGANIZATION

a. NAME Letterman Army Institute of Research

b. ADDRESS Presidio of San Francisco, CA 94129

c. RESPONSIBLE INDIVIDUAL

NAME Marshall, J. D., COL, MS

TELEPHONE (415) 561-3600

21. GENERAL USE

Foreign Intelligence Not Applicable

22. PERFORMING ORGANIZATION

a. NAME Letterman Army Institute of Research

b. ADDRESS Department of Dermatology Research
Presidio of San Francisco, CA 94129

c. PRINCIPAL INVESTIGATOR (Provide Last Name, First Name, Middle Initial)

NAME Kerbs, Sharon, Ph.D., DAC

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SOCIAL SECURITY ACCOUNT NUMBER

d. ASSOCIATE INVESTIGATORS Hutton, Robert D., LTC, MC, USAF

NAME

NAME POC: DA

23. KEYWORDS (Provide Last One Security Classification Code) (U) Troop Ineffectiveness; (U) Prevention-skin diseases; (U) Fungus; (U) Lymphocyte Transformation (LT); (U) Chemotaxis; (U) Laboratory Animals

24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Provide individual paragraphs identified by number corresponding to entries in items 24 and 25. Security Classification Code)

23. (U) Tinea corporis (body ringworm) has produced unacceptable non-effectiveness of combat troops in previous military conflicts. This problem may be anticipated within the D + 15-30 time frame of possible future conflicts unless adequate preventive measures are found. Prevention of infection is the major goal; determining how to decrease the severity of disease by drug therapy or alterations of the micro-environment will be steps toward that goal.

24. (U) The approach will include studies on (a) the influence of the local skin environment and skin condition on the severity of disease, (b) the effectiveness of different methods of therapy, (c) factors responsible for decreasing or increasing the severity of the disease.

25. (U) 77 10 - 78 09. Under controlled conditions with experimental infections on the skin of guinea pigs, the route of fungal invasion was defined for primary infections and work is now being completed on defining the route of invasion during secondary infections. On hairless dogs, abrasion is necessary for initiating infection. On the hairy skin of a guinea pig, abrasion is not necessary. Healing of a fungal lesion is delayed by 3-5 weeks in guinea pigs deficient in folic acid, but ascorbic acid deficiency does not influence the rate of healing. The micromethod of assaying fungal growth was adapted to test the sensitivity of fungi to griseofulvin. For adminstrative reasons, this work unit has been terminated and the research incorporated within Work Unit No. 067, DA 0C 6733.

* available to contractors upon [illegible]

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

ABSTRACT

PROJECT NO. 3M762772A810 — Other Tropical Medicine

WORK UNIT NO. 001 — Prevention of Fungal Infections of the Soldier's Skin

The following investigations have been conducted under this work unit:

STUDY NO. 1 Host-parasite relationships in dermatophyte infections

EX-1 Fungal infections in guinea pig skin

EX-4 The role of the physical condition of the skin in determining whether fungal infections develop

EX-5 Initiation of fungal infections on hairless skin

STUDY NO. 3 Immunology of fungal infections

EX-6 Evaluation of Oct 77 lot of trichophytin

STUDY NO. 4 Effect of vitamin deficiencies on the severity of fungal infections

STUDY NO. 1

EX-1 Studies were completed on the invasion of guinea pig skin by fungi. These studies showed that fungi invaded through the hair follicle and then spread out into the stratum lucidum.

EX-4 Intact guinea pig skin requires 10^6 spores to initiate a fungal infection, while abraded or occluded skin only requires 10^2 spores to initiate a fungal infection.

EX-5 When skin is hairless, it is difficult to initiate a fungal infection on the skin if it is intact. Fungal infections are more easily initiated on hairless skin when the skin is abraded.

STUDY NO. 3 The last lot of trichophytin antigen prepared in Oct 1977 was unstable and not suitable for use in any studies.

STUDY NO. 4 Studies are in progress to evaluate the effect of ascorbic acid and folic acid deficiencies on the severity of fungal infections. Preliminary results show that folic acid deficiency delays healing by several weeks and also interferes with cell-mediated immunity.

BODY OF REPORT

WORK UNIT NO. 001 — Prevention of Fungal Infections of the Soldier's Skin

STUDY NO. 1 — Host-parasite relationships in dermatophyte infections

EX-1 — Fungal infections in guinea pig skin

PROBLEM

In the combat soldiers' enviornment, disease produced by Trichophyton mentagrophytes may increase in severity and result in epidemic disabling body ringworm. By microvisual techniques, we investigated when and where in the skin the fungus invades. We hope to determine the fungal forms and their exact micro-anatomic pathways of invasion under various local skin conditions. These patterns may indicate inherent strengths or weaknesses of the host and parasite species as they interact, which may lead to improved prophylactic or therapeutic measures or agents.

RESULTS AND DISCUSSION OF RESULTS

Trichophyton mentagrophytes invasion of guinea pig skin was examined by scanning electron microscopy (SEM). Biopsies were obtained daily for twelve days from experimental infection sites. Dermatophyte invasion, examined in detail by SEM of cross-sectioned, prefixed skin was evidenced by the appearance of hyphae within the stratum corneum, follicular invasion by hyphae which remained initially within the follicle wall, emergence of the hyphae from the wall into the follicular canal, proliferation of the fungus down the follicle with furrowing of the follicle wall and hair shaft cuticle, penetration of hyphae into the hair shaft by subcuticular and transcuticular routes, and massive peripilar hyphal proliferation with arthrosporogenesis. A three-dimensional perception of the invasion sequence of a dermatophyte in guinea pig skin has been obtained by SEM. We are now preparing sections of lesions of secondary fungal infections to determine if they differ from primary lesions in respect to proliferation.

CONCLUSIONS

We have definitely shown that fungi invade guinea pig skin through the hair follicle. Work is now in progress to determine the method of invasion in secondary infections.

RECOMMENDATIONS

The work on secondary lesions should be completed to determine the route of invasion, and to determine the extent and location of fungal proliferation.

PUBLICATIONS

1. HUTTON, P.D., S. KEBBS, and E. YEH. Scanning electron microscopy of experimental Trichophyton mentagrophytes infections in guinea pig skin. Infect Immun 21: 247-253, 1978

MX-4 — The role of the physical condition of the skin in determining whether fungal infections develop

PROBLEM

We do not know if the condition of the skin (normal, abraded or occluded) influences the number of spores needed to initiate an infection.

RESULTS AND DISCUSSION OF RESULTS

We infected guinea pigs with spores of Trichophyton mentagrophytes. Three different methods of infection were compared. The first method, called occlusion, was performed by placing spores on unabraded skin and occluding the site. The second method of infection consisted of placing spores on abraded skin and gently rubbing in the inoculum. The third method was performed by placing spores on intact skin and gently rubbing in the inoculum. We found that fungal infections were initiated with 100 spores when the skin was occluded or abraded. If the skin was intact, 1,000,000 spores were needed to initiate infections in almost all animals.

Work is currently being completed to determine if spores can germinate on the surface of abraded (or occluded) skin and directly penetrate the stratum corneum. We know fungi do not penetrate the stratum corneum directly if the skin is intact.

CONCLUSIONS

Skin which is occluded or abraded is much more susceptible to fungal infections.

RECOMMENDATIONS

Any effort made to reduce abrasion, scratching, or occlusion of soldiers' skin through improved clothing or footwear should help to reduce fungal infections. The work on the site of invasion and areas of proliferation should be completed. This work should then be published.

PUBLICATIONS

None

EX-5 Initiation of fungal infections on hairless skin

PROBLEM

We know that on hairy guinea pig skin, occlusion or abrasion is not necessary for initiating an infection. The route of invasion appears to be the hair follicle. On hairless skin, such as man's or hairless dog's, we do not know if fungi can initiate an infection on intact skin.

RESULTS AND DISCUSSION OF RESULTS

We placed ten-fold dilutions of fungal spores (from 10^2 to 10^6 spores on 1 cm^2) on the backs of 3 hairless dogs at sites where the skin was either intact or abraded. Duplicates were performed on each animal. Fungal infections developed in abraded areas of skin with all spore doses. Fungal infections did not develop on intact skin unless 10^6 spores were used.

CONCLUSIONS

On hairless skin of dogs, abrasion is essential for initiating fungal infections with less than 10^5 spores per cm^2.

RECOMMENDATIONS

Reducing abrasion should help eliminate fungal infections in soldiers. These data should be published.

PUBLICATIONS

None

STUDY NO. 3 Immunology of fungal infections

EY-6 Evaluation of Oct 77 lots of trichophytin prepared under Work Unit 067

PROBLEM

Fungal antigen is needed for experiments in Work Units 001 and 067. A new lot of fungal antigen was prepared in Oct 1977

from fungal rats prepared under contract. This lot had to be evaluated to determine if it was suitable to be used as a standard antigen for these two work units.

RESULTS AND DISCUSSION OF RESULTS

Trichophytin lot Oct 77 was tested for its suitability to be used as a standard antigen. The standard antigen should give positive responses in infected animals to the following tests: (1) skin tests, (2) lymphocyte blastogenesis, and (3) cutaneous basophilic hypersensitivity. The antigen should also give negative responses in control animals to the above tests and additionally should not sensitize control animals.
We found that trichophytin lot Oct 77 was unstable. After 6 months' storage, none of the tests were positive in infected animals. Also, this antigen sensitized control animals. Therefore, this lot is unsuitable for use.

CONCLUSIONS

Trichophytin lot Oct 77 cannot be used as a standard antigen.

RECOMMENDATIONS

LAIR does not have a standard fungal antigen. This antigen should be obtained.

PUBLICATIONS

1. GREENBERG, J., and S. KEEBS. Cutaneous basophilic hypersensitivity responses to fungal antigens. Submitted for publication.

STUDY NO. 4

Effect of vitamin deficiencies on the severity of fungal infections

PROBLEM

The influence of vitamins on susceptibility to or recovery from fungal infections is unknown. We want to know whether guinea pigs marginally deficient in ascorbic acid or folic acid are more susceptible to fungal infections or require a longer time to heal than controls. Also, there is not agreement in the literature on the effects of this deficiency on the animals' cell-mediated and antibody responses, or on the level of saturated transferrin in their blood.

RESULTS AND DISCUSSION OF RESULTS

Guinea pigs were made marginally deficient in ascorbic or folic acid. Preliminary results show healing is greatly delayed (by 21-35 days) in animals marginally deficient in folic acid. Folic acid deficient animals also do not have normal cell-mediated responses, but antibody responses appear normal. Work is currently being done on determining the effects of ascorbic acid deficiency and on analyzing the iron-binding capacity of the serum.

CONCLUSIONS

Healing is delayed with marginal deficiencies in folic acid.

RECOMMENDATIONS

This work should be completed and published.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY							
				A. DE 3104	78 10 01		DD-DR&E(AR)636
77 10 01	Termination	U	U	NA	NL	YES NO	
	62772A	3M162772A810	00		044		
	KARDS 114f						

(U) Prevention of Skin Disease Caused by Environmental Assaults on Skin

003500 - Clinical Medicine

76 10	78 10	DA		C
			78	3.0
Not Applicable			79	0.0

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Foreign Intelligence Not Applicable

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Department of Dermatology Research
Presidio of San Francisco, CA 94129

Akers, William A., COL, MC

Prystowsky, Stephen, D., MAJ, MC
Grekin, David A., MAJ, MC POC: DA

(U) Ultraviolet light; (U) Sweat; (U) Contact Dermatitis; (U) Skin; (U) Friction; (U) Blisters; (U) Water Immersion; (U) Miliaria; (U) Occlusion

23. (U) The objectives are to develop prophylactic measures against the assaults of heat, humidity, friction, pressure, occlusion, water immersion, sweat, and ultraviolet light alone and in combination on soldiers' skin. Diseases like friction blisters, paddy foot, prickly heat rash, athlete's foot, jock itch, skin infections, and sunburn result in epidemics among soldiers and cause high morbidity, man-days lost, and interfere with performance of mission.

24. (U) The natural history of the diseases will be studied. Promising prophylactic and therapeutic measures will be tested in the laboratory and the field.

25. (U) 77 10 - 78 09. Epidemiologic study of friction blisters in recruits of all the services was completed. Marine recruits had bigger blisters that occurred earlier in basic training than in other services. There was no correlation between boot fit and blister development. Blistering was caused by stiffness of new boots, combined with vigorous running and forced marching. Preliminary studies of use of 10% glutaraldehyde solution to reduce foot and ankle blisters revealed mild transient irritation in a few subjects and an allergic contact dermatitis with one soldier. Sample size was too low to evaluate efficacy of the procedure. This work unit has been terminated due to departure of the investigators and lack of qualified investigators (dermatologists) to do the research.

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

ABSTRACT

PROJECT NO. 3M62782A810 — Military Skin Disease

WORK UNIT NO. 002 — Prevention of Skin Disease Caused by Environmental Assaults on Skin

The following investigations have been conducted under this work unit:

STUDY NO. 1 Epidemiology of friction blisters in soldiers

STUDY NO. 2 Prevention of friction blisters in soldiers with gluteraldehyde

STUDY NO. 1 Data on the natural history of the epidemiology of friction blisters in recruits of all the services (Army, Navy, Air Force, Marines) have been collected and analyzed. The Phase V study in 290 Marine recruits revealed that their blisters had occurred earlier in basic training (during the first 2 weeks) and were somewhat larger than in recruits of the other services. In Phase VI, the feet of 213 recruits were measured for correct boot fit and the fit was considered good in 87% of the cases. There was no correlation between improper boot fit and developing friction blisters. The culprits producing blisters in recruits are stiff new boots, vigorous running, and forced marching.

STUDY NO. 2 We received approval (from the Federal Drug Administration) of our investigational new drug (IND) application to determine the efficacy of a gluteraldehyde solution as an agent to reduce the incidence of foot and ankle blisters in recruits in basic combat training. In the study, the 10% gluteraldehyde solution will be applied one time daily, 5 days a week, for 5 weeks. A preliminary clinical trial (Phase I) was conducted for a period of 8 weeks to support the application. Twelve normal healthy volunteers participated. Mild transient irritation occurred in a few subjects; on one soldier, an allergic contact dermatitis was produced by the third week on the thin skin of the ankles. The nylon mohair-top plastic applicator tested in Phase I will be used in the efficacy study (Phase II) which will begin when a physician-investigator becomes available.

BODY OF REPORT

WORK UNIT NO. 002 — Prevention of Skin Disease Caused by Environmental Assaults on Skin

STUDY NO. 1 — Epidemiology of friction blisters in soldiers

PROBLEM

Friction blisters cause high rates of disability in the military, especially among recruits in training and among seasoned troops suddenly mobilized to stressful environments of sustained operations. Friction blisters may render large numbers of soldiers unfit for service rapidly; thus the effective force will be diminished even at the beginning of the campaign. As many as 25 to 30% of troops may sustain injury to the feet during the first few days of forced marching. Development of a prophylaxis for friction blisters has a high priority in the recently published STOG-78. Blisters must be prevented early (day 15) to permit greater strategic mobility and improve troop performance in the stressful environment of sustained operations.

RESULTS AND DISCUSSION OF RESULTS

The complete results of this 6-phase study have been analyzed. In the FY 77 annual progress report (pages 142-143) results on Phases I, II and III were reported.

In Phase IV we studied 105 recruits who were hospitalized with friction blister related cellulitis at Fort Leonard Wood. We found that 89% were hospitalized during the first 5 weeks of training. The median number of days hospitalized were 3.3 days. Retrospective study of hospital records revealed that 40 recruits/1000/year were hospitalized for infected blisters. Thus, friction blister related cellulitis is second only to acute respiratory disease as a cause for hospitalization in basic combat training. This proved true in the Army, Navy, Air Force, and Marine Corps basic training centers.

Phase V was a prospective podiatry clinic study of 290 marines. We found that 81% of the blisters occurred during the first 3 weeks of training. Basic training in Marines is more vigorous than the other services. Blisters occur earlier, are somewhat larger, and are more likely to become infected in the Marine recruits than other recruits, perhaps because of the more intensive training. In a collaborative study from Fort Leonard Wood, the Navy and Marine Recruit Training Center San Diego, and Lackland Air Force Base, we

observed that, of all recruits hospitalized for blisters and complications, 88% were hospitalized during the first 5 weeks of basic training. Friction blisters and associated cellulitis usually occurred early with forced marching during the second and third weeks of basic training. Severe frictional stress of a forced march in a new stiff boot is probably related to friction blisters. Apparently, after 5 weeks, the boots are sufficiently broken in and sufficient callus has formed on the soldiers' feet to make severe blisters unlikely. Marines developed blisters in the first and second weeks, whereas the Army, Navy, and Air Force recruits tended to get blisters in the second to fourth weeks. If some agent, such as glutaraldehyde, can protect the soldiers for the first 5 weeks and allow the foot to toughen while the boots are being broken in, recruits probably will have fewer blisters during the rest of basic training. Of 202 recruits with cellulitis, only 3 (1.5%) were black. Since black soldiers comprise more than 1.5% in all services, our observation suggests that black soldiers may have other protective factors preventing them from getting severe blisters, such as the stratum corneum, which in blacks is more compact and more resistant to frictional stress.

In the Phase VI study, we measured the size of both feet in 213 recruits to evaluate the association of improper boot fit and foot size as a cause of friction blisters. The boot fit was considered "good" in 87% of the group. There was no correlation between improper boot fit and friction blisters. The myth that "soldiers are not fitted properly with boots, and this is a major cause of friction blisters" can be put to rest. The causes of friction blisters appeared to be early forced marches and stiff new boots on the feet of recruits who did not have sufficient callus to protect them from blistering.

CONCLUSIONS

Friction blisters of the feet and blister-related cellulitis are second only to acute respiratory disease as medical problems in basic combat training in the peace-time army. History documents the magnitude of the impact of friction blisters in military operations. At least 60% of recruits develop blisters; 400/1000/year report for treatment and 40/1000/year are hospitalized for a mean of 3.3 days. Blisters in recruits cost DoD 1.46 million dollars in 1976. Black recruits develop fewer blisters than white recruits. Marine recruits have larger blisters that occur earlier in their training than the blisters recruits in the other services have on their feet. New stiff boots, vigorous running, and forced marching in weeks 1, 2, and 3 seem to cause the most difficulty. After week 5 of training, few blisters form; the boots are broken in and protective calluses on the feet have formed. We discovered only a small percentage (±13%) of poorly fitting boots. There was no correlation between improper boot fit and development of friction blisters. Blisters around the heel, the Achilles tendon, and the front of the ankle are the ones that readily become infected.

RECOMMENDATIONS

The boots should be broken in either mechanically and/or chemically before issue. The basic combat training schedule should be modified to permit a slower and more gradual increase in physical stress through the fifth week. Topical protective agents should be developed to lessen the frictional forces on the skin of the foot and ankle. Redesign of the combat boot in the heel counter and lace area over the anterior ankle should be considered.

PUBLICATIONS

None

STUDY NO. 2 — Prevention of friction blisters in soldiers with gluteraldehyde

PROBLEM

Friction blisters and friction blister-related cellulitis are well documented military medical problems. Friction blisters are prevalent among recruits of all services. If the primary causes of friction blisters are from stiff, new boots and early forced marching, then a preventive measure, which toughens the skin while the boots are being broken in and while the recruits are developing calluses, would be a genuine asset to military readiness. For these reasons, we looked at gluteraldehyde, a tanning agent with strong antimicrobial activity. Topical application of gluteraldehyde decreases sweat formation, which, in turn, reduces the coefficient of friction. Gluteraldehyde has been effective in friction blister prophylaxis in controlled testing.

RESULTS AND DISCUSSION OF RESULTS

Gluteraldehyde does not produce cross-sensitivity to formaldehyde. Gluteraldehyde penetrates the human stratum corneum in vitro poorly because it binds avidly to the horny layer. Even in patients highly allergic to gluteraldehyde, skin reactions do not occur when it is placed on the thick stratum corneum of the palm or sole.

In the Phase I clinical trial for skin irritation and sensitization to support the approved investigational new drug application (IND), gluteraldehyde (GLAD) was applied unoccluded to the posterior ankle and heel and the anterior ankle of 12 volunteers daily for 3 weeks, then 3 times a day for 5 weeks. Eleven of the 12 subjects had no reaction except for a few with transient, minimal irritation. One subject did develop a contact allergy to GLAD, and this was limited to the thin skin of the ankles. In future studies, GLAD will be applied to the thicker skin of the posterior heel and ankle and the medial and lateral heel. The degree of staining (a yellowish

brown color) varied greatly. All subjects commented on the lack of sweating and on a slipperiness of the skin of the treated areas. No one rubbed a blister in the treated area, although some engaged in team sports during the study.

The optimal concentration and the optimal frequency of application of GLAD to reduce the incidence of blisters have not been determined. We suspect that 10% GLAD solution applied 3 times a week for 5 weeks may suffice, since that dosage works in patients with epidermolysis bullosa. A 60 ml polypropylene applicator bottle with a nylon mohair top with flow valve, worked nicely to give a controlled amount rapidly on a limited area. Sufficient toxicological data were obtained from the manufacturers and the literature to support the IND.

The efficacy study (Phase II) was approved for application 5 days a week, for 5 weeks, in 4 companies of recruits at Fort Leonard Wood.

CONCLUSIONS

One of 12 volunteers developed a contact sensitization on the thin skin of the ankle, but not on the thicker areas of the heel during 3 weeks of daily application. Only mild transient irritation occurred in several other subjects during the 8 weeks. In reported studies in over 700 patients using topical gluteraldehyde frequently, no cases of allergic contact dermatitis were reported. A nylon mohair top and plastic applicator bottle performed most satisfactorily, and should perform nicely in larger trials.

RECOMMENDATIONS

Phase II clinical trials of 10% GLAD solution applied once daily, 5 days a week for 5 weeks to the posterior ankle and heel and to the lateral and medial heel should be performed. If the results are encouraging, Phase III clinical trials in 2 battalions of recruits (1800-2000 subjects) should be done.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

a. AGENCY ACCESSION	b. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6800	78 10 01	DD-DRAE(AR)636

b. DATE PREV SUMRY	c. KIND OF SUMMARY	d. SUMMARY SCTY	e. WORK SECURITY	f. REGRADING	g. GROUP INTERV	h. SPECIFIC DATA-CONTRACTOR ACCESS	i. LEVEL OF SUM
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A810	00	003
b. CONTRIBUTING				
c. [illegible]	CARDS 114f			

11. TITLE (Procede with security classification code)

(U) More Effective Topical Repellents Against Malaria - Bearing Mosquitoes

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 11	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: — b. EXPIRATION:
c. NUMBER:
d. TYPE: Not Applicable — e. AMOUNT:
g. KIND OF FUND — f. CUM. AMT.

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
78	2.4	51
79	2.4	80

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: Letterman Army Institute of Research
b. ADDRESS: Presidio of San Francisco, CA 94129
c. RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION

a. NAME: Letterman Army Institute of Research
b. ADDRESS: Division of Cutaneous Hazards
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c. PRINCIPAL INVESTIGATOR (Provide Last Name, First Name, Middle Initial)
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SOCIAL SECURITY ACCOUNT NUMBER
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NAME: Schmid, Peter, Ph.D., DAC
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21. GENERAL USE

Foreign Intelligence Not Applicable

POC: DA

22. KEYWORDS (U) Tropical Diseases; (U) Topical Repellents; (U) Human Volunteers; (U) Insect Repellent; (U) Mosquito; (U) Skin; (U) Stratum Corneum; (U) Polymer formulations

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs indicated by number. Proceed from left margin. Do not exceed the space provided.)

23. (U) The objectives are to discover a long-lasting water and abrasion resistant topical repellent formulation that will protect soldiers against malaria-bearing mosquitoes and other vectors of militarily important diseases; to develop in vitro test methods to determine physical properties of repellent formulations which are tested in the in vivo screening program, and by correlating the in vitro and in vivo test results to predict evaporation, penetration, and repellent-skin interactions which will aid in designing new repellents.

24. (U) Physical properties and efficacy of repellents and formulations will be determined by partition coefficient, relative solubility, volatility, surface activity, and duration on animals and man. With the use of a computer base, for statistical analyses of repellent test results, individual characteristics which can be enhanced to promote longer duration of repellent protection will be determined.

25. (U) 77 10 - 78 09. Eight known insect repellents tested for duration on the hairless dog yielded data comparable to analagous test results in human volunteers. A total of 9 new candidate repellent chemicals and 30 repellent formulations were screened for duration on the hairless dog. Based in part on this data, two repellent chemicals were selected for advanced testing. A computer data management system for the hairless dog test data and a computer system for relating individual volunteer characteristics and physical properties of repellents to repellent efficacy have been designed and are near completion. Gas and thin layer chromatographic methods have been developed for qualitative and quantitative assay of repellents.

* CLASSIFY BY SECURITY AND CONTROLLED GROUPS

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

ABSTRACT

PROJECT NO. 3M762772A810 — Military Skin Diseases

WORK UNIT NO. 003 — More Effective Topical Repellents Against Disease Bearing Mosquitoes

The following investigations have been conducted under this work unit:

STUDY NO. 1 Mosquito repellent data management systems

STUDY NO. 2 Further testing of the hairless dog as an animal model for repellent studies

STUDY NO. 3 Evaluation of candidate repellents and repellent formulations

STUDY NO. 1 Effort is directed for the development of ECLIPSE C300 based computer data systems for the management of mosquito repellent data derived from animals and volunteers.

STUDY NO. 2 Additional tests were performed on the hairless dog and man to support the use of the hairless dog as an animal model in repellent studies.

STUDY NO. 3 The results of testing new repellent chemicals and formulations on the hairless dog show that two compounds are candidate repellents for human volunteer testing.

BODY OF REPORT

WORK UNIT NO. 003 — More Effective Topical Repellents Against Disease Bearing Mosquitoes

STUDY NO. 1 — Mosquito repellent data management systems

PROBLEM

At the end of FY78, the Department of Dermatology Research has on record the results of approximately 250 separate repellent tests involving 275 different volunteers, in which 130 different repellent chemicals or formulations were evaluated. Also, 90 separate tests with 50 different repellents or formulations are on record for our animal model (hairless dog) testing. Data have been collected for the physical properties of repellents, individual volunteer and animal characteristics, and information on test conditions. Management and statistical analysis of these data require computer management systems. In the past, a portion of the human test data had been loaded into a Remote File Management System (RFMS) at Lawrence Berkeley Laboratory (LBL); however, this system lacks several important programmed capabilities; i.e., data editing during data entry, sort capability, real time sharing and interactive processing at LBL. Although RFMS can provide effective retrieval, the system has limited statistical options. No provisions were made for the management of animal test data.

RESULTS AND DISCUSSION OF RESULTS

Human test data. The design of an ECLIPSE C300 based system to manage mosquito repellent human test data was completed in cooperation with the Department of Information Science. This system allows for management of time-dependent and -independent volunteer characteristics, repellent physical-chemical characteristics, test condition information, and repellent test results. With the recent implementation by the Department of Information Sciences of the MINITAB interactive general statistical program on the LAIR central minicomputer, local processing is preferred to allow greater user control over data entry, maintenance and analysis. The task of unloading the existing RFMS test data to Statistical Package for Social Sciences for statistical analysis at LBL no longer exists. Instead, programs are under development to unload the data base to the LAIR ECLIPSE C300 minicomputer.

The design and programming of a repellent code and formulation specification dictionary has been accomplished for the purpose

of the human repellent management system. This allows the user short descriptive designations for test substances as well as ready access to directions for preparing formulations.

The entire data set in RFMS has been manually checked against laboratory notebooks to assure correct transcription, and all tests have been ordered, coded, and sequenced in preparation for the ECLIPSE C300 based system.

Animal test data. The design of an ECLIPSE C300 based system to manage repellent test data from our hairless dog animal model has been accomplished. The system manages animal characteristics, test conditions, repellent efficacy data and physical properties of candidate repellents. User control over data entry, maintenance have been stressed. Interface with generalized software statistical packages has been designed.

In addressing the problem of creating a data base for the dog data base, several goals were identified, and these goals are enumerated below:

- Develop a program to read essential information concerning repellent tests into files, and creation of appropriate files
- Develop a program capable of reading these files, and reporting the contents of those files
- Develop a program capable of indexing these files
- Create a separate file for storage of index parameters
- Develop a program capable of storing new parameters for inclusion in the data base
- Develop a program to calculate values from within the data base, the resultant new values to be included in the data base.
- Develop a program which will merge all previously created files into a single unified file
- Create such a single unified file
- Develop a program which has the capability of reading such a file and reporting its contents
- Develop programs to sort through the unified file and tabulate specified data in a temporary file to be used for statistical analysis

- Develop programs to perform specific statistical computations on data in temporary files

The first nine goals have been completed, and the majority of the test data has been entered into the system.

CONCLUSIONS

The data systems for animal and human repellent test data will provide an accurate historical file for future investigators, allow ready access and interaction by the user, and provide needed statistical options.

RECOMMENDATIONS

Work on both data management systems should continue with completion in FY 79. Statistical analysis of the animal model data is required for decisions concerning which repellents should be advanced for toxicity and human testing. Advanced statistical analysis of human test efficacy data, individual characteristics, and repellent physical properties will identify exploitable factors for the design of better insect repellents.

PUBLICATIONS

1. SPENCER, T.S., K.L. ZELLER, W.A. AKERS, and W.H. LANGLEY. Data storage and retrieval system for a mosquito repellent test program. Interim Report, June 1977. San Francisco, California: Letterman Army Research Institute. (Submitted for publication)

STUDY NO. 2 — Further testing of the hairless dog as an animal model for repellent studies

PROBLEM

The development of more effective mosquito repellents presently requires the screening of large numbers of compounds. Since testing on humans requires extensive toxicity tests of candidate compounds, in vitro and animal tests are necessary to select only the most promising compounds for testing on humans. In particular, an animal model is needed which can screen rapidly the duration of effectiveness of new repellents. The guinea pig is useful for repellent protection time determination; however, the animal has to be shaved and restrained during the test period. Because of the size (four repellents can be tested simultaneously), ease of handling, and lack of hair, the hairless dog was investigated as an animal model for determination of mosquito repellent efficacy in FY 77. Further tests were performed in FY 78 to compare the efficacy of repellents in man and dog.

RESULTS AND DISCUSSION OF RESULTS

Two major types of tests were conducted with the dog. One was the minimal effective dose (MED) test, or the minimal amount of repellent needed to repel mosquitoes. The second test conducted was the dry protection time (DPT) test, which is a measure of repellent duration. Analagous tests were performed with human volunteers. Testing procedures used are documented in the LAIR annual progress report FY 77.

Eight different mosquito repellents were tested for duration in the hairless dog and man. With the exception of SRI-6, the dogs ranked the repellents in the same order as man. At a dosage level of 0.32 mg/cm^2, the repellent DPT (in hours) in hairless dog versus man (Mean DPT ±S.D., N= number of replicates) for the following repellents were: Indalone^R: 0.69 ± 0.54 (16) vs 2.2 ± 1.8 (8); carbamide: 1.0 ± 0.75 (40) vs 2.1 ± 1.7 (23); dimethylphthalate: 2.7 ± 1.6 (16) vs 2.2 9 ± 0.9 (8); ethylhexanediol: 3.3 ± 2.3 (24) vs 3.6 ± 1.2 (40); SRI-6: 4.7 ± 3.1 (72) vs 2.5 ± 2.4 (23); and sulfonamide 8.3 ± 4.3 vs 7.9 ± 4.4 (23).

Many factors affect the duration of repellent protection, but one that was found most important was the MED. ME. tests were done on 5 compounds in both hairless dogs and man. The MED (mg/cm^2) in the hairless dog vs man (mean MED ± S.D., N= number of replicates) for the following repellents were sulfonamide: 0.024 ± 0.022 (24) vs 0.036 ± 0.06 (15); m-deet: 0.066 ± 0.047 (24) vs 0.026 ± 0.02 (24); ethylhexanediol: 0.065 ± 0.021 (7) vs 0.046 ± 0.028 (16); SRI-6: 0.12 ± 0.08 (24) vs 0.064 ± 0.06 (24); and carbamide: 0.61 ± 0.18 (24) vs 0.16 ± 0.06 (24). Both the dog and man ranked the repellents similarly, and it can be seen that, generally, compounds with lower MEDs last longer.

As a variation of the usual DPT method, we applied repellents at staggered pretreatment intervals (4 dogs per interval) to dogs and tested all of the animals (one-time challenge) with mosquitoes at the end of the test day. Sulfonamide provided at least 75% protection in each of the four test groups, whereas m-deet provided equivalent protection only through the 4.5-hour period. SRI-6 appears to provide borderline protection after the 2.5-hour pretreatment interval and carbamide, at a dose of 0.32 mg/cm^2, provides no useful protection throughout the entire test period. These results are in agreement with the duration test results reported above.

CONCLUSIONS

The hairless dog is a good model for insect repellent studies. The animal ranks repellents similarly to man in both MED

and duration tests (linear regression analysis of Dog DPT vs Human DPT reveals a correlation coefficient, r = 0.91). Percutaneous absorption studies with radiolabeled repellents suggest the permeability of the dog's skin does not differ markedly from man (Study No. 1, Work Unit 066). The hairlessness, ease of handling, and large size of the dog allow rapid screening of simple repellents and formulations.

RECOMMENDATIONS

The hairless dog should be used as an animal model for the routine screening of simple repellents and formulations of repellents.

PUBLICATIONS

1. HILL, J.A., P.B. ROBINSON, D.L. McVEY, W.A. AKERS and W.G. REIFENRATH. Evaluation of mosquito repellents on the hairless dog. (Submitted for publication)

STUDY NO. 3 — Evaluation of candidate repellents and repellent formulations

PROBLEM

Duration testing in animals is required for new candidate repellents synthesized on contract and for formulations of existing repellents developed in-house against mosquitoes. Toxicological evaluation of new candidate repellents is required before human testing can be accomplished. Development of in vitro methods to screen formulations before evaluation against mosquitoes is required.

RESULTS AND DISCUSSION OF RESULTS

- Duration testing in the hairless dog for new simple repellents and toxicological testing of repellents.

Eight candidate repellents, 835-29C, 835-27C, 835-25B 165-67, 835-23A, 835-19C, 835-17A, and 835-9B were synthesized by Stanford Research Institute (SRI). These compounds were part of a larger group of compounds sent to USDA Gainesville Laboratories for cloth tests. These eight compounds selected from the larger group had greater duration on cloth tests than did m-deet. Approximate lethal dose determination and rabbit eye and skin irritancy tests were conducted on these compounds by SRI. Based on these tests, one compound (835-27Q) was held back from consideration for volunteer tests pending additional information derived from its homologs.

Based on the hairless dog tests for duration, compound 165-67 provided the longest duration time among the group of 8 single repellents, albeit, shorter than m-deet; however, 165-67 provided greater

duration than m-deet (9 days vs 6 days) in USDA cloth tests and greater duration than m-deet on the mouse test performed by Mr. Rutledge, Department of Tropical Medicine, LAIR. Again, based on the hairless dog duration test, compound 835-23A was the poorest performer and provided shorter duration than m-deet in the mouse test for duration (Department of Tropical Medicine); however, 835-23A had a duration of 30 days on the USDA cloth test (m-deet = 6) and provided lower median effective doses against Aedes aegypti, Culex pipiens, Anopheles albimanus, and Anopheles stephensi mosquitoes in tests conducted by Mr. Rutledge. Since these compounds represent two distinct structural classes and have exhibited performance superior to m-deet in at least one type of screening procedure, it was decided to advance these two compounds for human testing. An Ames test for mutagenicity was performed on all of the 8 compounds (under the direction of Dr. Steven Taylor, Department of Nutrition, LAIR). All of the compounds (including m-deet) were negative in this test. Larger quantities of the compounds, 835-23A and 165-67, are being synthesized for additional mammalian toxicity tests (LD_{50}, rat: subacute feeding, rat; and guinea pig sensitization tests) before volunteer testing is considered.

- Repellent formulation testing on the hairless dog.

The goal of the repellent formulation endeavors conducted under this work unit is to incorporate toxicologically acceptable additives with established insect repellents to improve their performance: that is, reduce penetration into the skin, limit excessive evaporation, improve water wash resistance, improve user or cosmetic acceptability, etc. The formulations whose test results against mosquitoes are presented below incorporate additives (various types of CARBOSET and silicone polymers, mainly) which have been found useful in waterproofing various topical preparations.

With the development of the apparatus described in Study No. 2, Work Unit 066, for the measurement of repellent evaporation and penetration from excised skin, in vitro, as well as from in vitro procedures under development to evaluate the drying time, film thickness, film hardness (flexibility), and resistance to water wash off of formulations, the rational elimination of unacceptable formulations can be accomplished in the future before time-consuming animal and human testing is done.

- Testing of CARBOSET formulations on the hairless dog against Aedes aegypti mosquitoes.

Six different formulations of m-deet in combination with various CARBOSET polymers (C-526, C-525, C-514, and C-515) at repellent dosage levels of 0.5 and 0.32 mg/cm^2 were tested on the hairless dog model for duration of protection against Aedes aegypti mosquitoes. None of the CARBOSET-type formulations with m-deet decreased the duration of m-deet. One formulation (CARBOSET 515), based on the results of two separate tests, showed longer duration than other types of formulations

and unformulated m-deet. Tests were conducted on m-deet/CARBOSET-type formulations for a preliminary assessment of cosmetic properties. Component ratios of CARBOSET 526 to deet (maintaining the dose of repellent at 0.32 mg/cm^2) higher than 1:1 crack and peel from the surface, and are likely to be cosmetically unacceptable to humans.

- Testing of silicone formulations on the hairless dog against Aedes aegypti mosquitoes.

Eleven different formulations of m-deet, p-deet, and ethylhexanediol in combination with various silicone polymers (MDX 4-4142, OF-1-3593A, Dow Corning 200 fluid, 350 cs and 1000 cs) at repellent dosage levels of 0.5 and 0.32 mg/cm^2 were tested on the hairless dog model for duration protection against Aedes aegypti mosquitoes. None of these formulations increased the protection time of the repellents; however, the silicone formulations may provide increased wash resistance of the repellent, a factor to be addressed in FY 79.

- Miscellaneous formulations.

Six different formulations of the repellents m-deet, ethylhexandediol, and SRI-6 in combination with a latex and a fluorocarbon formulation at a repellent dosage level of 0.32 mg/cm^2 were tested for a duration of protection on the hairless dog. The latex formulations had unacceptably long drying times. None of the above formulations provided improvement over unformulated m-deet with regard to duration for protection.

CONCLUSIONS

1. On the basis of animal and in vitro repellency tests conducted by the Departments of Tropical Medicine and Dermatology Research, USDA cloth tests, and emerging toxicity tests, two candidate repellents are being advanced for human testing.

2. A variety of CARBOSET and silicone polymer formulations of m-deet and other insect repellents were tested for duration on the hairless dog. With the possible exception of the m-deet/CARBOSET 515 formulation, all formulations provided duration protection equal to or less than unformulated m-deet.

RECOMMENDATIONS

1. Mammalian toxicity tests for compounds 165-67 and 835-23A should proceed to allow volunteer testing.

2. Development of in vitro screening methodology for formulations, so that cosmetically unacceptable formulations can be eliminated early in the testing sequence, should be accomplished.

3. The hairless dog is a useful animal model for screening the repellent

repellent efficacy of formulations following suitable in vitro screening procedures.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OB 6912	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DRA&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: H.Termination	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. WORK UNIT: WA	8. CONTR/GRANT: NL	9. SPECIFIC DATA CONTRACTOR ACCESS: ☒ YES ☐ NO	10. LEVEL OF EFF: A. TYPE UNIT
11. AGENCY CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62772A	3M162772A810	OC	OC3			
b. CONTRIBUTING							
c. ESTIMATING	CARDS 114f						

12. TITLE: (U) Biochemical Studies on Prevention and Control of Skin Disease in Military Personnel

13. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 - Clinical Medicine

14. START DATE	15. ESTIMATED COMPLETION DATE	16. PERFORMING AGENCY	17. PERFORMER TYPE
68 07	78 10	DA	C. In-House

18. CONTRACT/GRANT: a. DATE/EFFECTIVE: b. NUMBER: c. TYPE: Not Applicable d. AMOUNT: e. KIND OF AWARD: f. CUM. AMT.

19. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR 78 (INCUMBENT)	3.0	83
FISCAL YEAR 79 (CURRENT)	0.0	00

20. RESPONSIBLE DOD ORGANIZATION
Name: Letterman Army Institute of Research
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Responsible Individual
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Telephone: (415) 561-3600

21. GENERAL USE: Foreign Intelligence Not Applicable

22. PERFORMING ORGANIZATION
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Social Security Account Number
Associate Investigators
Name:
Name: POC: DA

23. KEYWORDS: (U) Skin Disease; (U) Fungal Infection; (U) Biochemistry; (U) Protective Agents; (U) Environmental Conditions; (U) Antifungal Agents (U)Miconazole

24. TECHNICAL OBJECTIVE: (U) None of the allied armies in World War II correctly anticipated the large amount of disability caused by skin diseases. 20 years later, in Vietnam, extremely high rates of disability due to skin diseases focused command attention to dermatological problems. The probability for high rates of disability due to skin disease is just as high today as then, because the basic problems underlying combat related skin diseases have not been solved, nor are they studied by University researchers.

It is the purpose of this work unit to use biochemical, biophysical and other appropriate methods to develop effective preventive measures to reduce skin disease in soldiers under combat conditions.

25. APPROACH: (U) The project will focus on surface aspects of skin diseases. Efforts will be made (1) to develop or improve biochemical and biophysical methods, (2) to apply the methods to the study of bacterial and/or fungal infection and to causes of casualties such as trench foot or miliaria (which, by themselves, may not be incapacitating, but may be predisposing to infections and other skin diseases), and (3) to apply the evolved techniques to influence the retention and/or efficacy of insect repellents.

26. PROGRESS (Previous Work and Accomplishments): (U) 77 10 - 78 09. Work on the biological activity of skin lipids in relation to fungal disease continued. An electronic cell counting technique was perfected and antifungal agents such as miconazole, and skin lipid undecylenic acid were tested. Sodium choloride and urea at concentrations comparable to those on the skin surface were found to inhibit growth of *Trichophyton mentagrophytes*. Epidemiological aspects of fungal disease in relation to potential military dermatological problems in Europe and other countries were studied and it is suggested that the dermatopathogenicity of a number of fungi should be studied. For administrative reasons, this work unit has been terminated and the research incorporated within Work Unit No. 006.

27. PUBLICATIONS (Publications resulting from this effort):

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498A, NOV 68 AND 1498-1, 1 NOV 68 IF FOR ARMY USE: ARE OBSOLETE. 118

ABSTRACT

PROJECT NO. 3M162172A810 — Military Skin Diseases

WORK UNIT NO. 004 — Biochemical Studies on Prevention and Control of Skin Disease in Military Personnel

The following investigations have been conducted under this work unit:

STUDY NO. 1 Biological activity of human skin lipids in relation to fungal disease

EX-2 Inhibition of Trichophyton mentagrophytes by chloride ions. An ancillary finding relevant to skin infections in Vietnam

EX-3 Influence of urea on the growth of Trichophyton mentagrophytes

EX-4 Application of the electronic cell counting method to the testing of antifungal agents and skin lipids

STUDY NO. 3 Dermatological problems of the soldier during World War II as documented in confiscated German documents

STUDY NO. 4 Epidemiological aspects of fungal disease in relation to potential military dermatological problems in Europe and other countries

STUDY NO. 1, EX-2 Sodium chloride at concentrations likely to be found on the skin completely inhibits Trichophyton mentagrophytes.

STUDY NO. 1, EX-3 Urea within the range of concentrations likely to be found on the skin either can stimulate or completely inhibit growth of Trichophyton mentagrophytes.

STUDY NO. 1, EX-4 Conditions to recognize artifacts and to count and size spores of Trichophyton mentagrophytes have been worked out. The new method has been applied to a detailed study of the kinetics of growth of control cultures and cultures in the presence of miconazole, griseofulvin, undecylenic acid and skin lipid.

STUDY NO. 3 Confiscated documents (a total of 9,672 pages) from the "German Oberkomando der Wehrmacht" from World War II were scanned for relevant information on such topics as dermatology, chemical warfare, and repellents to skin insects. Over 500 pages have been identified for further evaluation.

STUDY NO. 4 A zoophilic variety of Trichophyton mentagrophytes, a strain not ordinarily isolated in the United States, caused epidemic inflammatory dermatosis in Vietnam. It is likely that some of the fungal pathogens now isolated in Eastern and Central Europe may cause significant problems in troops in a potential future conflict.

It is recommended that lessons learned in Study No. 3 and Study No. 4 be used to design and test preventive strategies for the benefit of combat troops.

BODY OF REPORT

WORK UNIT NO. 004	Biochemical Studies on Prevention and Control of Skin Disease in Military Personnel
STUDY NO. 1	Biological activity of human skin lipids in relation to fungal disease
EX-2	Inhibition of Trichophyton mentagrophytes by chloride ions. An interesting ancillary finding relevant to skin infections in Vietnam.

PROBLEM:

In preliminary studies in FY 77, two different media were used to grow Trichophyton mentagrophytes. It was noted that significant amounts of acid, in particular, hydrochloric, were needed to adjust the pH of the medium to 6.5. The data suggested that chloride ions might inhibit germination of spores or development of hyphae. If this inhibition of fungal growth due to chloride could be substantiated, an explanation for a puzzling and costly problem which occurred during the Vietnam war, might be found. That is, in 1967 and 1968, combat commanders reported a large number of men unavailable for duty after they had spent extensive periods of time fighting in inundated areas. It was noted that a marked difference in the prevalence and severity of cutaneous infections existed between combat and support troops. The problem was partially corrected, but not prevented, by issuing Regulation 40-29, which reads in part:

"Preventive measure: Limiting the duration of operation in watery terrain. The tactical situation permitting, a 48 hr limit should be placed on operations involving continuous exposure to water. If this is not possible, casualties from fungus infection may be disabled for 2 or more days after a five-day operation."

These continued observations lead to the testing of the hypothesis that chloride concentrations on the skin surface are not sufficiently high to inhibit dermatophytes to grow.

RESULTS AND DISCUSSION OF RESULTS

The hypothesis that chlorides inhibit growth of dermatophytes was tested in vitro. Spores of T. mentagrophytes, deposited on the surface of purified agar containing mainly bacteriological peptones

and 0.5 mg/ml sodium chloride, produce well-defined colonies. With increasing concentrations of sodium chloride, fewer and fewer colonies were formed. At 7.5 mg/ml a significant reduction in the number of colonies, as well as a reduction in the diameter of individual colonies was observed. Above 24 mg/ml, no colonies were formed, even after extended periods of time. The chloride effect was reproducible.

Under conditions of profuse sweating, the sodium chloride concentration ranges from 2 mg/ml to 6 mg/ml. However, due to evaporation of water, the concentration at the skin surface should be considerably higher. It is, thus, quite likely that the sodium chloride concentration on the skin surface of support troops in Vietnam was high enough to prevent dermatophytosis. The concentration on the skin surface of combat troops was obviously low because of constant contact with water over large areas of the body.

CONCLUSIONS

Sodium chloride in low concentrations (0.75%) partially inhibits, in higher concentrations (2.5%) completely inhibits, T. mentagrophytes. This observation may explain, in part, the relative low incidence of dermatophytosis in support troops and the high incidence of dermatophytosis in combat troops in Vietnam.

RECOMMENDATIONS

The in vitro finding that rather low concentrations of sodium chloride are able to inhibit T. mentagrophytes should be tested in a suitable animal, such as a mini-pig.

PUBLICATIONS

None

EX-3 — Influence of urea on the growth of Trichophyton mentagrophytes

PROBLEM

Severity of dermatophytosis in combat troops in Vietnam was associated with prolonged exposure of the skin of soldiers to water. It was suggested that heat and humidity were not the only important environmental factors contributing to the epidemic. The reasons for the epidemic have not been explained satisfactorily, however.

It is expected that extensive exposure of skin to water would result in removal of water soluble compounds such as urea from the skin.

It has been shown that skin urea is normally deposited in substantial amounts on the skin surface, even without sweat secretion, and the antimicrobial properties of urea have been known for centuries. It has also been shown in this laboratory that lipids extracted from feet of patients with fungal disease contain statistically significantly lower quantities of urea.

It was therefore hypothesized that urea deposited on the skin surface may be a controlling factor in dermatophyte infections. In vitro testing of this hypothesis may shed some light on control of fungal disease.

RESULTS AND DISCUSSION OF RESULTS

Spores of Trichophyton mentagrophytes deposited on the surface of purified agar containing mainly bacteriological pentones produced well-defined colonies at 30 C. No inhibition of the formation of visible colonies was observed when urea was added to the medium at a concentration of 10 mg/ml. At 20 mg/ml a delay of 2 days in the onset of growth was observed. This delay increased to 6 days at 30 mg/ml. At 50 mg/ml or higher, no colonies were observed even after 40 days. Urea thus produces an inhibitory effect on growth of spores of T. mentagrophytes which in some way can be overcome by spores provided the concentration is not very high. It is known that many, but not all, strains of T. mentagrophytes give a urease positive test, i.e. that they hydrolize urea. In contrast, most strains of T. rubrum are usually urease negative. At this time it is not known if the strain of T. mentagrophytes which was used in these experiments, and which was isolated in Vietnam, was urease positive. It is also not known if and at which concentration, strains of T. rubrum are inhibited by urea. A direct comparison of the urea concentration (mg/ml) in agar medium with the surface concentration (mg/cm^2) on the skin cannot be made; nevertheless, model calculations suggest that urea concentrations which were inhibitory in the in vitro assay are comparable to those observed in skin lipids of volunteers.

CONCLUSIONS

This in vitro study demonstrates conclusively that urea at relatively low concentrations can inhibit growth of a Vietnam strain of Trichophyton mentagrophytes. It is, thus, quite reasonable to postulate that small molecular weight molecules from sweat glands, sebaceous glands, or the epidermis may have a significant controlling effect on the growth of dermatophytes on the skin. These in vitro observations may further explain the epidemic outbreak of T. mentagrophytes infections in combat, but not in support troops in Vietnam.

RECOMMENDATIONS

1. In view of the low toxicity of urea, the role of this agent in the control of the growth of dermatophytes should be further explored.

2. In vitro tests should be extended to other dermatophytes which are significant pathogens in Europe, the Near East and, perhaps, Africa.

3. Model experiments on mini-pigs should be initiated to confirm in vitro experiments.

4. Commercial products, such as "Aquacare/HP" the skin lotion made by Herbert, should be tested as inexpensive nontoxic preventive agents against dermatophyte infections.

PUBLICATIONS

None

EX-4 Application of the electronic cell counting method to the testing of antifungal agents and skin lipids

PROBLEM

The activity of antimicotics currently in use is limited against a well-defined group of dermatophytes. While several compounds, such as undecylenic acid, have been used topically, only griseofulvin can be administered orally at present. Although griseofulvin was used prophylactically in Vietnam, it was later found that it may do no more than delay the outbreak of a fungal infection, that fungi are able to detoxify the medication, and furthermore that yeast infection cannot be controlled by griseofulvin.

Evaluation of more effective and safe antifungal agents to treat formulation of better prophylactic measures, and more rapid evaluation of susceptibility testing of fungi are still important objectives in military medical research.

RESULTS AND DISCUSSION OF RESULTS

Preliminary results of the shake culture method, which makes use of the Coulter Counter ZB, Channelizer, and Teletype system were reported in FY 77. Several additional experiments were required to clarify a number of questions. For example, signal amplification had to be studied so it could be optimized and so that contaminating

particles and bacteria (if present) are not interfering with spore counts and size distribution measurements.

The influence of particle density on count and size distribution was also evaluated. It was shown that above a count rate of 25,000, size distributions become distorted, presumably due to formation of aggregates. Reproducibility was checked; it is excellent. Multiple measurements over extended periods of time in samples where growth was inhibited indicated that size distributions were stable and highly reproducible.

In earlier experiments, spore preparations were made with 0.9% sodium chloride solutions. Subsequently, it was shown that sodium chloride inhibits the formation of colonies of Trichophyton mentagrophytes. For this reason, an experiment was done in which part of a culture was dispersed in Tween 80 and the other part in 0.9% NaCl. The results indicate that there is no significant difference between the two spore preparations, that development of hyphae is similar, and that changes in size distribution with time are similar.

Studies with Control Cultures

Phase contrast photomicrography revealed that an extensive network of hyphae was produced over a period of 24 hours. During this period growing spores utilized the aminoacids from peptones. As a result, the pH of the medium increased above 8. As spores germinated and increased in size beyond the upper threshold setting, they were no longer counted. As a result, spore counts decreased exponentially with a half life of about 10h. Early events of germination were seen in a 50% increase in size of the larger spores over a period of about 7 h. Thereafter, size distributions shift towards smaller sizes.

Studies with griseofulvin

Visual observations of liquid shake cultures indicated that after 24 h some spores were enlarged, and that some had germinated and formed short hyphae. The changes in size distribution over the period of 24 h were small and amounted to about 15% for the larger spores. Since the culture medium contains no antibiotics, multiple sampling in the presence of griseofulvin occasionally resulted in overgrowth with bacteria. This could be easily detected by a large increase in counts and a concomitant shift in the size distribution towards smaller particles.

Studies with Undecylenic acid

Visual observation of liquid shake cultures after 24 and 50 h indicated no growth of mycelia in the undecylenic acid treated

cultures. Phase contrast microscopy indicated that no spores had germinated, and that no hyphae could be observed. Over a period of 24 h the pH of undecylenic acid treated cultures decreased by over 1 unit and became acid. Size distributions indicated that larger spores decreased exponentially. The decrease was of the order of 40% in the first 10 h.

Studies with Skin lipids

A detailed kinetic analysis of counts and size distributions suggests the following:

Overall, the percentage of spores that produce hyphae and the speed at which they are produced are approximately the same for skin lipid treated and control cultures.

It appears that skin lipids decrease the time necessary for growth of spores before hyphae begin to grow; this observation suggests that germination is facilitated by the skin lipids. This could be due to the lipids acting on nutrients.

The rate of growth of hyphae appears significantly lower for skin lipid treated cultures than for control cultures; this observation suggests that skin lipids inhibit hyphal growth.

Studies with miconazole

Phase contrast photomicrography revealed that miconazole stops the formation of hyphae. Duration inhibition is long; no evidence for germination could be found. During incubation, spores in the presence of miconazole produced acidic metabolites. This resulted in lowering of the pH in contrast to control cultures, where the pH always increased. Although larger spores in the presence of miconazole did not form hyphae, they increase by 80% over a period of approximately 28 h. No bacterial overgrowth in the presence of miconazole was ever detected.

CONCLUSIONS

Size distribution studies on spores of Trichophyton mentagrophytes can be used effectively to monitor germination and formulation of hyphae. The time required for such studies appears considerably shorter than conventional assays, such as those used for drug susceptibility testing. The method is applicable to study inhibitors during the early phases of development of spores of dermatophytes and possibly for yeasts. It can effectively replace more time-consuming methods, such as the usual turbidimetric methods.

RECOMMENDATIONS

The Coulter counter method should be used to evaluate the efficacy of new antifungal agents. It should be used to detect inhibitors and growth promoting agents which have been suggested in skin lipids. It should be used in susceptibility testing of fungi (prevalent in potential war theaters) to currently available and newer more effective antifungal agents.

PUBLICATIONS

None

STUDY NO. 3 — Dermatological problems of the soldier during World War II as documented in confiscated German documents

PROBLEM

American records of militarily relevant dermatological problems during World War II are rather sketchy. As reported in FY 1977, preliminary screening of confiscated German documents of the Oberkommando der Wehrmacht identified 9 microfilms. These films were purchased in FY 78 from the National Archives in Washington D.C. for the purpose of analyzing German documentation from World War II with regard to relevance of potential dermatological problems of the U.S. soldier; and, possibly, for providing a basis for planning dermatological counter measures in order to maintain a healthy force structure in Central Europe. The study is done in two phases. In the first phase, reported here, the microfilms are scanned for relevant dermatological information of W.W. II documents.

RESULTS AND DISCUSSION OF RESULTS

In scanning the confiscated German war documents, it became obvious that the microfilms were films of everything as it would be found in a file cabinet. For this reason, each film had to be scanned in its entirety; unimportant material was mixed in with highly relevant documents. Consequently more time was required than expected. Scanning of the films involved assessment of relevance of 9,672 pages. From these pages, 563 were printed and identified for further evaluation. Most of the material is in the form of short, typed memoranda, reports, etc. The contents give a tremendous amount of insight into the medical aspects of conducting war in Russia, North Africa, Eastern Europe, North Africa, and Western Europe. Secret documents of a more general nature relating to medical statistics and tactics, regulations on treatment of diseases at

various levels, i.e., company aidmen or physicians at the division level, as well as organizational documents of the medical corps were identified. Extensive files were maintained by the German HQ on foreign intelligence with regards to military medicine; for instance, a German translation of a Russian document on chemical warfare agents was found. A total of 156 pages were identified which deal with specific dermatological and venerological topics. Much of that material is in the form of reports by consultants who dealt with dermatological and venereal disease problems. One hundred twenty-seven pages were identified which relate to "chemical warfare" decontamination measures and regulations. Many documents relate to infectious disease and 63 pages were identified which, in one form or other, relate to mission requirements of the Department of Dermatology and Tropical Medicine. Sixty-nine pages relate to "repellents" and measures related to the prevention of epidemics due to Sarcoptes Scabei, mosquitoes, Rickettsiae, and other disease organisms that vary in different countries.

CONCLUSIONS

Five hundred and sixty-three selected pages of confiscated documents from the HQ of the German Army constitute a valuable source of information for identifying potential medical problems for US Army forces in the European and Mediterranean theater.

RECOMMENDATIONS

Evaluation of identified German War documents should begin in Phase II of this Study Protocol.

PUBLICATIONS

None

STUDY NO. 4 — Epidemiological aspects of fungal disease in relation to potential military-dermatological problems in Europe and other countries

PROBLEM

Inadequate documentation of the etiological agents that cause skin disease due to fungi makes it almost impossible to estimate man-days lost in U.S. troops during peace-time and makes it equally difficult to predict risks in potential future conflicts in Europe or other theaters.

Will Trichophyton mentagrophytes, which caused major outbreaks of skin disease in Vietnam, be a problem for US troops in Europe, or other geographical areas, in a potential conflict? Will other etiological agents, such as Trichophyton violaceum be a problem with U.S. troops, since it is known to have a relatively well defined geographic area in Eastern Europe and the USSR?

Information on the occurrence and prevalence of many skin pathogens in foreign countries is sketchy in the American medico-mycological literature, and it appears that the foreign literature has not been evaluated at all. It also appears that no analysis of the type and frequency of skin pathogens in Eastern Europe and other countries has been made in recent years. As a result, the potential impact of these skin pathogens on U.S. soldiers is largely unknown. For this reason, a thorough review and analysis of the foreign literature is important. The review and analysis is also necessary in order to select and test newer antifungal agents so that they can be made available to military physicians and company aidmen, and to design and test up-to-date and effective counter measures and prophylactic strategies for U.S. troops.

RESULTS AND DISCUSSION OF RESULTS

An extensive review of reports published in the German journal "Mykosen" was made on epidemiological studies in Eastern Europe, Western Europe, the Middle East, and Africa. It was learned that throughout Central and Eastern Europe extensive changes in the type of fungal pathogens have occurred over the past 25 years. In particular, it was found that much more virulent strains of Trichophyton rubrum have replaced more benign strains of T. mentagrophytes. Extensive surveys by the East German commission on experimental mycology reveal that T. rubrum increased from 59.2% in the period 1955 to 1966 to 73.9% in the period of 1967 to 1971. During the same time interval T. mentagrophytes decreased from 26.2% to 19.7%. It appears that in most European countries the frequency of T. rubrum is now above 70%. A German military study revealed that 24.5% of soldiers suffered from dermatophyte infections attacking mainly the foot and groin. No similar study exists for U.S. troops in Europe, and thus, figures can only be compared with a recent U.S. study indicating dermatophyte prevalence of 87.7 per 1000 male persons. Other studies from East Germany and Poland suggest that the frequency of T. verrucosum, T. violaceum, T. tonsurans and E. floccosum can be significant depending on the geographic region. Although T. soudanense is quite common in Nigeria, Cameroons, and other countries of West and East Africa (Somalia) it is also found occasionally in Eastern Europe. It has not caused any epidemics as of to date. M. audouinii, which was identified in 40.4% of dermatophyte infections in USSR during World War II , had declined to 0.04% in the period of 1956-1968. It may, however, not be safe to conclude that dermatophytes which

are now rare in European populations may not pose a significant potential risk to U.S. troops.

Yeast infections due to Candida albicans appear to have increased in number and in severity in many parts of the world. A mycological study on Polish railroad workers indicates a 36.8% infection rate with yeasts (mainly Candida) against 12.5% with dermatophytes. A random sample of patients attending an outpatient clinic in Egypt were tested for maceration of toe webs. Yeasts of the genus Candida were isolated from 64% and only 4.6% had dermatophytes. The fact that Candida constituted the major causative agent explained the disappointing result with griseofulvin therapy.

CONCLUSIONS

Whereas a zoophilic variety of Trichophyton mentagrophytes, a strain not ordinarily isolated in the United States, caused epidemic inflammatory dermatophytosis among U.S. combat forces in Vietnam, the review of available literature of Eastern Europe, Middle East, and Africa suggests that other fungal pathogens may cause significant potential problems to U.S. combat forces. Potential problems due to these pathogens have not been assessed, and the virulence and infectivity of these pathogens have not been studied. Newer medications, available at present or in various stages of development, have not been tested as to their efficacy vis-à-vis these pathogens.

RECOMMENDATIONS

A review of the literature on new antifungal agents should be made with particular emphasis on pathogens that may be a real or potential threat to the combat effectiveness of U.S. soldiers in Europe, the Middle East and Africa.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

				DA OB 6915	78 10 01			DD-DR&E(AR)636
77 10 01	D. Change	U	U	NA	NL	YES	NO	
	62772A	3M162772A810		00	006			
	CARDS 1147							

(U) Skin Diseases Among Soldiers

003500 - Clinical Medicine

68 01	CONT	DA	C. In-House

	FISCAL YEAR 78	2.0	20
	FISCAL YEAR 79	2.0	40

Not Applicable

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Foreign Intelligence Not Applicable

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POC: DA

(U) Human Volunteers; (U) Occupation; (U) Diagnosis; (U) Skin; (U) Survey; (U) Soldiers; (U) Morbidity

23. (U) The objectives are to determine the type and frequency of potentially disabling skin diseases among soldiers in various environments, to conduct trials of potential preventive and therapeutic agents against the common disabling dermatoses that afflict military personnel, and to develop or improve methods of studying militarily relevant skin diseases under field conditions.

24. (U) Conduct a 3-year study of all outpatient visit diagnoses at the 4 Army dermatological centers; determine prevelence of contact sensitivities to materials that may produce contact dermatitis in soldiers; develop and test performance characteristics of new techniques for diagnosing skin diseases in the field.

25. (U) 77 10 - 78 09. Analysis was completed and a technical report prepared for data from outpatient visits to dermatological centers at Fitzsimmons, Letterman, Brooke and Walter Reed Army Medical Centers during FY 1973, 1974 and 1976. Patch tests to determine sensitivities to neomycin, benzocaine, nickel and ethylenediamine were performed on 1158 paid adult volunteers from a general population and the data have been completely analyzed.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498-1 (NOV 72) AND 1498-1 (MAR 80) (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M762782A810 Military Skin Disease

WORK UNIT NO. 006 Skin Diseases Among Soldiers

The following investigations have been conducted under this work unit:

STUDY NO. 1 Skin diseases among soldiers

STUDY NO. 10 Contact sensitivity to nickel, neomycin, ethylenediamine, and benzocaine in a general population: relationships between age, sex, history of exposure, and reactivity to standard patch test

STUDY NO. 1 A 3-year survey of all outpatient visit diagnoses at the 4 Army dermatological centers was completed, the diseases tabulated, and the 50 most common diagnoses have been stratified as to the hospital, and the patients as to age, race, sex, and military status. A technical report containing the data is in its third draft. When published, readers can ascertain what is contained in the computer data base and can order special analyses. Except for miliaria and water immersion foot syndromes, soldiers in peacetime suffer the 12 most common diseases as do soldiers in combat.

STUDY NO. 10 The prevalence of contact sensitivity to benzocaine, ethylenediamine, neomycin, and nickel was determined in a general population of 1158 adults. The prevalence and 95% confidence limits of of positive patch tests were for nickel 5.8% (women, 7.0% to 11.4%; men 0.24% to 2.2%), neomycin 1.1% (0.55% to 1.8%), ethylenediamine 0.43% (0.13% to 1%), and benzocaine 0.17% (0.02% to 0.64%). Except for nickel, patch test reactivity was unaffected by age, sex, race, or occupation. These four substances pose no significant threat toward producing contact dermatitis in soldiers.

BODY OF REPORT

WORK UNIT NO. 006 Skin Diseases Among Soldiers

STUDY NO. 1 Skin diseases among soldiers

PROBLEM

Ninety-five percent of dermatological patients are treated as outpatients, which is the American tradition of trying not to hospitalize patients with skin diseases. The Army efficiently collects elaborate data on diagnosis, treatment, and disposition on hospitalized patients, but little demographic data are saved on outpatients. Little data are available in the literature on the skin diseases on the soldiers, on the incidence, morbidity, disposition, and frequency of dermatological diagnoses in the military dermatology clinics. A computer supported dermatological outpatient data system was implemented to provide the physician and the administrator with information concerning the (1) outpatient load, (2) diagnostic and therapeutic problems encountered, (3) need for varying types of supporting personnel, (4) medical equipment and drug requirements, and (5) disposition of patients. All outpatients attending the dermatology clinics at Brooke, Fitzsimmons, Letterman, and Walter Reed Army Medical Centers completed an outpatient survey card for each visit and entered demographic information; the physician entered the diagnosis, initial or return visit, body area involved, special procedures, and an estimate of the time lost from duty because of the visit and the disease. Data were collected for FY 1973, 1974, and 1976. All cards were returned to LAIR where they were coded, key punched, and entered into a computer. Information was retrieved at monthly, quarterly, and yearly intervals. A compilation for the 3 fiscal years has been made of the frequency distribution of all diagnoses by initial and return visits, and by active duty and other personnel. The 54 most frequent diagnoses have been stratified by diagnosis, hospital, age groups, race, sex, initial visits, and military status (active duty, retired, and dependents).

RESULTS AND DISCUSSION OF RESULTS

The survey revealed 146,395 initial visits, 197,550 total visits, and 51,155 return visits. Since dermatologists are often kidded about never getting their patients well, the low number of return visits was checked by two independent methods, and so it is reasonably accurate: 82.9%, one visit, 11.6%, two visits, 5.1%, three to six visits, and 0.4%, seven or more visits. Compliance for filling out cards on each outpatient was 97%. Of first visits, active duty personnel accounted for 16.2% retired military personnel 24.4%, and dependents of military personnel 58.8%. Age grouping revealed the following: 1 day-2 years, 2.3%; 2-11 years, 5.4%; 12-17 years, 13%; 18-30 years, 29.3%; 31-45 years, 14.5%; 46-60 years, 20.4%; and over 61 years, 15%. Males totaled 47.1%; females, 52.9%; blacks, 10.4%; whites, 83.5%; orientals 2.5%; and other races, 3.5%.

The table lists the frequency of the 50 most common diseases for all patients. The 10 most frequent diagnoses among active duty military personnel in a temperate zone during peacetime are as follows: acne vulgaris (13.8%), warts (8.5%), pseudofolliculitis of the beard (5.3%), dermatophilic fungal infections (4.8%), allergic contact dermatitis (4.1%), seborrheic dermatitis (3.9%), no diagnosis made at time of visit (2.9%), nevi (moles) (2.7%), venereal diseases (2.7%), and dermatitis of unknown etiology (2.6%). The Army's experience in World War II and Vietnam revealed dermatophytosis, pyoderma, miliaria, immersion foot syndromes, allergic contact dermatitis, insect infestations and bites, warts, and over-treatment dermatitis to be the common causes for large man-day losses among combat and support troops. In those wars, common causes for hospitalization included pyoderma, infected eczematoid dermatitis, acute dermatitis, cystic acne, cellulitis, and urticaria. So there is considerable overlap among common skin diseases seen in soldiers in peacetime and wartime. Miliaria and immersion foot syndrome occur in restricted geographical areas. Fortunately, better topical and systemic medicaments and trained dermatologists have almost eliminated over-treatment dermatitis as a cause of morbidity. The soldier lost an average of 2.2 h for each outpatient visit, from leaving his unit to leaving the medical facility.

CONCLUSIONS

A 3-year proportional rate survey of all dermatology outpatient visit diagnoses at 4 Army dermatological training centers has been completed and the data tabulated. Data on the 54 most frequent skin diseases have been stratified as to age, race, sex, hospital, and military status. A technical report presenting the data is in its third draft. These data will be most helpful for planning, teaching, and management purposes for the military, federal agencies, and civilian groups, since much of the information is not presently available.

RECOMMENDATIONS

When the technical report is finished and published, readers will become acquainted with what is in the data base, and analyses can be made according to their wants and needs. Two training centers have published two articles already from the data. Selected findings from the technical report will be published in the medical literature.

TABLE

INITIAL VISITS ALL PATIENTS N= 129,275

RANK	DIAGNOSIS	NUMBER	%
1.	Acne vulgaris	19,573	15.1
2.	Verruca vulgaris	9,119	7.1
3.	Actinic keratoses	7,019	5.4
4.	Seborrheic keratoses	5,015	3.9
5.	Seborrheic dermatitis	4,995	3.9
6.	Allergic contact dermatitis	4,741	3.7
7.	Nevi (moles)	4,250	3.3
8.	Dermatitis, unknown etiology	4,107	3.2
9.	Basal cell carcinoma	3,942	3.0
10.	No diagnosis made	3,529	2.7
11.	Atopic dermatitis	3,529	2.7
12.	Asteatosis	3,013	2.1
13.	Psoriasis (all)	2,690	2.1
14.	Pyoderma (staph/strep)	2,326	1.8
15.	Tinea pedis	1,854	1.4
16.	Dyshidrosis	1,848	1.4
17.	Cysts, epidermal, sebaceous	1,768	1.4
18.	Pseudofolliculitis barbae	1,762	1.4
19.	Tinea, other	1,695	1.3
20.	Lichen simplex chronicus	1,618	1.3
21.	Primary irritant contact dermatitis	1,568	1.2
22.	Herpes simplex, all	1,449	1.1
23.	Venereal diseases, all	1,372	1.1
24.	Skintag, acrochordon	1,233	1.0
25.	Plantar warts	1,170	0.9
26.	Acne rosacea	1,160	0.9
27.	Urticaria, all	1,138	0.9
28.	Tinea versicolor	1,116	0.9
29.	Pityriasis, rosea	1,051	0.8
30.	Precancerous skin lesions	893	0.8
31.	Nummular eczema	840	0.6
32.	Tinea cruris	695	0.5
33.	Insect bites	672	0.5
34.	Venereal warts	652	0.5
35.	Skin cancer, other	617	0.5
36.	Herpes zoster	595	0.5
37.	Dermatofibroma	565	0.5
38.	Alopecia areata	538	0.4
39.	Scabies	530	0.4
40.	Candidiasis	549	0.4

TABLE concluded

RANK	DIAGNOSIS	NUMBER	%
41.	Alopecia, unknown etiology	480	0.4
42.	Periorbital dermatitis	480	0.4
43.	Pruritus	451	0.3
44.	Lichen planus	404	0.3
45.	Pityriasis alba	384	0.3
46.	Squamous cell carcinoma	370	0.3
47.	Drug eruption	360	0.3
48.	Keloid	348	0.3
49.	Discoid lupus erythema	320	0.2
50.	Vitiligo	308	0.2

PUBLICATIONS

None

STUDY NO. 10

Contact sensitivity to nickel, neomycin, ethylenediamine, and benzocaine in a general population: relationships between age, sex, history of exposure, and reactivity to standard patch test

PROBLEM

A recent report on the epidemiology of contact dermatitis has been widely quoted and misinterpreted by many physicians and some persons in regulatory agencies. Some have assumed that a certain percent of the American adult population are allergic to nickel (11%), ethylenediamine (7%), neomycin (6%), and benzocaine (5%).

Soldiers may come in contact with all four of these common antigens. Neomycin seems to reduce the infection rate of minor skin injuries and is widely used and accepted by military physicians. Impetigo and pyoderma are common military medical problems for which neomycin is used. Pretreatment with neomycin appears to prevent the development of miliaria rubra. Miliaria and post-miliarial hypohidrosis are well-documented common causes of military disability. Dog tags and their chains, stainless steel watch bands, and the snaps on boxer shorts contain nickel. Ethylenediamine is used as a preservative in some widely used topical antimicrobial formulations. Over 70 tons of benzocaine are used each year in the U. S. as a topical or mucosal anesthetic. This study was designed to determine the true prevalence of neomycin, nickel, ethylenediamine, and benzocaine sensitivity in a general population.

RESULTS AND DISCUSSION OF RESULTS

The data have been completely analyzed from 1158 paid adult volunteers from a general population who were patch tested in the currently standard manner to 20% neomycin sulfate, 5% benzocaine, 2.5% nickel sulfate, and 1% ethylenediamine. The patch test (AL test) was removed at 48 h and the sites were read at 5 days. The prevalence of the positive reactions and 95% confidence limits of these positive patch tests were for nickel 5.8% (0.24% to 11.4%); neomycin , 1.1% (0.55% to 1.8%); ethylenediamine, 0.43% (0.13% to 1%); and benzocaine, 0.17% (0.20% to 0.64%). Nine percent of women (7.0% to 11.4%) were allergic to nickel compared to 0.9% of men (0.24% to 2.2%). Nickel sensitivity has a strong correlation with a history of pierced ears, earlobe rash, and jewelry rash. Ten of 12 neomycin positive subjects had used neomycin for one week or longer on an inflammatory dermatosis as compared with 6 of 36 age, race, and sex match controls. This was highly significant ($P<0.001$) and yielded a relative risk of 13.

By history, 85% had been exposed to benzocaine, 48% to neomycin, and 15% had used Mycolog cream. All had been exposed to nickel. No definite age related patterns of exposure or patch test reactivity were found. Women had higher rates of exposure by history than men for all antigens tested. Only nickel showed a striking difference by sex. Age and sex adjusted rates of patch test reactivity for those employed by a medical institution (466 subjects) differed little from those with a non-medical occupation (692 subjects). For each contactant, subjects with a positive history of exposure had higher rates of patch test reactivity than those with a negative history. With respect to neomycin sensitivity 7 of 8 subjects with strong positive neomycin patch tests had positive use-tests when neosporin cream or ointment was applied. However, only 2 of 4 subjects with a weakly positive neomycin patch test had a positive use-test. The reactions by and large were mild in the use tests. Twenty-one applications of neosporin G cream or neosporin ointment over 7 days produced isolated pruritic papules. Neosporin ointment produced less intense confluent dermatitis than neosporin G cream.

Day 5 is the best time to read patch tests, at least with these 4 antigens; i.e., significant positive tests on day 5 had far more clinical relevance than those on day 2. Weak positive tests on the second day may become negative by day 5. We reclassified the old criteria of positive vs negative tests. We determined that a weak positive reaction has an induration of less than 75% of the patch test site and a strong positive has an induration of greater than 75% of the patch test site.

When more than one test is positive on a given individual, each test should be repeated on separate occasions. A strong positive reaction can greatly influence the outcome of a weaker or false-positive test. Spurious false-positive reactions may result from this phenomenon, known as the "angry-back" response.

CONCLUSIONS

This is the first epidemiologic study done where patch tests were performed in a large group of volunteers in a general population. Important findings in this study show that the prevalence of neomycin sensitivity in a general population is approximately 1%, far lower than the 6% figure reported from a selected patient population with contact dermatitis. When subjected to a use-test with drugs containing neomycin, 3 of 12 subjects allergic to neomycin applied the antibiotic 21 times in 7 days without any reaction. The other 9 subjects suffered a minimal dermatitis from repeated use with no severe reactions. We conclude that topical formulations containing neomycin, when used for minor burns and injuries, pose a small risk to the consumer. Superficial bacterial infections are common among soldiers, and topical antibiotics containing neomycin are frequently used. Benzocaine and ethylenediamine products would appear to cause little, if any, risk to the soldier. Nickel seems to have little military significance although it is impossible not to come in contact with nickel daily, since nickel is present in at least trace quantities in almost all metals. Benzocaine, ethylenediamine, neomycin, and nickel pose no significant threat for producing epidemics of allergic contact dermatitis in soldiers.

RECOMMENDATIONS

None

PUBLICATIONS

1. PRYSTOWSKY, S.D., A.M. ALLEN, R.W. SMITH, J.H. NONOMURA, R.B. ODOM, M.J. BUDNIK, and W.A. AKERS. Allergic contact hypersensitivity to nickel, neomycin, ethylenediamine and benzocaine in a general population: Relationships between age, sex, history of exposure, and reactivity to standard patch tests and use tests. (Submitted to Publications Review Committee, LAIR)

2. PRYSTOWSKY, S.D., J.H. NONOMURA, R.W. SMITH, and A.M. ALLEN. Allergic hypersensitivity to neomycin: Relationships between patch test reactions and "use tests." (Submitted to Publications Review Committee, LAIR)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6095	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: H.Termination	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. GEOGRAPHY: NA	8A. GROUP INTER: NL	8B. SPECIFIC DATA: CONTRACTOR ACCESS ☐ YES ☐ NO	9. LEVEL OF EFF: A. WORK UNIT

10 MD /CODES:	PROGRAM EL. ENCT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
A. PRIMARY	62772A	3M162772A810	00	010
B. CONTRIBUTING				
XXXXXXXXX	CARDS 1141			

11. TITLE (Procede with security classification code)

(U) Microbial Interactions on Healthy and Infected Skin of Soldiers

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 - Microbiology, 003500 - Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMING METHOD
75 04	78 10	DA			C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: — EXPIRATION:
b. NUMBER:
c. TYPE: Not Applicable — d. AMOUNT:
e. KIND OF AWARD: — f. CUM. AMT.

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDING (In Thousands)
FISCAL YEAR 78	2.2	76
FISCAL YEAR 79	0.0	00

19. PERFORMING ORGANIZATION

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RESPONSIBLE INDIVIDUAL
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20. GENERAL USE

Foreign Intelligence Not Applicable

21. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Provide last name, first name, middle initial)
NAME: Keith, William, Jr., Ph.D.,CPT,MS
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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Bibel, D.Jan, Ph.D., DAC
NAME: POC: DA

22. KEYWORDS (Provide at least three keywords or phrases separated by semicolons)

(U) Normal Flora; (U) Skin Ecology; (U) Microbiology; (U) Human Skin; (U) Human Volunteers; (U) Pyoderma; (U) Animal Models

23. TECHNICAL OBJECTIVE

23. (U) One objective is to study bacterial skin infections (pyoderma disease that can occur in epidemic proportions among combat personnel) with the use of animal models in an effort to develop prophylactic measures against pyoderma which are presently not available. The role of normal cutaneous flora in host resistance against microbes causing epidemic skin infections in soldiers will be investigated.

24. APPROACH

24. (U) Bacteria isolated from active lesions will be used in these studies. Animal models will be developed to determine the pathogenesis, prevention, and prophylaxis of pyoderma. By identifying and quantitating normal cutaneous microorganisms on healthy and infected skin of soldiers, topical bacterioprophylactic measures will be developed to augment natural resistance.

25. PROGRESS (Previous paragraphs identified by number)

25. (U) 77 10 - 78 09. In support of insect repellent studies, the normal flora of human skin has been determined. Experimental skin infections in hamsters and guinea pigs have been produced. Hamsters are more susceptible than guinea pigs to intradermally injected Group-A-beta-hemolytic streptococci or *Staphylococcus aureus*. The effect of minor trauma simulating that experienced by combat soldiers on *Staphylococcus aureus* or Group-A-beta-hemolytic streptococci infection has been determined on hamsters. A study in conjunction with the Academy of Dermatology to determine the frequency, description, and causative agents of pyoderma lesions has been undertaken. The antibiotics effects of a dermatophyte on micrococci growing on porcine skin was studied by using scanning electron microscopy. Survival and interaction with normal flora of four strains of *Enterobacteriaceae* were monitored on the forearms of human volunteers. This research performed under this work unit has proved to be of limited value. Therefore, the work unit has been terminated and the resources transferred to projects with more immediate priority.

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

ABSTRACT

PROJECT NO. 3M762772A810 Military Skin Diseases

WORK UNIT NO. 010 Microbial Interactions on Healthy and Infected Skins of Soldiers

The following investigations have been conducted under this work unit:

STUDY NO. 1 Sampling of microorganisms from skin

STUDY NO. 2 Microbial ecology of bacterioprophylaxis and bacteriotherapy

STUDY NO. 3 Animal models for studying the pathogenic mechanisms of bacterial pyodermas

STUDY NO. 1 Because of the lack of statistical agreement between bacterial counts found on the "same region," a sampling experiment was devised to determine whether or not microorganisms inhabiting the volar forearm were evenly distributed. Our findings indicated that on the volunteer we sampled the mesophilic aerobic microorganisms were not evenly distributed. Thus, bacterial counts obtained from one region of the skin cannot be used reliably to predict the number of bacteria on an adjacent skin site.

The mechanical linear sampler, a manual method (Williamson and Kligman), and the timed-cotton-swab method were compared by using aerobic plate counts to determine which was most reliable for removing a known concentration of bacteria from guinea pig skin. The manual method was most reliable, while the timed-cotton-swab proved to be the least reliable of the three.

STUDY NO. 2 The survival on skin of a bacitracin-negative mutant of Bacillus licheniformis 10716 and its interactions with normal flora were studied. The survival curve from pooled data was statistically similar to that of the parent strain, although strong variation was observed among individual volunteers. Normal flora remained essentially unchanged during the colonization period.

Scanning electron microscopy was used to search for local or limited synthesis of antibiotics by a strain of Trichophyton mentagrophytes. Micrococcus luteus displayed morphological alterations on skin cultures with the fungus.

STUDY NO. 3 To study the effectiveness of therapeutic and prophylactic agents against bacterial pyodermas, we have developed a reproducible method for producing bacterial skin infections in hamsters and guinea pigs. By injecting 10^9 colony-forming-units/0.1 ml of either coagulase positive Staphylococcus aureus or Group-A-beta-hemolytic streptococci intradermally into the clipped backs of hamsters and immediately occluding the site for 48 h (23.3 C and PH 487), pustules are produced. Occlusion alone does not enhance lesion production in the guinea pig. Although either coagulase positive Staphylococcus aureus or Group-A-beta-hemolytic streptococci can induce pustule formation in hamsters, guinea pig skin is more sensitive to Staphylococcus infection than to Group-A-beta-hemolytic streptococcal infection.

This animal model will be used to determine the pathogenesis, prevention, prophylaxis and therapy of pyoderma.

BODY OF REPORT

WORK UNIT NO. 010 — Microbial Interactions on Healthy and Infected Skin of Soldiers

STUDY NO. 1 — Sampling of microorganisms from human skin

PROBLEM

Contagious skin infections need to be monitored in military personnel in order to prevent spreading the disease organisms. Causative agents of bacterial skin infection reside on the skin along with many other microorganisms which are not pathogenic. Because the sample is no better than the sampling method, we have searched continually for ways of improving our sampling methods. Many of the methods used to sample microorganisms found on skin fail to produce reliable results. Many papers have been published which describe a variety of sampling techniques; however, few of those investigations have adequately evaluated the most used sampling procedure, swabbing. In addition to evaluating the sampling methods utilized in our laboratory, we have investigated the bacterial flora on one volunteer to determine if we can expect to obtain statistically similar bacterial counts from adjacent skin sites located on the volar forearm.

RESULTS AND DISCUSSION OF RESULTS

Our data, and that of other laboratories, have reported on the wide spectrum of microorganisms inhabiting the stratum corneum. Although the species and numbers of microorganisms on various anatomic sites, e.g., forearm, have been reported in numerous publications, there is little agreement among bacterial counts made on the same person on the same day. To study this problem, the volar forearm was delineated into three regions, wrist, mid-forearm and the antecubital fossa area. Each site was assayed for bacteria on the same day at the same time over a 128-day period. Examination of the data by the Kruskal-Wallis test and by the Chi Square "Goodness of fit" test led to the rejection of the null hypothesis that the bacterial counts were statistically the same in a supposedly similar region, the volar forearm. Our findings that bacteria inhabiting the skin were not evenly distributed on the volunteer's forearm we sampled suggest that bacterial counts obtained from one region on the skin cannot be used to predict reliably the number of bacteria found in an adjacent area.

Various methods for estimating microbial levels on skin have been introduced. Despite the number of methods available, few

have been standardized. Since we have sampled skin for bacteria using various methods and devices, and because none of the procedures have been adequately tested, we compared the mechanical linear sampler (a device developed at LAIP), a manual method (Kligman and Williamson), and the timed-cotton-swab for quantitatively removing bacteria from guinea pig skin. To make our comparisons, known concentrations of Micrococcus luteus or Staphylococcus epidermidis were placed on the clipped backs of guinea pigs. After the inoculum was spread by pipette tip and allowed to dry, each method was tested at random. The recovery fluids were measured and analyzed for the number of bacteria present. The most reliable method, based on the mean colony-forming-units, was the manual method. Timed-cotton-swab produced the greatest amount of variation. Although the statistical analysis of the data is not complete, it is accurate to conclude that timed-cotton-swab method cannot provide reliable quantitative bacterial counts.

CONCLUSIONS

Bacterial counts obtained from one skin site are not reliable for predicting the number of bacteria on an adjacent skin site. Of methods used to remove surface bacteria from guinea pig skin, the manual method (Williamson and Kligman) was the most reliable and the timed-cotton-swab the least reliable.

RECOMMENDATIONS

Information gained from this study should be used to determine effects of topical insect repellents and antimicrobial agents on the bacterial flora of normal skin.

PUBLICATIONS

1. KEITH, W.A., Jr., R.J. SMILJANIC, W.A. AKERS, L.V. KEITH, and J.D. NESMITH. Comparative statistical evaluation of the mechanical sampler, manual method and timed-cotton-swab technique for harvesting microorganisms from guinea pig skin. Abstract. Spring meeting of Northern California American Society of Microbiology, San Francisco, 1978.
2. KEITH, W.A., Jr., R.J. SMILJANIC, W.A. AKERS, and L.V. KEITH. Uneven distribution of aerobic mesophilic bacteria on human skin. Appl Environ Microbiol (in press), 1979.

STUDY NO. 2 — Microbial ecology of bacterioprophylaxis and bacteriotherapy

PROBLEM

Among the novel ecologic approaches for the prevention and treatment of bacterial and fungal diseases of the skin of soldiers is altering the indigenous microbial flora to include strains which are especially antagonistic to common pathogenic agents. Such a modification of normal flora has yielded generally poor results in the past, but this was largely due to an unsophisticated understanding of ecology, and to the use of improperly selected test strains of bacteria. In vitro results may not necessarily relate to the specific environmental conditions in situ on stratum corneum. The production and effects of antibiotics and other competitive agents in the cutaneous habitat is particularly in need of investigation.
Previous experiments suggested that the in situ production of bacitracin by Bacillus licheniformis ATCC 10716 may be important to its survival among competing flora. Different strains of the same species not known to synthesize antibiotics survived poorly in comparison with ATCC 10716. Because strain variation might have been responsible for these results, a nitrosoguanidine-derived bacitracin-negative mutant of ATC 10716 was attained to serve as a more appropriate control for the study of survival and interactions of bacilli. The production by dermatophytes of antibiotics (penicillin, streptomycin, and azalomycin) is dependent on species and strain and on culture conditions. The low sensitivity of agar diffusion biological assays might have been responsible for the frequent failure to observe antibiotic synthesis on stratum corneum culture, although the dermatophytes tested were abundant producers in broth. Because local or limited production of antibiotics may still be important in dermatophytosis, the interactions of a dermatophyte and micrococci growing in vitro on stratum corneum were examined by scanning electron microscopy.

RESULTS AND DISCUSSION OF RESULTS

Spores of the bacitracin-negative mutant of B. licheniformis ATCC 10716 were applied to the forearms of the same set of 11 volunteers who had participated in earlier studies. General results conformed to those obtained from the previous three strains of the bacillus. No statistically significant differences in survival, measured by population and duration, were found between ATCC 10716 and its mutant. However, the mutant tended to be eliminated at a faster rate, and strong variation in bacillus survival was noted among volunteers. Population densities of normal skin flora were unaltered. In contrast to the effects of ATCC 10716, no changes in carriage, composition, and bacitracin-sensitivity of resident flora were detected. Micrococcus luteus, whose growth was inhibited on agar media by penicillin

and by Trichophyton mentagrophytes var. granulosum, was placed on small squares of hydrated porcine skin kept moist in their own individual Petri dishes. Each skin sample was also inoculated with microconidia of the dermatophyte, and at daily intervals samples were prepared for scanning electron microscopy. A staphylococcus resistant to penicillin and to the dermatophyte served as a control strain. Only the micrococcus in presence of the fungus displayed morphological alterations of flattened walls, invagination, enlargement, and collapse. Penicillinase did not neutralize these effects. Germinating fungi showed positive tropism to the bacteria.

CONCLUSIONS

The superior survival of ATCC 10716 seen with earlier grouped data was not necessarily due to its synthesis of bacitracin as a competitive factor with resident flora because the antibiotic-negative mutant survived almost as well. However, among individuals, differences in survival between ATCC 10716 and the mutant may still be explained by antibiosis, because, in some instances, antagonists may have been eliminated, and for some subjects, cooperative flora may have been suppressed.

Effective, but limited, antibiosis does occur on stratum corneum. Morphological alterations of susceptible bacteria, patterns of dermatophyte growth, and regional variation of skin demonstrated the profound influence of microenvironment on antibiosis.

RECOMMENDATIONS

Studies of bacillus survival on different areas of skin should be continued. Use of the animal model of pyoderma for prophylactic and therapeutic testing of bacilli should begin. Scanning electron microscopy and the stratum corneum-microbe system to assay dermatophyte inhibitory agents should be used.

PUBLICATIONS

1. BIBEL, D.J., D.J. LOVELL, and R.J. SMILJANIC. Survival of Bacillus licheniformis on human skin. Appl Environ Microbiol 35:1128-1135, 1978

2. BIBEL, D.J., R.J. SMILJANIC, and D.J. LOVELL. Interactions of Bacillus licheniformis ATCC 10716 and normal flora of human skin. Appl Environ Microbiol 35:1136-1144, 1978

3. BIBEL, D.J. and R.J. SMILJANIC. Interactions of Trichophyton mentagrophytes and micrococci on skin culture. J Invest Dermatol 1979 (in press)

STUDY NO. 3

Animal models for studying the pathogenic mechanisms of bacterial pyodermas

PROBLEM

Pyodermas, bacterial skin diseases, can occur in epidemic proportion among combat personnel, and thereby reduce combat effectiveness. Under the ravages of war, the exact extent of combat man-days lost from pyodermas can not be predicted; however, in one survey, 18% of the soldiers fighting in the paddies in the Mekong Delta of Vietnam were infected. Since there is no currently acceptable preventive measure against the streptococcal-staphylococcal skin diseases observed in combat soldiers, and since there is no suitable animal model on which these preventive measures can be tested, we have developed a reproducible bacterial skin infection in hamsters and guinea pigs.

RESULTS AND DISCUSSION OF RESULTS

Initially, all strains of Group-A-beta-hemolytic Streptococci (GABHS) and Staphylococcus aureus (CPSA) were tested in hamsters and guinea pigs by injecting each culture simply into a skin site. Of the eight strains of GABHS and CPSA tested, two strains of GABHS and one strain of CPSA were chosen for further investigation.

The skin reaction in guinea pigs injected intradermally with various strains of GABHS was similar in most instances. Approximately 24 h after the infection, a large erythematous area with a dark colored lesion about the size of a pencil head was presented at the site of injection. In contrast to the limited local infection caused by GABHS, coagulase positive Staphylococcus aureus produced a readily identifiable lesion which, on removal of the intact roof yielded a small quantity of pus. These preliminary data suggested that guinea pig skin was more resistant to GABHS than to CPSA.

On testing various strains of GABHS in hamsters, we found hamster skin more sensitive than guinea pig skin to lesion development. At low cell densities, 10^5 - 10^6 colony-forming-units/0.1 ml, few pustules were produced in hamster skin. To increase the sensitivity of hamster skin, the intradermally inoculated site was occluded for 48 h. Although the true function of occlusion remains to be determined, it appears to decrease water loss from the occluded skin site. We found that 48 h of occlusion following intradermal injection of either CPSA or GABHS increased the number of lesions observed. In a subsequent experiment, we examined the effect of high cell

density, 10^9 CFU/0.1 ml, on hamster skin without occlusion. No differences were noted.

To rule out the effects of the spent media, 0.1 ml of membrane-filtered media was inoculated intradermally into hamster skin under occlusion and without occlusion. No lesions resulted. To determine whether or not the lesions produced required viable cells, heat killed cells were inoculated intradermally into hamster skin and occluded for 48 h. Again, no lesions developed. Washed cells produced lesions similar to those produced by unwashed cells. These studies demonstrated that viable cells were necessary for lesion formation.

The results presented demonstrate that our technique is capable of producing pyoderma in hamster skin. These bacterial skin infections are reproducible. The significance of these results is twofold: first, this animal model can be used to evaluate the efficacy of new and currently used antibacterial agents for treatment of pyoderma. Second, and perhaps, more important, selected therapeutic regimens can be tested directly with this model.

CONCLUSIONS

A method for producing bacterial pyoderma in the hamsters has been developed. This method employs high cell density of either coagulase positive Staphylococcus aureus or Group-A-beta-hemolytic streptococci injected intradermally, followed by 48 h of occlusion.

RECOMMENDATIONS

The effects of humidity and temperature on the development of bacterial pyoderma should be determined. Selected therapeutic and prophylactic measures should be tested for effectiveness against bacterial pyoderma.

PUBLICATIONS

1. KEITH, W.A., Jr., P.J. SMILJANIC, and F.V. CHAPMAN. An occlusive method for producing pyoderma in laboratory animals. Abstract. American Academy of Dermatology Annual Meeting. San Francisco, 1978

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA OE 6119	2. DATE OF SUMMARY: 78 10 01						
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. DOD UNDET: NL	9. SPECIFIC DATA: ☒ YES ☐ NO	10. LEVEL OF AGG: A. WORK UNIT
11. PRIMARY: 62772A	PROGRAM ELEMENT	3M162772A810	PROJECT NUMBER	00	TASK AREA NUMBER	015	WORK UNIT NUMBER
12. CONTRIBUTING							
13. APPROPRIATION/LIMITATION CODE: CARDS 114f							

14. TITLE (Protect with Security Classification Code): (U) Development of Improved Insect Repellents for Military Use

15. SCIENTIFIC AND TECHNOLOGY AREAS: 005900 Environmental Biology; 002600 Biology

16. START DATE	17. ESTIMATED COMPLETION DATE	18. PERFORMING AGENCY	19. PERFORMING ORGANIZATION
76 10	CONT	DA	C. In-House

20. CONTRACT/GRANT

a. DATE/EFFECTIVE: Not applicable
b. NUMBER:
c. TYPE:
d. AMOUNT:
e. END OF AGMT:
f. CUM. AMT.:

21. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
78 (PREVIOUS)	2.0	98
79 (CURRENT)	2.0	160

22. PERFORMING ORGANIZATION(S)

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ASSOCIATE INVESTIGATORS

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NAME: Wirtz, R. A., CPT, MSC POC: DA

25. GENERAL USE: Foreign Intelligence Not Applicable

26. KEYWORDS (Provide keywords, phrases or codes that describe the content of the work unit): (U) Repellent; (U) Diethyl toluamide; (U) Mosquito; (U) Sand Fly; (U) Tick; (U) Flea; (U) Assassin bug.

27. TECHNICAL OBJECTIVE, APPROACH, AND PROGRESS (Provide brief statements in paragraph form keyed to the specific work unit):

23. (U) To develop improved insect repellents for use by military personnel to insure combat effectiveness in units exposed to vector-borne diseases in areas or operational situations where effective vector control measures are impractical.

24. (U) Selected major vectors of disease will be colonized; improved repellent testing procedures will be developed; repellent compounds and formulations will be tested and evaluated in the laboratory and the field; basic and related studies will be conducted.

25. (U) 77 10 - 78 09. The colony of the tick *Ornithodoros parkeri* was brought to production strength and a colony of the bug *Rhodnius prolixus* was initiated. Quantitative methods were developed for testing repellents against mosquitoes and sand flies in human and animal test systems. Selected conventional repellents were tested against mosquitoes (*Anopheles, Aedes, Culex*), sand flies (*Lutzomyia*), fleas (*Xenopsylla*) and ticks (*Ornithodoros, Dermacentor*). New repellents obtained from the Stanford Research Institute and the U. S. Department of Agriculture were screened against *Anopheles albimanus, Anopheles stephensi, Aedes aegypti* and *Culex pipiens*. Respective tolerances of 6 inbred strains of *Aedes aegypti* to 4 standard repellents were determined.

DD FORM 1498 (REV 74) PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498A, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M6227A810 — Military Skin Disease

WORK UNIT NO. 015 — Development of Improved Insect Repellents for Military Use

The following investigations have been conducted under this work unit:

STUDY NO. 1 Colonization of selected insect vectors of disease

STUDY NO. 2 Genetic aspects of mosquito repellency

STUDY NO. 3 Development of improved tick repellents for military use

STUDY NO. 4 Development of duration testing methods for mosquito repellents

STUDY NO. 6 Entomological evaluation of insect repellents

STUDY NOS. 1,2,3,4, and 6 The colony of the tick *Ornithodoros parkeri* was brought to production strength and a colony of the bug *Rhodnius prolixus* was initiated. Quantitative methods were developed for testing repellents against mosquitoes and sand flies in human and animal test systems. Selected conventional repellents were tested against mosquitoes (*Anopheles, Aedes, Culex*), sand flies (*Lutzomyia*), fleas (*Diamanus*), and ticks (*Ornithodoros, Dermacentor*). New repellents obtained from the Stanford Research Institute and the U. S. Department of Agriculture were screened against *Anopheles albimanus, Anopheles stephensi, Aedes aegypti* and *Culex pipiens*. Respective tolerances of 6 inbred strains of *Aedes aegypti* to 4 standard repellents were determined.

BODY OF REPORT

WORK UNIT No. 015 — Development of Improved Insect Repellents for Military Use

STUDY NO. 1 — Colonization of selected insect vectors of disease

PROBLEM

Laboratory investigations relating to the development of improved insect repellents depend on the availability of an adequate stock of representative insect species for experimental use. Laboratory colonies provide an economical in-house resource for repellent bioassay in lieu of travel to distant areas where medically important species are known to occur. The present study encompasses research relating to the development of methods for the laboratory production of experimental insects required for the repellent development program. Emphasis is placed on the development of practical, efficient methods for the mass-production of selected, representative species.

RESULTS AND DISCUSSION OF RESULTS

The mosquito species now available for use are (1) *Anopheles (Anopheles) quadrimaculatus* Say, the historical vector of malaria in Eastern North America, (2) *Anopheles (Nyssorhynchus) albimanus* Wiedemann, the major vector of malaria in Central and South America, (3) *Anopheles (Cellia) stephensi* Liston, a major vector of malaria in Southern Asia and the Middle East, (4) *Aedes (Stegomyia) aegypti* (Linnaeus) (3 strains), the primary vector of yellow and dengue fevers, (5) *Aedes (Ochlerotatus) taeniorhynchus* (Wiedemann), a major pest species on the East and West coasts of the U.S., and (6) *Culex (Culex) pipiens* Linnaeus, the primary vector of periodic Bancroftian filariasis and a major vector of viral encephalitis. These species were selected for colonization on the basis of importance to military preventive medicine, taxonomic distance, and ease of rearing and handling. The flea, *Diamanus montanus* Baker, which has been implicated in the transmission of sylvatic plague, and the phlebotomine sand fly *Lutzomyia longipalpis* (Lutz and Neiva) which is a major vector of leishmaniasis in South America are also reared for use in repellent testing. This is the only established colony of sand flies in the U.S. Improvements in larval rearing containers have increased the productivity of the colony.

Colonies of the ixodid tick *Dermacentor variabilis* (Say), a major vector of Rocky Mountain spotted fever and tularemia, and the argasid tick, *Ornithodoros parkeri*, a vector of relapsing fever, are also reared for use in repellent testing. The latter colony was brought to production strength for the first time during the year.

A colony of the kissing bug *Rhodnius prolixus* Stal, a major vector of Chagas' disease in Central and South America, was established from material obtained from the Department of Entomology, U. C. Berkeley, Berkeley, CA. To date, the colony has not reached a strength to permit repellent testing; however, it should do so within the next 4 to 6 months. Procedures have been initiated to obtain a colony of chigger mites (*Leptotrombidium spp.*) and a collembolan food source from Walter Reed Army Institute of Research. Chigger mites (fam. Trombiculidae) are the vectors of scrub typhus.

For economic, humane and practical reasons, several laboratories in the U.S. and elsewhere have developed in vitro blood-feeding systems for use in lieu of laboratory animals for mosquito colony maintenance. An in vitro blood-feeding system suitable for use in the LAIR insectary is currently under development. The system is based on the use of collagen sausage casings filled with outdated human blood and warmed to 44 C in a water bath. The mosquitoes fed freely on the blood, but egg productivity dropped in 3 colonies after several weeks of using this system exclusively, and the use of live rabbits for blood feeding has been resumed. Although the methodology developed is satisfactory, further work is necessary to determine the deficiencies of outdated human blood and research in this area will continue.

CONCLUSIONS

A comprehensive battery of test species for use in repellent research has been established and maintained at production levels. Significant advances have been made in laboratory production methods for mosquitoes and sand flies.

RECOMMENDATIONS

Research on insect rearing methods should be continued with a view toward effecting further economies and benefits for the repellent development program. Emphasis should be placed on improved methods for rearing mosquitoes, because of their usage and importance, and on improved methods for rearing sand flies, because of their difficulty and relatively high cost in manpower.

PUBLICATIONS

1. RUTLEDGE, L.C., M.A. LAWSON, L.L. YOUNG and M.A. MOUSSA. Rearing of *Diamanus montanus* (Siphonaptera, Ceratophyllidae) and *Hoplopsyllus anomalus* (Siphonaptera, Pulicidae). J Med Entomol (in press)

STUDY NO. 2 Genetic aspects of mosquito repellency

PROBLEM

The USAMRDC supports an extensive program of research on chemical repellents for the protection of military personnel from mosquito-borne diseases. Investigators evaluating candidate repellents frequently have reported disparate or conflicting results. It is believed that genetic factors relating to the test mosquitoes may account, in part, for those discrepancies. The present study provides for a quantitative assessment of the relative importance of environmental and genetic factors in determining the responses of mosquitoes to repellents and for the development of genetically homogeneous strains of the yellow fever mosquito, *Aedes aegypti* (Linnaeus) for evaluation as standardized repellent test strains.

RESULTS AND DISCUSSION OF RESULTS

Six inbred strains of *Aedes aegypti* produced earlier in the study from the MASAKA (diethyl toluamide-sensitive) and MOYO INDOOR (diethyl toluamide-tolerant) strains were tested to determine the effects of inbreeding on the sensitivity of the daughter strains to diethyl toluamide, ethyl hexanediol, dimethyl phthalate and Indalone®. Results of the tests indicated that levels of sensitivity to particular repellents was substantially reduced in some of the inbred strains and substantially increased in others. These results demonstrate the genetic basis of the sensitivity trait and reflect the independent assortment of the genetic factors involved. Since levels of sensitivity to the different repellents were not correlated, it was concluded that different genetic factors were involved in the case of each of the repellents tested.

Intrastrain variability in the response to repellents was not reduced in the inbred strains. Accordingly, such strains are no better than normal laboratory strains for repellent testing purposes. However, since the F_1 hybrids of inbred strains are both heterozygous and genetically uniform, they might be expected to combine the separate advantages of inbred strains and normal laboratory strains for bioasssay work. The production of such hybrids for evaluation as test strains is currently in progress.

It is known that most repellents have insecticidal properties and, conversely, that many insecticides show repellent activity. Accordingly, it is possible, in principle, for cross-resistance between insecticides and repellents to develop in populations of mosquitoes in the field. Genetic linkages of this type would have practical importance in the selection of repellent compounds for use against insecticide-resistant populations of the vector species. In the present study, comparative data were obtained on the respective tolerances of 12 laboratory strains of *Anopheles albimanus, An. quadrimaculatus, An. stephensi, Aedes aegypti, Ae. taeniorhynchus* and *Culex pipiens* to the repellent diethyl toluamide and the insecticides DDT and malathion. Results obtained in the study indicate that the responses of mosquitoes to the standard military repellent (diethyl

toluamide) are not correlated with their respective tolerances to chlorinated hydrocarbon insecticides (DDT) or organophosphorus insecticides (malathion).

CONCLUSIONS

The responses of mosquitoes to different repellents are controlled by specific factors in the genetic makeup of the species. Isolation of such factors by inbreeding techniques does not significantly reduce intrastrain variation in the responses of mosquitoes to repellents. The behavioral responses of mosquitoes to diethyl toluamide are not genetically linked with their respective physiological responses to DDT or malathion.

RECOMMENDATIONS

It is recommended that the F_1 hybrids of inbred strains be evaluated for efficacy as standard repellent test strains in the repellent testing program.

PUBLICATION

RUTLEDGE, L.C., R.K. SOFIELD and M.A. MOUSSA. A bibliography of diethyl toluamide. Bull Entomol Soc Amer 24(4): (in press)

STUDY NO. 3 — Development of improved tick repellents for military use

PROBLEM

Military personnel are often required to enter areas which are endemic for tick-borne diseases. It is often impracticable to treat such areas with an effective insecticide to control the tick population prior to the time that troops are required to enter the region. Protection of troops from tick-borne diseases in such situations depends on the use of effective tick repellents. The current standard repellents, M-1960 for clothing application and deet for topical application, are not totally effective against ticks. There is a current need for longer-lasting repellents having high intrinsic repellency for ticks.

RESULTS AND DISCUSSION OF RESULTS

In FY 1977, the 4-hour ED_{50} of four major repellents were determined for nymphs of the Ixodid tick *Dermacentor variabilis* in a new white mouse test system. Diethyl toluamide and Indalone® were more effective repellents than dimethyl phthalate and ethyl hexanediol. During FY 1978, similar results were obtained in tests against the adult stage of *Dermacentor variabilis*. Diethyl toluamide and Indalone® were even more effective against the adult ticks than they had been

against the nymphs. Dimethyl phthalate, which had been comparatively ineffective against the nymphs, was even less effective against the adults. Tests of the four repellents against the larval stage of the tick are currently in progress.

The exact significance of the foregoing results depends on the specific epizootiology, epidemiology, and mode of transmission of the different tick-borne diseases. Zoonoses of small and large animals may be transmitted by different life stages of the tick because of the different host preferences of the larvae, nymphs and adults. The larval stage of the tick can transmit only those diseases that can be acquired transovarially from the female parent. Transtadial and transovarial transmission are important factors in the epidemiology of most tick-borne diseases, including Russian spring-summer encephalitis, Central European encephalitis and other tick-borne diseases of the Eurasian continent.

Tests of the military standard repellent, diethyl toluamide, against the Argasid tick *Ornithodoros parkeri* were initiated during the year. Various species of *Ornithodoros* are vectors of relapsing fever in North America, Central Africa, the Middle East and elsewhere in the world. Little is currently known of their sensitivity to the insect repellents in common use. Preliminary test results indicate that *Ornithodoros parkeri* is moderately sensitive to diethyl toluamide.

CONCLUSIONS

Insect repellents in current use are not equally effective against all the life stages of ticks. A given repellent will not be equally effective in preventing diseases that are transmitted by different life stages of the same tick.

RECOMMENDATIONS

The repellent testing program should be extended to provide additional baseline data on the effectiveness of currently available repellents to the different life stages of important vector species.

PUBLICATIONS

None

STUDY No. 4 — Development of duration testing methods for mosquito repellents

PROBLEM

While many chemicals are known to repel mosquitoes effectively when applied to the skin at practical dosages, relatively few persist on the skin for extended periods of time after application. Persistence

is critical to the military application, since military operations may require continuous exposure of personnel to mosquitoes for prolonged periods of time. Conventional methods are inadequate for unequivocal determination of the persistence properties of new mosquito repellents. Quantitative statistical methods based on modern bioassay techniques are critically needed in the repellent development program supported by USAMRDC.

RESULTS AND DISCUSSION OF RESULTS

In line with current efforts to minimize the use of human subjects in scientific research, activity in Study No. 4 was directed toward extension and refinement of the LAIR white mouse test system. Temperature and dosage effects on the effective halflives of 10 repellents were studied in the white mouse test system, and it was concluded that the initial tests of new or untried repellents in that test system should be designed to determine the 4-hour ED_{50} of the test repellent on mice maintained at 37 C. The 4-hour ED_{50} provides a measure of the combined effects of the intrinsic repellency and the persistence properties of the material under test on its efficacy as a repellent. It also provides approximate information on the dosages needed to determine the effective halflife and the ED_{50} proper. The 4-hour time period was chosen to correspond with that used in tests of repellents against the tick *Dermacentor variabilis* (Study No. 3). The 37 C temperature was chosen to correspond with the human body temperature.

In the 4-hour ED_{50} test procedure, suckling mice are treated by immersion in ethanol (control) and 4 serial dilutions of the test repellent in ethanol (repellent treatments). The treated mice are held separately in an incubator at 37 C for 4 h and then are transferred to individual compartments in a cage containing the test mosquitoes. The numbers of mosquitoes feeding on each mouse are recorded at 2-min intervals for 20 min, and the totals of the 10 counts made on each mouse are recorded as the test results. In subsequent replicates, the range of dosages used is adjusted as necessary to bracket the 50% end point. The 4-hour ED_{50} of the test repellent is calculated by probit analysis of the results obtained in tests that bracket the 50% end point. The 4-hour ED_{50} test has been proved in more than 90 trials of 26 different repellents during the year. The results obtained are given in the report for Study No. 6.

A frequent criticism of conventional methods of testing mosquito repellents on human subjects in the laboratory has been ambiguity and incompleteness of the data obtained. Several hundreds or thousands of mosquitoes are used in the conventional type of repellent test. Because of the biting potential of such large test populations, repellents are ordinarily tested at high dosages only, and the untreated control is almost invariably neglected. It would seem that the use

of smaller numbers of mosquitoes would permit the investigator to employ accepted methods and principles of biological assay. Accordingly, reform of traditional test methods along these lines was initiated in FY 1978.

The experiments were conducted with the mosquito *Aedes aegypti* and the sand fly *Lutzomyia longipalpis*. It was determined in trials with the latter species that approximately 40% of the test insects could be expected to bite within a ½-h test period. Thus, between 4 and 16 bites would be expected if the test population were limited to the range of 10 to 40 insects per test. These bites would be distributed on the control and the repellent treatments in conformance with the statistical model applicable to the experimental design employed in the test. Replication of such a test on a "sequential sampling" basis would insure maximal benefit/cost in terms of the amount of information obtained per bite received. These conclusions were confirmed in 150 dose-response trials of 6 standard repellents against *Lutzomyia longipalpis*. The test insects (10 to 40 per test) were confined in a small (600 cc) test cage which could be strapped to the forearm and opened to expose 5 circular test areas previously outlined on the arm and treated in accord with standard ED_{50} test procedures. Fifteen replicates of the test were sufficient for accurate determination of the ld-p regression line. The results of the tests are given in the report for Study No. 6.

CONCLUSIONS

New repellents should be screened and evaluated on experimental animals prior to testing on man. The established principles of biological assay are applicable in testing repellents in animal and human test systems. The conventional "first bite" type of test should be regarded obsolete.

RECOMMENDATIONS

White rabbit and guinea pig test systems should be developed to complement the white mouse test system currently used. Current procedures for testing repellents against *Lutzomyia* on the forearm should be extended to tests of repellents against *Anopheles*, *Aedes* and *Culex*.

PUBLICATIONS

None

STUDY No. 6 — Entomological evaluation of insect repellents

PROBLEM

Insect-borne diseases are among those of paramount importance to

military forces in the field. Insect repellents, together with certain vaccines and chemoprophylactic drugs, are the only practical and effective means available for preventing insect-borne diseases in field units operating in areas or situations where the reduction of insect populations is impracticable. Such situations include assault, movement, bivouac, patrol, and unsettled or uncertain military situations in general.

The complete baseline data needed for effective use of insect repellents in any possible military action are not currently available. Intrinsic repellency, range of efficacy and persistence properties of the standard repellents are poorly known, and the merits of many prospective new repellents have not yet been fully explored. In addition, many completely new compounds and formulations with potentially superior repellent properties are available for trial. The present study encompasses a systematic comprehensive program for testing standard and prospective repellents against major vectors of insect-borne diseases of known or potential importance to the Army in field operations.

RESULTS AND DISCUSSION OF RESULTS

Extended testing of 8 standard repellents (diethyl toluamide, ethyl hexanediol, Indalone^{R}, dimethyl phthalate, dibutyl phthalate, butoxy polypropylene glycol, MGK Repellent 11^{R}, and MGK Repellent 326^{R}) was initiated during FY 1978. Immediate goals are to test each compound against mosquitoes (*Anopheles albimanus, Anopheles stephensi, Aedes aegypti* and *Culex pipiens*), sand flies (*Lutzomyia longipalpis*), fleas (*Diamanus montanus*) and ticks (*Dermacentor variabilis* and *Ornithodoros parkeri*). The results obtained will represent the beginnings of the data base required for accurate prescription of specific repellents for use against specific vectors in a military emergency. Diethyl toluamide and ethyl hexanediol are available within the military supply system, and the remaining 6 repellents are available from commercial sources. Testing of diethyl toluamide, ethyl hexanediol, Indalone^{R} and dimethyl phthalate is well advanced, and testing of the remaining 4 repellents is well under way.

The before-mentioned tests include the first known trials of any repellents against *Lutzomyia longipalpis* (vector of visceral leishmaniasis), *Diamanus montanus* (vector of plague), and *Ornithodoros parkeri* (vector of relapsing fever). Currently, testing against *Lutzomyia longipalpis* is more advanced. Of the 6 repellents tested against this species to date, Indalone^{R} and diethyl toluamide are decidedly superior.

Except for a few minor reports in the foreign literature, the 8 repellents have not been previously tested by modern quantitative methods. In most cases, the median effective dosages were totally unknown, even for a single vector species. Comparative persistence on the skin was known only from crudely measured "protection times." In this respect,

the present study provides data that are more definitive, as well as more extensive, than any previously available.

Twenty coded repellent compounds obtained from the U.S. Department of Agriculture were tested against the mosquito *Aedes aegypti* during FY 1977. These were subsequently determined to include 17 prospective and 3 standard repellents. The ED_{50}s of 8 prospective repellents were better than those of the 3 standard repellents. During FY 1978 the tests of the 8 superior repellents were repeated, and the results obtained in FY 1977 were confirmed. If additional quantities of these materials can be obtained, they will be tested more extensively in FY 1979. The compounds in question include 4 quinolines, 2 piperidines, an oxazine and an oxazolidine.

During FY 1976-78, 101 new chemical compounds synthesized at the Stanford Research Institute were tested for repellent activity against *Aedes aegypti*. The ED_{50}s of 31 of these compounds were better than that of the standard repellent, diethyl toluamide. During FY 1978, 28 of the 31 were re-tested, and the superior status of 24 of those re-tested was confirmed. These 24 prospective new repellents are currently being tested more extensively. The immediate goal is to determine the 4-h ED_{50} for *Aedes aegypti* of each repellent. Those materials with better 4-h ED_{50}s are being further tested to determine their respective ED_{50}s for *Anopheles albimanus*, *Anopheles stephensi* and *Culex pipiens*. It is already apparent that no single repellent in the series is likely to prove superior for all species of mosquitoes to be tested.

CONCLUSIONS

Certain standard repellents not currently in the Army inventory may prove to be superior for use against some insects. Several prospective new repellents obtained from the U. S. Department of Agriculture and the Stanford Research Institute have given promising results in tests against *Anopheles*, *Aedes* and *Culex* mosquitoes.

RECOMMENDATIONS

The old idea of a single all-purpose insect repellent should be abandoned as a conceptual framework for the repellent development program. Repellents can best be used selectively against different vector insects in the same way that antibiotics are used selectively against different microorganisms.

PUBLICATIONS

SKINNER, W.A., H.T. CRAWFORD, L.C. RUTLEDGE and M.A. MOUSSA. Topical mosquito repellents. XII. N-substituted ureas and cyclic ureas. J Pharm Sci (in press).

SKINNER, W.A., H.T. CRAWFORD, L.C. RUTLEDGE and M.A. MOUSSA. Topical mosquito repellents. XI. Carbamates derived from N,N'-disubstituted diamines. J Pharm Sci (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				3. AGENCY ACCESSION DA OE 6300	4. DATE OF SUMMARY 78 10 01	REPORT CONTROL SYMBOL DD-DRAE(AR)636
5. DATE PREV SUMRY 77 10 01	6. KIND OF SUMMARY D. Change	7. SUMMARY SCTY U	8. WORK SECURITY U	9. ASSIGNMENT NA	10. SEC/DG DETER NL	11. SPECIFIC DATA-INSTRUCTOR ACCESS YES ☒ NO ☐
12. DOD / COSATI CODE	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER	
a. PRIMARY	62770A	3M162770A807		00	121	
b. SECONDARY	62772A	3M162772A810		00	015	
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13. TITLE (Procedure, if any, for Security Classification of Title)

(U) Diagnosis and Prevention of Leishmaniasis in Military Personnel

14. DESCRIPTIVE AND TECHNOLOGICAL AREAS

0010100 Microbiology; 002600 Biology

15. START DATE	16. ESTIMATED COMPLETION DATE	17. FUNDING AGENCY	18. PERFORMING ORGS
76 10	CONT	DA	C. In-House

19. CONTRACT/GRANT

a. DATE EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: Not Applicable
d. AMOUNT:
e. END OF AGMNT: f. CUM. AMT.:

20. RESOURCES ESTIMATE

	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
FISCAL YR 78	3.5	144
FISCAL YR 79	1.5	75

21. PERFORMING ORGANIZATION

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22. GENERAL USE

Foreign Intelligence Not Applicable

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23. KEYWORDS (U) Antigen; (U) Diagnosis (U) Immunity; (U) Disease Vector; (U) Leishmania; (U) Serology; (U) Epidemiology

24. TECHNICAL OBJECTIVE, APPROACH, AND PROGRESS (Precede with Security Classification Code)

23. (U) To develop improved methods for the diagnosis and prevention of cutaneous leishmanial infections encountered by service personnel deployed or stationed in endemic areas overseas. The absence of protective vaccines and the lack of satisfactory treatment renders these studies essential to the protection of the health of the field soldier.

24. (U) Mass culture of *Leishmania* will be instituted to provide sufficient cells for biochemical isolation of cellular components. Immunochemical analysis will be conducted to define components which offer greater sensitivity and specificity for utilization in serodiagnosis and skin testing methods. In vivo immune responses to purified components and in vitro immunochemical reactions will be conducted to characterize humoral and cell mediated mechanisms and to evaluate antigens for their potential protective value against *Leishmania*.

25. (U) 77 10 - 78 09. Enzyme immunoassay (ELISA) was shown to be extremely sensitive in following the immune response of rabbits sensitized to *Leishmania* and for detecting leishmanial antibody in sera from experimentally infected hamsters or in sera from humans with naturally acquired leishmaniases. Whole killed promastigotes or soluble antigens of *L. braziliensis* elicited initial delayed-type hypersensitivity responses 3-7 days after the appearance of lesions (14 days after infection) in guinea pigs infected with *L. mexicana*. Maximum responses were demonstrated 8-12 weeks after infection. The occurrence of circulating immune complexes in leishmaniases was confirmed by the detection of antigen and antibody simultaneously in the sera of hamsters infected with *L. braziliensis* or *L. donovani*.

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ABSTRACT

PROJECT NO.	3M762772A810	Military Skin Disease
WORK UNIT NO.	016	Diagnosis and Prevention of Leishmaniasis in Military Personnel

The following investigations have been conducted under this work unit:

STUDY NO.	1	Serodiagnosis of American Leishmaniasis
STUDY NO.	2	Improved skin test antigen for the diagnosis and prognosis of leishmaniasis
STUDY NO.	3	Immune mechanisms in leishmaniases

STUDY NO. 1 The response to immunization against *L. braziliensis* was followed in rabbits by measuring antibody by enzyme-immunoassay (ELISA), passive hemmagglutination (PHA), complement fixation (CF), and counter-current immunoelectrophoresis (CCIE). PHA and CF titers were interpreted according to standard methods, whereas ELISA exact titers were derived by the equation Y - a-b logX which describes the straight line that results when absorbances are plotted against test serum dilutions. Peak titer was measured at 32·1 days after initial injection, irrespective of the assay method. Seven days post-infection antibody titer was 1130 by ELISA, 0 by PHA and 8 by CF. Precipitin bands were shown by CCIE only at peak titer. With sera from hamsters experimentally infected with *Leishmania*, 94% showed leishmanial antibody by ELISA, compared to 92% by CF and 65% by PHA. Thirty-one samples of sera from human patients with cutaneous leishmaniasis were tested only by ELISA. Of these, 23 were positive for leishmanial antibodies.

STUDY NO. 2 Studies concerning the preparation, evaluation and standardization of skin testing antigens for diagnosis and prognosis of leishmaniasis were continued. Guinea pigs infected with *L. enriettii* produced sensitivity responses when tested with whole killed promastigotes or soluble antigens of *L. braziliensis*. Mild positive delayed-type skin responses were demonstrated 5 to 7 days after the earliest parasite lesions had formed. Maximum responses were obtained when resolution of lesions had begun, 8 weeks after infection, and were maintained for at least 4 additional weeks. The data suggest skin testing may have potential use for early diagnosis of leishmaniasis.

STUDY NO. 3 Leishmanial antibody of the IgG immunoglobulin class was

demonstrated in the sera of 6 hamsters 22 to 51 days after infection with *L. braziliensis*. One animal showed both complement fixing antibody and antigen before infection and 36 days after infection. A second animal demonstrated both components only at 29 days post-infection. Two animals infected with *L. donovani* showed both components 24 days after infection. The data confirm results of earlier experiments which suggested the occurrence of immune complexes during infection with *Leishmania* by the marked increase in anticomplementary activity of sera from *L. mexicana* infected subjects. The demonstration of the simultaneous occurrence of circulating antigen and antibody offers an explanation for the formation of immune complexes.

BODY OF REPORT

WORK UNIT NO. 016 — Diagnosis and Prevention of Leishmaniasis in Military Personnel

STUDY NO. 1 — Serodiagnosis of American leishmaniasis

PROBLEM

Standard serological methods are not sensitive and/or specific enough to detect low-level circulating antibodies in the sera of patients during or after infection with *Leishmania*. As a consequence, treatment is delayed until the appearance of a distinct lesion from which a positive diagnosis can be made. The development of a simple reliable method for the serodiagnosis of leishmaniasis will permit early treatment, thereby decreasing complications which may arise from mucocutaneous or visceral involvement. The introduction of enzyme-immunoassay (ELISA) has provided a method which is being investigated for its potential usefulness as a tool for the serodiagnosis of leishmaniasis. However, quantitative relationships in ELISA need to be defined and established to arrive at an accurate and reproducible assessment of leishmanial antibody in the sera of immunized and/or infected subjects. This study was designed to modify established serodiagnostic methods and/or develop new methodology for the detection of early infections among military personnel serving in, or returning from, endemic areas.

RESULTS AND DISCUSSION OF RESULTS

ELISA results are currently reported in the literature as absorbance readings which are obtained at a single dilution of a test sample, or as a titer obtained from the serum dilution giving a specified absorbance. An assessment based on a single result may be misleading, especially with multiple antigen-antibody systems in which, at all dilutions, some may be in antigen excess and others in antibody excess. Thus, carrying out a complete titration and reporting ELISA data in terms of exact end point titers decreases the chance of false negatives and is more meaningful in light of accepted methods for reporting and interpreting serodiagnostic results. In our studies with rabbits we noted that sera drawn on different days after immunization with *Leishmania* often gave end point absorbances of different values at the same dilution of sera. Therefore, it was necessary to derive exact titers to obtain characteristic and reproducible antibody response curves.

In the ELISA, anti-leishmanial sera quantitated by incubating serial two-fold dilutions in microtiter plates coated with leishmanial antigen. Enzyme-labelled specific antiglobulin is reacted with

the immune complex formed and a specific enzyme substrate is then added. The entire complex is finally assessed by spectrophotometric determination of the absorbance of the colored hydrolysis product of the enzyme-substrate reaction. This absorbance is proportional to the amount of conjugate specifically bound to leishmanial antibody and is consistent with an immune reaction in which increasing amounts of antigen precipitate antibody from a constant amount of serum until maximum antibody is bound at optimum antigen-antibody proportions, and decreasing antibody is bound at antigen excess. Thus, absorbances in ELISA represent antigen-antibody ratios in which conjugate antiglobulin is the antibody and leishmanial antibody is the antigen. When these ratios (absorbances) are plotted against antigen (test sera dilutions) a straight line results which follows the equation $Y = a - b \log X$, where a equals the intercept of the Y axis, and b equals the slope of the line. Our data were applied to the equation by deriving a and b for three or more absorbances obtained in each serum titration and then adjusting all end point titers to 0.01 absorbance. ELISA baseline values for two-fold dilutions of sera from normal rabbits, hamsters, and humans were established and subtracted from corresponding dilutions of respective test sera. ELISA exact end point titers were then derived by $Y = a - b \log X$.

The derivation of exact end point titers according to the method described above resulted in rabbit antibody response curves which closely paralleled those produced by passive hemagglutination (PHA), and complement fixation (CF). However, the superior sensitivity of ELISA was demonstrated by its early detection of significant antibody and markedly higher titers when compared to measurements by PHA and CF.

Rabbit immune sera. The response to immunization with soluble *L. braziliensis* antigen (LB016) was followed in rabbits by collecting sera before and at frequent intervals after immunization and determining ELISA exact end point titers by the method described in the foregoing section. PHA and CF titers were measured according to standard methods. The presence or absence of precipitating antibody was determined qualitatively by counter-current immuno-electrophoresis (CCIE). Antibody in two rabbits reached peak titer approximately 32 ± 1 days after initial injection, irrespective of the assay method, which suggests similar antibody was measured. However, antibody measured 7 days after initial injection showed a titer of 1130 by ELISA, as compared to titers of zero by PHA, and 8 by CF. The extreme sensitivity of ELISA was also shown by the marked differences between peak titers measured by ELISA (20,000), PHA (480), and CF (16). These differences were further amplified by the anamnestic response in which an ELISA peak titer of 66,957 was obtained compared to 5000 by PHA and 20 by CF, 9 to 12 days after a booster injection. Precipitin bands were produced by CCIE only with sera collected at peak titer, 32 to 34 days after initial injection, and could not be demonstrated again until the peak anamnestic response.

Hamster sera. Sera collected from hamsters 3 to 44 weeks after infection with *L. braziliensis* or *L. mexicana* were assayed for antibodies

by ELISA, PHA, and CF. Forty-eight hamsters out of 51 (94%) showed antibodies by ELISA, 33 out of 51 (65%) were positive by PHA, and 36 out of 39 (92%) demonstrated CF antibody. A high degree of sensitivity was apparent by the markedly high titers obtained by ELISA compared to those obtained with PHA or CF. Furthermore, of 33 sera positive by PHA, 17 were in the range of titers obtained with sera from some normal hamsters, which questions the validity of a positive diagnosis for these samples. Of 36 sera positive by CF, one showed a questionable titer. It is also significant that in PHA and CF tests, antibody titers appeared to remain at constant low levels, whereas ELISA demonstrated an increase in antibody through the 44th week after infection. There were no significant differences between antibody titers in sera from hamsters infected with *L. braziliensis* or *L. mexicana* which suggests that LB016 antigen cannot discriminate between *L. braziliensis* and *L. mexicana* infections.

Human sera. ELISA exact titers were obtained with sera from humans infected in Panama with *Leishmania*. The sera tested were from male and female patients, 9 to 44 years of age, who demonstrated *Leishmania* in skin scrapings and/or culture of skin scrapings. Of 31 sera examined, 23 (74%) showed titers which varied from 5 to 3429. In most cases, exact titers were calculated from titrations in which all or most serum dilutions produced absorbances. However, seven sera demonstrated absorbances in only one or two dilutions. In these cases, the titer was taken as the highest dilution of serum showing a net absorbance.

These results appear excellent when one considers that the human sera were randomly collected 5 to 10 years before they were assayed and were not identified relative to the time they were collected following infection and cure, which may account for some negative findings.

The low PHA titers obtained with hamster sera are not consistent with the markedly higher titers usually obtained in our laboratory in PHA determinations in which the antigen used is prepared by sonication and extraction with isotonic saline. This suggests that our antigen which was prepared with 3.5 M NaCl considerably decreases the antigenic determinants necessary for an optimal PHA assay and/or that antibody specific for predominant antigens in our preparation were lacking in infected hamsters.

We are currently attempting to prepare and apply purified antigens to ELISA in order to avoid drawbacks inherent with the use of mixed antigens in a quantitative system. Nevertheless, our data support the conclusions that the application of quantitative principles to the sensitive measurements provided by ELISA with 3.5 M NaCl extracted antigen, makes it an ideal tool for following the level of leishmanial humoral antibody and for determining variations in antibody responses among different individuals. In vitro and in vivo immunogenicity of antigens may be compared with greater sensitivity, and the method offers a procedure for the precise standardization of

conjugate and antigen reagents for serodiagnosis.

CONCLUSIONS

The micro-ELISA technique appears to offer considerable promise as a rapid in vitro procedure for the serodiagnosis of leishmaniasis. The method is more sensitive than PHA, CF, or CCIE for detecting leishmanial antibodies in the sera of immune or infected subjects. However, the sera tested does not permit conclusions to be drawn concerning cross-reactivity. It is anticipated that further refinements in antigen reagents will result in greater specificity and sensitivity. The appeal of the ELISA is that it is similar in design to radio-immunoassay and appears to have comparable sensitivity without the problems of unstable reagents or radiation hazards.

RECOMMENDATIONS

Further investigations should be carried out to determine the extent of cross-reactivity between leishmanial antigens and antibodies related to diseases other than leishmaniasis. These studies should be extended to include the biochemical and immunochemical purification and characterization of a specific leishmanial antigen to obtain optimal specificity and sensitivity in the serodiagnosis of leishmaniasis by ELISA.

PUBLICATIONS

LUZZIO, A.J., McROBERTS, M.J., and EULISS, N.H. Quantitative estimates of serum antibody titers in the serodiagnosis of American leishmaniasis by ELISA. Submitted for publication.

STUDY NO. 2 — Improved skin test antigens for the diagnosis and prognosis of leishmaniasis

PROBLEM

Leishmanial infections represent a group of parasitic diseases encountered by military personnel stationed in endemic areas. Since immunity to the leishmaniases is due principally to cell-mediated immune responses, skin testing to demonstrate delayed hypersensitivity is important to diagnosis and prognosis. Although skin testing procedures are widely used in epidemiologic surveys, a universally acceptable skin test antigen has not been developed and standardized. This study is directed toward preparation of a standard leishmanial antigen of known efficacy, potency, and safety which is suitable for skin testing U.S. military personnel.

RESULTS AND DISCUSSION OF RESULTS

Skin hypersensitivity tests based on the Montenegro reaction (intradermal injections of whole killed organisms as a means of eliciting

delayed skin reactions in sensitized subjects) were used to evaluate the ability of *L. enriettii* infected guinea pigs to respond to (1) leishmanin (promastigotes of *L. braziliensis* suspended in saline containing 0.4% phenol), and (2) soluble antigens of *L. braziliensis* prepared by sonication and extraction of promastigotes with 3.5 M NaCl. In addition, experimental animals were observed to determine the development of a period of optimum sensitivity, after infection, during which skin test antigens may be evaluated.

In guinea pigs which developed lesions 7 to 9 days after infection with $1x10^5$ *L. enriettii* amastigotes, mild responses were elicited by leishmanin or soluble antigen 14 days after infection. Soluble antigen containing 3.2 µgm total protein (TP) and injected intradermally in 0.05 ml volume produced a response which appeared to be comparable to that produced by leishmanin, which consisted of $1x10^6$ promastigotes or 4.3 µgm TP 0.05 ml. The highest dose of soluble antigen tested, 32 µgm TP produced significantly greater responses.

Intense delayed-type responses were obtained with soluble antigen containing 32 µgm TP when healing of cutaneous lesions began (8 weeks after infection). These responses were maximal at 24 h after antigen injection and were characterized by pronounced induration, and erythema with central pallor. Skin tests at 10 and 12 weeks yielded responses of equal intensity to those obtained at 8 weeks. Inoculation of antigens into uninfected control guinea pigs produced minimal non-specific reactions which were not typical of delayed-type responses. The early appearance of delayed-type skin responses indicates the potential usefulness of the skin test for early diagnosis of cutaneous leishmaniasis. The responses elicited during early infection did not approach the intensity of responses exhibited during the healing phase of the infection. However, 32 µgm TP of soluble antigen evoked responses during early infection which were of sufficient intensity to be clearly recognizable and which were markedly greater than those produced by leishmanin. Evaluation of the potency of antigen preparations would appear to be most suitably performed during the period of stable and maximal sensitivity at 9 to 12 weeks after infection.

CONCLUSIONS

L. enriettii infected guinea pigs exhibited delayed-type hypersensitivity skin responses to antigen preparations of *L. braziliensis* and may be useful models for the evaluation of skin test antigens. Delayed hypersensitivity skin responses to *L. braziliensis* antigens appear to be maximal and stable from 8 to at least 12 weeks after infection and appear to be optimal for the evaluation of skin test antigens.

RECOMMENDATIONS

The onset of delayed-type responses elicited by leishmanin and soluble antigen should be established and related to the development

of cutaneous lesions. The stability of skin sensitivity during the healing phase of infection should be further characterized. The intense responses obtained with soluble antigens suggest further evaluation with purified antigens as a means of increasing sensitivity and specificity.

PUBLICATIONS

None

STUDY NO. 3 Immune mechanisms in leishmaniases

PROBLEM

Leishmaniases constitute a group of vector-borne protozoan diseases among which some forms are mutilating and/or life-threatening to man. At present, there are no adequate means to protect military personnel required to operate in endemic areas. Immunization of troops before their deployment will be one of the best means of conferring protection. The long-lasting immunity conferred by healed cutaneous and visceral leishmaniases shows the potential of vaccine research. However, the many antigens of the organism present a major problem. These antigens must be isolated, purified and characterized, and their capacity to stimulate immunity must be ascertained in order to establish their potential protective value. Also, the roles of cell-mediated and humoral immunity in these diseases remain to be clearly delineated. The identification and characterization of circulating immune complexes will add considerable knowledge in understanding the regulation of the immune response of the parasitized host. Prerequisite to this approach is the necessity of establishing that immune complexes do indeed occur in leishmaniasis. The present study is designed to explore immunity in leishmaniases to provide a better understanding of the mechanisms involved.

RESULTS AND DISCUSSIONS OF RESULTS

In this study, blood was collected from hamsters once before infection with *L. braziliensis* and at frequent intervals thereafter. Gamma globulin was then separated from the sera and passed through a Sephadex G-200 gel filtration column at pH 10.5 to elute antigen-antibody components dissociated from immune complexes. The eluted fractions were then tested for leishmanial antigens or antibody and anti-complementary activity by microtiter complement fixation.

Of six animals with cutaneous leishmaniasis, all showed leishmanial antibody of the IgG immunoglobulin class 22 to 51 days after infection. Antigen and antibody were detected in the sera of one animal before infection and were not detected again until 36 days after infection. A second animal of this group showed both components only at 29 days after infection. Both of two animals infected with *L. donovani* showed

antigen and antibody in sera collected 24 days after infection. However, even though free antibody was detected in some fractions of eluted gamma globulin, the presence of free antigen was not confirmed because of anticomplementary activity of the fractions suspected to contain leishmanial antigen. The data indicate that immune complexes may not have dissociated by gel filtration at pH 10.5, or that dissociated components of similar molecular weights were eluted in the same fraction. In the latter case, it is likely that immune complexes were re-established when the pH of the fractions was readjusted to the pH (7.4), required for carrying out the complement fixation test, thus rendering such fractions anti-complementary.

The data support earlier work in which immune complexes of unknown composition were found in the glomeruli of human patients with visceral leishmaniasis and confirm our earlier work in which the presence of immune complexes in sera from hamsters infected with *L. mexicana* was suggested by the marked increase in anti-complementary activity. In addition, the evidence suggests that leishmanial antigen and/or antibody may be detected separately or simultaneously in the sera of infected subjects. The findings are consistent with the hypothesis that chronic parasitemia results in persistent shedding of antigen into the circulation whereby free antibody is bound in immune complexes.

CONCLUSIONS

Leishmanial antigens and/or antibody were detected simultaneously or separately in the sera of experimentally infected hamsters. This finding confirms our earlier studies in which the presence of circulating immune complexes were indirectly demonstrated by the marked increase in anti-complementary activity of sera from *Leishmania* infected subjects.

RECOMMENDATIONS

Studies of the immune response to infection with *Leishmania* should be extended to include the detection of specific antigen and/or antibody by enzyme-immunoassay. This will eliminate problems inherent with complement fixation and provide significantly greater sensitivity.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6313	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWN GRADING	7. GEOGRAPHY	8a. DISPOSITION	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A810	00	017
b. CONTRIBUTING				
c. [redacted]	CARDS 114¢			

11. TITLE (Procede with Security Classification Code)

(U) Area Repellents for Collective Protection of Troops from Vector-borne Diseases

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

005900 Environmental Biology; 002600 Biology

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

17. CONTRACT/GRANT

a. DATE/EFFECTIVE — EXPIRATION
b. NUMBER
c. TYPE: Not Applicable — d. AMOUNT
e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS ($ Thousands)
FISCAL YEAR 78	1.0	21
FISCAL YEAR 79	1.0	26

19a. PERFORMING ORGANIZATION

a. NAME: Letterman Army Institute of Research
b. ADDRESS: Presidio of San Francisco, CA 94129

19b. PERFORMING ORGANIZATION

a. NAME: Letterman Army Institute of Research
Division of Cutaneous Hazards
b. ADDRESS: Presidio of San Francisco, CA 94129

20. RESPONSIBLE INDIVIDUAL

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21. GENERAL USE

Foreign Intelligence Not Applicable

PRINCIPAL INVESTIGATOR (Provide Name, Grade, Title, Address, and Telephone)

a. NAME: Hooper, R. L., CPT, MSC
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c. SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

a. NAME: Wirtz, R. A., CPT, MSC
b. NAME: Rutledge, L. C., DAC POC: DA

22. KEYWORDS (Provide Security Classification Code)

(U) Repellent (U) Mosquito (U) Disease (U) Prevention (U) Formulations (U) Dispersal Equipment (U) Vector (U) Protection of Troops

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide Privacy Act data cited for human factors, man-machine interface or environmental studies)

23. (U) Protection of troops from vector-borne diseases is essential to maintaining an effective combat force. Repellents currently available for use by military personnel are not totally effective nor readily accepted by the user. This study is designed to test and evaluate repellents for field application to provide collective protection of troops on an area basis.

24. (U) Candidate chemical compounds suitable for area repellency and compatible with the environment will be identified. Formulations appropriate for field dissemination will be made. Dispersal equipment will be selected and evaluated for field use. Repellent formulations will be field tested to determine application rates, biological effectiveness, duration of protection and economy of use.

25. (U) 77 10 - 78 09. Methods for testing candidate compounds for area repellency have been developed. A series of natural products and petroleum-based materials have been tested in the laboratory for biological effectiveness against the yellow fever mosquito, *Aedes aegypti*. The most effective of the natural products tested to date have been geraniol and citronellal. The most active of the petroleum fractions listed to date have been identified as substituted naphthalenes. Laboratory testing of candidate compounds indicates that the concept of area repellents is feasible.

* Continued on continuation sheet(s)

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

ABSTRACT

PROJECT NO. 3M162172A810 — Military Skin Diseases

WORK UNIT NO. 017 — Area Repellents for Collective Protection of Troops from Vector-borne Diseases

Protection of troops from vector-borne diseases is essential to maintaining an effective combat force. Repellents currently available for use by military personnel are not totally effective nor readily accepted by the user. Methods for testing compounds for area repellency have been developed, and a series of materials has been tested in the laboratory for biological effectiveness against the yellow fever mosquito, *Aedes aegypti*. The most effective natural products tested to date have been geraniol and citronellal. The most active petroleum fractions have been identified as substituted napthalenes.

BODY OF REPORT

WORK UNIT NO. 017

Area Repellents for Collective Protection of Troops from Vector-borne Diseases

PROBLEM

The need to protect the military population from vector-borne diseases is essential for the maintenance of an effective combat force. Arthropod-borne diseases continue to be major health problems to military forces in overseas areas where troops are, or may be deployed, and where endemic foci of disease exist. The use of repellents appears to be the most practical means of interrupting arthropod-borne disease transmission, especially diseases for which vaccines or chemoprophylactic drugs are not available. The development of an effective area repellent could protect troops in areas of increased personnel activity without the disadvantages and shortcomings of skin repellents currently available in the military supply system.

RESULTS AND DISCUSSION OF RESULTS

Methods for the testing of candidate compounds for area repellency have been developed utilizing a 60 x 60 x 60 cm Plexiglas®-screen cage and suckling white mice. Candidate compounds in the following categories, which have exhibited area repellency in literature reports, have been procured for testing: (1) natural products, (2) petroleum distillate fractions between kerosene and fuel oil, and (3) repellents currently used for topical and cloth application. A series of 21 natural products and petroleum-based materials have been tested in the laboratory for biological effectiveness against the yellow fever mosquito, *Aedes aegypti*. The most effective of the natural products tested to date have been geraniol and citronellal. The most active of the petroleum fractions have been identified as substituted naphthalenes.

CONCLUSIONS

Laboratory testing of candidate compounds indicates that the concept of area repellency is feasible under certain environmental conditions.

RECOMMENDATIONS

Laboratory testing of candidate compounds against *Aedes aegypti* should be continued and the testing procedures should be expanded to include *Anopheles stephensi*, *An. albimanus*, and *Culex pipens*. Field testing of the compounds exhibiting the highest biological activity in laboratory tests should be initiated.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

			DA 08 6302	78 10 01		DD-DR&E(AR)636	
77 10 01	D. Change	U	U	NA	NL	Yes / No	

	62772A	3M162772A811	00	001
	CARDS 114f			

(U) Nutrition Studies in Support of DoD Food Program

002300 Biochemistry; 003500 Clinical Medicine; 009700 Mathematics and Statistics; 012900 Physiology; 006500 Food

73 07	CONT	DA		C. In-House

	78	10.0	383
	79	6.0	525

Not Applicable

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Foreign Intelligence Not Applicable

Letterman Army Institute of Research
Division of Nutrition Technology, Information Sciences Group, and Administration

Lauberlich, H.E. and Johnson, H.L.
415-516-4323 and 415-561-5066

Schnakenberg, D.D., MAJ, MS
Szurek, J.L., CPT, MS, Nelson, R.A., DAC
Skala, J.H., Kretsch, M.J., DAC POC:DA

(U) Military Rations; (U) Nutritional Requirements; (U) Military Medicine; (U) Clinical Chemistry; (U) Analytical Biochemistry; (U) Diet

23. (U) Capabilities will be provided to respond to the requirements of the DoD Food RDT&ENG Program (AR 70-3) for nutritional studies for all military services. Objectives are (a) to provide essential information on the nutritional adequacy of feeding systems, rations, and dietary standards for military personnel in all environs; (b) to evaluate the nutritional intakes and status, physical status, body composition, and work capacity of military to ensure that performance is not impaired by improper nutrition; (c) to evaluate the chemical and nutrient composition of present and future rations; and (d) to provide statistical and computer support for these objectives.

24. (U) Information is obtained through clinical examinations, dietary diaries, food analyses and intake measurements, blood and urine biochemistries, anthropometry and physical performance tests. Data are mathematically and statistically evaluated by computer programs. Unique military nutrition problems submitted by the services are studied through workshops, symposia, or research.

25. (U) 77 10 - 78 09 An Institute Report was published comparing average dining hall nutrient intakes and wastes before and after changing feeding systems at Loring AFB and nutrients for individuals are being evaluated. The Institute Report of diary-interview intakes from NAS-Alameda personnel was published and individual intakes from dining hall meals are being processed. Biochemical analyses of blood, urine, and foods from NAS-Alameda and Twentynine Palms MCB were completed. Two interim reports were prepared for evaluations of dining hall meal nutrients and individuals' daily intakes for Twentynine Palms. An interim report on the present afloat feeding system and plans for a study of the modified system were completed. Further progress was made in updating the LAIR Nutrient Factor File and the computer programming for nutrient calculations.

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

ABSTRACT

PROJECT NO. 3M6277A011 Military Nutrition and Food Hygiene

WORK UNIT NO. 001 Nutrition Studies in Support of DOD Food Program

The following investigations have been conducted under this work unit:

STUDY NO. 3 Nutrition survey at Loring AFB, Maine

STUDY NO. 4 Nutrition survey at Naval Air Station, Alameda

ADDENDUM A Phase II of study to evaluate changes in nutrient intakes after renovation of dining facility and initiation of a la carte feeding system

ADDENDUM B Phase III of study to evaluate the biochemical and clinical indices of nutritional status and survey food intakes (in dining hall, other sources, and total) of sailors on the COMRAT a la carte system

STUDY NO. 5 A series of nutrition surveys to evaluate the effects of changing feeding systems (to an all COMRAT/a la carte system) at Twentynine Palms, California, upon nutrition consumption and nutritional status of the Marine

STUDY NO. 6 A before-and-after series of nutrition surveys to evaluate the effects of modifications to Navy food service system afloat on nutrient intake

STUDY NO. 8 Evaluation of the MCI and MRE rations as the sole subsistence for an extended period

STUDY NO. 9 Nutrient intake data system, development and maintenance

STUDY NO. 3 A study of the nutritional impact of initiating an all BAS/a la carte item pricing system of feeding enlisted personnel was conducted at Loring Air Force Base by monitoring average food intakes and wastes, and obtaining individual tray inventories from each patron before and after initiating the new system. An Institute Report was published comparing average nutrient consumptions and food wastes before and after implementing this feeding system. Computer programming and data verification of the individual tray data were completed, and total nutrients for each tray and for each person during the individual studies have been computed and are being statistically analyzed. These data will provide for a thorough evaluation of the effects of the food

service changes upon the nutritional intakes of these patrons.

STUDY NO. 4 An additional study of the effects of a BAS/cash a la carte feeding system on the nutritional adequacy of enlisted personnel's diet was conducted at Naval Air Station, Alameda. Dietary diary-interview data from the conventional and the cash a la carte systems have been evaluated and an Institute Report has been submitted for publication. Computer programming and data verification of individual tray inventories from two 17-day studies of the dining hall are progressing.

STUDY NO. 5 The initial report summarizing the nutrient intakes of the enlisted Marine population at the Twentynine Palms Marine Corps Base, California, prior to food service system modification has been revised and reviewed again. Revision included further statistical analysis of the data and inclusion of demographic and anthropometric data. The reason(s) for the reported low energy intakes of the Marines (FY 77 Annual Progress Report, pp 202-204) remain(s) unknown. None of the Marines in the study population was below the military weight for height standards, and the percentages of body fat were within the normal range. A protocol addendum has been written and approved to investigate the energy requirements of the Twentynine Palms Marine population. Plans have been formulated for the second nutrition study to assess the nutritional impact of the food service system changes implemented by NARADCOM on the enlisted personnel.

STUDY NO. 6 The analyses of the dietary, demographic, and anthropometric data obtained from 203 Naval personnel messing afloat have been essentially completed. Recommendations to improve the nutrient intakes and nutritional health of sailors at sea have been incorporated into the new fast-food service system being tested on the USS Saratoga. It is imperative that a follow-up study be conducted to evaluate the effectiveness of the nutrition intervention program and the overall nutritional impact of the new system.

STUDY NO. 8 A protocol was prepared to determine how long personnel could subsist upon present and future combat rations, what the nutritional, physiological, and psychological effects of long-term (up to 90 days) subsistence on rations would be, and how to improve or supplement these rations to assure that personnel's health would not be detrimentally affected by sole subsistence on rations. This study cannot be initiated under present budgetary restrictions.

STUDY NO. 9 Nutrient Intake Data System (NIDS) is a system of computer programs for calculation of nutrient contents of dining hall meals and of the total daily intakes of individuals. The major programs of the system have been completed or are nearly completed, and several parts of the system have been used through "one-time" programs to accomplish specific goals. The LAIR Nutrient Factor file was extensively updated by adding the concentrations of cholesterol, protein from animal/plant sources, and fat from animal/plant/marine sources contained in

approximately 2500 food items. A significant number of vitamin E, vitamin B_6, vitamin B_{12}, folic acid, magnesium, copper, zinc, manganese, pantothenic acid, and fatty acid values were added to the file also.

BODY OF REPORT

WORK UNIT NO. 001 — Nutrition Studies in Support of DOD Food Program

STUDY NO. 3 — Nutrition Survey at Loring AFB, Maine

PROBLEM

The health, combat effectiveness, physical fitness and performance capabilites, and mental functioning of military personnel are affected by the nutritional status of the individual. Formerly, our military forces were assured a nutritionally adequate diet by the provisions of well-balanced meals in the dining halls, which were the major source of the soldier's food intake. As the enlisted personnel became more affluent and had greater liberties under the concepts of the Modern Army, the contribution of the military dining halls to his/her total nutrition decreased. To counteract the reduced utilization of military dining facilities, the military services began modernizing the feeding system. Included in the changes were menu alterations which gave minimal considerations to the nutritional adequacy of the diets. One of these new feeding concepts that is favored by the Air Force is the BAS/a la carte (item priced) system. This concept was tested at Loring Air Force Base, and LAIR was requested to evaluate the adequacy of nutritional consumptions that would occur with the new feeding system.

RESULTS AND DISCUSSION OF RESULTS

To determine the effects of changing feeding systems upon nutritional balance of meals consumed, 2 studies were conducted at Loring AFB. A 3-day evaluation of the conventional feeding system was conducted in October 1974, and a 5-day study of the BAS/a la carte system was done in November 1975. An Institute Report comparing average nutrient consumptions from dining hall meals was published in December 1977. In audition, the Air Force took photographs of all individual trays of food during both of these studies and provided copies of these films to LAIR. Dietitians extracted the information on individuals' food selections and the Department of Information Sciences provided programming and data processing to obtain nutrient consumptions for each person eating in the dining hall. These data are being statistically analyzed and will be published as another report.

CONCLUSIONS

The conclusions from the evaluations of average meals consumed in the dining hall were presented in the FY 77 Annual Progress Report and the Institute Report No. 48. The results of the individuals' nutrient consumptions are being evaluated and are not yet available.

RECOMMENDATIONS

The BAS/a la carte feeding system should not be broadly expanded until more studies have been evaluated. Further recommendations for improvement of this feeding concept should be forthcoming from the evaluation of individuals' food and nutrient intakes.

STUDY NO. 4 — Nutrition Survey of the Naval Air Station, Alameda, California

ADDENDUM A — Phase II of study to evaluate changes in nutrient intakes after renovation of dining facilities and initiation of a la carte feeding system

ADDENDUM B — Phase III of study to evaluate the biochemical and clinical indices of nutritional status and survey food intakes (in dining hall, other sources, and total) of sailor on the COMRAT a la carte system

PROBLEM

(Same as for Study No. 3, and in addition) The Department of Defense directed all services to test the all COMRAT (BAS)/cash a la carte feeding concept. The Navy Food Service System Office submitted a requirement under OPNAVINST 3900.26B (AR 70-3) for an evaluation of Navy feeding systems, and Natick Army Research and Development Command (NARADCOM) responded to this requirement and requested that LAIR conduct a nutritional evaluation of the systems in conjunction with their studies.

RESULTS AND DISCUSSION OF RESULTS

A nutrition study was conducted at Naval Air Station, Alameda (NAS/Alameda), California to evaluate the nutritional impact of conversion from the existing mixed ration-in-kind (RIK) commuted ration (COMRAT) standard dining hall system to an all-COMRAT Cash a la Carte system. Phase 1 was conducted in March 1975 to study the existing mixed RIK/COMRAT system. Phase 2 was conducted in June 1976 as an interim study following conversion to the Cash a la Carte system which was implemented on 1 March 1976. Phase 3 was conducted in August 1976, following a 5-month stabilization period. Phases 1 and 3 encompassed dining hall, biochemical/clinical, and total dietary intake aspects. Only the total dietary intake aspect was assessed in Phase 2. Dining hall attendance, nutrient intake per dining hall meal, and total daily nutrient intake data were obtained by a diary-interview technique from 445 male enlisted personnel. As previously reported in 1976 and 1977 Annual Progress Reports, the increased rate of dining hall utilization by single and married COMRAT status personnel did not completely compensate for the marked decrease in utilization by RIK personnel converted to COMRAT status.

The major nutritional impact of the item-pricing policy was to reduce milk and milk-product consumption markedly and, consequently, calcium and riboflavin intakes per dining hall meal. Item-pricing also reduced citrus juice and citrus fruit consumption and, consequently, ascorbic acid intakes per dining hall meal. The average energy, protein, phosphorus, iron, and thiamin intakes per dining hall meal also decreased. Item-pricing and modifications to the menu were not effective in reducing the percent of calories derived from fat sources. Although nutrient intakes per dining hall meal decreased under the new system, the mean intakes (with the exception of energy) still met or exceeded one-third of the military daily allowances for these nutrients.

The Cash a la Carte system had an undesirable nutritional impact upon the total daily nutrient intakes of former RIK personnel. This was due to the combination of reduced dining hall utilization and reduced nutrient intakes per dining hall meal. The total daily milk and milk-product consumption of the RIK group markedly decreased with a corresponding decrease in calcium and riboflavin consumption. Furthermore, the percentage of RIKs with calcium and riboflavin intakes below the recommended daily allowances increased after implementation of the Cash a la Carte system. The changes associated with item-pricing did not significantly affect the total daily nutrient intakes of single and married personnel on COMRAT because these groups consumed less than 12% of their daily calories in the dining hall.

Significant progress has been made in developing computer programs to process the individual in-dining hall food selection and plate waste data obtained during Phases 1 and 3 of this study. This large volume of data (approximately 35,000 individual meals) has required an extensive data verification effort which is nearing completion. These individual tray selection and waste data, when coupled with an analysis of the clinical, biochemical, and anthropometric data will contribute to a more extensive and complete evaluation of the nutritional impact of the Cash a la Carte system.

CONCLUSIONS

The conclusion that conversion of all RIK personnel to COMRAT status will markedly reduce dining hall attendance, as observed at NAS/Alameda, can be projected to other military installations that offer many readily available alternative food outlets. A decline in dining hall utilization is a potential threat to the training base for food service personnel necessary for feeding afloat. Decreased dining hall utilization was a primary factor contributing to the less than desirable nutrient intake patterns of the RIK group after conversion to COMRAT status. Item-pricing of milk in the dining hall at a price identical to that of less nutritious competing beverages reduced milk consumption and subsequently reduced the total daily calcium and riboflavin intakes of patrons who consumed a substantial number of meals in the dining hall.

RECOMMENDATIONS

We recommend a discontinuation of converting all RIK status personnel to COMRAT status. However, item-pricing could be used to promote improved food habits and nutrient intakes.

STUDY NO. 5

A series of nutrition surveys to evaluate the effects of changing feeding systems (to an all COMRAT/a la carte system) at Twentynine Palms, California upon nutrient consumption and nutritional status of the Marine

PROBLEM

The effects of changing feeding systems upon nutrient consumptions and nutritional status of the Marine were evaluated to assure that his/her military capabilities are not adversely affected by poor nutrition. Any change in the feeding system has the potential of influencing the nutritional status of personnel. The Marine Corps submitted a requirement to the DOD Food RDT & Eng Program for an analysis of the food service system at Twentynine Palms Marine Corps Base. The analysis was to include alternatives for improving this system. Operational Research and Systems Analyses Office (OR/SA) of NARADCOM is conducting a test of a new system (several types of dining facilities, e.g., A-ration dining halls, short-order dining halls, steak houses, and specialty houses serving ethnic foods). The test is a multi-year effort with individual goals of changing decors of dining facilities, updating kitchen equipment and cooking methods, modernizing serving concepts, and evaluating Marine Corps operation of the system after test personnel have relinquished control. A definitive evaluation of the nutritional impact of the new system upon the Marine would require a series of surveys, one of the conventional system for control data, and one after each goal has been attained.

RESULTS AND DISCUSSION OF RESULTS

Total daily nutrient intake, nutrient intake per dining hall meal consumed, and dining hall attendance data were obtained by a diary-interview technique from 279 male and 36 female enlisted personnel during a nutriton study conducted at Twentynine Palms Marine Corps Base, California during March 1977. This information will serve as baseline data to measure the nutritional impact on Marine personnel of food service system changes designed to improve customer acceptance and utilization. The results of this study have been revised and reviewed. Revision included further statistical analysis of the data and inclusion of demographic and anthropometric data.

Only one of the groups in the diary-interview portion of the study reported daily energy intakes adequate to meet the military energy

allowances established for moderately active persons living in a temperate environment. Between 65 and 85% of the personnel of the other four groups were 500 kcal or more below the allowance. None of the Marines studied were below the military weight for height standards, and although the percentages of body fat were significantly lower than values of previously studied military populations, they were within the normal range. However, since energy expenditure and weight change were not monitored during the first study, the reason(s) for the low energy intakes is(are) unknown. The accuracy of the reported energy intake values is critical to the interpretation of the other nutrient consumption values. Therefore, in order to evaluate the Marines' total daily nutrient intakes, and in order to propose valid recommendations based on this evaluation, energy expenditure and weight change will be studied in future Twentynine Palms nutrient intake studies.

In addition to reported low energy consumptions, between 20% and 60% of the population consumed diets low in vitamin A. The diets of the women were low in iron, protein, and thiamin for 78%, 33%, and 20%, respectively, of the group. Approximately 20% of the Rations-in-Kind (RIK) personnel from the Force Troops units consumed diets with low thiamin density. Average dining hall utilization was between 40% and 50% for RIK men, and 28% for RIK women.

CONCLUSIONS

Results of the study and the suggested menu modifications to elevate vitamin A content and lower the percentage of fat calories in the meals served in the dining halls have been communicated to MARADCOM and to the Twentynine Palms Marine Food Service System Officer. However, the food service is limited in its capability to improve the nutrition of individuals. If nutritionally adequate and acceptable food is provided, it then becomes the Marine's responsibility to improve his/her nutrition. A Marine program to provide nutritional guidance in altering food selection and food habits must be developed for Marines in order to correct and prevent nutritional problems.

RECOMMENDATIONS

1. Energy requirements of male and female Marines at Twentynine Palms Marine Corps Base should be investigated.

2. The vitamin A content should be increased and the percentage of fat calories should be lowered in meals provided by enlisted dining hall facilities.

3. Iron supplements should be free and easily available to the female population and their use encouraged.

4. Increased usage of base dining hall facilities by RIK personnel, particularly the women, should be encouraged.

5. A Marine Nutrition Education and Awareness Program to prevent and correct individual nutritional problems should be developed.

STUDY NO. 6

A before-and-after series of nutrition surveys to evaluate the effects of modifications to Navy food service systems afloat on nutrient intake

PROBLEM

The Department of the Navy requested that a study be conducted aboard a Forrestal class aircraft carrier to define the problems that exist with Navy afloat food service systems. They requested that qualitative and quantitative alternatives to the existing system be developed to achieve improvements in user acceptance, greater efficiency in operations, reduced costs and manpower requirements, and that architectural and design concepts be proposed to improve the total food service environment. NARADCOM has implemented a test of a fast-food service system in the forward galley of the USS Saratoga as a solution to some of the problems of food delivery. LAIR has conducted a study of the adequacy of the nutritional intakes of the crew prior to modification of the food service system. There remains the requirement to evaluate the nutritional impact of the new system which, if accepted for use Navy-wide, will affect 112,000 enlisted personnel messing afloat each day.

RESULTS AND DISCUSSION OF RESULTS

The July-August 1977 nutrition survey, conducted aboard the USS Saratoga during operations in the Mediterranean area, identified a number of areas of nutritional concern that existed prior to modifications of the food service system. These concerns included:

- The ship's supply of milk was depleted after 4 to 6 days at sea due to insufficient chill space. The lack of milk directly contributed to an increased incidence of low and marginal calcium, riboflavin, and vitamin A intakes.

- Meals (mostly short-order) offered and consumed in the Forward Galley were low in vitamin A and vitamin C. Poor food habits (infrequent consumption of dark-green and yellow vegetables and citrus fruits) of some individuals also contributed to a relatively high incidence of low and marginal intakes of certain nutrients, especially vitamin A.

Twenty percent of the ship's company reported consuming diets low in vitamin A and 8% selected diets low in vitamin C. Low vitamin A intakes will increase the risk that some individuals may develop problems with night blindness and dark adaptation, and impair their abilities to perform duties in darkness (night flight operations). Low vitamin C intakes may limit the capacity to cope with severe stress and may slow recovery from disease or injury.

The body weights of 25% of the studied population (203 enlisted, E-6 and below) exceeded the BUMED weight for height standards.

CONCLUSIONS

Results of the study and recommendations to improve nutrient intakes and nutritional health have been communicated to Navy Food Service Systems Office (NAVFSSO) and the USS Saratoga. A number of these recommendations have been incorporated into the new fast-food service system being tested on the USS Saratoga. LAIR and NARADCOM personnel have collaborated to try to enhance the nutritional quality of the fast-food type menu with a program of menu expansion and nutrient fortification of selected food items. The milk availability and vitamin A problems will be addressed by offering a low-fat milk shake, reconstituted aboard ship from a dry, shelf-stable mix, fortified according to LAIR and NARADCOM specifications, to provide 1800 IU of vitamin A per serving. This product is predicted to reduce markedly the incidence of low and marginal vitamin A intakes and to promote adequate intakes of calcium and riboflavin when milk supplies are depleted. Fresh green salads will be offered at all Forward Galley dinner and supper meals; however, the acceptance of this product and its beneficial effects upon vitamin and mineral intakes will be limited by the quality, quantity, and variety of produce available, and by the food habits of the individuals. The use of beverage base fortified with vitamin C for preparing non-carbonated beverages, and offerings of citrus fruits and citrus juices at Forward Galley meals will be used to improve vitamin C intakes. There has been little progress, however, in establishing and implementing a Nutrition Education and Awareness Program that will effectively improve food habits and deal with the problem of developing obesity. None of the involved organizations (NAVFSSO, BUMED, NARADCOM, OTSG, MRDC, or LAIR) appear to be willing or adequately staffed and funded to develop such a program. The Secretary of the Army is responsible for developing nutrition education programs for use by the Services.

RECOMMENDATIONS

1. It is absolutely essential that a follow-up study be conducted after implementation of the new fast-food service system in the Forward Galley of the USS Saratoga. This study should answer the question of whether a fast-food service system forward will improve, have no effect, or have a detrimental effect upon nutritional health of Navy afloat personnel.

2. Based upon the findings of the follow-up study, recommendations should be made to the Department of the Navy on what aspects of the test system should be implemented on other ships in the fleet.

3. A research effort should be initiated at LAIR to develop an effective Nutrition Education and Awareness Program.

STUDY NO. 8 — Evaluation of the MCI and MRE rations as sole subsistence for an extended period

PROBLEM

No long-term study has been conducted on the use of either the present MCI or future MRE combat ration as sole source of subsistence; however, military contingency plans for the future include this concept. Although each meal of these rations contains at least one-third of an adult's allowance of all known required nutrients (excluding water) when procured, both rations are comprised of only 12 meals and long-term stability of some vitamins are not assured. With only 12 meals available (and food preferences of the individuals would reduce the number acceptable to him or her), the menu would become monotonous and total nutrient consumptions would be expected to be drastically reduced. Deterioration of labile vitamins could result in nutritional deficiencies in the consumer that would adversely affect his/her mental and/or physical performance. Therefore, it is imperative that this study be conducted before we have a military operation with only rations available. Excessive casualties due to inadequate nutrition may compromise the entire operation.

RESULTS AND DISCUSSION OF RESULTS

Further progress on this study is awaiting the availability of the new MRE rations and the status of funds for this project area.

CONCLUSIONS AND RECOMMENDATIONS

A long-term feeding study with each ration as the sole source of subsistence should be conducted. Personnel's food consumption, nutritional status, body weight and composition, mental and physical capacities, and military performance of duties should be monitored. This study should be conducted before rations are accepted as the only food available during a military operation.

STUDY NO. 9 — Nutrient intake data system, development and maintenance

PROBLEM

A nutrition study includes a detailed monitoring of foods consumed by individuals and groups, in military dining facilities and away

from them, both from observations and from the analysis of diaries kept by individuals. The analyses of these data require the use of a computer because of their large volume and complexity. Programs are needed to maintain files and process data collected during studies, maintain files of nutrients in foods, and to calculate nutrients consumed. The validity of these nutrient calculations depends upon current information on the foods consumed. Therefore, considerable updating of the nutrient factor file is needed to reflect additional foods and the latest information, both from published values and from laboratory analyses, on nutrient contents of food items.

RESULTS AND DISCUSSION OF RESULTS

Two major efforts have been ongoing in this area, computer programming and updating the nutrient factor file. A system of programs (Table 1) has been designed for data base management and for nutrient calculations. Some programs have been completed and others are being coded and tested. NIDSNF was completed first, since data calculations require a file of nutrient concentrations. NIDSDB has been completed, although it needs major modifications to increase its utility. Programs that are nearing completion are NIDSDH (will require major modifications to utilize LAIR-analyzed data), NIDSGC and NIDSDR. Programs which have not been started are NIDSTI, NIDSPW, NIDSIS, NIDSIC, and NIDSSC; however, most of these have been simulated with "one-time" programs and system libraries to process specific data. The programs that have been completed or are nearly completed represent the major programs of the system and the majority of the total effort. The nutrient factor file that is maintained by NIDSNF has had several recent enhancements (Table 2). This computerized file describes approximately 2500 food items, including nutrient concentrations. These data were derived from standard food composition handbooks, published literature, in-house laboratory analyses, and food manufacturers. The file requires continual input for maintenance of accuracy, revising old values, and the addition of values for new foods. During FY 78, complete nutrient files were established for cholesterol, the quantity of protein from animal/plant sources, and the quantity of fat from animal/plant/marine sources. A significant number of values have been added to the file for vitamin E, vitamin B_6, vitamin B_{12}, folic acid, magnesium, copper, zinc, manganese, pantothenic acid, and fatty acids. In addition, a master recipe file was established to utilize recipe data to calculate nutrients for a specific food dish when values were not available.

CONCLUSIONS

The Nutrient intake data system (NIDS) is nearing completion with the major programs either completed or nearly completed. The nutrient factor file has been updated with the most current nutrient values and additional new foods. Therefore, future nutrition investigations can be evaluated with greater efficiency and results will be the most current available.

RECOMMENDATIONS

1. The remaining programs of NIDS should be completed and the system evaluated for efficiency and versatility for all types of nutrition studies.

2. The nutrient factor file should be continually updated and expanded as analyzed nutrient values become available.

3. Annual editing of the file should be continued to maintain accuracy.

4. Users should be encouraged to utilize this file in their programs.

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Table 1 - Nutrient Intake Data System (NIDS) Functional Outline

Routine	Function	Input	Output
NIDSDB	NIDS Data Base performs data base management	Raw data	Headcount/Attendance Tray Inventory Observations Cash Register Observations Serving Data Recipes Individual Plate Waste Observations Dietary Diary - Recall Data Posters and Demographic Data
NIDSTI	NIDS Tray Inventory formats tray inventory data and matches trays, meal cards, Diner ID, etc.	Headcount Tray Inventory Observations Cash Register Observations Gross Serving Data	Headcount Individual Serving Data Attendance Patterns
NIDSPW	NIDS Plate Waste edits and accumulates plate waste data	Individual Plate Waste Observations	Individual Plate Waste Gross Plate Waste
NIDSIS	NIDS Individual Servings develops data of the food served to each individual	Individual Serving Data Individual Plate Waste	Portion Data Individual Consumption Data
NIDSNF	NIDS Nutrient Factor File maintains a data base of nutritional information on foods	Food Description and Nutrient Concentration Data	Nutrient Factor File
NIDSDM	NIDS Dining Hall displays serving and recipe data and develops a file of the concentration of nutrients consumed by diners and the quantity of each food consumed	Gross Serving Data Recipes Portion Data Gross Plate Waste Nutrient Factor File	Serving Factor File Serving Data Display Recipe Data Display

Table 1 (cont) - Nutrient Intake Data System (NIDS) Functional Outline

Routine	Function	Input	Output
NIDSGX	NIDS Gross Consumption displays and accumulates nutrient consumption data from the serving line factor file	Serving Factor File	Gross Nutrient Consumption
NIDSGS	NIDS Gross Summary summarizes the gross consumption data	Gross Nutrient Consumption	Gross Consumption by Food and Nutrient Type
NIDSIC	NIDS Individual Consumption develops a file of nutrient consumption data by individual	Serving Factor File Individual Consumption Data	Individual Nutrient Consumption from Tray Inventory
NIDSDR	NIDS Diary/Recall determines the nutrient intake of individuals as recorded in dietary diaries or recalled	Diary - Interview Data Nutrient Factor File Serving Factor File	Individual Nutrient Consumption from Diary Data
NIDSSC	NIDS Selected Consumption selects data for analysis with specific programs or statistical libraries	Individual Nutrient Consumption Rosters and Demographic Data Recalled Nutrient Consumption	Individual Nutrient Consumption Analysis Food Selection

Table 2 - Enhancements to the LAIR Nutrient Factor File

PROXIMATE ANALYSIS		
Total animal protein	Total lipids	Alcohol
Total plant protein	Total animal fat (non-fish)	Dietary fiber
	Total fish fat	Sucrose
	Total plant fat	

AMINO ACIDS		
Tryptophan	Valine	Aspartic acid
Threonine	Histidine	Glutamic acid
Isoleucine	Arginine	Tyrosine
Leucine	Cysteine	Proline
Lysine	Cystine	Hydroxyproline
Methionine	Alanine	Serine
Phenylalanine	Glycine	

FATTY ACIDS		
Total Saturated	Polyunsaturated	Cholesterol
4:0	18:2	Phytosterols
6:0	18:3	Total Monounsaturated
8:0	18:4	16:1
10:0	20:4	18:1
12:0	20:5	20:1
14:0	22:5	22:1
16:0	22:6	
18:0	Fatty acid ratio (Polyunsaturated/Saturated)	

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Routine	Function	Input	Output
NIDSDB	NIDS Data Base performs data base management	Raw data	Headcount/Attendance Tray Inventory Observations Cash Register Observations Serving Data Recipes Individual Plate Waste Observations Dietary Diary - Recall Data Rosters and Demographic Data
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NIDSPW	NIDS Plate Waste edits and accumulates plate waste data	Individual Plate Waste Observations	Individual Plate Waste Gross Plate Waste
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NIDSDH	NIDS Dining Hall displays serving and recipe data and develops a file of the concentration of nutrients consumed by diners and the quantity of each food consumed	Gross Serving Data Recipes Portion Data Gross Plate Waste Nutrient Factor File	Serving Factor File Serving Data Display Recipe Data Display

Table 1 (cont) - Nutrient Intake Data System (NIDS) Functional Outline

Routine	Function	Input	Output
NIDSGC	NIDS Gross Consumption displays and accumulates nutrient consumption data from the serving line factor file	Serving Factor File	Gross Nutrient Consumption
NIDSGS	NIDS Gross Summary summarizes the gross consumption data	Gross Nutrient Consumption	Gross Consumption by Food and Nutrient Type
NIDSIC	NIDS Individual Consumption develops a file of nutrient consumption data by individual	Serving Factor File Individual Consumption Data	Individual Nutrient Consumption from Trav Inventory
NIDSDR	NIDS Diary/Recall determines the nutrient intake of individuals as recorded in dietary diaries or recalled	Diary - Interview Data Nutrient Factor File Serving Factor File	Individual Nutritent Consumption from Diary Data
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Table 2 - Enhancements to the LAIR Nutrient Factor File

PROXIMATE ANALYSIS

Total animal protein	Total lipids	Alcohol
Total plant protein	Total animal fat (non-fish)	Dietary fiber
	Total fish fat	Sucrose
	Total plant fat	

AMINO ACIDS

Tryptophan	Valine	Aspartic acid
Threonine	Histidine	Glutamic acid
Isoleucine	Arginine	Tyrosine
Leucine	Cysteine	Proline
Lysine	Cystine	Hydroxyproline
Methionine	Alanine	Serine
Phenylalanine	Glycine	

FATTY ACIDS

Total Saturated	Polyunsaturated	Cholesterol
4:0	18:2	Phytosterols
6:0	18:3	Total Monounsaturated
8:0	18:4	16·1
10·0	20:4	18·1
12:0	20:5	20·1
14:0	22:5	22·1
16:0	22:6	
18:0	Fatty acid ratio (Polyunsaturated/Saturated)	

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6111	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8A. GROUP MARK: NL	8B. SPECIFIC OR TA CONTRACTOR ACCESS: ☒ YES ☐ NO
9. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER	
a. PRIMARY	62772A	3M162772A811		00	002	
b. SECONDARY						
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10. TITLE: (U) Nutritional Requirements of Military Personnel in Health, Injury and Disease

11. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine; 002300 Biochemistry; 006500 Food

12A. START DATE	12B. ESTIMATED COMPLETION DATE	13. FUNDING AGENCY	14. PERFORMANCE METHOD
76 10	CONT	DA	C. In-House

15. CONTRACT/GRANT
a. DATE/EFFECTIVE: — EXPIRATION:
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e. KIND OF AGENCY: — f. CUM AMT:

16. RESOURCES ESTIMATE

	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
FISCAL YEAR (PROGRAM) 78	10.0	308
FISCAL YEAR (CURRENT) 79	12.0	600

17. RESPONSIBLE DOD ORGANIZATION
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19. GENERAL USE: Foreign Intelligence Not Applicable

20. KEYWORDS (U) Military Nutrition; (U) Military Rations and Feeding; (U) Human Nutrient Requirements; (U) Nutritional Assessment; (U) Nutrient Intakes

21. TECHNICAL OBJECTIVE, 22. APPROACH, 23. PROGRESS (Provide brief statement of work performed in support of each technical objective)

23. (U) Military men and women may be placed in stressful situations that can significantly alter their nutritional requirements and influence their military effectiveness and health. One general objective is to conduct research pertaining to the adequacy of military diets and nutrient requirements, intake and nutritional status of all military personnel. The investigations are aimed to provide increased knowledge concerning (a) nutritional requirements, (b) nutrient content of modern military foods, and (c) effects of certain food or food items on metabolism.

24. (U) Through use of animal experiments and controlled human studies, we will seek to (a) define the nutrient requirements of military personnel in terms of age, sex, and activity levels; (b) establish the nutritional parameters essential for military personnel to perform with maximum physical and mental capability under all environmental and military stresses; (c) develop methods for determining nutrients and biochemical components in blood, tissues, urine, and foods for application in military medicine, nutrition surveys, and rations studies; (d) develop techniques which reflect the nutritional status; (e) determine whether or not various stresses, diseases, or injuries increase nutrient requirement.

25. (U) 7o 10 - 78 09 Procedures were developed for the measurement of brain catecholamine without vitamin C interference, for the measurement of whole blood vitamin C by oxidation reduction methods, for the micro-determination of copper, iron and total iron, and for the measurement of tissue vitamin C metabolites and riboflavin derivatives. The biochemical mechanism of anemia in scurvy was investigated in separate experiments, by determining the influence vitamin C has on folate status, copper and iron status, and heme degradative pathways. Interrelationships between brain catecholamine and vitamin C, copper deficiency-vitamin C status and serum ferritin were studied. The vitamin C requirement of monkeys was investigated.

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

ABSTRACT

PROJECT NO. 3M762772811 Military Nutrition and Food Hygiene

WORK UNIT NO. 002 Nutritional Requirements of Military Personnel in Health, Injury and Disease

The following investigations have been conducted under this work unit:

STUDY NO. 1 Requirements, functions, interactions and metabolism of ascorbic acid (vitamin C)

STUDY NO. 2 Riboflavin - metabolism and analytical methods

STUDY NO. 3 Pharmacology, toxicology, interactions and functions of nutrients

STUDY NO. 4 Evaluation and development of procedures and techniques for the assessment of the trace mineral nutritional status of military personnel

STUDY NO. 5 Nutritional iron deficiency

STUDY NO. 7 Techniques to evaluate nutritional status of humans

STUDY NO. 1 The role of vitamin C was studied in guinea pigs and monkeys. In the guinea pig, vitamin C deficiency results in elevated serum copper and ceruloplasmin and liver copper levels. However, iron dependent functions like liver cytochrome P-450 and cytochrome b_5 and liver iron levels as well as blood heme were decreased. Vitamin C deficiency in guinea pigs did not seem to influence brain catecholamines. Studies to determine the ascorbate requirements of male monkeys suggested that a minimum of 3 ascorbate pools exist and that young monkeys may require twice the amount of ascorbate as adult monkeys. In addition, oxidation-reduction assay systems for ascorbate were modified to measure whole blood ascorbate.

To investigate the relationship between vitamin C intake and folate status, guinea pigs were maintained on diets adequate or marginal in folate and supplemented with high or low ascorbic acid. Preliminary evidence confirms an increased need for ascorbic acid to prevent folate deficiency symptoms when folic acid intake is marginal.

STUDY NO. 2 A rapid, sensitive, fluorometric titration assay using a riboflavin specific apoenzyme has been developed for measurement of riboflavin in urine and dietary samples.

STUDY NO. 3 As ascorbic acid deficiency progressed, decreases in hepatic aminopyrine N-demethylase activity and cytochrome P-450 levels seem to be related to a decrease in the cytosol ascorbic acid

(100,000 x g supernatant) in guinea pigs. Hepatic microsomal heme oxygenase activity and cytochrome P-450 level are directly related to the dietary or liver ascorbate levels. After the incorporation of δ-aminolevulinic acid-3, 5-^{3}H, the disappearance of radioactivity from the cytochrome P-450 heme (CO-binding pigments) was not dependent on the ascorbic acid status of the guinea pigs. Ascorbic acid deficiency does not seem to influence the degradation of hepatic heme.

STUDY NO. 4 Procedures have been evaluated and techniques developed for the microanalysis of copper, iron, and total binding capacities. These procedures require only 50 µl of serum for each analysis. Application of these methods has been made to other studies in this work unit and in work unit 056.

STUDY NO. 5 Studies on ferritin and iron metabolism have included a statistical study of the influence of race upon ferritin and other parameters of iron status. Preliminary results suggest that blacks have higher serum ferritin levels than whites and that individuals at elevated altitudes have higher ferritin levels. Efforts are underway to characterize monkey ferritin and to study the effect of copper deficiency upon ferritin levels in monkeys.

STUDY NO. 7 Comparison of whole blood folate analyses by radioassay and microbiological assay methods of samples from 2 military nutrition surveys has been completed. Because of qualitative and quantitative differences in the radioassays, neither of the 2 commercial kits tested on whole blood samples was considered comparable to the microbiological assay in terms of diagnostic capability.

BODY OF REPORT

WORK UNIT NO.	002	Nutritional Requirements of Military Personnel in Health, Injury and Disease
STUDY NO.	1	Requirements, functions, interactions and metabolism of ascorbic acid (vitamin C)

PROBLEM

The experiments reported in this study were initiated in order to advance further the fundamental knowledge concerning the nutrition and biochemistry of ascorbic acid. Research is needed to provide improved means to assess the ascorbate adequacy in military personnel. Means for assessment must be sensitive, relatively free, and specific. Unfortunately, basic information on the mechanism and function of ascorbic acid is incomplete and fragmentary; subsequently, it is difficult to recommend intakes of ascorbate to military personnel, especially under adverse or stress situations. Therefore, basic research must be undertaken to provide the understanding of ascorbate's biological function. Research should include the metabolism and nutrient interactions as well as the pharmacology and toxicology of ascorbate. Once some insight into these functions is determined, recommendations of ascorbic acid intakes can be made for optimal health and performance of military personnel.

RESULTS AND DISCUSSION OF RESULTS

Vitamin C, iron and copper. Experiments were conducted to determine the effects of low and high intakes of vitamin C on plasma and liver concentrations of iron and copper. Vitamin C-deficient guinea pigs with plasma values of <0.6 ug/ml demonstrated significantly higher plasma and hepatic copper concentrations, 99.8% and 94% respectively, compared to animals dosed with a saturating level of the vitamin. Also, plasma ceruloplasmin values were concurrently 102.3% higher in the vitamin C-deficient animals. In contrast, plasma and liver concentrations of iron were 38.2% and 57.9% respectively lower in vitamin C-deficient animals. Hepatic microsomal heme and blood heme parameters were also signficantly ($P < 0.05$) reduced in vitamin C deficiency. In the second experiment, in a group of guinea pigs dosed with the National Research Council (NRC) recommended level of vitamin C as a control group, plasma ceruloplasmin and copper were similar to animals dosed with a saturating level of the vitamin. However, hepatic copper and iron levels in this control group were more similar to animals receiving no vitamin C. Plasma iron levels seemed to be related directly to vitamin C intake. The relative amounts of ceruloplasmin and ascorbate in the system exert a strong influence on the relative levels of ferrous iron and ferritin. This, in turn, can control iron utilization, transport, and heme synthesis.

Brain catecholamine and ascorbic acid. A high pressure liquid chromatography technique was developed which allowed the investigator to measure the amounts of catecholamines (CAs) without interference by ascorbic acid (AA). The investigator using the newly developed technique determined CA content of brain tissue in control and AA-deficient guinea pigs. No differences were found in CA content in the two dietary conditions; however, this experiment examined CA equilibrium in an unchallenged manner. Further research is currently being conducted to elucidate a possible protective role of ascorbic acid in the maintenance of CAs after a biochemical challenge.

Cultured neuroblastoma as a model for study of the role of ascorbic acid in neural tissue. The cell line and most of the techniques necessary for the study have been set up in the laboratory. Preliminary results indicate that ascorbic acid is toxic to these cells. The mechanism of this tocicity is currently under investigation.

Experiments are being conducted into the possible role of ascorbic acid in the neurotoxicity of 6-hydroxydopamine, but no conclusive results are yet available.

Methods of analysis for ascorbic acid. Modifications of an older manual method, which depends upon reduction of the dye 2,6-dichlorindophenol, have been made. These make it more convenient and sensitive and allow the use of whole blood, which was not possible with the original method. A second assay, depending upon reduction of iron by ascorbic acid and its subsequent measurement with the dye, 6,6'-dipyridyl, has also been modified so that it can be used with whole blood.

Metabolism of ascorbate. The relationship of plasma ascorbate levels of male monkeys (M. fascicularis) to varying levels of oral dietary ascorbate was measured during chronic ascorbate deficiency and gradual ascorbate repletion. Two plateaus in the plasma ascorbate levels were measured during the repletion phase. Active juvenile male monkeys required twice the amount of ascorbate as did the mature males to sustain similar plasma levels. The oxidation of ascorbate to CO_2 was shown to be a minor metabolic pathway of ascorbate in monkeys as it is in man. Oxidation of ascorbate was independant of the animal's nutritional ascorbate status.

Vitamin C and folic acid. Megaloblastic anemia (symptomatic of folate deficiency) is sometimes noted in clinical vitamin C deficiency. Some studies have suggested that ascorbic acid is necessary for normal folate metabolism and that vitamin C deficiency may cause a secondary folate deficit. In an effort to evaluate this interaction, the following experiment was conducted to investigate the relationship between vitamin C intake and folate status under conditions of adequate or marginal folate intake.

Guinea pigs were fed semi-purified diets adequate or marginal in folic acid and supplemented with high or low ascorbic acid. Hematological parameters were monitored and at the end of the experiment, tissues were removed for folate and ascorbate assays. When folic acid intake was low, there appeared to be an increased requirement for vitamin C to maintain normal blood cell counts (especially white cells). Tissue analyses have not been completed yet (personnel have been diverted to another project).

CONCLUSIONS

Dietary copper and vitamin C must be in the diet of guinea pigs in balanced amounts for proper iron utilization. Strong imbalances in either direction interfere with iron utilization and may cause anemia.

The high pressure liquid chromatography (HPLC) technique as developed in this protocol is superior to the standard fluorescence assay for the measurement of catecholamines because the HPLC technique eliminates a source of false positive values.

The fate of ascorbate metabolism is similar in monkey and man only when the monkeys are trained to the procedures to be used prior to the study. Therefore, the trained monkey is an excellent model for study of ascorbate metabolism in man.

Data collected in the guinea pig study suggest that a deficiency in ascorbic acid is detrimental to folate-requiring processes when folate intake is marginal.

RECOMMENDATIONS

1. The interactions of ascorbic acid with other trace minerals should be studied in animal models.

2. Further studies on the copper, ascorbate, iron interrelationships, where levels of dietary copper and iron are varied simultaneously with the ascorbate level are needed to define an optimum balance.

3. Although steady-state levels of catecholamines (CAs) are not effected by a scorbutic state, the dynamic metabolism of CA may be influenced by ascorbic acid. Rather than observations in the steady-state, a dynamic test of CA metabolism should be studied.

4. Investigation of cultured neuroblastoma should be continued to determine whether or not ascorbic acid may be toxic to these cells and, if so, what the mechanism might be. Such information could prove useful in the understanding of the interaction of ascorbic acid with nervous tissue.

5. The possible effect of ascorbic acid on synthesis of acetylcholinesterase in cultured neuroblastoma should be investigated. Through such studies a role for ascorbic acid in prophylaxis or treatment of nerve agent poisoning might ultimately be found.

6. The use of trained monkeys to elucidate the requirement and fate of ascorbate in monkeys should be continued. These data should establish criteria for judging the course of ascorbate metabolism in man.

7. The evaluation of the ascorbic acid-folic acid interaction should be repeated with guinea pigs and terminated earlier. There is reason to believe that (because folate requirements decrease with age) treatment differences in tissue folate levels would have been greater at an earlier phase of the experiment.

STUDY NO. 2 — Riboflavin - metabolism and analytical methods

PROBLEM

The riboflavin nutritional status of military personnel is determined by measurement of urinary and/or plasma riboflavin. Present assays are time consuming and nonspecific. Recently, a riboflavin specific protein was isolated from egg white. The fluorescence of riboflavin is quenched when it binds with equal molar quantities of the riboflavin specific apoprotein. A fluorometric titration with the apoenzyme may be a quantitative measure of riboflavin.

RESULTS AND DISCUSSION OF RESULTS

A technique for the isolation and purification of the riboflavin specific apoprotein from egg white has been developed in this laboratory. This apoenzyme has been used to measure quantitatively riboflavin in urine and dietary extracts. The sensitivity of the assay is similar to the microbiological procedures but the specificity is greater. Samples can be assayed immediately without extraction of the flavins and the apoprotein isolated from 1 egg will measure 200 to 300 samples.

CONCLUSION

The utilization of this assay will decrease the time and expense required for measurement of riboflavin with increased specificity.

RECOMMENDATION

Continued development of procedures for isolation of the flavin mononucleotide specific apoprotein. This protein will permit the measurement of the flavin nucleotides without hydrolysis, therefore decreasing time and expense.

STUDY NO. 3 Pharmacology, toxicology, interactions and functions of nutrients

PROBLEM

Optimal nutrition requires sufficient intake of all essential nutrients to meet minimum physiological needs. However, nutrient intakes must be less those levels considered detrimental to health. Optimal response of various nutrient-dependent functions may vary considerably over the relatively wide range of nutrient intakes. Various environmental, pharmacological and toxicological stress situations may influence these nutrient-dependent functions and alter the optimal nutrient intake levels. Alternatively, various nutritional regimens may provide protection from such stress situations. Excessive intakes may elicit pharmacological effects or toxicological syndromes that may or may not be related to known functions of that substance. If separate physiological, pharmacological, and toxicological effects exist, the relationship between such effects, the closeness of levels at which these effects occur, and the specific effects produced must be determined. These studies will produce information of direct value in maintaining maximal physiological performance of the military member. Knowledge of the requirements for specific nutrients under various stressful circumstances, along with their potential therapeutic or prophylactic uses, would be of great value in this regard. Troops may be exposed to a number of toxicants in the course of their military duties; for this reason, knowledge of detoxification mechanisms and the effect of nutrition upon them is of great importance. Finally, once a military member is undergoing medical treatment for a specific disease or injury, obviously an understanding of nutritional and pharmacological interactions will contribute to the effectiveness of his care.

RESULTS AND DISCUSSION OF RESULTS

The influence of ascorbic acid on microsomal heme oxygenase (MHO) (E. C. 1.14.99.3), drug metabolism and ascorbate distribution was studied in livers and liver cytosols isolated from guinea pigs. Aminopyrine N-demethylase and cytochrome P-450 content were examined in guinea pigs on 0, 6, 12, and 19 days after being placed on a basal diet deficient in ascorbate. Plasma ascorbate seems to reflect total liver ascorbate; however, ascorbate from the liver cytosol (100,000 x g soluble fraction) component decreased at a slower rate than total liver ascorbate as deficiency progressed. The decrease in hepatic aminopyrine N-demethylase and cytochrome P-450 content paralleled the decrease in cytosol ascorbate. Perfusion of livers from guinea pigs not depleted of ascorbate resulted in 71% loss of extracellular ascorbate and a 26% decrease in cytochrome P-450 content. Ascorbate-supplemented (25 mg/100 g) guinea pigs had a 16% decrease in cytochrome P-450 content. MHO activity was decreased significantly ($p < 0.005$). These results suggest that a labile soluble pool of ascorbate may have some influence on cytochrome P-450 content and drug metabolism. Also, these

results suggest a dose-related dependence of MHO on ascorbate and that ascorbate deficiency does not produce an increase in hepatic heme catabolism via increased MHO activity.

Degradation of hepatic cytochrome P-450 heme in ascorbate-deficent and ascorbate-dosed (P.O. 25mg/100g/day) guinea pigs was investigated by determining the turnover of radioactive cytochrome P-450 and the formation of in vivo expired carbon monoxide (^{14}CO), a specific degradation product of the labeled heme. Hepatic levels of cytochrome P-450 and cytochrome b_5 were decreased significantly, 61%, $p < 0.001$, and 29%, $p < 0.01$, respectively, in scorbutic animals compared to scorbate-adequate animals. After the administration of δ-aminolevulinic acid-3, 5-^{3}H, the disappearance of radioactivity from the fast-phase and slow-phase components of cytochrome P-450 heme (CO-binding pigments) exhibited half-lives of 4.2 h and 30.8 h respectively in the ascorbate-depleted guinea pigs. The turnover of cytochrome P-450 heme was similar for both phases in animals fed the ascorbate-deficient diet and dosed with ascorbate or animals fed a normal stock diet. In guinea pigs deficient in ascorbate and injected with [5-^{14}C] δ-aminolevulinic acid, the cumulative expired ^{14}CO was similar to guinea pigs adequate in ascorbate. These studies demonstrate that the decrease in hepatic cytochrome P-450 content in ascorbate deficiency is not due to a defect in cytochrome heme catabolism.

CONCLUSIONS

Essentially, ascorbic acid deficiency in guinea pigs did not appear to influence the degradation of microsomal cytochrome P-450 heme. These findings, along with the past investigations on heme synthesis, suggest that ascorbate has no influence on heme catabolism or heme synthesis. The possibilities still remain that ascorbate may influence apo-cytochrome P-450 synthesis, the incorporation of ferrous iron into the heme moiety of cytochrome P-450, or the binding of heme and apo-cytochrome P-450 to form active cytochrome P-450.

RECOMMENDATIONS

1. Experiments need to be designed to develop more understanding in how ascorbate is utilized in drug metabolism.

2. Purification of cytochrome P-450 from guinea pigs will be needed in order to provide standards for apo-cytochrome P-450 synthesis studies.

3. Methods for the specific identification of the iron oxidation state in tissues must be developed in order to determine the effect of ascorbate on the incorporation of ferrous iron into the heme moiety.

4. Methods to study the binding of heme to cytochrome P-450 must be developed in order to determine whether or not ascorbate influences the formation of active cytochrome P-450.

STUDY NO. 4 Evalulation and development of procedures and techniques for the assessment of the trace mineral status of military personnel

PROBLEM

Little is known about the trace element requirements of man or current dietary intakes of these elements. Rapid changes in food resources and food processing portend alterations in the levels, ratios, and availability of essential mineral elements in military feeding systems. Thus, it is important to know the requirements and levels of essential trace minerals in foods consumed by the military in order to assure optimum performance, health, and recovery from injury. It is also important to develop procedures to assess the status of military populations with regard to their trace mineral nutriture.

RESULTS AND DISCUSSION OF RESULTS

Procedures have been developed for the analysis of copper, iron, and total iron binding capacity for small (50 µl) serum samples.

For copper, the 50 µl of serum is diluted with 450 µl of 10 mM HNO_3. Twenty µl of diluted sample is then injected into the graphite furnace of an atomic absorption spectrophotometer and copper levels are determined by comparison with suitable standards. This procedure compared favorably with conventional flame methods for copper, which require a much larger (1 - 3 ml) sample size.

Iron and total iron binding capacities were also determined by micro methods using flameless atomic absorption spectroscopy. Heme iron and serum protein were precipitated in 50 µl of serum by adding an equal volume of 10% trichoroacetic acid. After heating, the sample iron further diluted by adding 100 µl of deionized water, then the sample was centrifuged. Five µl of the supernatant was then injected into graphite furnace and iron levels were determined. Total iron binding capacities were determined by adding 100 µl of 5 ppm iron standard to 50 µl of serum. After precipitation with magnesium carbonate to remove the excess iron, iron levels were analyzed as described above.

These procedures compared favorably with more conventional methods which require much larger samples. The major problem in the iron and total iron binding capacity analyses was that erroneously high values were obtained in badly hemolyzed samples.

These methods offer a considerable advantage when a large number of analyses are required on a limited quantity of sample.

These procedures have been applied in other studies in this work unit and in a study in Work Unit 056.

Additional food samples are being analyzed for copper, zinc, and manganese for inclusion in the LAIR nutrient factor file.

CONCLUSION

None

RECOMMENDATIONS

1. The data base for trace mineral contents of foods should be expanded to cover more food items and other essential and toxic minerals.

2. Other military populations need to be surveyed as to their trace mineral intakes in order to determine if potential problems exist relating to trace element nutriture.

3. The effects of marginal or low intakes of various essential trace minerals on performance and recovery from injury should be more thoroughly investigated.

4. Human requirements for the essential trace minerals need to be more carefully defined, particularly under various stress situations.

STUDY NO. 5 Nutritional iron deficiency

PROBLEM

Previous studies with human subjects have indicated that both altitude and race may play some role in the metabolism of iron. The objective of this study was to determine if either race or altitude influence the storage levels of iron as measured by serum ferritin.

While anemia has been indicated as being associated with scurvy in man, monkeys, and guinea pigs, the literature contains contradictory results. The cynomolgus monkey was chosen as an animal model to evaluate the interactions between iron status and copper and ascorbic acid nutriture.

RESULTS AND DISCUSSION OF RESULTS

Race and altitude effects. As a continuation of the ferritin studies begun last year, a statistical analysis of parameters of iron status was undertaken. The study was expanded from the original surveys of Twentynine Palms Marine Corps Base, Alameda Naval Air Station I and III, and Ft. McClellan to include data from surveys performed at Ft. Meyer, Ft. Lewis, and Ft. Benning. This study was a retrospective study of the influence of race upon the parameters of iron status. Because of the similarity in economic factors and availability of mess halls, the military population is relatively free of economic or dietary influences upon nutritional indicators.

The total population being analyzed consisted of 1860 individuals, of which 729 had serum ferritin analyses. Preliminary results indicate that blacks have lower hemoglobin levels than whites (15.9 vs 16.1 gm/dl). This difference is statistically significant ($p < 0.005$). However, serum ferritin exhibits a reverse of this situation with blacks having a serum ferritin level of 94 µg/ml and whites having a level of 57 µg/ml. This difference is statistically significant ($p < 0.00002$). It would appear that the lowered hemoglobin levels of the black population are not due to lowered iron stores. Studies of other parameters of dietary intake, plasma ascorbate, plasma vitamin A, and whole blood folate showed no statistically significant differences; these observations suggest that there were no gross differences in overall dietary intake.

A second study was undertaken to determine the effect of altitude upon iron stores as measured by serum ferritin. A total of 132 normal male serums from Leadville, CO were analyzed for serum ferritin and the results compared to the population discussed above. Information on the hemoglobin and hematocrit values indicated that, as expected, individuals at higher altitude have higher values (Hgb = 17.3 gm/dl, Hct = 52.1%) than do individuals at lower elevations (Hgb = 16.1 gm/dl, Hct = 46.8%). Serum ferritin levels also exhibited this increase with individuals at altitude having an average serum ferritin level of 87 µg/ml as compared to individuals at lower altitude with 64 µg/ml.

Ferritin and iron status in monkeys. Studies on monkey ferritin were carried out in 2 separate experiments. In the first study, the iron status of monkeys on a diet deficient in copper with 2 levels of ascorbate (1 mg/day and 100 mg/day) was investigated. Analysis of the data is not yet complete, but preliminary indications are that neither the level of copper deficiency nor the amount of ascorbate ingested has any effect upon the red cell count, hemoglobin, hematocrit, or serum ferritin levels.

As a corollary for the development of a ferritin assay for monkey serum, preliminary work has been conducted on the Model E analytical ultracentrifuge in an effort to establish the molecular weight of rhesus monkey ferritin. The purification of the ferritin has been monitored using gel electrophoresis. It appears that a single protein has been isolated which migrates as a single peak in the ultracentrifuge. No further results are available at this time and work is in progress to characterize further monkey ferritin.

CONCLUSIONS

Preliminary results indicate that blacks have higher serum ferritin levels than whites and that individuals at elevated altitudes have higher ferritin levels. Thus, if serum ferritin is to be used as an indicator of iron stores, both race and altitude will have to be considered.

RECOMMENDATIONS

Further study of the incidence of iron deficiency in military force needs to be undertaken. In addition, more information is required concerning the influence of race upon iron metabolism. The conflicting findings of lower hemoglobin levels in blacks as compared to whites and higher iron stores as measured by serum ferritin indicate that factors other than dietary intake may be important in adequate iron metabolism. Further studies in this area should be undertaken. Finalization of the data processing on the monkey study is recommended as well as completion of the monkey ferritin characterization.

STUDY NO. 7 — Techniques to evaluate nutritional status of humans

PROBLEM

The purpose of this study was to evaluate and improve current methods for assessing folic acid nutritional status. Radioassays of serum and red cell folate are now widely being used instead of the traditional microbiological assays. However, their diagnostic reliability has not been established, particularly with respect to red cell folate. The purpose of the first experiments was to compare commercial folate radioassay kits with the L. casei microbiological assay with particular attention being paid to responses at concentration ranges which have diagnostic significance.

RESULTS AND DISCUSSION OF RESULTS

Four kits have been tested on whole blood and plasma samples from Alameda III and Twentynine Palms military nutrition surveys. All but one agreed reasonably well with the microbiological assay when compared on serum samples (discussed in progress report last year).

Both kits (New England Nuclear (NEN) and Bio-Rad) tested on whole blood samples gave means which were significantly higher than the microbiological assay and different from each other ($p < 0.01$). In addition, there were distinct differences in the frequency distribution curves. Although the Bio-Rad curve was displaced to the right of the microbiological, they both showed distinct positive skewness. In contrast, NEN data were clustered in a sharp peak with little skewness. Thus, the estimated normal range for red cell folate was much narrower according to the NEN kit than either the Bio-Rad or the microbiological assay.

CONCLUSION

Because of the qualitative and quantitative differences in the radioassay kits, neither of those tested on whole blood samples was considered to be comparable to the microbiological assay in terms of diagnostic capability.

RECOMMENDATIONS

Although radioassays appear to be suitable for serum samples, the microbiological assay is recommended still as the most reliable method for future analyses of whole blood folate at this institute.

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3. TILLOTSON, J.A., M.E. SHEA, and A.A. SOMERA. Ascorbate metabolism in trained primates. (Abstract) Fed Proc 37:448, 1978

4. AMOS, W.H. and H.E. SAUBERLICH. Serum ferritin and its relation to other parameters of iron status. (Abstract) Fed Proc 37:892, 1978

5. AMOS, W.H. and G.C. VAUGHN. Ascorbic acid and iron metabolism in the macaca fascicularis (cynomolgus) monkey. (Abstract) Clin Chem 24:1014, 1978

6. OMAYE, S.T. and J.D. TURNBULL. Influence of ascorbic acid (AA) on hepatic microsomal heme oxygenase (MHO) and cytochrome P-450 in the guinea pig (GP). (Abstract) The Pharmacologist 20:181, 1978

7. McGOWN, E.L., C.M. LEWIS, H.H. DONG, and H.E. SAUBERLICH. Comparison of commercial radioassay kits with microbiological assay of serum and red cell folate. (Abstract) Fed Proc 37:494, 1978

8. MILNE, D.B., D.D. SCHNAKENBERG, and H.L. JOHNSON. Dietary intakes of copper, zinc, and manganese by military personnel. (Abstract) Fed Proc 37:894, 1978

9. MILNE, D.B., D.D. SCHNAKENBERG, H.L. JOHNSON, and G.L. KUHL. Dietary intakes of selected trace elements by military personnel: preliminary observations. (Submitted for publication)

10. OMAYE, S.T., M.D. GREEN, J.D. TURNBULL, W.J. AMOS, and H.E. SAUBERLICH. Influence of ascorbic acid and erythorbic acid on drug metabolism in the cynomolgus monkey. (Submitted for publication)

11. OMAYE, S.T., J.D. TURNBULL, and H.E. SAUBERLICH. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol (In press)

12. GREEN, M.D., W. BELL, C. KRAUT, and S. OMAYE. Failure of ascorbic acid to influence brain catecholamines in the guinea pig. Experientia (In press)

13. OMAYE, S.T. and J.D. TURNBULL. Heme oxygenase activity, drug metabolism, and ascorbic acid distribution in the livers of ascorbic acid-deficient guinea pigs. Biochem Pharmacol (In press)

14. TURNBULL, J.D., H.E. SAUBERLICH, and S.T. OMAYE. Effects of ascorbic acid deficiency and of erythorbic acid on blood components in the cynomolgus monkey. Int J Vit Nutr Res (In press)

15. McGOWN, E.L., C.M. LEWIS, M.H. DONG, and H.E. SAUBERLICH. Comparison of commercial radioassay kits with microbiological assay of serum and whole blood folic acid. Clin Chem (In press)

16. BASHOR, M.M., and J.A. TILLOTSON. Isolation of a riboflavin-binding apoprotein from chicken egg white and its use in a radioassay for urinary riboflavin. (Abstract) Fed Proc 37:672, 1978

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22. OMAYE, S.T.. and J.D. TURNBULL. Degradation of cytochrome P-450 heme in ascorbic-acid deficient guinea pigs. Arch Biochem Biophys (In press)

23. MILNE, D.B., and S.T. OMAYE. Effect of vitamin C on copper and iron metabolism in the guinea pig. (Submitted for clearance)

24. SAUBERLICH, H.E. Biochemical parameters (infants, children, women). In: Proceedings "Nutrition Assessment of Children and Youth Workshop," (Lansing, Michigan, 2-4 May 1977). pp 33-66

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OA 6337	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWN GRADING	7. REGRADING	8. KIND OF INVEST	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM	10. WORK UNIT
77 10 01	H. Termination	U	U	NA	NL	☒ YES ☐ NO		

11. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A811	00	003
b. [illegible]				
c. [illegible]	CARDS 1147			

12. TITLE (Procedure, Method, or Technique Only)

(U) Analytical Biochemistry

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 003500 Clinical Medicine

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
66 07	78 09	DA	C. In-House

18. CONTRACT/GRANT

a. DATE/EFFECTIVE — EXPIRATION
b. NUMBER* Not Applicable
c. TYPE — d. AMOUNT
e. KIND OF AGENCY — f. CUM AMT

19. RESOURCES ESTIMATE

FISCAL YEAR	a. MANPOWER (In-House)	b. PROFESSIONAL MAN YRS	c. FUNDS (In-Thousands)
PREVIOUS 78		3.0	144
CURRENT 79		0.0	00

20. PERFORMING ORGANIZATION

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Presidio of San Francisco, CA 94129
b. ADDRESS*

c. RESPONSIBLE INDIVIDUAL
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TELEPHONE 415-561-3600

21. GENERAL USE

Foreign Intelligence Not Applicable

22. PERFORMING ORGANIZATION

a. NAME* Letterman Army Institute of Research
Biochemistry Division
b. ADDRESS* Department of Nutrition
Presidio of San Francisco, CA 94129

c. PRINCIPAL INVESTIGATOR (Person who conducts project)
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TELEPHONE 415-561-5871
SOCIAL SECURITY ACCOUNT NUMBER

d. ASSOCIATE INVESTIGATORS
NAME Sauberlich, H.E., DAC
NAME POC:DA

23. KEYWORDS (Precede Each with Significant Classification Code)

(U) Analytical Biochemistry; (U) Instrumentation; (U) Automated Analyses; (U) Nutrition Surveys; (U) Clinical Chemistry; (U) Mil Medicine

24. TECHNICAL OBJECTIVE, APPROACH, AND PROGRESS (Precede Each Paragraph with Significant Classification Code)

23. (U) The objectives are to develop and adapt new concepts in analytical biochemistry, to provide reliable and advanced procedures and services to military-oriented research programs at LAIR and, on occasion, to approved cooperating agencies; to innovate or develop analytical procedures to meet specific needs of such research as, for example, the development of micro-automated assay procedures for enzymes related or altered during nutritional deficiencies, disease states, or stress conditions; to develop procedures applicable to military nutrition surveys, ration test studies, and food wholesomeness evaluations.

24. (U) Analytical support will be provided to studies in military medicine requiring routine analyses in volume or unique equipment and special techniques for assays of physiological specimens obtained during military nutrition surveys or field studies. Specific analyses will be originated or adapted, as required, to meet the needs of specific studies and to improve the economy and efficiency of laboratory operations. Research will be conducted on a continuing basis in support of the objectives indicated to provide new methods. Whenever feasible and practical, the methods will be automated and linked to computer systems.

25. (U) 77 10 - 78 09 Analytical support requiring more than 16,000 analyses was provided to 25 studies originating in 5 departments at LAIR and 2 services of LAMC. Problems in applying dye-binding techniques for determining serum albumen in different species were partially resolved. This work unit is terminated due to directed realignment of the LAIR research program. Certain functions have been transferred to another division.

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

ABSTRACT

PROJECT NO. 3M762772A811 Military Nutrition and Food Hygiene

WORK UNIT NO. 003 Analytical Biochemistry

The following investigations have been conducted under this work unit:

STUDY NO. 1 Analytical support and services

STUDY NO. 2 Development of analytical biochemistry procedures

STUDY NO. 1 Analytical support requiring 16,326 individual analyses was provided to 25 research projects.

STUDY NO. 2 Preliminary study was initiated on the applicability of analytical reagent systems to different species of animal models. Sedimentation velocity and meniscus depletion sedimentation equilibrium studies were performed on ferritin preparations from two species.

BODY OF REPORT

WORK UNIT NO. 003 Analytical Biochemistry

STUDY NO. 1 Analytical support and services

PROBLEM

Chemical analysis of various physiological specimens and diet or ration items is a fundamental adjunct to the majority of the research objectives of the many research protocols active at LAIR. The Analytical Biochemistry Branch has the responsibilities for providing service to the investigating staff in the form of automated analyses and special chemistries.

RESULTS AND DISCUSSION OF RESULTS

The Branch provided support to 25 research projects, including 3 field studies, which resulted in a total of 16,326 automated, semi-automated, and manual analyses. These analyses were distributed by type as follows:

Analytical Service	Number of Analyses
Blood Chemistry:	
(1) Automated electrolytes, glucose, total protein, urea nitrogen, creatinine, iron, cholesterol, triglycerides, vitamin C, lactate, pyruvate, uric acid, hemoglobin, and various enzymes	10,635
(2) Semi-automated lipid phosphorus and total iron binding capacity	170
(3) Manual electrophoresis, hemoglobin, hematocrit, free fatty acids	388
Urine Chemistry:	
(1) Automated electrolytes, creatinine, and urea nitrogen	2,022
(2) Semi-automated methylmalonic acid and total nitrogen	802
(3) Manual titrable acidity	28
Food and Tissue Chemistry:	
(1) Semi-automated minerals	1,556
(2) Manual proximate analyses	231
Special Chemistry:	
Amino Acids	494

These analyses were distributed among the Departments as follows: Surgery, 31.0%; Nutrition, 28.6%, Medicine, 23.1%; Comparative Medicine, 15.3%; and Blood Research, 1.9%. Special projects accounted for the remainder of service consisting of 158 analyses for the Neonate Service (LAMC), 709 analyses for 3 field studies (Work Unit 001, Nutrition Studies in Support of DoD Food Program) and 383 analyses for the Irradiated Food Program.

CONCLUSIONS

The branch staffing was reduced to 40% of normal at the start of the fiscal year. Fortuitously, the demands for service declined to 52% of the total analyses for the previous year. The relative proportion of semi-automated and manual analyses increased over the previous year. This included elimination of a small percentage of the backlog which stands at 14,079 analyses.

The branch rendered service to all investigations requesting analyses which were automated or semi-automated. Unfortunately, a support request originating in the Department of Surgery could not be serviced because of the extensive manual workload it would have placed on the limited staff.

RECOMMENDATIONS

Knowledge of the concentration of various constitutents of physiological and diet specimens is essential to the conduct of mission-oriented research programs at the Institute. It is recommended that a central analytical facility be maintained for automated analyses and special chemistries.

PUBLICATIONS

ASKEW, E.W., S.S. KULINSKI, J.R. LOWDER, and W.R. WISE, JR. Comparison of turnover rates of four adipose tissue depots as influenced by exercise. (Abstract) Fed Proc 37:428, 1978

STUDY NO. 2 Development of analytical biochemistry procedures

PROBLEM

Automation or simplification of analytical procedures is required to expand analytical support capabilities and maintain operational efficiency. Improvement of method quality is a continuous concern and innovation of procedures is occasionally required. Staff must be prepared to advise investigators on analytical approaches.

RESULTS AND DISCUSSION OF RESULTS

One staff member was trained in the operation of an analytical ultracentrifuge. The schlieren system was tested and evaluated. Studies of horse ferritin and monkey ferritin preparations were initiated. These included sedimentation velocity series to determine if the preparations were monodispersed, and if so, the actual sedimentation velocity. Meniscus depletion sedimentation equilibria were determined to facilitate estimation of molecular weights. The results indicated that the preparation provided did not contain ferritin.

Of primary concern has been the suitability of reagent systems developed for human blood components when applied to specimens from various animal species used as experimental models. The problem may also be masked by the use of human sera for quality control. In establishing baseline values for the owl monkey colony such a problem occurred. Serum albumin values obtained by using a proprietary dye which has an absorbance maximum at 505 nm with human albumin yielded satisfactory quality control recoveries (human sera) but approximately 60% of the value for albumin indicated by the electrophoretic pattern of the unknown specimens. Evaluation of a randomly selected number of specimens (N=22), by using bromcresolgreen and the proprietary dye, yielded values (mean ± standard deviation) of 4.1 ± 0.3 and 2.7 ± 0.3, respectively.

CONCLUSIONS

Training of staff to utilize properly available sophisticated instruments not only assures accurate results but appropriate application and utilization of the equipment.

Method performance in application to animal species must be monitored carefully when trying to establish baselines, particularly when assaying protein constituents with reagent systems developed for human specimens.

RECOMMENDATIONS

Continuous surveillance of operations with the objectives of automating or improving efficiency of performance and assurance of the applicability of methods is required.

PUBLICATIONS

SAUBERLICH, H.E., W.C. GOAD, J.H. SKALA, and P.P. WARING. Procedure for mechanized (continuous-flow) measurement of serum ascorbic acid (vitamin C). In: Part VII, Vitamins, Selected Method of Clinical Chemistry, Volume 8. American Association for Clinical Chemistry, Washington, D.C., 1978. pp 191-197

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OA 6370	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DRAE(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. GROUP MARK: NL	9. SPECIFIC DATA-CONTRACTOR ACCESS: [X] YES [ ] NO
10. NO./CODES: PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER	
a. PRIMARY	62772A	3M162772A811		00	004	
b. [illegible]						
c. [illegible]	CARDS 114f					

11. TITLE (Protocol code and security classification code):
(U) Military Food Hygiene

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
006500 Food, 007800 Hygiene and Sanitation, 016800 Toxicology, 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
70 04	CONT	DA			C. In-House

17. CONTRACT GRANT
a. DATE(S) EFFECTIVE: EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: d. AMOUNT:
e. KIND OF GRANT: f. CUM AMT:

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS ($ Thousand)
FISCAL YR: 78	4.7	195
FISCAL YR: 79	2.0	150

19. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
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RESPONSIBLE INDIVIDUAL
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TELEPHONE: 415-561-3600

20. GENERAL USE
Foreign Intelligence Not Applicable

21. PERFORMING ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Robert Wirtz, CPT, MS
NAME: Linda S. Guthertz, DAC POC:DA

22. KEYWORDS (Precede each with security classification code):
(U) Wholesomeness; (U) Data Collection; (U) Microbiological Limits; (U) Safety; (U) Foodborne Disease; (U) Food Contaminants; (U) Methodology

23. TECHNICAL OBJECTIVE: (U) The objectives are to monitor the microbiological quality of food items through laboratory analysis; to review the literature for trends; to report findings; to determine toxicological quality of foods; to establish suggested guidelines for items of potential health hazard; to review wholesomeness and safety requirements for DoD procured subsistence and to make recommendations to OTSG; to evaluate and develop new and existing methodology and equipment in the areas of food microbiology, virology and toxicology; and to collect microbiological data for food items analyzed by DoD laboratories.

24. (U) Examine various bacterial species and strains for their ability to produce histamine in media supplemented with histidine at levels comparable to high risk food products. Test various segments of intestinal mucosa for the histamine-catabolizing enzymes, diamine oxidase and histamine-N-methyltransferase in an effort to learn the mechanism of foodborne histamine toxicity. Study the speed and accuracy of automated colony counters as opposed to manual counting to determine if the time savings and precision of automated colony counters are suitable for routine usage. Microbiological data collected during the analysis of foods with high potential for transmitting foodborne disease are collected and reported. Chemicals secreted by insects are quantitated and assessed for toxicity in model systems.

25. (U) 77 10 - 78 09 Initial experiments indicate that the lethal effects of microwave cookery on pathogenic microorganisms showed microwaves to be slightly less effective than conventional oven or slow cooking but presented no hazard. The survey of the histamine content of foods indicate normal levels to be in the range of less than 1 mg per 100 g. A number of chemicals have been discovered that possess varying degrees of cryoprotective properties for *Clostridium perfringens*. Microbial analysis of the comminuted pork revealed a diverse flora which included low levels of pathogens.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. [illegible]

ABSTRACT

PROJECT NO. 3M762772A811 Military Nutrition and Food Hygiene

WORK UNIT NO. 004 Military Food Hygiene

The following investigations have been conducted under this work unit:

STUDY NO. 11 Investigations of microbiological methodology performed in conjunction with the Chapter Chairman, agar plate method, for development of the 14th edition of Standard Methods for the Examination of Dairy Products

EX-5 A comparison of analyst and automated colony counters

STUDY NO. 12 Investigations into the microbiological quality of food items purchased by DoD for the purpose of establishing tentative microbiological guidelines for military subsistence

EX-7 A survey of the microbial flora of comminuted pork and a comparative study of microbiological analytical techniques

STUDY NO. 13 Investigations into the use of Group D streptococci as indicators of the hygienic quality of frozen food products

STUDY NO. 15 The effect of cryoprotective agents in the survival of *Clostridium perfringens* type A vegetative cells in selected meat products after freezing and thawing

STUDY NO. 17 Investigations into potential toxicological problems associated with food items purchased by DoD: Identification of hazardous substances, evaluation of food safety, alleviation of toxicological problems, and establishment of tentative toxicological guidelines for military subsistence

EX-1 Histamine production by food spoilage microorganisms and development of an analytical method for detection of histamine in food products

EX-2 The toxicity of histamine and other biogenic amines as related to food poisoning, the presence of inhibitors of histamine-metabolizing enzymes in foods, dietary conditions, and other gastrointestinal factors

EX-3 A survey of commercially packed scombroid fish products, luncheon meats, cheese, and sauerkraut for histamine content

STUDY NO. 19 Investigations into potential insect induced toxicological problems in foods

EX-1 Insect toxic products in military subsistence - induction and quantitation

EX-2 Benzoquinones from the secretory glands of *Tribolium confusum* and *Tribolium castaneum* - Assay, reactions, effects, and acute toxicity

STUDY NO. 21 Enterotoxigenic *Escherichia coli* from foods

EX-1 Assessment of methods for the identification of enterotoxin-producing *Escherichia coli* strains

EX-2 Methodology for isolation of EEC from foods

STUDY NO. 22 Survival of pathogenic organisms in food prepared in a microwave oven

STUDY NO. 11, EX-5 Laboratory equipment manufacturers have developed automatic colony counters (ACCs) which are designed to reduce manpower requirements, to count plates with large numbers of colonies, and to reduce human error. Complete evaluation of the ACCs is essential to determine their overall suitability for DoD use.

STUDY NO. 12, EX-7 Microbiological analyses of several types of comminuted pork products have been accomplished. Data collected are added to the data base which will be used in the establishment of microbiological guidelines for pork products. The gram negative segment of the microbiological flora of a portion of the samples examined has been completely characterized. Due to the composition and physical structure of pork, various means of homogenizing samples have been used.

STUDY NO. 13 The presence or absence of *E. coli* in foods has been the basis for several criteria developed in regulatory food microbiology. The use of the Group D streptococci as indicator organisms instead of *E. coli* has been suggested for the analysis of frozen food items. During this study, it was shown that while *E. coli* and the Group D streptococci both survive in the frozen state, the streptococci do not suffer freezing injury as does *E. coli*. The streptococci are recoverable on selective media while *E. coli* cannot be enumerated in that fashion.

STUDY NO. 15 *Clostridium perfringens* is extremely sensitive to freezing and to frozen storage. Thus when investigating the etiology of food poisoning outbreaks, frozen specimens may not provide the true microbial flora for characterization. Recent investigations indicate that peroxides formed during freezing may account for at least a portion of *C. perfringens* cryofragility. A number of chemicals possess cryoprotective qualities.

STUDY NO. 17, EX-1 A total of 162 bacterial strains representing 28 species were examined for the potential to elicit food poisoning outbreaks via histamine formation in food products. Of the strains examined, *Proteus morganii* and *Enterobacter aerogenes* demonstrated a quantitative superiority in terms of histamine production when grown in either trypticase soy broth containing histidine or a tuna fish infusion broth. Additionally, bacterial histamine formation was examined in several isolates of raw tuna involved in an outbreak of scombroid poisoning.

STUDY NO. 17, EX-2 Intestinal catabolism has been suggested to explain the lack of toxicity when histamine is orally administered to animals. The enzymes, diamine oxidase and histamine-N-methyltransferase have identified in the intestinal contents of several animal species. Inhibition of these enzymes by substances present in spoiled scombroid fish products may explain the oral toxicity of histamine in these outbreaks.

STUDY NO. 17, EX-3 Surveys of the histamine levels in several foods such as sauerkraut, mild cheddar cheese, sausages and tuna fish were conducted to assess the potential these foods might have for involvement in histamine poisoning outbreaks.

STUDY NO. 19, EX-1 A total of 12 different species of stored-product pests of military subsistence have been developed in support of studies to determine the potential hazard from insect induced toxins in foods. Gas liquid chromatography (GLC) methods have been completed for the quantitative analysis of 2-methyl-1,4-benzoquinone (MBQ), 2-ethyl-1, 4-benzoquinone (EB), and 1-pentadecene (PD) in two additional species of *Tribolium*. Results of GLC analysis of *Cyangus angustus* extracts appear to have principal components other than MBQ, EBQ, and PD.

STUDY NO. 19, EX-2 Chemicals excreted by insects suspected of being potentially hazardous to man were tested for mutagenicity by using the Ames *Salmonella typhimurium* assay. Procedural difficulties occurred because the chemicals and solvents were incompatible with the Ames assay medium. Efforts are underway to find suitable alternative solvent systems. Although quantitation was uncontrolled because of the solvent incompatability it appears that the chemicals tested were lethal to the *Salmonella* test strains.

STUDY NO. 21, EX-1, EX-2 The potential of bacterial species other than *Salmonella* and *Shigella* to cause acute gastroenteritis has been recently recognized. Several *E. coli* strains have demonstrated capability for the elaboration of enterotoxins shown to be involved in gastrointestinal disease. This study was designed to assess methods for selective isolation and identification of such strains of *E. coli* and to screen military subsistence to determine the existence of enterotoxigenic strains of *E. coli*.

STUDY NO. 22 The generation of heat in a microwave oven is the result of molecular excitation caused by the penetration of microwaves through a substance. Consequently, normal cooking temperatures can be reached quite rapidly because the heat is generated within the product and does not have to penetrate by normal conduction which requires a considerable amount of time at conventional oven temperatures. One of the most important of the many reasons for cooking foods is to kill pathogenic microorganisms. The destruction of microorganisms is based on cumulative time-temperature relationships. Because cooking by microwave energy is more rapid than by conventional means, questions arise as to the safety of foods from microbiologic hazards when cooked by microwave means. Initial findings indicate that microwave cooking is not quite as effective in the destruction of pathogenic microorganisms as conventional means but sufficient to render food safe when suggested cooking temperatures are achieved.

BODY OF REPORT

WORK UNIT NO. 004 Military Food Hygiene

STUDY NO. 11 Investigations of microbiological methodology performed in conjunction with the Chapter Chairman, agar plate method, for development of the 14th edition of Standard Methods for the Examination of Dairy Products

EX-5 A comparison of analyst and automated colony counters

PROBLEM

Scientific equipment manufacturers have developed automatic colony counting machines (ACCs) intended for use when one is determining the microbial content of food items. These machines reduce the amount of technician time required for counting plates. Their accuracy and precision have not been fully evaluated.

Food standards, specifications, and guidelines refer to Standard Methods for the Examination of Dairy Products (SMEDP) or some similar official publication for analytical procedures. SMEDP prescribes that standard methods agar (SMA) plates will be counted when estimating the microbial population of foods. This publication prescribes standards of accuracy, i.e. "laboratory workers who cannot duplicate their own counts on the same plate within 5% and the counts of other analysts within 10% should discover the cause(s) and correct such disagreements." Earlier experiments under this study have demonstrated the SMEDP plate count accuracy standards are unknowingly frequently exceeded.

Consequently, an evaluation of the ACCs with regard to speed, accuracy, and precision compared to the speed, accuracy, and precision of analysts counting manually was undertaken. If the ACCs prove to be suitable instruments, substantial analyst time can be saved in DoD food testing facilities. If the ACCs are unsatisfactory, DoD facilities will be spared the expense of the machines and the possibility of erroneous analytical results.

RESULTS AND DISCUSSION OF RESULTS

The data collection phase of this study has been completed. As expected, the ACCs provided for a significant reduction of manpower required in the counting of agar plates. When counting plates manually, analysts require considerably more time plates with high number colonies, i.e. greater than 200 colonies per plate; however, no additional time is required for counting high count plates with the ACCs. The variation in manual counting time between analysts is great; however, variation in ACC counting is slight. There is variation in counting time between

ACCs which comes primarily from two sources: the actual time that the machines require to arrive at a count varies, and the physical configurations of the plate counting surfaces which made positioning the plates more difficult on one ACC.

Analysis of the data collected when enumerating colonies on selective bacteriological media which included violet red bile agar, KF streptococcal agar, sulfite-polymyxin-sulfadiazine agar, and standard methods agar with 2,3,5 triphenyltetrazolium chloride added. Analysts' ability to count colonies on selective media by the manual method was superior to results obtained with the use of the automatic colony counters.

Analysis of data collected when enumerating SMA plates is incomplete. Initial observations indicate accuracy and precision by ACCs are not equal to that of analysts. Further statistical analysis is required.

CONCLUSIONS AND RECOMMENDATIONS

ACCs save considerable amounts of analyst time but apparently lack both accuracy and precision. The current state of the art in ACCs makes them suitable for special purpose counting only. One application is for plates having too many colonies to count manually, i.e. Ames *Salmonella* assay plates.

PUBLICATIONS

GUTHERTZ, L.S., and J.T. FRUIN. Colony count accuracy on selective media: Analysts versus automatic colony counters. J Food Prot (In press)

STUDY NO. 12	Investigations into the microbiological quality of food items purchased by DoD for the purpose of establishing tentative microbiological guidelines for military subsistence
EX-7	A survey of the microbial flora of comminuted pork and a comparative study of microbiological and analytical techniques

PROBLEM

A large volume of comminuted pork is procured annually for DoD consumption. Microbiological analysis of several types of these products to establish a data base suitable for supporting microbial guidelines is under way. Methods used for analysis of these products by DoD food testing laboratories have been modified and compared to established procedures in paired tests. The effect of dilution water ion

content is being evaluated. The enteric gram-negative flora of a portion of samples tested have been completely characterized. Because of the composition and physical structure of pork, two different methods of homogenizing samples were compared. A conventional blender and a newly developed machine (Stomacher™) that macerates by repeatedly crushing the product, were used to homogenize portions of the same sample prior to analysis. The microbial content of aerosols created by the conventional belnder and the Stomacher™ were collected and characterized with an Andersen™ air sampler.

RESULTS AND DISCUSSION OF RESULTS

The collection of aerosols during sample homogenization showed the Stomacher™ to produce virtually no aerosols; however, because of the high fat content of the samples, the actual homogenization was inadequate. Conventional blending in stainless steel cups did create bacterial laden aerosols. Characterization of these aerosols are in progress to determine if their bacterial content varies by size of particle and to determine how closely the bacterial content reflects the content of the samples. Data collection for other phases of the study are still in progress.

CONCLUSIONS AND RECOMMENDATIONS

It is recommended that when homogenizing samples for bacterial analysis, the pipetting port be utilized and that material be drawn into the pipet with a suction bulb rather than by mouth.

PUBLICATIONS

FRUIN, J.T., L.S. GUTHERTZ, F.J. TILLMAN, J.F. FOSTER and H.E. SAUBERLICH. The bacterial content of aerosols associated with a conventional blender. Abstracts of the annual meeting of the American Society for Microbiology (Las Vegas, Nevada, May 1978), p 186

STUDY NO. 13 — Investigations into the use of Group D streptococci as indicators of the hygienic quality of frozen food products

PROBLEM

The methodology in current use for the evaluation of the hygienic quality of frozen food products is based upon the detection and enumeration of *Escherichia coli*. *E. coli*, however, is subject to sublethal cellular injury following several treatments to which foods are subjected. One of the ways this cellular injury is manifested is by the inability of the organism to grow on the selective media routinely used for its enumeration.

This study was undertaken to investigate the use of other organisms, such as the Group D streptococci, as hygienic quality indicator organisms in frozen food products.

RESULTS AND DISCUSSION OF RESULTS

The 7 species comprising the Group D streptococci and *E. coli* have been studied for their ability to survive freezing at temperatures of -4, -20 and -80 C in water, 0.2 M phosphate buffer, pH 7.0, 10% sucrose, 5% glycerol, and a 15% slurry of mashed potatoes. The organisms were also compared as to their ability for enumeration on selective bacteriologic media. The non-enterococcal portion of the Group D streptococci, including *Streptococcus bovis* and *Streptococcus equinus*, exhibited marked sensitivity to all parameters measured. *Escherichia coli* was able to survive freezing and frozen storage, however cellular injury prevented its enumeration on selective media. The enterococcal portion of the Group D streptococci demonstrated resistance to the sublethal effects of freezing and frozen storage. Enumeration of the enterococci with the use of selective media was unaffected by either freezing or frozen storage.

CONCLUSIONS

Based on the findings in this study, it can be concluded that *E. coli*, *S. bovis*, and *S. equinus* are poorly suited as hygienic indicator organisms for frozen food products. *S. faecalis*, *S. faecium*, or *S. durans* due to their ability to survive frozen storage and be enumerated on selective media may be acceptable substitutes as hygienic indicators.

RECOMMENDATIONS

It is recommended that microbial guidelines be developed for frozen food products with the use of either *S. faecalis*, *S. faecium*, or *S. durans* as the indicator organism.

If *E. coli* is to remain the indicator organism of choice, analytical methodology for the selective repair of injured organisms in mixed culture systems must be developed.

PUBLICATIONS

GUTHERTZ, L.S., S.L. TAYLOR and J.L. FOWLER. Hygienic Indicator Organisms: A Comparison of Survival and Enumeration of the Group D Streptococci and *Escherichia coli* following Freezing and Frozen Storage. Report No. 52. Presidio of San Francisco, California: Letterman Army Institute of Research, April 1978

STUDY NO. 15

The effect of cryoprotective agents in the survival of *Clostridium perfringens* type A vegetative cells in selected meat products after freezing and thawing

PROBLEM

Clostridium perfringens type A is now known to be the cause of a significant portion of foodborne disease outbreaks. In addition, *C. perfringens* is thought to be responsible for a disproportionately large share of the foodborne disease outbreaks of undetermined etiology because of its unique cultural characteristics. The organism is anaerobic. It is also susceptible to destruction at low temperature, particularly freezing, the recommended method of preserving samples suspected of causing foodborne illness when submitted to diagnostic laboratories. Anaerobic organisms present special problems for laboratory isolation. Consequently, the collection, handling, shipment, storage and laboratory preparation of samples has a detrimental effect in recovery of *C. perfringens*. Cryoprotective agents and agents that reduce the oxidation reduction potential of food samples, could result in a more precise quantitative isolation of *C. perfringens*.

RESULTS AND DISCUSSION OF RESULTS

Chemicals showing cryoprotective properties for *C. perfringens* are glycerol, methanol, dimethyl sulfoxide, propylene glycol, and ethylene glycol. Survival of cells between strains and replicas is highly variable. During the freezing of complex substrates such as food peroxides are formed. Peroxides are known to be effective in causing either injury or death to anaerobic microorganisms. Catalase has been added to culture medium and causes a dramatic increase in the number of *C. perfringens* enumerated.

CONCLUSIONS

Glycerol, methanol, dimethyl sulfoxide, propylene glycol, and ethylene glycol possess cryoprotective properties for *C. perfringens*.

RECOMMENDATIONS

Catalase and peroxidases should be evaluated in conjunction with the chemicals found to be cryoprotective.

PUBLICATIONS

None

STUDY NO. 17 — Investigations into potential toxicological problems associated with food items purchased by DoD: Identification of hazardous substances, evaluation of food safety, alleviation of toxicological problems, and establishment of tentative toxicological guidelines for military subsistence

EX-1 — Histamine production by food spoilage microorganisms and development of an analytical method for detection of histamine in food products

PROBLEM

In several foodborne disease outbreaks, tuna and other scombroid fish products, cheese and sauerkraut have been implicated due to their possibility for containing high levels of histamine. The methodology available for the routine analyses of these foods is cumbersome and tedious. In this study an evaluation of fluorometric detection methods which are sensitive and specific will be made. Various extraction procedures for the selective removal of histamine from food samples will be assessed. An evaluation of the use of chromatographic procedures for their potential in removing interfering substances will be made. Microbial decarboxylation of histidine contained in foods results in the production of histamine. Although numerous microbial species have been reported to contain the required histidine decarboxylase enzyme, few quantitative comparisons have been made. This study will make quantitative comparisons of bacterial histamine production in tuna fish media as well as a synthetic broth containing histidine. The optimal conditions for histamine production will be determined for several strains producing high histamine levels.

RESULTS AND DISCUSSION OF RESULTS

Evaluation of numerous amine detection methods showed that a fluorometric assay based on o-phthalaldehyde was the most sensitive and specific for detection of histamine. A selective extraction step (using n-butanol) was used to extract histamine from interfering histidyl dipeptides. These two procedures were combined to form an analytical method for histamine analysis in foods that will detect histamine at levels as low as 0.02 mg histamine/100 g food. Results of pilot surveys indicated that sauerkraut and tuna fish had the highest histamine contents of seafoods, comminuted meats, luncheon meats, cheeses, and sauerkraut products which were analyzed.

Over 100 different strains of bacteria were tested for their ability to produce histamine in trypticase soy broth with histidine and a broth media made from a tuna fish infusion. *Proteus morganii* and *Enterobacter aerogenes* displayed a quantitative superiority in

terms of histamine production in both media. Although several strains of *Hafnia alvei*, *Providencia alcalifaciens*, *Enterobacter cloacae*, *Proteus rettgeri* and *Citrobacter diversus* strains were found capable of histamine production, it appeared as though *P. morganii* and *E. aerogenes* were the only strains capable of producing sufficient histamine to elicit a food poisoning episode.

Analysis of samples of tuna "sashimi" involved in an outbreak of scombroid fish poisoning in San Francisco revealed 10 gram-negative bacterial strains present in the product. When the ability of each organism to produce histamine in tuna fish infusion broth was monitored, a strain of *Klebsiella pneumoniae* capable of producing 732 mg% histamine in 7 hours was revealed. None of the other organisms isolated were capable of equivalent histamine production. Examination of 50 strains of *K. pneumoniae* isolated from foods indicated 12 of these strains were capable of equivalent levels of histamine production.

CONCLUSIONS

The method developed for histamine analysis in foods, by using a selective extraction procedure and a specific detection step, has advantage over other reported methods with respect to specificity, sensitivity, and accuracy.

Proteus morganii, *Enterobacter aerogenes*, and *Klebsiella pneumoniae* have the ability to produce histamine in quantities sufficient to elicit outbreaks of scombroid fish poisoning.

RECOMMENDATIONS

None

PUBLICATIONS

TAYLOR, S.L., and E.R. LIEBER. Specificity and sensitivity of seven histamine detection methods. J Food Sci 42:1584-1586, 1978

TAYLOR, S.L., E.R. LIEBER, and M. LEATHERWOOD. A simplified method for histamine analysis of foods. J Food Sci 43:247-250, 1978

LIEBER, E.R., and S.L. TAYLOR. Thin layer chromatographic screening methods for histamine in tuna fish. J Chromatogr 153:143-152, 1978

TAYLOR, S.L., L.S. GUTHERTZ, M. LEATHERWOOD, F.J. TILLMAN, and E.R. LIEBER. Histamine production by food-borne bacterial species. (Submitted for publication)

TAYLOR, S.L., L.S. GUTHERTZ, M. LEATHERWOOD, and E. LIEBER. Histamine production by *Klebsiella pneumoniae* and an incidence of scombroid fish poisoning. Applied Environ Microbiol (In press)

LERKE, P.A., S.B. WERNER, S.L. TAYLOR, and L.S. GUTHERTZ. Scombroid poisoning - report of an outbreak. West J Med, 129:381-386, 1978

GUTHERTZ, L.S., S.L. TAYLOR, M. LEATHERWOOD, and E.R. LIEBER. Bacterial histamine production and an incident of scombroid fish poisoning. Abstracts of the annual meeting, American Society for Microbiology (Las Vegas, Nevada, May 1978) p 188

TAYLOR, S.L., L.S. GUTHERTZ, M. LEATHERWOOD, and E.R. LIEBER. Histamine levels in fermented foods and identification of histamine-producing bacteria. Abstracts of Thirty-eighth Annual Meeting of the Institute of Food Technologists (Dallas, Texas, June 1978)

LIEBER, E.R., and S.L. TAYLOR. Comparison of thin-layer chromatographic detection methods for histamine for food extracts. J Chromatogr 160: 227-237, 1978

EX-2

The toxicity of histamine and other biogenic amines as related to food poisoning, the presence of inhibitors of histamine metabolizing enzymes in food, dietary conditions, and other gastrointestinal factors

PROBLEM

Consumption of foods containing abnormally high levels of histamine, tyramine or other biogenic amines has resulted in outbreaks of food-borne disease. Histamine, however, has been orally administered to rats in large doses without apparent development of the chemical symptoms of toxicity. Three enzymes found in the cells of the gastrointestinal tract are capable of histamine catabolism to non-toxic substances. These enzymes are monoamine oxidase, diamine oxidase (DAO) and histamine-N-methyltransferase (HMT). Upon ingestion of histamine with a food, some defect in the detoxification mechanism must occur. Possible presence of naturally occurring or additive foodborne inhibitors of these enzymes requires investigation.

RESULTS AND DISCUSSION OF RESULTS

Rats, guinea pigs, and rhesus monkeys appear to have the capability for intestinal histamine metabolism. Histamine-N-methyltransferase from jejunal mucosa of rats, guinea pigs, and rhesus monkeys was localized in the soluble fraction. Diamine oxidase (DAO) from jejunal mucosa of rats and rhesus monkeys was localized in the soluble fraction. Guinea pig jejunal mucosa had no observable DAO activity. The dissociation constants (K_m) observed for jejunal HMT were 33.2 μM for rat, 114 μM for rhesus monkey and 7.1 μM for guinea pig. The K_ms observed for jejunal mucosa for DAO were 0.21 mM for rat and 0.26 mM for rhesus monkey. Substrate inhibition was encountered at high substrate concentrations with rat HMT and rat and monkey DAO.

Species differences occur in ability of intestinal tissue to catabolize histamine. The lack of DAO in guinea pig jejunum and the substrate inhibition observed with rat HMT and with rat and monkey DAO may have profound implications in terms of oral histamine toxicity in these species. The lack of oral toxicity with histamine demonstrated with guinea pigs and in humans seems to indicate a vital role for histamine catabolism in protection from the effects of ingested histamine.

CONCLUSIONS AND RECOMMENDATIONS

None

PUBLICATIONS

TAYLOR, S.L., and E.R. LIEBER. Subcellular distribution and properties of intestinal histamine-metabolizing enzymes from rats, guinea pigs and Rhesus monkeys. Comp Biochem Physiol (In press)

TAYLOR, S.L., and E.R. LIEBER. In vitro inhibition of rat intestinal histamine-metabolizing enzymes. (Submitted for publication)

LIEBER, E.R., and S.L. TAYLOR. Inhibition of intestinal histamine-metabolizing enzyme by amines known to occur in scombroid fish. (Abstract) Fifth International Congress of Food Science and Technology (Kyoto, Japan, 17-22 September, 1978)

EX-3 — A survey of commercially packed scombroid fish products, luncheon meats, cheese, and sauerkraut for histamine content

PROBLEM

Pilot surveys indicated that the highest histamine contents could be found in scombroid fish products, certain luncheon meats, cheese, and sauerkraut. A survey of histamine levels in these food groups can be used to assess the potential of histamine poisoning, to set guidelines for subsistence foods, and to determine the incidence of potentially toxic levels of histamine in foods. Histamine levels greater than 100 mg/100 g should be considered harmful..

RESULTS AND DISCUSSION OF RESULTS

Among 50 samples of sauerkraut tested, the range of histamine level was 0.91 mg/100 g to 13.0 mg/100 g and the average histamine content was 5.06 mg/100 g. Such levels are much lower than those associated with histamine poisoning so it can be concluded that sauerkraut is a low risk product for this type of foodborne outbreak. When luncheon meat-sausages were examined it was found that fermented sausages had

much higher histamine levels than did the cooked and semi-dry sausages. Cooked sausages had the lowest mean histamine levels with bologna - 0.55 mg/100 g, cooked salami - 0.83 mg/100 g, and Kosher salami - 0.50 mg/100 g. Semi-dry sausages had slightly higher mean histamine levels than cooked sausages with beef summer sausage - 1.07 mg/100 g, thuringer-cervelat - 2.35 mg/100 g, and thuringer 1.19 mg/1000 g. By comparison to cooked and semi-dry sausages, dry sausages had larger and more variable amounts of histamine. Brand specific variations in histamine content were noted for dry sausage, Italian dry salami and pepperoni. Statistical analyses of results would indicate that the natural fermentation process used with dry sausages can result in the accumulation of high histamine levels. Since histamine accumulation is most likely due to the presence of undesirable bacteria in the early stages of fermentation, imposition of rigorous quality control procedures should circumvent any possible public health problems.

One-hundred cheese samples were examined for their histamine levels and data analysis is currently in progress.

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

TAYLOR, S.L., M. LEATHERWOOD, and E.R. LIEBER. A survey of histamine levels in sausages. J Food Prot 41:634-637, 1978

TAYLOR, S.L., M. LEATHERWOOD, and E.R. LIEBER. Histamine in sauerkraut. A Research Note. J Food Sci 43:1030-1032, 1978

TAYLOR, S.L., E.R. LIEBER, and M. LEATHERWOOD. A survey of histamine levels in commercially processed scombroid fish products. J Food Quality 1:393-397, 1977-78

STUDY No. 19	Investigation into potential insect induced toxicological problems in foods
EX-1	Insect toxic products in military subsistence - induction and quantitation

PROBLEM

The military, with its necessity for transport and extended storage of foods in environments favorable to insect infestation, has concern about the potential health hazards associated with chemicals released by insects into military subsistence. Several insect species have been recognized as important pests of military subsistence and many of these stored product insects are known to release chemicals into infested food products. The nature and toxicity of many of the chemicals remain to be determined. At present, no satisfactory methods are available for detecting insect-induced chemicals in the contaminated subsistence. Toxicological evaluation of these chemicals is impossible in the absence of standardized methods for identification and quantitation of the suspected material. The present study (a joint effort of Department of Tropical Medicine and Food Hygeiene Division, Department of Nutrition) is designed to establish an in-house resource of selected insects of military subsistence, to develop the required methodology, and to investigate conditions which promote the release of the toxic chemicals.

RESULTS AND DISCUSSION OF RESULTS

Three additional species of stored products insects have been added to the insectary: *Tribolium destructor* and *T. audax* (both Coleoptera, Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera, Bostrichidae). The total number of colonies is now 12. Obtaining the tenebrionids is particularly significant because of the release of p-benzoquinones by these insects. *R. dominica* is also reported to impart a characteristic odor to the material that it infests. Studies have been initiated for rearing these beetles on subsistence items and preliminary results look promising. Controlled rearing procedures will permit qualitative and quantitative gas-liquid chromatographic (GLC) analysis of the p-benzoquinones in sexed adults of known age.

The GLC analysis of 2-methyl-1,4-benzoquinone (MBQ), 2-ethyl-1,4-benzoquinone (EBQ) and 1-pentadecene (PD) in sexed *Tribolium brevicornis*, *T. castaneum*, *T. confusum*, and *T. madens* of known age postecdysis has been completed.

The GLC analyses of *Cynaegus angustus* (Coleoptera, Tenebrionidae) extracts for MBQ, EBQ and PD have been performed. The three major peaks detected did not correspond to those of MBQ, EBQ and PD. Since a quinone odor is detected from adult insects when disturbed, they are probably releasing substituted benzoquinones other than MBQ and EBQ.

Extracts of these insects were submitted to us for analysis by colleagues at the University of Minnesota where the frequency of infestation by this insect is increasing.

Approval has been obtained to conduct studies on the effects of hydrogen phosphide fumigation on benzoquinone release from Tenebrionides flour beetles. Aluminum phosphide, which generates hydrogen phosphide, is the Army's fumigant of choice and is currently used extensively for stored products insect infestation.

CONCLUSIONS

The in-house resource of coleopterous stored-products pests of military subsistence has been expanded to twelve species reared on selected subsistence items. These include six species of Tenebrionids, known or suspected of producing and releasing p-benzoquinones into the subsistence they infest. Studies were completed on the levels of p-benzoquinones and 1-pentadecene in four species of *Tribolium* flour beetles and have been initiated on two other species.

RECOMMENDATIONS

The investigation of p-benzoquinones and 1-pentadecene released by other species of *Tribolium* should be continued. Further work is also necessary on the release of these chemicals from flour beetles into infested commodities under different environmental and stress conditions. These data will provide information on the amount of p-benzoquinone present in infested flour which will be necessary for future toxicological analyses.

PUBLICATIONS

WIRTZ, R.A., S.L. TAYLOR and H.G. SEMEY. Concentrations of substituted p-benzoquinones and 1-pentadecene in the flour beetles *Tribolium confusum*, J. du Val and *Tribolium castaneum* (Herbst). Comp Biochem Physiol 61:25-28, 1978

WIRTZ, R.A., S.L. TAYLOR, and H.G. SEMEY. Concentrations of substituted p-benzoquinones and 1-pentadecene in the flour beetles *Tribolium madou* (Charp.) and *Tribolium brevicornis* (Lec.) (Coleoptera, Tenebrionidae). Comp Biochem Physiol 61:2870290, 1978

STUDY NO. 19	Investigation into potential insect induced toxicological problems in food
EX-2	Benzoquinonones from the secretory glands of *Tribolium confusum* and *Tribolium castaneum* - Assay, reactions, effects, and acute toxicity

PROBLEM

Stored products insect damage to DoD subsistence items is appreciable and costly. The current criteria for disposal of infested subsistence

materials are based on crude measurments of whole insects and or fragment counts. Some stored products insects release toxic products into the infested commodity which may not be detected by using current rejection criteria. The tenbrionid flour beetles release p-benzoquinones, and other compounds, which have been reported to be toxic and carcinogenic in laboratory animals. A need exists to verify these reports and to determine the toxicity and carcinogenicity/mutagenicity of the benzoquinones released by flour beetles into subsistence items which might be consumed by military personnel. The methods of choice for initial determination of benzoquinone toxicity and mutagenicity are the Ames *Salmonella typhimurium* assay and the sex-linked recessive lethal *Drosophila melanogaster* test.

RESULTS AND DISCUSSION OF RESULTS

Initial attempts to determine the mutagenic activities of synthesized p-benzoquiononones and insect secretory products were unsuccessful because of the incompatibility of extraction solvents used with the Ames assay medium. New extraction methods have been developed and pilot studies completed which indicate that beetle extracts with high levels of p-benzoquinones can be supplied in solvents routinely used with the Ames assay.

Although quantitation was uncontrolled in the initial tests because of solvent incompatibility, it appears that the tenbrionid beetle extracts and the individual p-benzoquinones tested were lethal to the *Salmonella* test strains. This experiment is a joint effort of personnel from Department of Tropical Medicine and Food Hygiene Division, Department of Nutrition.

CONCLUSIONS

None; this study has not been completed.

RECOMMENDATIONS

The toxic and mutagenic effects of tenebrionid beetle extracts and individual p-benzoquinones using the Ames assay should be completed. The effects of these materials should also be examined using the sex-linked recessive lethal *Drosophila* test. This information, as well as acute and chronic toxicity levels of the benzoquinones, will be necessary to establish guidelines for rejection criteria of tenebrionid-infested military subsistence.

PUBLICATIONS

None

STUDY NO. 21 — Enterotoxigenic *Escherichia coli* (EEC) from foods

EX-1 — Assessment of methods for the identification of enterotoxin-producing *Escherichia coli* strains

PROBLEM

Until recently, gastroenteritis of bacterial origin was most commonly associated with species of *Salmonella*, *Shigella*, and *Staphylococcus aureus*. Adequate methodology for isolation and identification of these organisms exists. However, adequate methods for the separation and identification of the recently recognized enterotoxigenic strains of *Escherichia coli* (ECC) have not been developed. Although this organism is frequently isolated from foods, the incident of strains with enterotoxigenic potential is unknown. Foods are a vehicle for transmission of such strains. Our ability to isolate and identify these strains is important for the protection of our troops' health.

Invasive as well as non-invasive strains of enterotoxigenic *E. coli* have been identified. Among the non-invasive strains which can produce diarrheal syndromes similar to salmonellosis, organisms may produce either an antigenic heat-labile enterotoxin (LT) or a non-antigenic heat-stable enterotoxin (ST) or both types. Although several clinical assays have been developed for the identification of LT-producing strains, the ligated intestinal loop is the only method available for the assay of ST.

This experiment will evaluate the use of these methods for identification of EEC in foods as well as for their routine use in food testing laboratories. An assessment of the incidence of EEC in military subsistence will be made.

RESULTS AND DISCUSSION OF RESULTS

None; this study has not been completed.

CONCLUSIONS AND RECOMMENDATIONS

None

PUBLICATIONS

None

EX-2 Methodology for isolation of EEC from foods

PROBLEM

Foods can serve as the vehicle for the transmission of enterotoxin-producing strains of *E. coli* (EEC). In 1971, the first documented case of such a transmission was reported when an isolate of *E. coli* serotype 0124:B17 was identified as the causative agent in an outbreak which occurred following the consumption of imported cheese. Following this outbreak, a protocol based on serotyping, use of elevated incubation temperatures, and enrichment media was proposed for isolation and identification of enterotoxigenic *E. coli* strains.

Recently published studies have shown, however, that no relationship exists between serotype and enterotoxigenic capability. This correlates with the biological nature of plasmids and the demonstration that enterotoxin production by *E. coli* is plasmid mediated.

E. coli is frequently isolated from foods and it is not uncommon for a food product to contain 6 or more biotypes of this organism. A determination of the enterotoxigenic capabilities of each isolated biotype would be expensive, time-consuming, and not feasible for a routine food testing laboratory.

There is a need for methodology outlining the selective isolation and identification of strains with enterotoxigenic potential. This experiment is to determine necessary conditions for the selective isolation of enterotoxigenic strains of *E. coli* present in foods.

RESULTS AND DISCUSSION OF RESULTS

None; this study has not been completed.

CONCLUSIONS AND RECOMMENDATIONS

None

PUBLICATIONS

None

STUDY NO. 22 Survival of pathogenic organisms in food prepared in a microwave oven

PROBLEM

Cooking by use of microwave energy is a feasible means of reducing overall cooking time, conserving food preparation space, reducing manpower requirements for final food preparation and, when properly

employed, can reduce the overall energy used in preparing foods for consumption. The Department of the Army (DA) has evaluated the use of microwave cookery in conventional, proposed garrison and proposed modular field facilities from an overall acceptance standpoint in addition to their resource conserving features.

Some important short-comings of microwave cookery have also been identified and include: the lack of browning and associated flavor formation, irregular heating of large or bulk food items, inability to heat products in metal or foil containers, excessive drying, and the inability to select slower heating rates are some of the disadvantages most often listed. Fortunately, except for the lack of browning and flavor development, which is irrelevant to this discussion, these deficiencies can be circumvented or negated with progressive food service management and/or oven design.

During the conventional cooking process, the death of microorganisms is directly related to cumulative time and temperature values. Because of the extremely rapid heat transfer into food items during microwave cooking, the time/temperature values attributed to conventional cooking are not achieved. This has led to questions concerning microwave cooking from a public health standpoint: Are foodborne pathogenic microorganisms and heat labile toxins destroyed?

RESULTS AND DISCUSSION OF RESULTS

In pilot experiments in which meatloaf was used as the contaminated medium, there was considerable variation in the temperature of a product after cooking. Temperatures were recorded at 9 positions in the medium immediately after cooking. A variation from position to position of approximately 60 C, 45 C and 30 C for microwave, conventional oven and slow cookery, respectively. With such a wide variation in temperatures, care was taken to assure the inoculating organisms were uniformly dispersed throughout the loaf and that samples for isolation were representative of all areas of the loaf. Microbial analysis taken immediately after cooking was compared to the inoculum. The log of the survival rate was then plotted against the temperature for *Clostridium perfringens, Escherichia coli, Streptococcus fascalis, Staphylococcus aureus* and total aerobic plate count. As expected, the most effective form of cooking with regard to microbial destruction was slow cooking followed by conventional oven and then microwave oven in all cases.

CONCLUSIONS

Further analysis of data and possibly more replicas of experiments is required.

RECOMMENDATIONS

None

PUBLICATIONS

None

ADDITIONAL PUBLICATIONS UNDER WORK UNIT 004

1. GUTHERTZ, L.S. and J.T. FRUIN. *Yersinia enterocolitica*: A foodborne pathogen. Army Veterinary Corps Memorandum. Washington, DC: Department of the Army, Office of the Surgeon General, November - December 1978

2. FRUIN, J.T., H.F. ALISHOUSE, and A.L. DUNGAN. Collection of Food Microbiological Data from the Central Food Preparation Facility Pilot Kitchen - 1976. Report No. 54. Presidio of San Francisco, California: Letterman Army Institute of Research, July 1978.

3. FRUIN, J.T., J.F. FOSTER, D.L. STUTZMAN, W.H. LANGLEY, J.L. FOWLER, and K.E. TREFZ. Report of 1976 Microbiological Data Collection Program. Report No. 55. Presidio of San Francisco, California: Letterman Army Institute of Research, 1978

4. FRUIN, J.T. Food microbiological criteria. Army Veterinary Corps Memorandum. Washington, DC: Department of the Army, Office of the Surgeon General (In press)

5. FRUIN, J.T., and S.L. TAYLOR. Acute food borne histamine toxicity. Army Veterinary Corps Memorandum. Washington, DC: Department of the Army, Office of the Surgeon General, July 1978

6. FRUIN, J.T. *Salmonella* update. Army Veterinary Corps Memorandum. Washington, DC: Department of the Army, Office of the Surgeon General, May 1978

7. FRUIN, J.T., J.F. FOSTER, and J.L. FOWLER. A survey of the bacterial populations of bologna products. J Food Prot 41:692-695, 1978

8. FOSTER, J.F., L.S. GUTHERTZ, R.C. HUNDERFUND, and J.L. FOWLER. A comparison of bacterial species isolated from ground beef textured soy protein and textured soy protein extended ground beef. J Food Prot (In press)

9. FOSTER, J.F., R.C. HUNDERFUND, J.L. FOWLER, J.T. FRUIN, and L.S. GUTHERTZ. Bacterial populations of ground beef, textured soy protein and textured soy protein extended ground beef after 3 and 10 days of refrigerated storage. J Food Prot 41:647-653, 1978

10. FOSTER, J.F., J.T. FRUIN, and L.S. GUTHERTZ. Bacterial survey of ground beef, textured soy protein, and textured soy protein extended ground beef. Abstracts of the Annual Meeting of the American Society for Microbiology (Las Vegas, Nevada, May 1978) p 188

11. FOSTER, J.F., J.L. FOWLER, J.T. FRUIN, L.S. GUTHERTZ, E.L. SHROYER, M.R. SHALABY, and R.C. HUNDERFUND. A Survey of the Microbial Flora of Ground Beef Textured Soy Protein and Textured Soy Protein Extended Ground Beef after 3 Days' and 10 Days' Storage at 4C. Report No. 43. Presido of San Francisco, California: Letterman Army Institute of Research, January 1978

12. FRUIN, J.T. Types of *Clostridium perfringens* isolated from selected foods. J Food Prot (In press)

13. FRUIN, J.T. Microbiological criteria for food. J Food Prot 41:481-482, 1978

14. GUTHERTZ, L.S., and R.L. OKOLUK. Comparison of minaturized multitest systems with conventional methodology for identification of *Enterobacteriocsae* from foods. Applied Environ Microbial 35:109-112, 1978

15. FOSTER, J.F., J.L. FOWLER, and W.C. LADIGES. A bacterial survey of raw ground beef. J Food Prot 40:790-794, 1977

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA 0A 6357	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: H. Termination	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. GRP/S INSTR: NL	8a. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO

9. NO / CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A811	00	005
b. CONTRIBUTING				
c. CONXXXXX	CARDS 114f			

9. LEVEL OF SUM: A. WORK UNIT

11. TITLE (Precede with Security Classification Code)

(U) Radioisotope Support for Military Medical Research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008500 Isotopes; 013900 Radioactivity; 011000 Nuclear Instrumentation

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY		16. PERFORMANCE METHOD
64 05	78 09	DA		C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: — EXPIRATION: —

b. NUMBER: Not Applicable

c. TYPE: — d. AMOUNT: —

e. KIND OF AWARD: — f. CUM. AMT: —

18. RESOURCES ESTIMATE

FISCAL YEAR	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
PREVIOUS 78	1.5	51
CURRENT 79	0.0	00

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

ADDRESS:

RESPONSIBLE INDIVIDUAL
NAME: Marshall, J.D., COL, MS
TELEPHONE: 415-561-3600

20. GENERAL USE

Foreign Intelligence Not Applicable

20. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Radioisotope Division
ADDRESS: Department of Nutrition
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Furnish SSAN and Additional Phone Numbers)
NAME: Zolock, D.T., CPT, MS
TELEPHONE: 415-561-4770
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME:
NAME:

POC:DA

22. KEYWORDS (Precede each with Security Classification Code) (U) Military Research Projects; (U) Radioisotopes; (U) Instrumentation; (U) Data Acquisition

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

23. (U) The objectives are to provide radioisotope support to all projects requiring the use of radioisotopes and improve procedures and counting techniques where needed, to conduct radioactive counting procedures for joint military medical research projects, to conduct research to improve technology, and to adapt existing technology to research areas of significance to the LAIR mission.

24. (U) Methodology research is conducted as required to improve existing procedures. Nine automatic sample changing radiation detection instruments are maintained for detection of beta and gamma radiation. All aspects of the radiological protection program, as required by Nuclear Regulatory Commission licensure, are conducted.

25. (U) 77 10 - 78 09 Research support has been provided to 39 investigators and their technicians utilizing radioisotopes during this period. Support provided includes procurement and storage of radioisotopes, radiation safety monitoring, decontamination of glassware, radioactive waste disposal and maintenance of logs and records as required by Nuclear Regulatory Commission and Army regulations. Consultation concerning proper use and application of radioisotope technology has been provided as needed. Through the cooperative effort of the Health Physics Officer, LAMC, the Physicist, LAMC, and the Radiological Protection Officer, LAIR, a 40-hour AMA accredited training course, entitled, "Safe Use and Handling of Radioisotopes," was presented in November 1977 and again in May 1978. This work unit is terminated due to directed realignment of the LAIR research program.

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

ABSTRACT

PROJECT NO.	3M16272811	Military Nutrition and Food Hygiene
WORK UNIT NO.	005	Radioisotope Support for Military Medical Research

Research investigators are currently being supported with radioisotope services, including procurement and storage of radioisotopes, radiation safety monitoring, decontamination of glassware, waste disposal, maintenance of appropriate logs and records, and maintenance of radiation detection instruments for investigator use. A 40-hour AMA accredited training course entitled, "The Safe Use and Handling of Radioisotopes," was presented on 2 separate occasions during the current fiscal year.

BODY OF REPORT

WORK UNIT NO. 005 Radioisotope Support for Military Medical Research

PROBLEM

Military medical research projects often require the use of radioisotopes. Adherence to governmental regulations to ensure safe usage of radioisotopes by investigators is essential. Nuclear Regulatory Commission and Army regulations describe rules for procurement and storage of radioisotopes, radiation safety monitoring, decontamination of glassware, radioactive waste disposal, and maintenance of logs and records. Maintenance of radiation detection instruments and training of personnel in the safe use of radioisotopes is necessary. In order to meet these requirements, centralized radioisotope support is essential.

RESULTS AND DISCUSSION OF RESULTS

The use of radioisotopes in biomedical research has proven useful. Radioactive substances may be used for tracer studies and therapy. Tracer studies may be utilized in either basic research or as diagnostic aids. Tracer procedures may be employed to follow the behavior of specific elements or compounds in the body or to follow the path of any molecule to which a radioactive atom has been attached, when the latter takes no part in the metabolism. Thus, the formation of metabolic products can be traced, the utilization of metabolic products can be observed, and turnover studies can be readily performed. The Radioisotope Division is responsible for procurement and storage of radioisotopes, radiation safety monitoring, decontamination of glassware, radioactive waste disposal, and maintenance of logs and regulations. All support functions delegated to the Radioisotope Division were maintained. Advice and counsel are given to investigators regarding the use of radioisotopes. Beta and gamma counting instruments are maintained for use of investigators at LAIR in support of military medical research. Current instrumentation includes 7 liquid scintillation counters and 4 gamma counting instruments.

The Department of Information Sciences is able to provide computer programming support such as disintegrations per minute for the beta counters and various other programmable systems. This computer support saves investigators and their technicians many man-hours which can be better utilized for furthering their research efforts.

Bulk procurement of radioisotope support supplies, such as liquid scintillation counting solutions and vials, results in price difference savings. Additional savings are accrued by processing a few large orders instead of numerous small orders from the 39 research investigators currently authorized to use radioisotopes. A charge back system

has been established to distribute these costs to the appropriate research work unit.

Through the cooperative effort of the Health Physics Officer, LAMC, the Physicist, LAMC, and the Radiological Protection Officer, LAIR, a 40-hour training course entitled, "Safe Use and Handling of Radioisotopes," was presented in November 1977 and again in May 1978. This course is now AMA accredited.

CONCLUSIONS AND RECOMMENDATIONS

The use of radioisotopes is essential to the mission of LAIR. It is recommended that the centralized support activity be maintained as the most economical and efficient means of making radioisotopes available to research investigators while maintaining adequate control of their use and thus protecting the health of laboratory personnel. Technological studies should continue to identify the most efficient, the most accurate, and the most economical laboratory procedures to be used in the routine use of radioisotopes.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY
DA OE 6311	78 10 01

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SECY	6. WORK SECURITY	7. PROGRAMING	8. COMPU ENTRY	9a. SPECIFIC DATA RESTRICTION ASCRIB	9b. LEVEL OF SUM	9c. WORK UNIT
77 10 01	M, Termination	U	U	NA	NL	☒ YES ☐ NO		

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A811	00	006
b. CONTRIBUTING				
c. CONTRIBUTING	CARDS 1141			

11. TITLE (Procede with Security Classification Code)

(U) Role of Nutrition in the Enhancement of Wound Healing in the Combat Casualty

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 002300 Biochemistry; 006500 Food

13. START DATE	14. ESTIMATED COMPLETION DATE	15. PERFORMING AGENCY			16. PERFORMANCE METHOD
77 10	78 10	DA			C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: d. AMOUNT:
e. END OF AGENCY f. CUM. AMT.

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
78	0.0	0.0
79	0.0	0.0

19. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
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RESPONSIBLE INDIVIDUAL
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20. GENERAL USE

Foreign Intelligence Not Applicable

21. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
ADDRESS: Biochemistry Division
Department of Nutrition
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR
NAME: Turnbull, J.D., CPT, MS
TELEPHONE: 415-561-5872
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Green, M.D., CPT, MS
NAME: Omaye, S.T., DAC POC:DA

22. KEYWORDS (Procede with Security Classification Code)

(U) Nutrition; (U) Wound Healing; (U) Military Nutrition; (U) Military Medicine

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Procede with Security Classification Code)

23. (U) Adequate nutrition is recognized as essential for the proper healing of wounds. Requirements for such nutrients as protein and calories are known to be higher than those required for normal maintenance. Other nutrients such as vitamin A, vitamin C, and zinc are essential to normal wound healing but the nature of their role remains unclarified. The objective of these studies is to investigate various nutrients such as amino acids, vitamins, and minerals, and determine their role in recovery from wounds, particularly combat wounds.

24. (U) The approach will be to investigate (a) effects of prior nutrition upon recovery from trauma; (b) effects of specific nutrients during the recovery phase; and (c) the biochemical roles of specific nutrients in tissue repair and recovery.

25. (U) 77 10 - 78 09 Due to losses in personnel and positions, studies on this work unit were not initiated.

* Applicable to contractor and subcontractor efforts only.

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

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6312	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GROUP MARK	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
77 10 01	H. Termination	U	U	NA	NL	☒ YES ☐ NO	A

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A811	00	007
b. CONTRIBUTING				
c. [illegible]	CARDS 114f			

11. TITLE (Precede with Security Classification Code)

(U) Basic Studies to Prevent or Speed Recovery from Bone Injuries

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 002600 Biology; 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 10	78 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE — EXPIRATION

b. NUMBER: Not Applicable

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
FISCAL YEAR 78 (PREVIOUS)	2.0	50
FISCAL YEAR 79 (CURRENT)	0.0	00

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
NAME: Marshall, J.D., COL, MS
TELEPHONE: 415-561-3600

20. GENERAL USE

Foreign Intelligence Not Applicable

19b. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Radioisotope Division
ADDRESS: Department of Nutrition
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Provide last name, first name, middle initial)
NAME: Zolock, D.T., CPT, MS
TELEPHONE: 415-561-4770
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Morrissey, R.L., MAJ, VC
NAME: POC:DA

21. KEYWORDS (Precede each with Security Classification Code)

(U) Combat Bone Injuries; (U) Vitamin D; (U) Calcium; (U) Phosphorus; (U) Mineral Metabolism

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

23. (U) In the past, approximately 25% of the operations performed in combat surgical hospitals were for bone and joint injuries. At current hospitalization costs of $168.00/day and a conservative estimate for soldier compensation of $20.00/day, the treatment of these bone and joint injuries in the Army between the years of 1964 and 1969 would cost $935,008,282.30. Also, Army dental facilities perform a tremendous amount of tooth restorations. For example, in FY 75, the Army performed 859,722 tooth restorations at a cost of $6,488,290.00. This research is directed at decreasing these numbers by increasing the absorption and retention of calcium and phosphorus through nutritional and medical therapeutic improvements.

24. (U) Vitamin D and its metabolites, especially the active hormonal form, 1,25-dihydroxycholecalciferol, are associated with metabolic increases in intestinal calcium absorption, increased bone calcium mobilization, and the induction of calcium binding protein (CaBP). In order to understand better the mechanism of calcium transport and homeostatic regulation of calcium, in vivo animal studies were performed by altering various nutritional conditions, such as dietary calcium, phosphorus, and 1,25-dihydroxycholecalciferol. Intestinal calcium transport, bone calcium uptake and mobilization, and CaBP concentrations were analyzed in these various studies.

25. (U) 77 10 - 78 09 Several in vivo animal studies have been designed and conducted during the year. The results from these studies have significant impact on understanding dietary and therapeutic regulation of calcium absorption and bone calcium uptake and bone calcium mineralization. Technological advances from these studies have been reported in 5 research journal articles (published or in press), 4 published abstracts, 5 articles sumitted to pusblishers, and 10 potential manuscripts currently in an advanced stage of laboratory data analyses. Work unit is terminated due to loss of personnel.

26. (U) PUBLICATIONS (References)

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

ABSTRACT

PROJECT NO. 3M76772A811 Military Internal Medicine

WORK UNIT NO. 007 Basic Studies to Prevent or Speed Recovery from Bone Injuries

The following investigation has been conducted under this work unit:

STUDY NO. 1 Basic mineral metabolism studies

Studies have demonstrated that 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) given to vitamin D-deficient chicks mediated an increase in serum calcium concentration (bone calcium mobilization), an increase in calcium transport across the intestine, an increase in bone calcium uptake, and induced a calcium binding protein (CaBP). All of the mediated effects except the increase in calcium transport were blocked by cycloheximide, a protein synthesis inhibitor. The results demonstrate that protein synthesis is necessary for the $1,25(OH)_2D_3$ mediated bone Ca uptake and bone Ca mobilization, but is not necessary for the mediated Ca transport across the intestine. Although CaBP is not necessary for this initial mediated increase in Ca transport by $1,25(OH)_2D_3$, it does appear to be a regulator of cellular Ca concentration since its synthesis parallels a decrease in cellular Ca accumulation. A study involving the influence of dietary Ca upon the synthesis of CaBP demonstrated a direct relationship between CaBP and the dietary Ca concentration. Except for at least the initial Ca transport across the intestine, $1,25(OH)_2D_3$ appeared to mediate its responses as a typical hormone via protein synthesis.

BODY OF REPORT

WORK UNIT NO. 007 — Basic Studies to Prevent or Speed Recovery from Bone Injuries

STUDY NO. 1 — Basic mineral metabolism studies

PROBLEM

Approximately 25% of combat trauma includes fracture wounds. The impact of fracture wounds is greater than that of flesh wounds because the degree of incapacitation is considerably greater, the patients usually must be evacuated, and the recovery time is usually prolonged. Fracture wounds accounted for 5.78 million man-days lost by the Army between 1964 and 1969. While considerable effort has been expended to develop and perfect methods of treatment for both flesh and fracture wounds, no specific efforts have been made at the field level to modify the susceptibility of soldiers to fracture wounds. Susceptibility to fracture wounds varies greatly. Some individuals are quite resistant even to heavy trauma. Others sustain fractures without apparent trauma (stress fractures). The degree of bone mineralization is a function of both the amount of calcium and phosphorus absorbed from the gut and that retained by the kidney. Vitamin D is intimately involved in both intestinal absorption and renal retention of calcium as well as in the mineralization process. To understand better the regulation of intestinal calcium absorption, the acute response to 1,25-dihydroxy vitamin D_3 ($1,25\text{-}OH_2D_3$) was studied in the intestine and bone of chicks with rickets.

RESULTS AND DISCUSSION OF RESULTS

Rachitic chicks were given 62.5 pmol of $1,25(OH)_2D_3$. Cycloheximide in 50 µg doses was administered starting 1 h before $1,25(OH)_2D_3$, and at subsequent 4-h intervals. The usual $1,25(OH)_2D_3$ mediated increase in Ca transport as measured by ^{45}Ca in the serum was observed. This mediated increase was not blocked by cycloheximide. ^{45}Ca uptake by bone was significantly increased by 3 h after $1,25(OH)_2D_3$ administration, maximized by 18 h, and declined to control level by 72 h. However, unlike transport, this $1,25(OH)_2D_3$ mediated bone response was blocked by cycloheximide. The $1,25(OH)_2D_3$ mediated increase in serum Ca was also blocked by cycloheximide. The data suggest that $1,25(OH)_2D_3$ induces protein synthesis which is necessary for both bone mineral uptake and mobilization. However, we cannot conclude whether or not both actions of $1,25(OH)_2D_3$ on bone are mediated by the same protein(s). The data also show that the $1,25(OH)_2D_3$ mediated mechanisms of the intestine and bone are independent of each other.

In order to determine dosage effects on serum Ca, intestinal ^{45}Ca transport, bone ^{45}Ca uptake, duodenal Ca accumulation, and intestinal CaBP, rachitic chicks were given various doses (0, 1, 10, 100, 1000 I.U.)

of $1,25(OH)_2D_3$ (1 I.U. = 62.5 pmol). The following results were observed 3 h after $1,25(OH)_2D_3$ was administered: 1) no apparent differences among serum Ca; 2) intestinal ^{45}Ca transport and bone ^{45}Ca uptake started to increase proportionally with dosage but then leveled off at the three higher doses; 3) Ca accumulation increased proportionally with the dosage except at the 1000 I.U. dose which remained at control (0 I.U.) level; and 4) CaBP was absent except at the high doses (100 and 1000 I.U.) where CaBP was barely detectable. At 18 h after $1,25(OH)_2D_3$ was administered, the following results were observed: 1) serum Ca was increased at all dosage levels over the controls; 2) intestinal ^{45}Ca transport and bone ^{45}Ca uptake increased proportionally with dosage of $1,25(OH)_2D_3$; 3) Ca accumulation initially remained at control level for the 1 I.U. dose and then decreased and leveled out for the 100 and 1000 I.U. doses; and 4) CaBP concentrations increased proportionally with dosage. It is apparent that the mediated effects of $1,25(OH)_2D_3$ are affected proportionally by the dosage of the metabolite at the longer time period (18 h). However, the higher doses at the shorter time period (3 h) apparently saturated the system and the effect remains constant at the higher dose levels.

A 3 X 3 factorial design was used to assess the influence of dietary calcium and dietary phosphorus on the mediated responses of 62.5 pmol of $1,25(OH)_2D_3$ in rachitic chicks. Intestinal calcium transport, CaBP, in vivo Ca accumulation by duodenal cells during transport, serum calcium and phosphorus concentrations, bone accumulation of transported ^{45}Ca and percent bone ash were assessed 24 h after administering the $1,25(OH)_2D_3$ dose and simultaneously changing the diet. We found a direct relationship between dietary calcium concentration and CaBP concentration rather than the inverse relationship characteristic of adaptation of normal chicks to low dietary calcium. Chicks fed normal dietary calcium and phosphorus levels responded to $1,25(OH)_2D_3$ by elevated serum calcium concentration as expected, but those fed low dietary calcium and high dietary phosphorus responded by a significant depression of serum calcium concentration. The results suggest that dietary calcium influences CaBP synthesis via a direct effect on the intestinal cell.

CONCLUSIONS

Based on our observations, we propose that $1,25(OH)_2D_3$, the active hormonal metabolite of vitamin D, exerts its mediated effects on bone calcium uptake and bone calcium mobilization via protein synthesis. However, at least the initial calcium transport across the intestine mediated by $1,25(OH)_2D_3$ is not dependent on protein synthesis. In fact, $1,25(OH)_2D_3$ probably exerts a direct effect on the permeability of the intestinal cell's membrane for calcium. The $1,25(OH)_2D_3$-dependent CaBP is not involved in the initial mediated increase of Ca transport across the intestinal cell, but is apparently somehow involved in the regulation of accumulation of Ca in the intestinal cell. Dietary calcium influences the $1,25(OH)_2D_3$-dependent CaBP synthesis probably

through a direct effect on the intestinal cell. The extent of these $1,25(OH)_2D_3$-mediated effects are proportional to the dosage of the metabolite.

RECOMMENDATIONS

Bone densitometry and ultrasound evaluation of military inductees should be performed. Inductees whose bone density is in the lower or subnormal range should receive dietary supplementation with calcium and magnesium as well as specialized exercise training programs during their basic combat training. Candidates should be prevented from entering military service if their bone density value is subnormal on the basis that a high probability exists that they will be included in the population of individuals medically retired due to bone and joint problems.

PUBLICATIONS

1. BIKLE, D.D., D.T. ZOLOCK, E.L. MORRISSEY, and R.H. HERMAN. Independence of 1,25 dihydroxyvitamin D_3 mediated calcium transport from de novo RNA and protein synthesis. J Biol Chem 253:484-488, 1978

2. MORRISSEY, R.L., D.T. ZOLOCK, D.D. BIKLE, R.N. EMPSON, JR., and T.J. BUCCI. Intestinal response to 1α,25-dihydroxycholecalciferol. I. RNA polymerase, alkaline phosphatase, calcium and phosphorus uptake in vitro and in vivo calcium transport and accumulation. Biochim Biophys Acta 538:23-33, 1978

3. MORRISSEY, R.L., R.N. EMPSON, JR., D.T. ZOLOCK, D.D. BIKLE, and T.J. BUCCI. Intestinal response to 1α,25-dihydroxycholecalciferol. II. Optimal study of this intracellular localization of calcium binding proteins. Biochim Biophys Acta 538:34-41, 1978

4. MORRISSEY, R.L., D.T. ZOLOCK, T.J. BUCCI, and D.D. BIKLE. Immunoperoxidase localization of vitamin D dependent calcium binding protein. J Histochem Cytochem 00:000-000, 1978 (In press)

5. BIKLE, D.D., R.L. MORRISSEY, D.T., ZOLOCK, and R.H. HERMAN. Dietary regulation of $1,25(OH)_2D_3$ stimulated calcium binding protein, alkaline phosphatase and calcium transport. (Abstract) Clin Res 26:580A, 1978

6. MORRISSEY, R.L., D.T. ZOLOCK, and D.D. BIKLE. Influence of dietary calcium and phosphorus on the induction of calcium binding protein (CaBP) in response to 62.5 pM of 1,25 dihydroxy vitamin D_3. (Abstract) Fed Proc 37:408, 1978

7. BIKLE, D.D., E.W. ASKEW, D.T. ZOLOCK, R.L. MORRISSEY, and R.H. HERMAN. Calcium accumulation by intestinal mitochondria from rachitic and $1,25(OH)_2D_3$ treated chicks. (Abstract) Fed Proc 37:1300, 1978

8. ZOLOCK, D.T., R.L. MORRISSEY, and D.D. BIKLE. 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) mediated bone mineral uptake and its blockage by cycloheximide (cyclo). (Abstract) Fed Proc 37:1316, 1978

9. BIKLE, D.D., C.C. PECK, R.L. MORRISSEY, D.T. ZOLOCK, and R.H. HERMAN. Pharmacokinetics of 1,25 dihydroxyvitamin D_3 in plasma and gut. Endocrinology (Suppl) 102:318, 1978

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6080	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: h. Termination	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. PROGRAM: NA	8. START/TERM: NL	9a. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	9b. LEVEL OF SUM: A. WORK UNIT
10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3M162772A811		00	010		
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 1147						

11. TITLE (Protect with Security Classification Code): (U) The Metabolic Response of Hepatic and Extra-Hepatic Tissues to Dietary Substances, Drugs and Hormones in Health and Disease.

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 12	78 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATE EFFECTIVE: Not Applicable
b. NUMBER:
c. TYPE:
d. AMOUNT:
e. KIND OF AWARD:
f. CUM AMT:

18. RESOURCES ESTIMATES

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
78	4.0	97
79	0.0	00

19a. RESPONSIBLE DOD ORGANIZATION

Name: Letterman Army Institute of Research
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20. GENERAL USE: Foreign Intelligence not Applicable

19b. PERFORMING ORGANIZATION

Name: Letterman Army Institute of Research
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Social Security Account Number:

Associate Investigators
Name: Hagler, L., COL, MC
Name:

POC: DA

21. KEYWORDS (Provide Key Technical Words for Publication Cross-Reference Code): (U) Hepatic Disease; (U) Hepatic Enzymes; (U) Enzyme Deficiency Disease, (U) Adaptive Responses of Hepatic Enzymes; (U) Laboratory Animals (U) Human Volunteers.

22. TECHNICAL OBJECTIVE, APPROACH, PROGRESS (Provide brief paragraphs identified by number corresponding to number entered in Item 21 with Security Classification Code):

23.(U) Military personnel require highly responsive adaptive mechanisms for the regulation of blood glucose and other substances which are essential for normal mental and muscular activity. The mechanism of glucose utilization during exercise, trauma, and septic shock are poorly understood. Muscle glucose utilization increases while insulin secretion is decreasing and being released from its muscle receptors. It is suggested that low molecular weight peptides called somatomedins are responsible for the regulation of muscle glucose utilization during exercise and other conditions of stress. Somatomedins mediate the action of pituitary growth hormone and account for greater than 93% of the non-suppressible insulin-like activity measured in vitro systems.

24.(U) The effect of somatomedin on gluconeogenesis in the perfused isolated rat liver obtained from fasted male rats will be investigated. Samples of arterial and venous blood will be obtained serially after the injection of somatomedin, somatomedin plus glucagon, somatomedin plus insulin, insulin, glucagon, or buffer. Blood will be measured for total glucose, total alanine, total lactate, ^{14}-C glucose, ^{14}-C lactate, ^{14}C alanine, and cyclic AMP. The perfusate will contain ^{14}C alanine. This substance and the ability of the perfused liver to produce ^{14}C glucose when stimulated with the various hormones will be measured.

25.(U) 77 09 - 78 09. This study has just been completed. The results are awaitir the completion of the various analyses.

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

ABSTRACT

PROJECT NO.	3M762772A811	Military Nutrition and Food Hygiene
WORK UNIT NO.	010	The Metabolic Response of Hepatic and Extra-Hepatic Tissues to Dietary Substances, Drugs and Hormones in Health and Disease

The following investigation has been conducted under this work unit:

STUDY NO. 12 Studies of non-insulin dependent mechanisms of glucose utilization during exercise and trauma

The effects of somatomedin on gluconeogenesis was examined in the isolated perfused rat liver. Somatomedin was infused into the isolated rat liver at 2 doses (100 and 1000 μU/ml) alone and in combination with insulin and glucagon. Arterial and venous samples of blood were obtained at zero and 20 min after which the various combinations of hormones were administered. The ability of the perfused liver to form ^{14}C-glucose and ^{14}C-lactate from ^{14}C-alanine was measured and the effect of the various hormone combinations was determined. Measurements were made every 5 min of radioisotopically labeled substances, total glucose, total alanine, total lactate, and cyclic-AMP. These analysis are currently in progress.

BODY OF REPORT

WORK UNIT NO. 010 — The Metabolic Response of Hepatic and Extra-Hepatic Tissues to Dietary Substances, Drugs and Hormones in Health and Disease

STUDY NO. 12 — Studies of non-insulin dependent mechanisms of glucose utilization during exercise and trauma

PROBLEM

The mechanism of glucose utilization during the stress of exercise and following trauma is not completely understood. Muscle glucose utilization increases dramatically in these circumstances while insulin secretion is decreasing and insulin is being released from its receptors on cardiac and skeletal muscle. Teleologically insulin would be detrimental for exercise since hypoglycemia might result from excess glucose use and since insulin in small amounts will inhibit hepatic gluconeogenesis. It would be reasonable to predict and the evidence suggests that insulin is not the primary mediator of glucose utilization during stress states such as exercise, severe trauma and during septic shock.

All of these circumstances have important military ramifications. Soldiers in training, maneuvers or combat must be capable of endurance exercise and the possibility of trauma and infection are ever present. Understanding the mechanisms of glucose utilization in such stress states may lead to improved methods of treatment for trauma and methods for increasing physical endurance.

In vivo studies during the 1950s in which cross-perfused dogs and rats were used, suggested that a humeral substance, that clearly is not insulin, can cause glucose utilization during exercise. Those studies suggested that the substance was a small molecular weight peptide, but it was incompletely purified and not identified. More recently, small molecular weight peptides (4000-7000 Daltons) have been found which account for greater than 93% of the insulin-like activity in human serum when measured in in vitro systems. These peptides have been called somatomedins (Sm) because they appear to mediate the anabolic growth activity of pituitary growth hormone (Gh). Most studies to date have focused on purification, amino acid sequencing, and the clinical changes that occur in acromegaly or hypopituitarism. Although the Sm are active on the insulin receptor the effects of these substances are thought to be minimal in in vivo situations since greater than 90% of these peptides are bound to a carrier protein in blood and thus are inactive. When perfused into heart muscle in a solution free of carrier protein, as little as 0.2 μU/ml causes maximal glucose utilization. (The normal amount in blood is arbitrarily defined as 1 unit/ml). The somatomedin

carrier protein system is unique in that the Sm are the only known peptide hormone with a specific carrier protein. An acid environment is required to separate the peptide from its carrier protein. It is not unreasonable to postulate that during exercise local muscle acidosis or a protease might cleave the peptide from its carrier and make it active. The somatomedins could be considered to be candidates for the humoral substance(s) responsible for non-insulin dependent glucose utilization.

RESULTS AND DISCUSSION OF RESULTS

In previous studies we demonstrated a small increase in somatomedin activity in blood from exercising subjects. The increase may have been small because there was no actual increase in total Sm, but rather an increased activity at the muscle site where there could be increased dissociation as postulated above. Such a mechanism would be protective in that the activity would persist only as long as the milieu of the muscle was that which occurs during exercise; hypoglycemia after exercise is prevented. There are other characteristics which the hormone should possess if it is to be active during exercise. If Sm is to be the exercise hormone, it should not inhibit gluconeogenesis as insulin does. We have examined the effects of Sm on gluconeogenesis by perfusing isolated rat livers from fasted 180 g male rats with Sm alone at 2 doses (100 and 1000 μU/ml) and in combination with insulin and glucagon. For 20 min the livers were perfused (4.0 ml/min) with washed human red cells suspended in Krebs-Ringer buffer containing amino acids in physiological concentrations, 4% bovine serum albumin and ^{14}C-alanine. Zero time arterial and venous samples were obtained at 20 min following which Sm (100 μl/ml), Sm plus glucagon (45 ng/ml), Sm plus insulin (100 μU/ml), insulin alone, glucagon alone or buffer was perfused for 2.5 min. Venous samples were collected at 5 min intervals for measurement of total glucose, total alanine, total lactate, ^{14}C-glucose, ^{14}C-lactate, ^{14}C-alanine, and cAMP for a total of 4 collections or 20 min. After this 20 min period the livers were perfused for another 2.5 min with the same combination of hormones except that the Sm was increased to 1000 μU/ml. Venous blood was collected at 5 min intervals for 20 min for measurement of the same parameters. Quadruplicate livers were perfused with each treatment group. The samples have been partially analyzed but it is not possible to draw conclusions until all the samples are analyzed and statistical evaluation has been made.

CONCLUSIONS

None

RECOMMENDATIONS

This is important work and should be continued. With the discontinuation of funding and the loss of investigative personnel it is not possible to continue this work.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				DA OE 6081	78 10 01	
77 10 01	h.Termination	U	U			
	62772A	3M162772A811		02	011	
	CARDS 114f					

(U) The Metabolic Response of Hepatic and Extra-Hepatic Tissues to Dietary Substances, Drugs and Hormones in Health and Disease.

003500 Clinical Medicine

74 12	78 09	DA		C. In-house
Not Applicable		FISCAL YEAR 78	4.0	208
		FISCAL YEAR 79	0.0	00

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POC: DA

(U) Gastrointestinal Disease, Jejunal Enzymes, Adaptive Responses of Jejunal Enzymes, Diarrheal Syndromes; (U) Human Volunteers; (U) Laboratory Animals.

23.(U) Acute and chronic gastrointestinal (GI) diseases affect military personnel. These diseases cause long-term morbidity, loss of duty time, and high costs of continuing in- and out-patient care. In 1966 in Vietnam (RVN), diarrheal diseases accounted for 12% of all hospital admissions. In World War II functional dyspepsia and chronic idiopathic diarrhea accounted for 15-20% of admissions to war zone hospitals and 62% of these patients were returned to CONUS for treatment. GI injury from combat wounds precludes enteral feeding and results in atrophy of the small intestine, pancreas, and liver. Atrophy may be due to absence of gastrin which results from lack of enteral feeding of traumatic loss of gastrin secreting tissue. Gastrin replacement may reverse GI atrophy and enhance rehabilitation. Jaundice is not uncommon in catabolic states. This so-called constitutional hyperbilirubinemia, or Gilbert's disease, is poorly understood.
24.(U) Patients with idiopathic diarrhea and subtotal gastrectomy were tested for the production of gastrin with a test meal and jejunal mucosa obtained by peroral biopsy was incubated in vitro to measure C-14 leucine incorporation into protein. Selected jejunal enzyme activities were assayed. The effect of a pentagastrin infusion on each of these parameters was determined. The effect of diet and drugs on the serum bilirubin of patient's with Gilbert's disease was studied.
25.(U) 77 09 - 78 09 Patients with idiopathic diarrhea had gastrin levels and C-14 leucine incorporation into protein similar to that of control subjects. No significant changes were found in disaccharidases and glycolytic enzyme activities after pentagastrin infusion. In other patients there was an inverse relationship between basal gastrin levels and the incorporation of C-14 leucine in jejunal mucosal protein after pentagastrin infusion. In Gilbert's disease serum bilirubin was decreased by small amounts of dietary fat or pharmacological amounts of nicotinic acid. This work unit is being terminated.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORM 1498A NOV 67 FOR 1469, 1 MAR 68 FOR ARMY USE ARE OBSOLETE

ABSTRACT

PROJECT NO.	3M762772A811	Military Nutrition and Food Hygiene
WORK UNIT NO.	011	The Metabolic Response of the Gastrointestinal Tract to Dietary Substances, Drugs and Hormones in Health and Disease

The following investigations have been conducted under this work unit:

STUDY NO. 6 In vivo study of the control of small intestinal enzymes in man

STUDY NO. 10 The effect of diet and drugs on Gilbert's disease

STUDY NO. 6 The effect of a pentagastrin infusion in normal subjects and selected patients on the ^{14}C-leucine incorporation into protein in jejunal enzyme activities has been studied. Patients with chronic idiopathic diarrhea had gastrin levels and ^{14}C-leucine incorporation into protein of jejunal tissue similar to that of control subjects. There was no significant change in disaccharidase and pyruvate kinase activities, a slight increase in fructose-1,6-diphosphatase aldolase, variable changes in fructose-1-phosphate aldolase, and a decrease in fructose-1,6-diphosphatase activities. In other patients there appeared to be an inverse relationship between gastrin levels after a test meal and the effect of a pentagastrin infusion on ^{14}C-leucine incorporation into jejunal mucosal protein. In one patient with high levels of endogenous gastrin there was no ^{14}C-leucine incorporation into jejunal mucosal protein while in another patient with no increase in serum gastrin after a test meal a pentagastrin infusion resulted in a marked increase in ^{14}C-leucine incorporation into jejunal mucosal protein. During studies done on one of the patients who also had chronic obstructive pulmonary disease it was found that intravenous glucose worsened pulmonary function. Administration of equivalent calories as fat (Intralipid®) had no effect on pulmonary function.

STUDY NO. 10 It has been demonstrated in jaundiced Gunn rats that dietary lipid reverses fasting hyperbilirubinemia. Agents, such as nicotinic acid, which block lipolysis have a similar effect. We have studied 3 patients with Gilbert's disease by comparing the rise in bilirubin during a series of 2-day fasts, intake of a 15% isocaloric diet as fat, or during administration of nicotinic acid every 3 to 4 hours. The bilirubin levels were decreased more than 50% by fat or nicotinic acid as compared to fasting levels. Free fatty acid levels were lower during the fat diet and showed fluctuating amplitude with a wide range during nicotinic acid administration.

BODY OF REPORT

WORK UNIT NO. 011 — The Metabolic Response of the Gastrointestinal Tract to Dietary Substances, Drugs and hormones in Health and Disease

STUDY NO. 6 — In vivo study of the control of small intestinal enzymes in man

PROBLEM

An important aspect of acute gastrointestinal disease involves combat-related abdominal injury and its sequelae. Abdominal injuries occur frequently in any military operation and serious complications may develop. In World War II, in one field hospital, wounds of the stomach comprised 416 of 3,154 cases of abdominal injury. The fatality rate was 40%. Approximately 30% of the abdominal injuries consisted of wounds of the small intestine. Approximately 20% of the total number of injuries required partial resection of the gastrointestinal tract. Many patients with abdominal injuries will have altered gastrointestinal function secondary to resection of portions of the intestinal tract. With improved techniques of first aid, evacuation, blood replacement, surgery, prophylaxis and treatment of infection, we can expect an increased number of combat-wounded soldiers to reach the post operative period. At this point only general supportive measures are available and no specific therapy is known which can hasten healing and restore function of the gastrointestinal tract. Food intake, intestinal hormones and intestinal adaptation all make considerable contributions to the recovery process after intestinal resection. Gastrin, a polypeptide hormone, synthesized in the gastric antrum regulates the metabolism and growth of gastrointestinal tract cells in animals. Evidence of the trophic action in human has been indirect. Mucosal hyperplasia has been noted in patients with hypergastrinemia in the Zollinger-Ellison syndrome and mucosal atrophy and abnormal protein and enzyme synthesis after subtotal gastric resection.

Two different laboratories have demonstrated the importance of food intake in regulating small intestinal enzymes. In rat, intravenous hyperalimentation decreased intestinal maltase and sucrase activities. Tissue gastrin fell concomitantly. The disaccharidases were restored to control levels by pentagastrin which suggests that gastrin may control intestinal disaccharidases which returned to normal after oral feeding. Previous studies in this laboratory have demonstrated increased activity of jejunal glycolytic enzymes in response to carbohydrate meals. Specific sugars caused adaptive changes in the enzymes

most concerned with the metabolism of the specific substrate and the changes were in addition to a generalized increase in enzyme activity attributed to calories alone. Since food intake influences gastrin and intestinal enzymes, and since gastrin has documented trophic effects in the gut, it is conceivable that gastrin has a generalized effect on protein synthesis in the gut. If the trophic effect of gastrin is required for normal intestinal protein synthesis, then gastrin deficiency states, occurring as a consequence of gastric or intestinal resection, could result in abnormal protein synthesis and subsequant maladaptation of intestinal enzymes. There is ample in vivo and in vitro support for a gastrin trophic effect. There is also evidence to suggest that food intake is important in determining the level of intestinal enzymes and the amount of tissue gastrin. The acutely injured soldier who has lost variable amounts of stomach and small intestine has reduced intestinal function by virtue of the surgical resection. The enzyme activities in the remaining gut are responsive to food intake and gastrin, both of which have been reduced by the surgical procedure. It is reasonable to believe that replacement of gastrin will restore intestinal enzymes to normal and hasten restoration of gastrointestinal function. We have studied the role of gastrin in the control of selected intestinal enzyme activities in selected patients with surgically altered gastric physiology. We have determined if any alteration of intestinal enzyme activity can be reversed by the administration of pentagastrin. Related studies on the dog have been done (Work Unit 043, Study No. 1).

RESULTS AND DISCUSSION OF RESULTS

We have studied the effect of pentagastrin on ^{14}C-leucine incorporation into protein in jejunal tissue incubated in vitro and the effect of pentagastrin on selected jejunal enzyme activities in 4 human volunteers and 11 patients with various gastrointestinal disorders (chronic idiopathic diarrhea, post gastroduodenostomy with and without vagotomy, and post gastrojejunostomy).

For each subject, maximal gastrin output was determined after a high protein gastrin test meal. Pentagastrin (6 ug/min/kg) was infused for 16 h. Peroral proximal jejunal biopsies were obtained before and for 3 days after infusion.

The chronic idiopathic diarrhea patients had gastrin levels and ^{14}C-leucine incorporation patterns similar to control subjects. There was an elevation of serum gastrin within 15 to 30 min after the gastrin test meal and a 10 to 15% stimulation of ^{14}C-leucine incorporation into protein. There was no significant change in disaccharidase and pyruvate kinase activities, a slight increase in fructose-1,6-diphosphate aldolase, variable results in fructose-1-phosphate aldolase, and a decrease in fructose-1,6-diphosphatase activities.

In one subject with diffuse gastritis who had no gastric surgery, the stimulated gastrin levels exceeded 500 pg/ml. There was no ^{14}C-leucine incorporation, a slight increase in fructose-1-phosphate aldolase and maltase activities, but a decrease in other enzyme activities.

In patients with gastroduodenostomies, there was a significant increase in ^{14}C-leucine incorporation and phosphofructokinase and fructose-1,6-phosphate aldolase activities but no significant change in disaccharidase activities.

In a patient with gastrojejunostomy (Billroth II with an 80% subtotal gastrectomy), there was no increase in serum gastrin after a test meal, a marked increase in ^{14}C-leucine incorporation and a significant diminution of glycolytic enzyme activities 24 h after pentagastrin infusion. There was no change in disaccharidase activities.

In the latter patient a study of the effects on jejunal enzyme and protein synthetic mechanisms during hyperalimentation was attempted. This aspect of gastrin activity has been extensively studied in vivo in rats. A Broviac catheter was surgically placed and the patient nourished parenterally with the Letterman Army Medical Center's LIVN solution[1] or Intralipid(R)[2] (Cutter Laboratories, Oakland). In addition, we were able to evaluate the effects of intravenous carbohydrate and lipid on respiratory parameters, because the patient coincidentally had emphysema. We observed during three separate studies that intravenous glucose infusion (240 to 504 gm/day) resulted in progressive increases in respiratory exchange rates, minute CO_2 and O_2 ventilation (V_{CO_2} and V_{O2}), and more importantly, decreased arterial pH and markedly increased P_{CO_2}. The studies using glucose as the caloric source had to be terminated because of impending patient deterioration. Thus, the jejunal biopsies during these periods were not obtained.

When isocaloric fat (816 kcal/day in 750 ml Intralipid(R)) infusion was begun, the respiratory parameters did not deteriorate as with carbohydrate.

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[1] LIVN soln: D_{25}, 4 g nitrogen, 40 mEq Na, 40 mEq K^+, 46 mEq Cl, 46 mEq acetate, 8 mEq MG, 5 mEq each Ca^{+2} and Zn^{+2}, elemental phosphorus 419 mg and 0.7 ml multivitamins.

[2] Intralipid(R): 54% linoleic acid, oleic acid 26%, palmitic acid 9%, linolenic 8%.

CONCLUSIONS

Our preliminary data suggest that pentagastrin increases the ability of jejunal epithelial cells of man to incorporate ^{14}C-leucine into protein in vitro, increases glycolytic enzymes in certain patients but has no effect on disaccharidase activity.

In addition, from observations of respiratory parameters during hyperalimentation, we conclude that intravenous carbohydrate loading can increase V_{CO_2}, P_{CO_2}, and decrease P_{O_2} in the face of compromised pulmonary function. The clinical significance of these findings is under investigation. The beneficial effects of an isocaloric lipid load may have far-reaching effects on the nutritional management of patients with chronic obstructive pulmonary disease.

RECOMMENDATIONS

These studies should be continued but because of lack of funding they will be terminated.

PUBLICATIONS

1. NYLUND, B., R. WICKS, and R. H. HERMAN. The action of pentagastrin on jejunal enzymes and protein synthesis in humans. Scand J Gastroenterol 13: 136, 1978.

2. NYLUND, B., T. D. CLEMMER, and H. D. CALDWELL. The effects of intravenous carbohydrate in a patient with chronic obstructive pulmonary disease (COPD). Surg Forum (in press)

STUDY NO. 10 — The effect of diet and drugs on Gilbert's disease

PROBLEM

The mechanism of fasting hyperbilirubinemia in Gilbert's disease is unknown. Studies in jaundiced Gunn rats show that blocking lipolysis with nicotinic acid during a fast will inhibit the rise in serum bilirubin. Dietary lipid in small quantities also inhibits the rise in bilirubin and free fatty acids seen in fasting Gunn rats. There appears to be a relationship between lipolysis and the mechanism of fasting hyperbilirubinemia. Oral fat appears to inhibit lipolysis by some as yet unexplained mechanism. Understanding the mechanism of control of lipolysis by dietary fat will enable us to explain the mobilization of energy during fasting or prolonged exercise. Control of total body fat in obese and lean persons seems to be an integral part of these metabolic processes.

RESULTS AND DISCUSSION OF RESULTS

To date 5 patients have been studied. Each patient fasted intermittently for 2-day periods with 4 to 5 control days between each fast. Free fatty acid levels and bilirubin (total and direct) were measured 4 times/day. The mean of all the morning values for control days was calculated for each patient and was considered to be 100%. The bilirubin and free fatty acid levels during fasting were compared to the mean control values and are expressed as percent above control.

After 36 h of fasting the bilirubin level was 210 ± 27% (SEM) and after 60 h it was 253 ± 33%. The bilirubin level with the 15% isocaloric diet supplied as fat was 109 ± 15% after 36 h and 143 ± 15% after 60 h. During administration of nicotinic acid (50 mg every 4 h) during fasting the bilirubin level was 105 ± 17% at 36 h and 125 ± 8% at 60 h. The free fatty acid levels rose to 194 ± 18% of control after 30 h and to 230 ± 15% after 60 h. During the fast with a small quantity of fat the free fatty acid level was 121 ± 11% of control after 30 h and 150 ± 22% after 60 h. The free fatty acid levels fluctuated widely while the patients were on nicotinic acid probably due to the rebound of free fatty acid levels seen about 3 h after ingestion of the drug.

These results suggest that bilirubin is released into the circulation during fasting from adipose tissue and causes hyperbilirubinemia. The release is blocked by dietary lipid. This lipid specific effect may participate in the dietary modulation of serum bilirubin levels and in the synthesis and catabolism of fat in the human.

CONCLUSIONS

We have applied the results of animal studies to man. Fasting hyperbilirubinemia is related to lipolysis and the rise in serum bilirubin can be prevented if lipolysis is blocked. We have shown that dietary lipid during fasting partially blocks lipolysis.

RECOMMENDATIONS

These studies should be continued in animals and humans. Animals should be studied with the use of double isotopic labeling of endogenous fat. The human studies would involve determining the type of dietary fat that is the most effective in blocking lipolysis.

PUBLICATIONS

1. MORRIS, R. E., E. ROSENTAL, R. H. HERMAN, and M. M. THALER: Mechanism of fasting hyperbilirubinemia in Gilbert's Syndrome:

Inhibition of lipolysis by dietary lipid. (Abstract) In: Program for American Association for the Study of Liver Disease, Chicago, IL, 8 November 1978.

2. ROSENTAL, E., R. E. MORRIS, R. H. HERMAN, and M. M. THALER: Opposing effects of nicotinic acid on bilirubin nutrition. (Abstract) Gastroenterology (In press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6116	2. DATE OF SUMMARY: 79 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. CHANGE	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. RESEARCH: NA	8a. ORDER NUMBER: NL	8b. SPECIFIC DATA CONTRACTOR ACCESS: ☐ YES ☒ NO	9. LEVEL OF SUM: A. WORK UNIT
10. NO. CODES: a. NUMBER	PROGRAM ELEMENT: 62772A	PROJECT NUMBER: 3M162772A812		TASK AREA NUMBER: 00		WORK UNIT NUMBER: 001	
b. CONTRIBUTING							
XXXXXXXXXX	CARDS 114 f						

11. TITLE (Procede with Security Classification Code):

(U) Studies to Assure a Supply of Non-human Primates for Research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

001700 Animal Husbandry; 012900 Physiology; 002600 Biology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY		16. PERFORMANCE METHOD
76 10	79 09 30	DA		C. In-house

17. CONTRACT GRANT

a. APPROPRIATION/LIMITATION:
b. NUMBER: Not Applicable
c. TYPE:
d. AMOUNT:
e. END OF AGRMT:
f. CUM AMT:

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR (PREVIOUS): 78	2.0	179
FISCAL YEAR (CURRENT): 79	1.0	143

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129
ADDRESS:

RESPONSIBLE INDIVIDUAL
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19b. PERFORMING ORGANIZATION

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Division of Research Support
ADDRESS: Animal Resources Group
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Person doing work in this assigned responsibility)
NAME: Goldsboro, J., LTC, VC
TELEPHONE: (415) 561-2000
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Kelley, S.T., CPT, VC
NAME:

20. GENERAL USE: Foreign Intelligence Not Applicable

POC:DA

21. KEYWORDS (Procede each with Security Classification Code): (U) Primate; (U) Reproduction; (U) Animal Colony; (U) Physiology; (U) Behavior; (U) Husbandry

22. TECHNICAL OBJECTIVE, APPROACH, PROGRESS (Furnish individual paragraphs identified by number. Procede each with Security Classification Code.)

23. (U) An assured supply of primates is essential for continued research to solve Army medical problems in infectious disease, new drug development, and a variety of other areas. Curtailment of export by the Colombian source has limited the overseas supply of owl monkeys (Aotus) required for ongoing malaria research. Owl monkeys are nocturnal primates and there are few data on their requirements in captivity or on the behavior and needs of their offspring. This work unit will develop information on reproductive biology, nutrition, disease control, and low-cost husbandry methods for domestic production of required owl monkeys.

24. (U) Owl monkey pairs will be matched by karyotype and housed in a variety of cage group arrangements. Reproductive potential will be evaluated by observation and physiological measurements.

25. (U) 7710-7909 At present there are 110 animals in the owl monkey colony, including 38 breeding pairs and 25 offspring. Most animals have been karyotyped and fecundity evaluated in terms of karyotype matching and adaptation to laboratory conditions. Growth of young animals was described in terms of age, body weight, and skeletal maturation. New techniques for pregnancy diagnosis and in vivo body composition analysis were developed. Seventy M. mulatta were accumulated as the nidus for the rhesus monkey production contract. A pilot in-house breeding program has produced 14 live births and 4 stillbirths. In-house breeding of this species has been curtailed due to a reduction in personnel and lack of caging facilities for juvenile primates. The contract for production of M. mulatta has not been awarded due to a shortage of funds.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498B, 1 MAR 69 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M6277A812 Military Research Animal Resources

WORK UNIT NO. 001 Studies to Assure a Supply of Nonhuman Primates for Research

The following investigations have been conducted under this work unit:

STUDY NO. 1 Basic studies in reproduction of owl monkeys

STUDY NO. 2 Development of husbandry methods for an outdoor colony of macaque monkeys

Nonhuman primates are essential for a number of areas of military biomedical research. Most notably they are used in infectious disease to establish pathogenesis of infections and tests of chemotherapeutic agents and vaccines. They are also needed for research in trauma, resuscitation, performance, and toxicology. All of these studies are designed to benefit military personnel who are exposed to disease or toxic substances or who suffer injury in performance of duty.

Nonhuman primates from foreign sources are becoming decreasingly available; future supplies depend upon development of domestic breeding colonies. Much needs to be learned so they can be produced in large numbers and as economically as possible.

This study has made a substantial contribution to the limited amount of data available about the husbandry of this nocturnal primate.

STUDY NO. 1 At present there are 110 animals in the owl monkey colony, including 38 breeding pairs and 25 offspring. Most animals have been karyotyped and fecundity evaluated in terms of karyotype matching and adaptation to laboratory conditions. Growth of young animals has been described in terms of age, body weight, and skeletal maturation. New techniques for pregnancy diagnosis and in vivo body composition analysis have been developed.

BODY OF REPORT

WORK UNIT NO.	001	Studies to Assure a Supply of Nonhuman Primates in Research
STUDY NO.	1	Basic studies in reproduction of owl monkeys

PROBLEM

There is a critical shortage of owl monkeys (*Aotus sp.*) for biomedical research because the countries of origin have severely limited exportation. In the near future owl monkeys will be placed on the list of endangered species. This will make importation of these animals impossible at any cost.

Owl monkeys are the only nonhuman primate in which falciparum malaria can be maintained. Consequently, the Army is dependent upon these animals for research in prevention and treatment of malaria.

The long-term solution to provide a source of these animals is a domestic breeding program. Little is known about the reproductive biology or behavior of owl monkeys. Until recently, consistent breeding and rearing of these animals in captivity had not been accomplished.

The objective of this study is to determine the environmental and husbandry conditions, physiological state, and behavior which contribute to successful laboratory breeding and rearing of owl monkeys. The specific investigations in progress are owl monkey husbandry, cytogenetic analysis, behavior patterns, hematology, blood chemistry, virology, pathology, growth, pregnancy diagnosis, nutrition, and urine analysis.

RESULTS AND DISCUSSION OF RESULTS

Since the arrival of the first group of owl monkeys in May 1975, the breeding program has produced 32 live progeny. At the present time, the colony contains 110 animals, including 25 offspring and 85 adults, of which 76 are paired for breeding and 9 are single males. The oldest LAIR-born animal is 2.8 years, too young for entry into the breeding program.

During the past year, certain modifications in colony management were instituted. Canned commercial monkey food was eliminated from the diet because it was not readily consumed, was expensive, and did not appear critical to optimal nutrition. Also, the use of instant breakfast drink to soak dry commercial monkey bisquets was discontinued because of its expense and lack of impact on acceptability. Subsequent to behavioral

analyses to assure that established characteristics would not be adversely affected, the light cycle was increased to 13 hours, 2100 to 1000 hours daily, and the dark cycle was correspondingly reduced; the 30-minute transition between cycles was continued. This change allowed better husbandry by a reduced technical staff. At present access to the colony is restricted to the principal investigator and two specific veterinary technicians. Such restriction was imposed to reduce environmental variables and so enhance colony productivity.

All animals imported from Bolivia and Panama and some LAIR-born offspring were karyotyped in FY 78. Results from other LAIR-born animals have yet to be received. The cytogenetic analysis was performed by Dr. T.C. Jones of Pathobiology, Inc., Marlboro, MA. Six karyotypes were identified.

Since 1 October 1977, 11 monkeys have died, including 6 adults and 5 LAIR-born young animals. All were necropsied and tissues were evaluated by light microscopy. In some cases, scanning and transmission electron microscopy were also used. Causes of death have been variable and unrelated. In young animals death could be associated with septicemia at or near parturition, stress of weaning, or behavioral factors, especially parental rejection and abuse. In adult monkeys, death could be associated with non-specific clinical signs of central nervous system (CNS) deterioration in 2 animals, reaction to ketamine in 1 animal, and severe renal disease in 2 animals. Anemia of unknown cause has been observed in some animals but has resolved spontaneously following symptomatic treatment and intensive nursing care.

On the basis of karyotype, the 38 breeding pairs in the colony can be divided into four breeding groups. Breeding by karyotype seemed necessary to examine those factors which seem critical to fecundity. The majority of the progeny have been produced from group I (karyotype pairs III x II, II x II, III x III, and IV x III) which are of Colombian origin, have been at LAIR for three years, and were acclimated to laboratory environment prior to arrival. The production rate for II x II karyotype pairs is almost twice that of III x III pairs. The frequency of abortions is about the same between the two groups, and only four pairs account for all abortions in group I. Group II is composed of pairs of different origin and karyotype (VI x VI, IX x IX, III x IV, and II x VIII). Production did occur in two pairs received from other laboratories (IX x IX, III x IX). All animals of Bolivian origin, group III (VI x VI) have failed to produce progeny or demonstrate positive pregnancy tests. Most of these animals were wild-caught; therefore, failure in production may be the result of slow acclimation to a laboratory environment. However, establishment of a production colony requires that the progeny also mate successfully. We estimate that these animals must be at least 3 years old before they reach sexual maturity.

After a behavioral taxonomy for owl monkeys was established, the activity distribution pattern was completed. It was demonstrated that

activity for adults of either sex, different sources, or juveniles was about the same pattern but varied with intensity. All animals came out of their nest boxes when lights were off and spent most of their time outside the box until the lights went on 11 hours later. Activity curves have been prepared for 18 behavioral categories for all observed groups, including all animals, all adults, all juveniles, male or female animals, adults from Bolivia, and adults from Colombia. Significant differences were observed between animals of Bolivian and Colombian origin. Those from Bolivia were more active, showing more locomotor and paly behavior. As would be expected, juvenile animals were more active than adults.

All animals showed three peaks in feeding behavior. The first just after the morning (after the light change to darkness) feeding, the second at the afternoon feeding, and a third smaller peak about two hours before the light change to daylight. This behavior was not altered by changes in feeding schedules such as three small meals per day or one large meal per day. Twice-a-day feeding, however, seems to provide better nutrition. The feeding activity pattern in the colony, therefore, seems to correspond to that reported for owl monkeys in the wild.

Continued collection of blood samples for hematology and serum chemistry gave results that did not change significantly from those reported last year. This confirms that the values previously reported are probably characteristic for our colony.

Swab samples from two sites, the oropharynx and rectum, and serum samples were taken for isolation and identification of viruses commonly carried by animals in this colony. This work was accomplished and reported under Project No. 3M762772A812, Work Unit 004, Study No. 3.

A project to determine owl monkey growth and skeletal maturation was undertaken and mostly completed physical growth was studied radiographically and by measuring body weights in 25 laboratory-born owl monkeys of known origin. All ossification centers were radiographically visible at approximately 300 days. Epiphyseal fusion began around 400 days of age and appeared completed by 900 days. In some instances, adult weight was obtained as early as 500 days of age, but skeletal maturation was still continuing. Therefore, body weight was not considered to be an accurate indicator of chronological age during the skeletal maturation period. It was determined that chronological age of owl monkeys could be best estimated by evaluation of the presence of ossification centers and the number and location of fused epiphyses.

An immunological pregnancy diagnosis technique, by using urine, has proven effective. Since the inception of the use of this technique in November 1977, no false negative results have been detected, no monkeys have had a negative result before parturition or abortion. However, some animals have converted from negative to positive to negative again. This is troublesome but can be reasonably explained by fetal resorbtion,

undetected abortion, or variation in interpretation of test results. Early detection of pregnancy allows for changes in management techniques to minimize stress to pregnant animals, thus abortions resulting from stress are decreased.

New techniques have been developed to measure body composition in owl monkeys. A pilot study was completed successfully and more data collection in the future is anticipated. Techniques for safe consistent collection of semen from male owl monkeys are also being determined. Successful completion will allow for combined studies of male fertility.

As of June 1978, the number of technical personnel assigned to owl monkey project was reduced from 3 to 2 technicians. With only two technicians to assist in conducting research and taking care of daily colony maintenance, it is impossible to both maintain the owl monkey colony in a satisfactory manner and perform research. In order to provide 7-day-a-week care, it is necessary that of every 5-day work week, there are only three days when both technicians are present. This does not make allowances, furthermore, for absences because of illness, extra or special duties, or leave. One man day is required to provide minimum daily care to the colony. Therefore, when the second technician is available, most of his/her time must be employed in performing more detailed maintenance procedures including collection of urine for pregnancy tests, monitoring animal health, treatment of sick animals, and record keeping. These procedures are needed just to maintain a healthy colony. Obviously, with only two technicians available, it is difficult to accomplish the minimum, let alone any research.

CONCLUSIONS

1. Wild-caught owl monkeys, particularly those of Colombian origin, can be maintained and mated effectively in a controlled environment.

2. Alterations in the environment adversely affect good health and reproduction.

3. Husbandry conditions in our colony are appropriate and efficient in terms of fecundity.

4. Behavior must be monitored continuously and daily, particularly at weaning and new-pair formation, to assure viability and maturation of offspring.

5. Growth and skeletal development for owl monkeys in an artificial environment has been described.

RECOMMENDATIONS

The provisions of the protocol should be completed to include establishment of nutrient requirements, identification of hematologic and

biochemical reactions to specific stresses, and delineation of behavioral and physiologic requirements for reproduction of wild-caught and laboratory-born monkeys.

PUBLICATIONS

1. KELLEY, S.T., R.S. MURRAY, N.L SAY. and G.S. WARD. Breeding owl monkeys (*Aotus sp.*) in a climate-controlled laboratory environment: Preliminary report on operations, diet, housing, and husbandry, October 1975 to June 1977. Report No. 49. San Francisco, California: Letterman Army Institute of Research, 1978

2. KELLEY, S.T., J. SIMPSON, B.C. LEIBRECHT. A behavioral taxonomy for owl monkeys (*Aotus sp.*). Report No. 53. San Francisco, California: Letterman Army Institute of Research, 1978

3. KELLEY, S.T., T.J. BUCCI. Blood chemistry in a colony of owl monkeys. In: Abstracts for the 28th Annual Session of the American Association for Laboratory Animal Science (Anaheim, California, 2-7 October 1977)

4. KELLEY, S.T., B.C. LEIBRECHT, R.S. MURRAY, N.L. SAY. Behavioral taxonomy for owl monkeys. In: Abstracts for the 28th Annual Session of the American Association for Laboratory Animal Science (Anaheim, California, 2-7 October 1977)

5. VANDERLOO, P., S.T. KELLEY, J. GRIMM. Hematologic values of owl monkeys. In: Abstracts for the 28th Annual Session of American Association for Laboratory Animal Science (Anaheim, California, 2-7 October 1977)

6. KELLEY, S.T., T.J. BUCCI, S. SILVERMAN. Skeletal maturation in owl monkeys (*Aotus sp.*). In: Abstracts for the 1978 Meeting of the Federation of American Societies for Experimental Biology (Atlantic City, New Jersey, 9-14 April 1978)

STUDY NO. 5 — Development of husbandry methods for an outdoor colony of macaque monkeys

PROBLEM

There is a critical shortage of rhesus monkeys (*Macaca mulatta*) for research because the countries of origin have restricted exports. The rhesus monkey is used by the US Army for many areas of research. The cynomolgus monkey (*Macaca fasicularis*) is a possible replacement for the rhesus monkey, or at least for some studies. However, the degree of interchangeability of the two species is not known. The cynomolgus monkey faces the same problems (i.e., about restrictions on exports) that have resulted in scarcity of rhesus monkeys. The Army must

provide for its own future needs for these species and must do so in the most expeditious and inexpensive fashion possible. There is also the real danger that no breeding stock will be available after the next 2 to 3 years.

The best solution is to establish domestic breeding colonies of these species. In order to do so, inexpensive, efficient, and scientifically sound methods of husbandry, medical management, and facility design must be developed. In the case of the rhesus monkey, considerable information is available, but little is known about the specifics of out-door breeding colony operations. The cynomolgus monkey has seen only limited use in this country as an experimental animal. Little is known of its reproductive biology or climatic adaptability.

The object of this study is to develop inexpensive techniques of outdoor colony management, husbandry methods, disease control and eradication, breeder selection, and rearing of offspring. For the cynomolgus monkey, ovulatory cycles, hormonal cycles, and social factors beneficial to reproduction must be discovered.

RESULTS AND DISCUSSION OF RESULTS

The majority of the rhesus and cynomolgus monkeys in the colony are approaching sexual maturity. Clinical laboratory examinations have been restricted to the minimum necessary to protect the health of the colony. Some cage breeding has been accomplished as a check for breeding maturity and the ability of males to copulate. To date, 14 live births and three abortions have resulted. An additional three females are currently pregnant. Currently the cage breeding of the macaques, both rhesus and cynomolgus, has been curtailed due to lack of housing and shortage of animal care personnel. The land reserved at Camp Parks, California, for the outdoor colony has not been developed, nor has the request for proposal (RFP) to obtain extramural research i outdoor production of macaques been advertised.

CONCLUSIONS

Import limitations of non-human primates and the increasing cost of laboratory-raised and laboratory-conditioned animals will reduce the use of these animals to a minimum. Army requirements for non-human primates is not expected to decrease. Thus, non-human primates will continue to be needed for current malaria research programs and several other experimental programs considered to be of critical need.

Studies to determine the husbandry methods and practices and the physiologic characters conducive to a successful outdoor breeding colony of *M. mulatta* monkeys have been severely restricted by a lack of funds to acquire adequate facilities and by a reduction of the professional and technical staff at LAIR. Because of the lead time required to establish a successful colony of non-human primates, each year lost postpones the

day when MRDC might be self-sufficient in the supply of these animals for research purposes.

RECOMMENDATIONS

Establishment by contract of an outdoor breeding colony of rhesus and cynomolgus monkeys has not been initiated due to shortage of funds. Reduction in professional staff and animal care personnel at LAIR will severely restrict attempts to identify and define physiological parameters that would lead to a successful breeding program. It is recommended, therefore, that this study be discontinued.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OE 6117	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWN GRADING	7. REGRADING	8. SMRY MATG	8a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM	9. WORK UNIT
77 10 01	H.TERMINATIO	U	U	NA	NL	YES ☐ NO		

10. NO CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A812	00	002
b. CONTRIBUTING				
c. CONTRIBUTING	CARDS 114 f			

11. TITLE (PROJECT AND SUBTASK CLASSIFICATION CODE)

(U) Development of Military Research Animal Resources

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

001700 Animal Husbandry; 012900 Physiology; 002600 Biology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMING METHOD
76 10	78 10	DA	C. In-house

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: — EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: — d. AMOUNT:
e. KIND OF AWARD: — f. CUM. AMT.:

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS (In thousands)
FISCAL YEAR 78 (PREVIOUS)	2.7	134
FISCAL YEAR 79 (CURRENT)	0.0	00

19a. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129
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RESPONSIBLE INDIVIDUAL
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20. GENERAL USE

Foreign Intelligence Not Applicable

19b. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME:
NAME: POC:DA

21. KEYWORDS (PROJECT AND SUBTASK CLASSIFICATION CODE)

(U) Animal Model; (U) Physiology; (U) Animal Diseases; (U) Laboratory Animal

22. (U) An estimated 75% of USAMRDC's budget is for studies in which laboratory animals are used. Animals have threefold importance as resources in medical research. (1) New knowledge of physiologic processes in soldiers can be derived through study of similar processes in the animal by using methods not feasible in man. It is imperative that the animals be in normal health and also that the correct animal species be used because interspecies variations occur. (2) Many diseases of animals may be studied as models to enhance prevention, detection, and treatment of counterpart diseases required by soldiers. (3) The animals are a major economic resource, so there is constant need for improved husbandry methods. These studies will contribute to improved animal care and to identification, development, and use of animal models to investigate normal and abnormal health processes in soldiers.

24. (U) Animal diseases which also occur in soldiers, such as dermatitis, will be characterized by modern laboratory methods to determine their appropriateness as model systems for the human condition. Costly laboratory animal diseases, e.g., respiratory diseases in cats, will be studied for eventual eradication. Normal processes, e.g., utilization of vitamin C, will be studied so as to improve animal care as well as for possible extrapolation to the soldier.

25. (U) 7710-7809 Viral isolates from 52 cats, 168 rhesus and 131 owl monkeys were identified. Attempts were made to correlate viral isolation with the presence of antibody titer in cats and rhesus monkeys and with karyotype and country of origin with owl monkeys. Knowledge of the epidemiology of viral infection within the animal colonies permits measures to be taken to minimize loss from disease and compromise of experimental results. Acid-base and blood gas characteristics of procine blood were found to be sufficiently close to those of human blood to serve as a useful model. This study will be terminated due to reorganization of LAIR.

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

ABSTRACT

PROJECT NO. 3M762772A812 Military Research Animal Resources

WORK UNIT NO. 002 Development of Military Research Animal Resources

The following investigations have been conducted under this work unit:

STUDY NO. 3 Infectious disease surveillance of laboratory animals

STUDY NO. 5 Physiological and biochemical characteristics of domestic swine used in military research

STUDY NO. 3 The owl monkey colonies, rhesus monkeys, and a group of newly purchased cats were studied to determine the viral infection(s) present in LAIR's laboratory animals. The study revealed that owl monkeys are frequently infected with three different serologic types of adenovirus and eight serotypes of cytomegalovirus. In some individual owl monkeys both viruses persisted for many months. Of 168 rhesus monkeys, seven serotypes of adenovirus, *Herpesvirus simiae*, and simian foamy virus were identified. From a group of 52 newly purchased cats, 237 oropharyngeal swabs were collected. Feline herpesvirus, feline calicivirus, and feline syncytial-forming virus were isolated from 26, 49, and 95 swab samples, respectively.

STUDY NO. 5 Acid-base and blood gas characteristics of porcine blood were found to be sufficiently close to those of human blood to serve as a useful model.

BODY OF REPORT

WORK UNIT NO.	002	Development of Military Research Animal Resources
STUDY NO.	3	Infectious disease surveillance of laboratory animals

PROBLEM

Laboratory animals represent a major economic resource in the US Army medical research effort. Outbreaks of infectious disease in an animal colony are economically wasteful and can be devestating to on-going research. Monitoring for the presence of infectious agents through clinical histories, laboratory tests, immunologic surveys, studies of gross pathology and histopathology, and microbiological studies is an effective method for the early recognition, diagnosis, and control of infectious diseases.

The objectives of this study are (1) to investigate the prevalance, cause, control, and elimination of infectious diseases among laboratory animals at LAIR, (2) to identify and characterize animal models of human disease from instances of spontaneous disease occurrence in the colony, (3) to identify and eliminate animals carrying dangerous zoonotic diseases.

RESULTS AND DISCUSSION OF RESULTS

Owl Monkeys. Three serotypes of adenovirus and eight serotypes of cytomegalovirus were isolated from owl monkeys.

Adenovirus. From a colony of 131 owl monkeys, adenovirus was isolated 68 times from 433 oropharyngeal and rectal swab sample pairs collected over a 3-year period. Sixty-three of 68 adenovirus isolates were grouped by neutralization into 1 to 3 types designated owl monkey adenovirus types I, II, and III. The OMAV Ty I and Ty II were identified by neutralization with antiserum to squirrel monkey adenovirus type I and adenovirus SV 11, respectively. The OMAV Ty III was partially neutralized by antiserum to OMAV Ty II.

The OMAV Ty I was isolated from 8 of 30 newly imported owl monkeys (group IV) within 3 weeks of arrival. Thirteen of 26 had four-fold or greater rise in serum neutralization (SN) titer with 25 of 27 developing SN titers of $\geq$ 1:80. Forty of 87 other owl monkeys in the colony had SN titers of $\geq$ 1:5.

The OMAV Ty II was isolated from 51 to 433 (12%) swab sample pairs. Virus was isolated from 26 owl monkeys with 13 having persistent infections. Viral isolations spanned a 10- to 17-month period for 5 of these owl monkeys. In 114 owl monkeys, 101 (86%) had SN titers to OMVA Ty II $\geq$ 1:20.

The OMAV Ty III was isolated from 3 owl monkeys. Ninety-three of 114 (82%) owl monkeys had SN titers to OMAV Ty III of $\geq$ 1:20. Although the distribution of SN titers was similar for OMAV Ty II and Ty III, 42 of 114 (37%) owl monkeys had eight-fold or greater differences in SN titers between OMAV Ty II and Ty III.

Cytomegalovirus. Cell-associated herpesvirus or cytomegalovirus was isolated from 74 to 131 owl monkeys. In 24 infected monkeys the infection persisted for over 12 months; 18 others shed virus for over 6 months. Eight distinct serotypes of virus have been identified as follows:

Serotype	No. of Isolates
A	12
B	15
C	57
D	6
E	1
G	67
I	4
K	5
C + G	8

Identification of 37 other isolates was not possible because of the lack of specific antisera.

Among owl monkeys at LAIR there are 6 different karyotypes:

Karyotype	Origin	Serotype of Virus Isolated
II	Colombia	B, C, G
III	Colombia	B, C, G
IV	Colombia	B, C, G,
VI	Bolivia	A,C,D,E, and I
VIII	Panama	K
IX	Panama	K

Except for 2 isolates of serotype C from 2 owl monkeys of karyotype VI, a given serotype was only isolated from owl monkeys of the same country of origin. Fifteen LAIR-born owl monkeys were also found to be infected with cytomegalovirus. These animals are of karyotype II, III, and IV, and they were infected with the same serotypes (B, C, and G) as their parents.

Rhesus and Cynomolgus monkeys . At the beginning of FY 78, the colony of Old World monkeys consisted of 134 rhesus and 20 cynomolgus monkeys (group 1). In addition, 24 rhesus monkeys arrived on 27 September 1977 (group 2) and were held in quarantine for 8 weeks. From group 1, 177 pairs of oropharyngeal and rectal swab samples were collected and tested. The following viruses were isolated and identified: adenovirus SV 20 (9 isolates), SV 23 (1), SV 33 (1), and SA 7 (1); entervirus SV 16 (1), and SV 26 (2); *Herpesvirus simiae* (1), and simian foamy virus (101). From group 2, swab sample pairs were collected at 1, 10, and 45 days after arrival. The following viruses were isolated and identified: adenovirus SV 20 (18), SV 23 (2), and SV 34 (2); and simian foamy virus (49).

Serum from monkeys of groups 1 and 2 was tested for percipitating (PPT) antibody to SFV by using the microimmunodiffusion tests. Antigens were prepared *in vivo* cell cultures to SFV isolates No. 7-387 (type 2) and 7-411 (type 1). From group 1, 133 of 154 (87%) were positive. The SFV was isolated from 93 of 133 (70%) of the PPT antibody-positive monkeys. From group 2, 21 of 24 (87.5%) monkeys had PPT antibody. In group 2, SFV was only isolated from PPT antibody-positive monkeys. It was isolated from 15 monkeys on day 1, from 19 on day 10, and from 15 on day 45. Accumulatively, SFV was isolated from 20 of 21 PPT antibody-positive monkeys.

Domestic cats. On 1 November 1977, 45 cats (Nos. 76-120) arrived at LAIR, and on 15 November 1977, 7 additional cats arrived (Nos. 121-127). All cats were inoculated with feline panleukopenia and rabies vaccines upon arrival. In addition, cats Nos. 121-127 were inoculated with a combined "modified-live" feline herpesvirus (FHV)-feline calcivirus (FCV) vaccine. Oropharyngeal swabs were collected for viral isolation and serum for serology. From the 52 cats, 237 oropharyngeal swab specimens were tested for virus. FHV was isolated 35 times from 26 cats. FeV as isolated 49 times from 19 cats. Feline cyncytia-forming virus (FeS. , changes were seen in cell cultures inoculated with 95 swab specimens from 34 cats. Based on multiple isolations of FeSFV from each cat and a positive immunodiffusion test, 23 cats are infected with FeSFV. Other cats may also be infected with FeSFV.

It was difficult to make useful conclusions from the virus neutralization titiers. FHV was isolated from several cats without an expected rise in antibody titer and, conversely, FHV was not isolated from other cats that had a rise in titer. However, swabs were not collected between 10 and 29 days in many of these cats. FHV infections could have occurred during this period and not have been detected. The presence of high antibody titers to FCV in the first serum samples did not preclude persistent shedding of FCV in several cats.

CONCLUSIONS

Owl Monkey

1. Owl monkeys are frequently infected with three serotypes of adenovirus and eight serotypes of cytomegalovirus.

2. Viral infections may persist for long periods of time (e.g., 12 months or longer).

3. The serotype of virus isolated was usually dependent on karyotype of the monkey and its country of origin. This may be due all or in part to management practices, isolation procedures, or host susceptibility.

Rhesus Monkey

1. Rhesus monkeys are frequently infected with many different serotypes of adenovirus, *Herpesvirus simiae*, and simian foamy virus.

2. Monkeys frequently have either percipitating antibody titers, neutralizing antibody titers, or both types of antibody to simian foamy virus.

Cats

1. Cats obtained for commerical sources are frequently infected with feline herpesvirus (FHV), feline calicivirus (FCV), and feline syncytial-forming virus (FeSFV).

2. One commercial vaccine was not effective in eliminating FHV or FCV from the small number of cats studied.

3. Some cats shed virus persistently despite high titers of antibody.

RECOMMENDATIONS

Although surveillance should continue, this study is being terminated because of the departure of the principal investigator and reorganization of LAIR.

PUBLICATIONS

1. SHROYER, E.L., and R.M. SHALABY. Isolation of feline synctial-forming virus from oropharyngeal swab samples and buffy cat cells. Am J Vet Res 39: 555-560, 1978

2. SHROYER, E.L., S.T. KELLEY, P.C. TAYLOR, P. VANDERLOO, and T.L. LESTER. Three serologic types of adenovirus infections of owl monkeys. (submitted for publication)

STUDY NO. 5

Physiological and biochemical characteristics of domestic swine used in military research

PROBLEM

Mongrel dogs have served as the predominant large animal species for medical research. Such usage is attributable to tradition and the ready availability of dogs at local pounds and animal shelters. In recent years, however, the use of dogs in medical research has come under increasing criticism from antivivisectionists in the general populace and from the scientific professions. Criticisms by antivivisectionists is mostly based on ethical or emotional grounds: the predominant theme is the unnecessary suffering or mutilation of pets in research projects of dubious merit. Criticism by scientists falls into two categories. One criticism concerns uncertainties about the age, the genetic, nutritional, and environmental background, and the disease characteristics of mongrel animals acquired from dealers; the other criticism concerns the applicability to humans of data acquired in experiments on dogs because, in many instances, canine response characteristics are vastly different from those observed in man.

The domestic pig offers an attractive alternative to the dog as a large animal model for human-oriented research. Its use has not elicited an emotional response from antivivisectionists, probably because swine are commonly slaughtered for meat and are not kept as life-long pets. Domestic pigs are readily available in all parts of the country. They are usually healthy and free of disease. They can be acquired in a variety of ages, sizes, and genetic backgrounds. Cost of acquisition and maintenance is usually far less than that of a dog of comparable size. A more important fact than these considerations exists, i.e., available information shows the pig is superior to the dog in terms of his physiological and biochemical similarities to man. Such information, however, is limited. Thus, if the pig is to become firmly established as a more appropriate large animal model than the dog for gathering experimental data which are applicable to humans, much additional research is needed to establish baseline data. It is to this problem that the present study is directed.

Two types of experiments are being conducted. In one, selected normal physiological or biochemical characteristics will be described. In the other, normal response characteristics of selected physiological or biochemical variables will be delineated.

RESULTS AND DISCUSSION OF RESULTS

Arterial and venous measurements in young swine during controlled acid-base steady state yield the following average values:

Component	F.A.	P.A.	A.V.C.	P.V.C.	I.J.	C.S.
P_{O_2} (Torr)	97	36	39	35	39	30
S_{O_2} (%)	94	51	58	49	52	42
C_{O_2} (ml/100 ml)	15.4	8.5	9.5	8.1	9.3	6.9
pH	7.40	7.34	7.33	7.34	7.33	7.34
P_{CO_2} (Toor)	47	57	58	57	58	57
$[HCO_3^-]$ (meq/l)	27.6	29.6	29.5	29.8	29.6	29.8
B.E. (meq/l)	+2.8	+3.9	+3.6	+4.3	+3.7	+4.7

F.A. = femoral artery; P.A. = pulmonary artery; A.V.C. = anterior vena cava; P.V.C. = posterior vena cava; I.J. = internal jugular; C.S. = coronary sinus.

At equivalent pH_a and Pa_{O_2} levels the foregoing data indicate that young pigs have a lower arterial Sa_{O_2} and Ca_{O_2} than humans. These effects are attributable to the lower oxyhemoglobin affinity and hemoglobin concentration in porcine as compared to human blood. Porcine blood has higher values of $P_{a_{CO_2}}$ and $[HCO_3^-]_a$ than human blood at an equivalent pH_a value. Thus, in acute experimental settings ventilatory regulation to establish the Pa_{CO_2} at 40 toor, the average human value, will lead to alkalosis in the pig.

CONCLUSIONS

The acid base and blood gas characteristics of porcine blood are sufficiently close to those of human blood to serve as a useful model.

RECOMMENDATIONS

Although this study has opened a tremendous area of potentially invaluable research, it is being terminated because of a planned reorganization of LAIR and loss of the principal investigator.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OE 6118	78 10 01	DD DR&E(AR)636
3. DATE PREV SUMM	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADE	8. DISTR INSTR	9. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM & WORK UNIT
77 10 01	H.TERMINATIO	U	U	NA	NA	☒ YES ☐ NO	
10. NO CODES	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3M162772A812		00	003		
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 114 f						

11. TITLE (Previous title and security classification code)

(U) Improvement of Health and Effectiveness of Military Dogs

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology; 012900 Physiology; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	78 09	DA	C. In-House

17. CONTRACT GRANT

a. DATE/EFFECTIVE — b. EXPIRATION

c. NUMBER: Not Applicable

d. TYPE — e. AMOUNT

f. KIND OF AWARD — g. CUM AMT

18. RESOURCES ESTIMATE

	FY	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PREVIOUS FISCAL YEAR	78	1.3	65
CURRENT FISCAL YEAR	79	0.0	00

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
NAME: Marshall, J.D., COL, MC
TELEPHONE: (415) 561-3600

20. GENERAL USE

Foreign Intelligence Not Applicable

19b. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Pathology & Comparative Studies Div
ADDRESS: Department of Comparative Medicine
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Former Name if a Student Investigator)
NAME: Hannon, J.P., DAC
TELEPHONE (415) 561-4004
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Buell, T.F., LTC, VC
NAME

POC:DA

21. KEYWORDS (Provide keywords and security classification code)

(U) Physiology; (U) Nutrition; (U) Bioenergetics; (U) Combat Patrols; (U) Metabolism; (U) Endurance; (U) Biological Detectors Performance

22. TECHNICAL OBJECTIVE 23. APPROACH 24. PROGRESS (Previous and anticipated accomplishments relative to number, project no. of work unit and security classification code)

23. (U) Dogs are used extensively by U.S. Armed Forces in conjunction with combat operations, as sentries for important installations in CONUS and WOUTS, as sensitive detectors of explosives and contraband, and in many other roles which cannot be performed reliably by any other detector system. A need exists for detailed information about how endurance may be improved by training, diet, or other procedures. Laboratory and field tests will be developed to evaluate nutritional and physiologic aspects of performance. Means will be sought to identify and ameliorate or eliminate chronic diseases which impair the effectiveness of trained dogs.

24. (U) Alterations in bioenergetics, metabolism, nutritional status, and cardiopulmonary functions will be studied in military working dogs during exercise and training. Food requirements and body composition changes associated with specific levels of physical activity will be delineated. Factors impairing physical performance, such as high temperature and humidity, will be investigated. Diseases which impair effectiveness will be characterized by advanced laboratory means.

25. (U) 7710-7809 Eight German Shepherd type dogs were physically conditioned to gradually increasing work loads on a motor-driven treadmill over a 4-month period. The four best performers were able to run 34 km (21 mi) daily at a rate of 11.3 km/hr (7 mph) on a 7% grade. Over the course of the training these four dogs had an average body weight loss of 1.5 kg (4.8%) and increased their daily food consumption by 31.5%. Adaptive changes associated with physical conditioning included increased values for plasma, erythrocyte, and total blood volume and decreased values for hematocrit and extracellular fluid volume. This work unit is being terminated.

25. KEYWORDS (Continued) (Provide keywords and security classification code)

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. AR FORM 1498 NOV 68 AND AMC FORM 68 FOR ARMY USE ARE OBSOLETE

ABSTRACT

PROJECT NO. 3M62772A812 Military Animal Resources Development

WORK UNIT NO. 003 Improvement of Health and Effectiveness of Military Dogs

The following investigation has been conducted under this work unit.

STUDY NO. 1 The bioenergetics of exercise and physical training in the German Shepherd

Eight German Shepherd type dogs were physically conditioned over a 4-month period to gradually increasing work loads on a motor-driven treadmill. The four best performers were able to run 34 km (21 mi) daily at a rate of 11.3 km/hr (7 mph) on a 7% grade. Over the course of training these four dogs had an average body weight loss of 1.5 kg (4.8%) and increased their daily food consumption by 31.5%. Adaptive changes associated with physical conditioning included increased values for plasma, erythrocyte, and total blood volume and decreased values for hematocrit and extracellular fluid volume.

This work unit is being terminated.

BODY OF REPORT

WORK UNIT NO.	003	Improvement of Health and Effectiveness of Military dogs
STUDY NO.	1	The bioenergetics of exercise and physical training in the German Shepherd

PROBLEM

The German Shepherd dog, because of his size, intelligence, and adaptability to physical and obedience training, has been extensively used for military patrol and sentry duties. Field reports, however, indicate that substantial numbers of military working dogs did not perform at anticipated levels during the Vietnam conflict. The factors responsible for such performance decrements are unknown, but speculative causes include an inadequate caloric intake and poor tolerance to hot humid environments. The objective of this study is to delineate the physiological, biochemical, and nutritional factors which limit physical performance of the German Shepherd dog.

RESULTS AND DISCUSSION OF RESULTS

Over a 2-month period eight German Shepherd type dogs were preconditioned to a 12-hour day-night lighting schedule, ad libitum feeding and exercise familarization (5 minutes daily) on a motor-driven treadmill. During the last week of the preconditioning period, daily food (Gaines Meal) consumption, body weight, hematocrit, plasma (Evans Blue) volume, and extracellular (sucrose space) volume were measured. Circulating erythrocyte and total blood volume were calculated.

Subsequent to the preconditioning period, the dogs were subjected at approximately 2-week intervals to progressively increasing levels of physical work. This was achieved on a 5-day per week schedule over a 4-month period by gradually increasing the grade and/or velocity of the treadmill. The dogs were allowed 15-minute rest periods after each 30-minute bout of exercise. During the course of this physical conditioning period one dog was withdrawn because of hip dysplasia, while desired levels of performance in three others were not achieved because of foot pad soreness or impaired physical work capacity of undefined nature. The four best dogs ultimately achieved a capacity for running 34 km (21 miles) per day at 11.3 km (7 miles) per hour on a 7% grade over a 3-hour period. During the final week of the physicial conditioning period all of the above indicated measurements were repeated. In the four best dogs the following data were obtained:

Dog	Body Wt.	Food Intake	Hct.	P.V.	E.V.	B.V.	E.C.V.
			Before Physical Conditioning				
01	29.7	92.5	46	42	36	78	255
02	26.0	99.4	49	44	42	86	288
97	35.6	70.9	50	34	34	68	235
98	36.8	59.9	49	38	37	75	243
Mean	32.0	80.67	48.5	38.5	37.2	76.7	255.2
			After Physical Conditioning				
01	28.0	113.8	42	48	43	91	209
02	25.8	143.3	46	54	46	100	238
97	33.1	95.0	45	52	42	94	261
98	[illegible].0	72.[illegible]	46	50	43	93	193
Mean	30.47	106.10	44.7	51.0	43.5	94.5	225.2

Body wt = kg; Food intake = kcal/kg/day; Hct = hematocrit, %; P.V. = plasma volume, ml/kg; E.V. = erythrocyte volume, ml/kg; B.V. = blood volume, ml/kg; E.C.V. = extracellular volume, ml/kg

These data show that the average energy cost associated with daily running at 11.3 km/hr on a 7% grade for 34 km is 25.4 kcal/kg body weight, or 31.5% over the sedentary value. On the basis of vertical distance achieved under such conditions, the apparent efficiency of food utilization for physical work was 21.1%. Adaptive changes associated with canine physical conditioning include increased values for plasma, erythrocyte and blood volume, and reduced values for body weight, hematocrit, and probably extracellular volume.

CONCLUSIONS

Heavy physical work by German Shepherd dogs would not appear to be limited by the capacity to consume adequate food energy to compensate for increased energy expenditure, thus, the food energy increment observed here, 31.5%, is less than one-half of that reported for sedentary dogs exposed out of doors to the Arctic winter. Hyperthemia would seem to be a more likely cause of the impaired canine work performance observed in hot humid environments. In this regard, limited measurements in this study showed that heavy work in the German Shepherd, even at a room temperature of approximately 21°C (70°F), regularly led to rectal temperatures in excess of 41°C (106°F) and frequently as high as 42.8°C (109°F).

BODY OF REPORT

WORK UNIT NO.	003	Improvement of Health and Effectiveness of Military dogs
STUDY NO.	1	The bioenergetics of exercise and physical training in the German Shepherd

PROBLEM

The German Shepherd dog, because of his size, intelligence, and adaptability to physical and obedience training, has been extensively used for military patrol and sentry duties. Field reports, however, indicate that substantial numbers of military working dogs did not perform at anticipated levels during the Vietnam conflict. The factors responsible for such performance decrements are unknown, but speculative causes include an inadequate caloric intake and poor tolerance to hot humid environments. The objective of this study is to delineate the physiological, biochemical, and nutritional factors which limit physical performance of the German Shepherd dog.

RESULTS AND DISCUSSION OF RESULTS

Over a 2-month period eight German Shepherd type dogs were preconditioned to a 12-hour day-night lighting schedule, ad libitum feeding and exercise familarization (5 minutes daily) on a motor-driven treadmill. During the last week of the preconditioning period, daily food (Gaines Meal) consumption, body weight, hematocrit, plasma (Evans Blue) volume, and extracellular (sucrose space) volume were measured. Circulating erythrocyte and total blood volume were calculated.

Subsequent to the preconditioning period, the dogs were subjected at approximately 2-week intervals to progressively increasing levels of physical work. This was achieved on a 5-day per week schedule over a 4-month period by gradually increasing the grade and/or velocity of the treadmill. The dogs were allowed 15-minute rest periods after each 30-minute bout of exercise. During the course of this physical conditioning period one dog was withdrawn because of hip dysplasia, while desired levels of performance in three others were not achieved because of foot pad soreness or impaired physical work capacity of undefined nature. The four best dogs ultimately achieved a capacity for running 34 km (21 miles) per day at 11.3 km (7 miles) per hour on a 7% grade over a 3-hour period. During the final week of the physicial conditioning period all of the above indicated measurements were repeated. In the four best dogs the following data were obtained:

RECOMMENDATIONS

The interrelationships of temperature and humidity on canine work capacity should be systematically investigated so that tolerance characteristics and animal handling procedures can be accurately delineated. This work unit, however, will be terminated because of the reorganization of LAIR and consequent loss of the principal investigator.

PUBLICATIONS

1. RODKEY, W.G., J.P. HANNON, J. DRAMISE, R.D. WHITE, D.C. WELSH, and B.N. PERSKY. Arterial capillary blood used to determine the acid base status of dogs. Am J Vet Res, 39:459-464, 1978

2. TURNIER, J.C., and S. SILVERMAN. Panosteitis: Comparison of radiographic and radioisotopic studies. J Am Vet Med Ass (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | DA OE 6110 | 78 10 01

1. DATE PREV SUMMARY	2. KIND OF SUMMARY	3. SUMMARY SECY	4. WORK SECURITY	5. AGGREGATE	6a. GROUP INDEX	6b. SPECIFIC DATA: CONTRACTOR ACCESS	7. LEVEL OF SUM
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. WORK CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3M162772A812	00	024
b. [redacted]				
c. [redacted]	CARDS 1147			

11. TITLE: (U) Neurobehavioral Investigations of Military Trauma

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 008800 Life Support; 012900 Physiology; 013400 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMING ORGANIZATION METHOD
76 10 01	Cont	DA	C. In-house

17. CONTRACT/GRANT
a. DATE/EFFECTIVE: — EXPIRATION:
b. NUMBER: —
c. TYPE: Not Applicable — d. AMOUNT:
e. END OF AGREED — f. CUM. AMT.

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
78	1.5	92
79	1.5	61

19a. RESPONSIBLE DOD ORGANIZATION
NAME: Letterman Army Institute of Research
ADDRESS: Presidio of San Francisco, CA 94129
RESPONSIBLE INDIVIDUAL
NAME: Marshall, J. D., COL, MS
TELEPHONE: (415) 561-3600

19b. PERFORMING ORGANIZATION
NAME: Letterman Army Institute of Research
Division of Biorheology
ADDRESS: Presidio of San Francisco, CA 94129
PRINCIPAL INVESTIGATOR
NAME: O'Mara, P. A., MAJ, MS
TELEPHONE: (415) 561-2905
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Pribyl, V. J., DAC
NAME:

20. GENERAL USE: Foreign Intelligence Not Applicable

21. KEYWORDS: (U) Neurophysiology; (U) Resuscitation; (U) Psychopharmacology

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

23. (U) Resuscitation from trauma in combat operations imposes problems not typically encountered in civilian medical operations. Among these are the occurrences of mass casualties, and physical limitations of field medical facilities. Short, violent conflicts will further limit field medical support of combat operations and medical casualties may be required to continue limited support of combat operations. There will be greater emphasis on methods of treating combat injuries which permit rapid return to duty. Therobjectives of this work unit are to assess the effects of military trauma on combat effectiveness and to evaluate functional recovery following the use of different resuscitating procedures.

24. (U) The methods used emphasize the detection and quantification of those functional changes which could limit adaptation to the environment following trauma. Both behavioral and neurophysiological techniques are employed in order to increase the changes of detecting effects which are of functional significance to the organism. Behavioral testing is used to evaluate basic sensory and motor processes as well as more complex cognitive processes. The additional data obtained through appropriate analysis of spontaneous and evoked electroencephalographic activity provides further evidence of possible changes in central nervous system functioning.

25. (U) (7710-7809). Behavioral recovery following massive transfusion with a plasma protein solution was found to be dose-dependent and also a function of the behavioral test employed. Data are being analyzed following an extensive neurophysiological study of the central nervous system effects of massive transfusion. A method has been developed for automatically regulating delivery of anesthetics according to computer monitored changes in physiological processes.

* Available to contractors upon sponsor's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND USED UNTIL EXHAUSTED FOR ARMY USE. ARE OBSOLETE

ABSTRACT

PROJECT NO. 3E762772A812 Military Research Animal Resources

WORK UNIT NO. 024 Neurobehavioral Investigations of Military Trauma

The following investigations have been conducted under this work unit:

STUDY NO. 1 Behavioral effects of massive transfusion

STUDY NO. 2 Neurophysiological effects of massive transfusion

STUDY NO. 3 Servoanesthesia

STUDY No. 1. The behavior of rats in operant conditioning modular test chambers in the open-field test was examined following exchange transfusion with a plasma protein solution. Subjects were divided into three groups: control, 50% replacement, and 70% replacement - the group denoting the amount of blood replaced. A significant decrement in operant performance was recorded on the first three days following transfusion. Behavioral recovery appeared to be nearly complete by the fourth post-transfusion day. There was no significant correlation between high performance levels in the operant test and activity in the open-field. Future research should include a manipulation of several transfusion doses to be correlated with various response levels of the subjects.

STUDY NO. 2. Physiological data were collected during several daily test sessions before and after massive transfusion with a cell-free plasma protein solution. The electrocardiogram, respiratory rate and rectal temperature were monitored by using detachable electrodes. Spontaneous and stimulus-evoked brain electrical activity was recorded from chronically implanted epidural electrodes. Baseline and post-transfusion data have been collected from several animals. Both time-domain and frequency analyses are being used to process these data. Further statistical analyses will be required to assess the significance of changes in brain electrical activity observed during this study.

STUDY NO. 3. Spontaneous and stimulus-evoked brain electrical activity was recorded from animals anesthetized with pentobarbital. Combinations of electroencephalographic and physiological indices were evaluated as indicators of the anesthetic level of the animal. These indices were derived through a principle components analyses of the physiological data. The effectiveness of these indicators in feedback regulating anesthesia will be examined.

BODY OF REPORT

WORK UNIT NO. 024 — Neurobehavioral Investigations of Military Trauma

STUDY NO. 1 — Behavioral effects of massive transfusion

PROBLEM

Relatively few clinical studies have addressed the physiological effects of transfusion on behavior and performance. Clearly, the maintenance of life of the exsanguinating patient, such as the massively injured combat soldier, depends upon transfusion. Human albumin is presently used in clinical situations, however, the behavioral effects of its use during the recovery of the patient have not been evaluated. The present study was undertaken to determine the behavioral effects upon exsanguinated rats transfused with an albumin concentrate.

RESULTS AND DISCUSSION OF THE RESULTS

Two test situations were used to measure behavior: 1) operant conditioning, and 2) the open-field test. A fixed ratio (FR) schedule provided reinforcement in direct proportion (1:20) to the number of barpress responses emitted by the animal. The FR performance is characterized by relatively high and stable response rates. The open-field test was carried out in a 3 X 3 ft field with a grid floor ruled into 16 nine-inch squares. All subjects were placed for 3 min in the open-field, during which time their scores on ambulation, rearing, grooming, freezing, defecation, and urination were recorded. Ambulation scores were determined by the number of grid lines each rat crossed during the 3-min interval.

Each rat was assigned at random to one of the following groups: control, 50% replacement, and 70% replacement - the group denoting the amount of blood replaced. Blood replacement was a 5% solution of human plasma protein fraction (Plasmanate®). Results from the analysis of covariance showed that effects of treatment produced the most significant effects on the subject's performance scores on the first three days following transfusion. From the fourth to the tenth days, the response differences among groups decreased and groups began to reassume their normal baseline levels. There was no significant correlation between high response rates on the FR task and a high level of activity in the open-field either before or after treatment.

CONCLUSIONS

The effect of the experimental treatment upon the subjects for all groups was dependent upon their level of transfusion. Data from the analysis of covariance indicate that the subjects were nearly recovered

after the third post-treatment day. Contrary to expectations, the open-field test did not appear to be an effective means of measuring the effects of transfusion upon the subjects. Data from the correlation analysis on open-field activity and FR performance have important theoretical implications regarding the nature of exploration and food acquisition. It may be concluded from these data that the level of exploratory drive in the rat is not related to its drive in the acquisition of food.

RECOMMENDATIONS

It would be advisable to determine if a manipulation of several transfusion doses might be correlated with various response levels of the subjects. A further consideration would be to study the effects of transfusion upon subjects transfused with various other materials.

Studies in which banked blood is used as the transfusion medium would be useful in obtaining baseline data from a standard transfusion medium. The comparison of these data with data obtained from studies in which other transfusion materials are used would be useful in the development and refinement of transfusion solutions.

Pathological studies may be performed on each experimental subject. The tissue samples would be used to corroborate with any data of physiological and cerebral damage.

PUBLICATIONS

1. ESCANDARIAN, G. E., J. S. SURINCHAK, P. A. O'MARA. Behavioral effects in rats following massive transfusion with plasma protein solution. (Submitted for publication)

STUDY NO. 2 — Neurophysiological effects of massive transfusion

PROBLEM

Examination of stimulus-evoked peripheral and central nervous system electrophysiological events can provide valuable information concerning the ability of the nervous system to process and respond to external events. Changes in the spontaneously occurring electrical activity of the brain may provide additional information concerning the general state of the organism. The objectives of this study are (1) to use electrophysiological data in assessing the effects of various resuscitating solutions and procedures on brain functioning and behavior and (2) to relate electrophysiological data obtained during resuscitation to the subsequent functional recovery of the subject. Suspected post-traumatic functional changes in the subjects will also be confirmed with behavioral measurements.

The identification of physiological variables which reliably predict overall functional recovery could lead to the development of diagnostic procedures for use in rapid screening of brain dysfunction in combat casualties where significant blood loss and replacement have occurred.

RESULTS AND DISCUSSION OF RESULTS

Baseline and post-transfusion visual, auditory, and somatosensory evoked potential data have been recorded from rats with the use of chronically implanted electrodes. Epochs of spontaneous electroencephalographic (EEG) activity were also recorded from these electrodes. The electrocardiogram, respiratory rate, and rectal temperature were also monitored during each data collection session. Data were obtained over a period of several days before and after transfusion with a cell-free albumin solution.

Data analyses have not been completed. Preliminary analyses suggested that changes in evoked potential waveforms and EEG and evoked potential power spectra may occur following transfusion. Multivariate statistical analyses will be used to assess the significance of the observed changes in the electrophysiological signals.

CONCLUSIONS

None

RECOMMENDATIONS

The studies which are in progress must be expanded to include studies of standard crystalloid and colloidal asanguinous resuscitating solutions. Whole blood transfusions may prove useful as a standard control condition. The functional significance of changes in brain electrophysiology which might occur following transfusion remains to be investigated.

PUBLICATIONS

None

STUDY NO. 3 Servoanesthesia

PROBLEM

Several anesthetics produce characteristic changes in the spontaneous electrical activity of the brain, and the changes can be correlated with the depth of anesthesia. It would appear feasible, therefore, to use selected features of the electroencephalogram to control automatically the delivery rate of anesthetic agents and consequently maintain a given depth of anesthesia. Historically, attempts to

implement servoanesthesia systems have met with limited success due to the complexities of biological signal processing and the construction of the control systems for anesthesia delivery. There were also unresolved questions concerning the selection of appropriate features of the biological signals for use in feedback regulation. Recent developments in digital signal processing offer practical solutions to these difficulties. This study was undertaken in order to re-examine the servoanesthesia problem by using the more advanced computer-assisted methods which are currently available. Servoanesthesia would be particularly valuable in forward medical support of combat operations.

RESULTS AND DISCUSSION OF RESULTS

Spontaneous and stimulus-evoked electroencephalographic activity were recorded from animals anesthetized with pentobarbital. Visual, auditory and somatosensory stimuli were used. Repeated baseline measures using chronic implants were conducted to examine reliability of the measures. Electroencephalographic data were combined with physiological measures of heart rate, temperature, and respiration rate. A principle components analysis was performed on these combined data. Several components which recover with changes in anesthetic level of the animal were identified.

CONCLUSIONS

Combinations of electroencephalographic and physiological data have been identified which, for a given subject, are related to anesthesia level.

RECOMMENDATIONS

The derived indicators should be evaluated for effectiveness in automatic feedback regulation of anesthesia levels. The reliability of these indicators across subjects must be evaluated. These methods should be expanded to include faster-acting intravenous anesthetic agents such as thiopental and inhalation anesthetics such as halothane.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY | DA OE 6079 | 78 10 01 | DD-DR&E(AR)636

1. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. SPAR SECURITY	7. PROGRAM	8a. GRADE/MOD	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. MAJ/CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS01	00	202
b. [redacted]	62772A	3E162772A813	00	021
c. [redacted]	CARDS 114f			

11. TITLE (Procedure and Technique Code)

(U) Determination of Threshold Data from Coherent and Incoherent Radiation Sources

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

09600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
7412	Cont	DA	C. In-House

17. CONTRACT GRANT

a. DATE/EFFECTIVE: — b. NUMBER: Not Applicable — c. TYPE: — d. KIND OF AWARD: — e. AMOUNT: — f. CUM. AMT.:

18. RESOURCES ESTIMATE

	FY	a. PROFESSIONAL MAN YRS	b. FUNDS ($ thousands)
FISCAL YEAR	PREVIOUS 78	2.5	111
	CURRENT 79	3.0	80

19a. RESPONSIBLE DOD ORGANIZATION

Name: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129
Address:

Responsible Individual
Name: Marshall, J. D., COL, MS
Telephone: (415) 561-3600

19b. PERFORMING ORGANIZATION

Name: Letterman Army Institute of Research
Division of Biorheology
Address: Presidio of San Francisco

Principal Investigator (Name, title, address, phone)
Name: Beatrice, E. S., COL, MC
Telephone: (415) 561-2732
Social Security Account Number

Associate Investigators
Name: Stuck, B. E., DAC
Name: POC:DA

20. GENERAL USE

Foreign Intelligence Not Applicable

21. KEYWORDS (Procedure and Technique Code): (U) Eye Protection; (U) Infrared Lasers; (U) Systems Safety; (U) Laser Hazard; (U) Eye Damage; (U) Skin Damage

22. TECHNICAL OBJECTIVE, APPROACH, PROGRESS (Previous work and anticipated progress must be numbered. Progress must be described chronologically.)

23. (U) The objectives are to experimentally determine dose response relationships for exposure to infrared laser radiation for exposure conditions relevant to DARCOM laser systems operation, for systems used for ECOM designation and guidance tasks and to recommend to the Army user community permissible exposure limits and field safety procedures.

24. (U) The ED_{50} (effective dose required to produce a change 50% of the time) for various exposure conditions and response criteria were determined. The cornea exposures are evaluated at various time intervals, by direct observation, histological techniques, and specular microscopy. Available dose-response relationship were evaluated with respect to permissible exposure limits.

25 (U) 7710-7809) After a review of available repetitive pulse exposure data for a wide range of exposure conditions, two methods were derived to calculate permissible exposures that provide "margins of safety" that are comparable to and consistent with those provided for continuous wave conditions. These methods were based on the observation that the ED_{50}s for repetitive pulse conditions expressed in terms of the total intraocular energy exhibit a dependence on the train duration of T.3/4 This analysis also indicated that the average pulse repetition frequency can be used for pulse coded trains in the computation of permissible exposures. Rhesus monkey endothelial cell size and appearance were evaluated in vivo and in vitro before and after single exposures to CO_2 laser radiation (10.6 microns) from the permissible exposure to doses 1.5 times those required to produce a corneal opacity. Exposed cornea were evaluated up to 6 months post exposure and no significant change in endothelial cell size was observed for doses that produced immediate epithelial opacities.

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ABSTRACT

PROJECT NO. 3E162772A813 — Health Effects of Military Lasers

WORK UNIT NO. 021 — Determination of Threshold Data from Coherent and Incoherent Radiation Sources

The following investigation has been conducted under this work unit:

STUDY NO. 1 Ocular and skin effects of infrared laser radiation

Rhesus monkey endothelial cell size and appearance were evaluated by corneal specular microscopy before and after single exposures to CO_2 laser radiation (10.6 microns) from the permissible exposures to CO_2 doses 1.5 times those required to produce corneal opacities. The cornea were evaluated at various intervals up to 6 months after exposure and no significant changes in endothelial cell size were observed for these exposure conditions.

After a review of available repetitive pulse exposure data for a wide range of exposure conditions, two methods were derived to calculate permissible exposures that provide "margins of safety" that are comparable to and consistent with those provided for continuous wave conditions. These methods were based on the observation that the ED_{50}s for repetitive pulse conditions expressed in terms of the total intraocular energy exhibit a dependence on the train duration of $T^{3/4}$. This analysis also indicates that the average pulse repetition frequency can be used for pulse coded trains in the computation of permissible exposures.

BODY OF REPORT

WORK UNIT NO. 021 — Health Effects of Military Lasers

STUDY NO. 1 — Ocular and skin effects of infrared laser radiation

PROBLEM

Many currently used and proposed military laser systems operate in the infrared region of spectrum beyond 1.4 microns. This region of the electromagnetic spectrum is commonly referred to as the "eye safe" region because the radiant exposure required to produce an observable change is 10 to 10000 times higher than that required for visible and near infrared wavelengths. For infrared wavelengths, the cornea and outer ocular structures are the primary absorption site and consequently the site of injury, whereas the retina is affected by the visible and near infrared wavelengths. Although permissible exposure limits have been defined in TB MED 279, there are exposure conditions relevant to military applications where no bioeffects data are available. Permissible exposure limits have been established by extrapolation for these conditions.

Most of the corneal dose-response experiments have evaluated the acute response by using the production of an epithelial opacity as the response criteria. Endothelial effects which may implicate chronic changes in corneal clarity have not been studied.

RESULTS AND DISCUSSION OF RESULTS

The corneal endothelium of rhesus monkeys was evaluated by using in vivo specular microscopy before and after exposure to CO_2 laser radiation at 10.6 microns. Since limited experimental data are available describing normal endothelial cell size, the following set of experiments were performed. A technique was developed for the measurement of endothelial cell size (i.e., the number of endothelial cells per unit area) from the photographs taken with the corneal specular microscope. The central endothelium of 17 rhesus monkeys was photographed and endothelial cell sizes were determined. The mean number of endothelial cells per mm^2 ranged from 3000 to 4200 cells/mm^2. The standard deviation of these measurements ranged from 160 to 230 cells/mm^2 for measurements on a given eye. The endothelial cell mosaic was uniform with little pleomorphism evident within a given photographed area (100 by 400 microns). Endothelial cell size determinations were made at intervals of several weeks over a 5 mo period. The effect of the dilating drops (2 drops phenylephrine hydrochloride and 2 drops of cyclopentolate hydrochloride) was evaluated by photographing the endothelium before and after ocular dilation. No effect on the endothelial cell size or appearance could be attributed to the dilating drops.

The eyes of 5 rhesus monkeys were then exposed to single 100 msec doses of CO_2 laser radiation. The peak irradiance was scaled from 3.0 W/cm^2 (permissible exposure for these conditions) to 33 W/cm^2. The intensity distribution was Gaussian with a 1/e diameter of 7.4 mm (full corneal exposure). The ED_{50} for the production of an epithelial opacity was previously determined to be 18.3 W/cm^2 for these conditions. These exposed animals were followed for a period of 5 mo after exposure. No significant changes in central endothelial cell size were observed for this range of exposures. Trypan blue staining of the endothelium has previously been observed beneath the lesion site and to 24 h after exposure to the higher doses used in this experiment. Although the epithelial opacity produced by the 26-33 W/cm^2 doses prevented corneal specular microscopy between one and 48 h after the exposure and resulted in a diffuse stromal scar at the nearest zone, no change in endothelial cell size up to 5 mo after the exposure was observed. At the time of enucleation, the cornea were excised and stained with trypan blue and alizarin red. No staining of the endothelium with trypan blue was observed. Photographs of the endothelial cells in vitro were taken and endothelial cell size measurements were made and compared with the in vivo measurements. The cell size measurements in vivo resulted in consistently larger cell size. An example of the measurements from two different eyes and the cell size variation with area is given in Table 1.

Table 1

Endothelial Cell Size

In Vitro: Cells/mm^2 ± SD

Location	M-3, OD	M-6, OS
Superior	3919 ± 150	4171 ± 106
Inferior	3950 ± 169	4315 ± 116
Temporal	3532 ± 63	4335 ± 40
Nasal	3791 ± 219	4300 ± 218
Central	3849 ± 87	4162 ± 132
In Vivo		
Central	3541 ± 185	3752 ± 160

Loss of endothelial cells as a result of trauma is thought to result in the enlargement of neighboring cells which eventually results in their inability to continue the maintenance of the delicate corneal-aqueous interface. Large endothelial cells were observed in the cornea of a rhesus monkey 2 years after the lens was removed and a 180 degree iridectomy was performed. The central endothelial cells were somewhat

irregular in shape but smaller and more regular in size than those near the location of the surgical incision in the cornea. If endothelial cells were lost because of the laser radiation insult, enlarged cells should have been observed; however, for the conditions evaluated, endothelial cell size several months after exposure was comparable to the pre-exposure measurement.

Two methods were derived for the calculation of permissible ocular exposure to repetitive pulse laser radiation. The rationale for these methods (the correction factor method and the alternate method) was based upon available experimental data from several laboratories. The ED_{50}s (effective dose for 0.50 probability of an ophthalmoscopically visible retinal lesion) for repetitive pulse exposures expressed in terms of the total intraocular energy are proportional to the pulse train duration to the 3/4 power. The continuous wave ED_{50}s exhibit the same dependence on the exposure duration; however, the repetitive pulse ED_{50}s were always some factor below the continuous wave ED_{50}s. This correction factor is dependent on the pulse repetition frequency and the duration of each individual pulse in the train. It was applied as a multiplicative correction to the continuous wave maximum permissible exposure to obtain the permissible exposure for repetitive pulse conditions.

A "margin of safety" comparable to those imposed for continuous wave conditions was obtained. The alternate method applied a multiplicative correction to a single pulse in the train. This correction was dependent only on the number of pulses in the train. Because of the limitations of the present standard, this method was not as consistent in providing an adequate "margin of safety" as the correction factor method; however, the alternate method does suggest that an average pulse repetition frequency can be used for pulse coded trains in the computation of permissible exposures.

A 360 degree scanned visual display system was evaluated with respect to permissible exposure limits and recent research data. Laser scanned visual display systems are currently being developed by the Project Manager's Office for Training Devices (PM Trade) at the Naval Training Equipment Center with Army and Navy funding support to enhance the training of military pilots. These systems require aviators to view and respond continuously to a scene generated by multiple scanned visible laser beams.

The wide angle laser scanned display was evaluated in three ways. (1) Instantaneously, the illuminated area can be considered as a finite point which, if continuously observed, will appear to pulsate at the frame rate. (2) The display can be considered as an extended source which is pulsating asynchronously at the framing rate. (3) The display can be considered as a continuously illuminated extended source with an average irradiance. Repetitive pulse exposure conditions are implied by the first two considerations, while the third implies a

continuous exposure condition. The most stringent restriction on the illuminance of the display screen results when the display is considered as a continuously illuminated extended source. The permissible irradiance on the screen for a 2-h exposure is 9.2 mW/cm^2. The average screen irradiance (26 $\mu W/cm^2$) of proposed laser scanned displays is well within acceptable limits based upon current permissible exposures. The work of Zwick et al (Work Unit 025 from this laboratory) suggests changes in visual function of primate subjects who were repeatedly exposed to a diffuse screen irradiated at the approximate levels proposed for use in laser scanned displays. Currently, the speckle pattern is being evaluated to determine the possible contribution to the observed effects.

CONCLUSIONS

No effects on endothelial rhesus monkey cell size or shape were observed for single 100 msec exposures to carbon dioxide laser radiation as evidenced by in vivo specular microscopy and in vitro staining techniques. The doses ranged from the permissible exposure to 1.5 times the ED_{50} for the production of an epithelial opacity. Corneas were evaluated for 6 mo after the exposures.

Methods derived to calculate permissible exposures for repetitive pulse more accurately reflect the trends exhibited by available experimental data than do current procedures.

RECOMMENDATIONS

It was recommended that the correction factor method be incorporated into the current Army laser protection standards. It was further recommended that the average pulse repetition frequency be used in the calculation of the permissible exposure for repetitive pulse conditions with a variable interpulse spacing.

Further research is required to determine the dose response relationships for wavelengths from 1.4 to 4.0 microns. The role of the endothelium in corneal injury and repair must be evaluated for different infrared wavelengths and exposure conditions. The effects of repetitive pulses and repeated exposure for infrared wavelengths need to be investigated. The permissible exposure limits must be evaluated with respect to current bioeffects research.

Laser scanned visual displays currently being developed to enhance pilot training were reviewed with respect to current permissible exposures and ocular effects research. Although the projected screen irradiances are well below permissible exposure limits, recent animal research indicates ocular effects at these doses. These effects may, in part, be attributed to the speckle pattern produced by the laser source.

PUBLICATIONS

1. STUCK, B. E., D. M. TALSMA, and F. S. BEATRICE. Vitreal syneresis in rhesus monkeys. Invest Ophthalmol Vis Sci 16: 1068-1070, 1977

2. STUCK, B. E. and D. J. LUND. Extrapolation of pulsed light data in scanned displays. In: Proceedings of the Society of Photo-Optical Instrumentation Engineers 162: 107-111, 1978

3. STUCK, B. E., D. J. LUND and E. S. BEATRICE. Repetitive Pulse Laser Data and Permissible Exposure Limits. Report No. 58, San Francisco, California: Letterman Army Institute of Research, April 1978

PRESENTATIONS

1. STUCK, B. E., D. J. LUND, and E. S. BEATRICE. Repetitive pulse laser data and permissible exposure limits. Presented to the American National Standards Institute Z136.1 Subcommittee on the Biological Effects of Lasers on the Eye, Dr. Myron Wolbarsht, Chairman (Meridan, New Hampshire, June 27, 1978)

2. STUCK, B. E. and D. J. LUND. Extrapolation of pulsed light data in scanned displays. Presented to the Society of Photo Optical Instrumentation Engineers. (San Diego, California, August 23, 1978)

3. SLINEY, D. H. Regulatory requirements of Manufacturers Associated with the ANZI Z136 Standard and International Regulations. Presented by B. E. Stuck for Mr. D. H. Sliney at the Society of Photo Optical Instrumentation Engineers (San Diego, California, August 24, 1978)

4. SLINEY, D. H. and L. LYON. The Defense Department exemption. Presented by B. E. Stuck for the authors at the Society of Photo Optical Instrumentation Engineers (San Diego, California, August 24, 1978)

5. STUCK, B. E. Acute ocular and skin effects of laser radiation. Presented to the National Aeronautics and Space Administration/ Ames laser user personnel as part of their laser safety instruction (Moffett Field, California, May 26, 1978)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

DA OE 6308 | 78 10 01

77 10 01	D. Change	U	U	NA	NL	YES	

	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS01	00	205
b. [illegible]	62772A	3E162772A813	00	022
c. [illegible]	CARDS 1141			

(U) System Developer Assistance Studies in Laser Bioeffects

009600 Masers and Lasers; 012900 Physiology

77 07	Cont	DA	C. In-House

	PROFESSIONAL MAN YRS	MANYEARS
FISCAL YEAR 78	1.3	95
FISCAL YEAR 79	2.3	80

Not Applicable

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POC:DA

(U) Erbium; (U) Repetitive pulse; (U) Ocular hazard; (U) Damage threshold (U) Laser Safety; (U) GaAs; (U) Neodymium;

23. (U)Objective: To provide bioeffect data base for safety documentation of gallium arsenide (GaAs) laser training devices. To study the ocular effects of frequency double Neodymium (Nd) laser irradiation.
24. (U) Approach: Determine the ocular damage threshold in rhesus monkeys for lasers which simulate GaAs training devices in pulse repetition frequency and/or wavelength. Expose ocular tissue to the emission of training device transmitters to determine the effect. Determine the ocular effect of Nd and frequency doubled Nd irradiation for ophthalmoscopic and functional criteria.
25. (U) (7710-7809) Ocular tissue was exposed to irradiation from MAGLAD, SIMFIRE, and MILES GaAs training devices. Subtle retinal changes were noted as a result of exposure to SIMFIRE and MILES. No effect was noted after exposure to MAGLAD. The nature of this retinal effect is not understood, but is under study via photographic and histopathologic techniques. The retinal doses obtained were less than the current maximum permissible exposure. Ocular tissue was exposed to irradiation with frequencydcoubled Nd(532nm) and combination Nd and frequency doubled Nd(1064nm and 532nm). The ED_{50} for 532 nm irradiation is 2.85uJ. Simultaneous exposure to 60uJ at 1064 nm reduces the 532nm ED_{50} to 2.23uJ.

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ABSTRACT

PROJECT NO. 3E162772A813 — Health Effects of Military Lasers

WORK UNIT NO. 022 — System Developer Assistance Studies in Laser Bioeffects

The following investigations have been conducted under this work unit:

STUDY NO. 1 Project MILES

STUDY NO. 3 Project A813

STUDY NO. 4 Electrophysiological evaluation of low level laser radiation

STUDY NO. 5 Electrophysiological evaluation of frequency doubled neodymium

STUDY NOs. 1, 3, 4, 5. The ocular hazard of a continuous wave GaAlAs semiconductor laser was determined in rhesus monkey eyes. The wavelength was 880 nm. The ED_{50} for 30-sec exposures was 230 millijoules total intraocular energy (TIE).

Three direct fire simulator training device transmitters were evaluated against ocular tissue. The three devices, MAGLAD, SIMFIRE, and MILES, all utilized GaAs lasers. MAGLAD and SIMFIRE produced no visible alterations following a single 30-sec exposure. The MILES M-16 ED transmitter produced subtle visible retinal alterations following exposures of 15 and 30 sec.

Rhesus monkey eyes were irradiated separately with 1064 nm laser irradiation and 532 nm irradiation from a neodymium laser and simultaneously with both wavelengths. The pulse durations were 180 nsec at 1064 nm and 140 nsec at 532 nm. The ED_{50} at 1064 nm was 128 μJ TIE. The ED_{50} at 532 nm was 2.85 μJ. When 60 μJ of 1064 energy was included in each exposure, the ED_{50} for 532 nm was reduced to 2.23 μJ.

Several experiments were performed to determine (a) whether or not charges previously reported in the evoked occipital potentials (EOP) following gallium arsenide radiation could be reproduced, (b) the cumulative effect of gallium arsenide radiation upon the EOP, and (c) the effects of frequency doubled neodymium upon the EOP. In addition, a technique is being developed to obtain a localized, small spot argon laser stimulated EOP and established a series of normal evoked potentials as a function of the retinal site stimulated. Data collected thus far have verified the earlier observation of changes occurring in the late stages of the EOP. A series of GaAs exposures in the same area in one eye of a rhesus monkey failed to show cumulative effects, however, late changes were observed in the evoked

potential. A single frequency doubled neodymium exposure of approximately 1×10^{-6} joules for 140 nsec was observed. Changes in the EOP were seen up to approximately 32 sec post exposure. A technique is being developed to obtain focal EOPs from various areas of the retina while ipsilateral and contralateral potentials are collected.

BODY OF REPORT

WORK UNIT NO. 022 System Developer Assistance

STUDY NO. 1 Project MILES

PROBLEM

Widespread deployment of "eye-safe" laser training devices has been in progress for the past two years. The laser of choice for this application has been the gallium arsenide (GaAs) diode laser. The proposed laser training device MILES (Multiple Integrated Laser Engagement Simulator) incorporates a GaAs laser operating in a variety of coded pulse repetition rates. The intended field use of the MILES system requires the direct intrabeam irradiation of personnel. TRADOC purposes to field 80,000 of these devices for training of friendly troops within one-and-a-half years.

The prototype MILES laser was reviewed by the US Army Environmental Hygiene Agency in January 1976. The output exceeded a safe level to the eye of 4.8×10^{-8} J per pulse obtained by multiplying the single pulse safe level by the pulse repetition frequency (PRF) correction factor listed in TB MED 279 for 100 Hz. The PRF correction for 100 Hz was somewhat arbitrarily chosen because data do not exist for non-uniform interpulse spacing. The device could not be classified as safe for intrabeam viewing. The dilemma presented by the USAEHA report was that while safety standards indicated that MILES laser emission levels were hazardous, available research results did not support this finding. Further research was required to clarify the effect of PRF on the ocular damage threshold and to determine the ocular hazard of the GaAs laser wavelength (850 nm to 905 nm).

RESULTS AND DISCUSSION OF RESULTS

Well-behaved lasers were used to establish the effects of pulse repetition rate and pulse duration at wavelengths neighboring the emission spectrum of the GaAs laser. One result of this research was the development of consistent relationships between the ocular damage threshold for exposure to continuous wave radiation of the same wavelength and for the same total exposure duration. This implied a requirement for knowledge of the ocular hazard of a continuous wave semiconductor laser. Previous research has provided data for cryogenically cooled GaAs laser pulsed at 120 kHz. This report provides ocular damage data for a true continous wave semiconductor laser device.

The device employed in these experiments was a GaAlAs stripe geometry CW laser diode, Model LCW-10, fabricated by Laser Diode Laboratories. The wavelength of maximum emission, measured in this laboratory, was 883 nm.

The laser was driven with a stable 385 mA source. A 5.5 mm focal length lens collimated the laser emission. The beam divergence was 0.65 milliradian (mrad) by 4.8 mrad.

The animals used in these experiments were rheaus monkeys (Macaca mulatta) weighing between 2 and 5 kg. Preanesthetic medication consisted of a sedative dose of phencyclidine hydrochloride (0.25 mg/kg) intramuscular and atropine sulfate (0.2 mg) subcutaneously. Anesthesia was induced with sodium pentobarbital (approximately 5 mg/kg) via the saphenous vein. A pediatric intravenous injection set was placed into the saphenous vein to administer fluids and to facilitate additional anesthetic. The pupils were dilated and sutures of 3-0 silk were placed in the upper eyelid to facilitate manipulation. While the eyes were open during the experiment, physiologic saline was used to maintain good corneal transparency.

The animals were positioned in the exposure system and the fundus examined via the Zeiss fundus camera. A horizontal row of 12 suprathreshold retinal exposures were made with the erbium laser to produce location markers. Three rows of GaAlAs laser exposures were located below the marker row. The doses were varied through one log unit below the maximum attainable. All GaAlAs exposures were of 30-sec duration, controlled by turning the laser on and off. The exposure sites were immediately recorded on a polaroid fundus photograph to insure subsequent identification, and were examined via ophthalmoscope one hour later. The criterion for damage was the presence of a visible opacity at this examination. The data were statistically evaluated to determine the ED_{50} and associated 95% confidence limits.

All exposures were of 30-sec duration. The ED_{50}, determined from 185 exposures in 6 eyes, was 230 mJ. The lower and upper 95% confidence limits were 202 mJ and 262 mJ respectively. This result is somewhat biased because the laser was unable to deliver a dose which produced damage 100% of the time. However, the deviation from a true ED_{50} should be small.

The 120 kHz GaAs and cw GaAlAs data are not for minimal image diameter. Extrapolation is possible from these data to the estimated threshold for worst case conditions. Data collected for a variety of pulse durations, wavelengths, and retinal irradiance diameters show that, on the average,

Equation 1. $\quad \log E = k = \log D$

where E is the retinal radiant exposure ED_{50} and D is the retinal irradiance diameter. From this equation can be derived the relationship:

Equation 2. $$\frac{TIE_1}{TIE_2} = \frac{A_1}{A_2}$$

where TIE is the total intraocular energy and A is irradiated area. The 120 kHz laser had a beam divergence of 11 mrad by 0.2 mrad. It is highly unlikely that the eye would be able to produce the approximately 3 micron (μm) spot predicted from 0.2 mrad divergence; a dimension of at least 20 μm is probable. The continuous wave (cw) diode produced a beam divergence of 4.8 by 0.6 mrad. The eye would again produce at least a 20 μm spot from the 0.6 mrad divergence. The 120 kHz diode irradiated a retinal spot of 165 X 20 μm and the cw diode irradiated a retinal spot of 72 X 20 μm. From Equation 2 the 120 kHz GaAs data must be reduced by a factor of 3.24 and the cw GaAlAs data reduced by a factor of 2.14 to present the worst case conditions (20 μm diameter spot).

Viewing multiple pulses is more hazardous than viewing a single pulse because of additivity of pulse effects. At question is the degree of additivity. Ocular damage thresholds were determined for exposure to pulse trains from a repetitively pulsed neodymium laser. These measurements were designed to determine the additivity of the effectiveness of pulses as a function of pulse number and pulse separation. The consistent relationship between pulse train data and cw data led to the following formulation for computing the maximum permissible exposure to pulse trains.

Equation 3. $MPE^{RP}(T) = CF\ MPE(T)$

$MPE^{RP}(T)$ is the maximum permissible exposure expressed as total intraocular energy for the pulse train of duration T, MPE(T) is the maximum permissible exposure for cw irradiation of duration T and CF is a correction factor which is independent upon the pulse repetition frequency and the duration t of an individual pulse in the pulse train.

Equation 4. for t greater than 2 μsec $CF = 3.5\ PRFt$

for t less than 2 μsec $CF = 1.8 \times 10^{-11} PRF t^{-1}$

This method has the advantage of applying the same margin of safety for pulse train exposures as is applied to cw exposures.

CF was derived by taking the ratio:

Equation 5. $CF = ED_{50}^{RP}(T)/ED_{50}(T)$

$ED_{50}^{RP}(T)$ and $ED_{50}(T)$ are the damage threshold doses for pulse trains of total duration T and cw exposures of total duration T respectively.

Applying CF to the estimated worst case for cw exposures yields a worst case for pulse train exposures.

Another result obtained from the evaluation of pulse repetition data is:

Equation 6. $MPE^{RP}(T) = n^{3/4}\ Q\ MPE(t)$

where n is the number of pulses in the pulse train, and MPE(t) is the maximum permissible exposure for a single pulse. Q is a constant for any given pulse duration and wavelength, but its numerical value is not readily predictable. This relationship says that MPE^{RP} is essentially independent of PRF, but depends only on the number of pulses. Therefore, the results of the foregoing section are valid when applied to the average PRF of a non-uniformly spaced train of pulses.

In addition to the above, experimental evaluation of three laser training devices was performed. These were the MAGLAD, SIMCAT, and MILES M-16 transmitters.

The MAGLAD laser is a direct fire simulator designed for use with a detector instrumented pop-up target. The transmitter utilizes a pulsed GaAs laser (905 nm). A prototype transmitter was provided to the Division of Non-Ionizing Radiation, LAIR by International Laser Systems, Orlando, Florida for evaluation of the effects of the emitted optical radiation on primate retina.

The device has two internally controlled modes of operation. In the single shot mode the laser will emit a group of 16 pulses (simulation of one round) each time the trigger switch is closed. In the automatic mode, the laser will emit groups of 16 pulses at a group repetition rate of 12.5 Hz until a full clip (20 rounds or 30 rounds, selectable) is simulated or until the trigger is released. For the purpose of the measurement reported here, the device was externally triggered at a group repetition frequency of 10 Hz so that 30-sec exposures could be provided.

The beam characteristics were evaluated prior to exposure of rhesus monkey eyes. These data are summarized in Table 1.

Table 1

Summary of MAGLAD Emission Characteristics

Pav	= 7.6×10^{-6} W
t	= 140 ns FWHM
PRF	= 1558 pps (within one shot)
PRF (AV)	= 200 pps (16 pulses/round, 12.5 rounds/sec)
Q	= 3.8×10^{-8} J/pulse
P_{peak}	= 0.271 W
Beam diameter	= 3 cm
Beam divergence	= 1 mrad circular

Rhesus monkey eyes were exposed to the MAGLAD laser beam. Marker burns were placed with the erbium laser to facilitate location of the subsequent exposures.

The total intraocular energy from the MAGLAD was limited by the animals ocular pupil diameter (7 mm). No retinal alterations were noted as a result of 30-sec duration ocular exposure to the MAGLAD laser.

The SIMCAT device employs a pulsed GaAs laser (905 nm) with emission characteristics that are typical of the entire SIMFIRE family. The device has two pulse repetition frequency (PRF) modes of operation. In the interrogation mode, the laser emits pulses of a frequency of 240 pps. In the kill mode the laser PRF oscillates between 280 pps and 359 pps. The duration of each mode is controlled by internal logic circuits which access the target via radio link.

The emission characteristics were measured. The results are tabulated in Table 2.

Table 2

Emission Characteristics

MODE	Pav	PRF	t(FWHM)	Q	P_{peak}
Interrogate	108 mW	235 Hz	160 ns	460 mJ	2.87 W
Kill	139 mW	285/361(325 Hz, avg)	160 ns	428 mJ	2.67 W

The beam divergence measured at the 1/e point is 1.0 mrad X 2.9 mrad. Q and P_{peak} for the kill mode are averaged over the two frequency ranges.

Pav = average power; PRF = pulse repetitition frequency; t(FWHM) = pulse duration measured at the full width half maximum points; Q = pulse energy; and P_{peak} = peak pulse power. Rhesus monkey eyes were exposed to the SIMCAT laser beam.

All exposures were 30-sec duration. In the first sequence, the dose was 4.3 X 10^{-8} J/pulse. The total intraocular energy for an exposure was 420 μJ. Twenty-six exposures were placed in two eyes. In the second sequence, the dose was 1.7 X 10^{-7} J/pulse. The total intraocular energy was 1680 μJ. Thirty-six exposures were placed in two eyes. There was no direct evidence of retinal alteration either by funduscopy or by direct ophthalmology one hour after exposures were completed.

A MILES M-16 engineering development transmitter was evaluated. This device emits a coded train of 905 nm pulses from a GaAs laser. Two levels of pulses are emitted: a kill pulse of 2.0 X 10^{-8} J/pulse and a near miss pulse of 8.3 X 10^{-8} J/pulse. The average PRF is 1636 Hz.

Rhesus monkey eyes were subjected to irradiation from this device for exposure durations of 15-sec and 30-sec. The 15-sec exposure dose was 1.92 mJ total intraocular energy. Retinal alteration (clouding) was observed at 4 of 8 exposure sites.

The 30-sec exposure dose was 3.85 mJ total intraocular energy. Retinal clouding was observed at 6 of 8 exposure sites.

CONCLUSIONS

The prototype MILES GaAs laser device is capable of producing retinal alterations. These alterations are not like the lesions typically seen as a result of laser exposure. Their nature and significance is not yet understood.

The procedures of TP MED 279 for calculation of the maximum permissible exposure to laser pulse trains does not accurately model existing damage threshold data. Recommendations for modification of these procedures will be formally submitted.

RECOMMENDATIONS

Further studies are needed with the pulsed GaAs diode to determine the nature and significance of the retinal alteration observed. The ocular damage threshold should be determined for the cw GaAs laser. More data are required on the ocular hazard of pulse trains at a variety of pulse durations, pulse repetition rates and wavelength. Especially essential are data for pulse durations of 1 to 10 μsec. Pulse durations in this time domain appear to be more hazardous than either longer or shorter pulse durations.

PUBLICATIONS

None

STUDY NO. 3 Project A813

PROBLEM

The Electronics Command (DARCOM) Ft. Monmouth, N.J., is actively pursuing the application of a laser device which produces simultaneous colinear beams of 1064 nm radiation (Nd) and 532 nm radiation (frequency doubled Nd). The application requires the laser output to consist of Q-switch pulses emitted either singly or at moderate pulse repetition frequencies (less than 100 Hz).

The employment of such a laser device poses the possibility of subjecting personnel to laser irradiation for which the ocular hazard is not yet known. While the ocular hazard of Q-switch pulses (ED_{50} based

on ophthalmoscopically visible damage end point) has been evaluated at 1064 nm and 532 nm no research has been performed on the effect of simultaneous irradiation by both wavelengths. Further, the Army safety standards, documented in AR 40-46 and TB MED 279, provide no guidance for the evaluation of the hazard of dual wavelength exposure.

Differences exist in the interaction of these wavelengths with ocular tissue. The focal distances do not coincide; the infrared focus is posterior to the visible focus. The 1064 nm radiation is more strongly attenuated in the media anterior to the retina, but less strongly absorbed by the primary absorption site, the melanin granules of the pigment epithelium. The result is a partial axial separation of the damage loci for the two wavelengths. The mechanism for production of ophthalmoscopically visible retinal damage by Q-switch pulses is assumed to be thermomechanical in nature. It is difficult to predict the effect of two axially displaced simultaneous epicenters on the total energy required to produce damage.

Less completely understood is the relative interaction with the retinal layers anterior to the pigment epithelium. Alterations in these layers are manifested through functional changes in visual evoked response (VER), electroretinogram (ERG), and visual acuity. It is in these interactions which may be photochemical rather than thermal in nature, that wavelength differences and the synergistic effects of multiwavelength irradiation may be most readily demonstrated. Little effort has been expended on the functional effects of single wavelength Q-switch exposures. Multiwavelength exposures have not been considered.

DARCOM has requested that DNIR conduct an experimental program to elucidate the ocular hazard of the two wavelength laser operating at 1064 nm and 532 nm.

RESULTS AND DISCUSSION OF RESULTS

Ocular tissue was subjected to simultaneous irradiation at 1064 nm and 532 nm. A continuously excited acoustooptic Q-switched neodymium-YAG laser operating in the TEM_{oo} mode was used. An intracavity frequency doubler provided simultaneous colinear emission at 1064 nm and 532 nm with a ratio of 90% infrared and 10% green.

A harmonic beamsplitter separated the laser output into two beams of different wavelengths so that each could be manipulated and attenuated independently. Blocking filters guaranteed the purity of each beam. The beams were recombined at a second harmonic beam splitter.

Photodiodes with appropriate blocking filters detected the laser pulses at each wavelength for display on an oscilloscope for dosimetry. Calibration was accomplished by reference to a radiometer at the eye exposure position. A goniometer mount provided rotation of the animal about the pupil of the eye to be exposed, which assured precise

positioning of the exposures on the retina. An accurately repositionable mirror, which directed the beam into the eye, was moved to permit fundus camera observation of the retinal exposure sites.

The animals used in these experiments were rhesus monkeys (Macaca mulatta) weight between 2 and 5 kg. Anesthesia was induced with sodium pentobarbital. The pupils were dilated and sutures of 3-0 silk were placed in the upper eyelid to facilitate manipulation. While the eyes were open during the experiment, physiologic saline was used to maintain good corneal transparency.

The animals were positioned in the exposure system and the fundus examined via the Zeiss fundus camera. Any abnormalities were noted. Thirty-six to 48 exposures were placed in a square array utilizing suprathreshold marker burns to locate accurately the rows and columns for subsequent examination.

Detailed ophthalmoscopic examination of the exposure sites was conducted at one hour postexposure. The criterion for damage was the presence of a lesion visible via this examination.

The data were evaluated by probit analysis to provide an ED_{50} and confidence limits. The ED_{50} is defined as the dose which will create a lesion in 50% of the exposures.

Threshold studies were performed for each of three exposure conditions. The first experiment was exposure to 1064 nm irradiation only. The second experiment was exposure to 532 nm irradiation only. The third experiment was simultaneous exposure to 1064 nm irradiation and 532 nm irradiation. In this experiment each exposure included 1064 nm irradiation at 50% of the dose previously determined to be the ED_{50} for 1064 nm irradiation. In addition, each exposure included 532 nm irradiation. The 532 nm dose was varied about the level required to produce a lesion with the combined energies.

All experiments utilized a beam divergence to irradiate a minimal retinal area. The results are shown in Table 1.

Table 1

Results of Threshold Studies

Wavelength	Pulse Duration	ED_{50}
1064 nm	180 nsec	128 μJ
532 nm*	140 nsec	2.85 μJ
532 nm*	140 nsec	2.23 μJ

* includes 60 microjoule at 1064 nm in each exposure

Let ED_{50} (532) = threshold dose at 532 nm alone

ED_{50} (1064) = threshold dose at 1064 nm alone

Dose (532), dose (1064) = doses at respective wavelengths which, when applied simultaneously, produce a threshold lesion

$$\text{Let } R = \frac{\text{Dose (532)}}{ED_{50}\ (532)} + \frac{\text{Dose (1064)}}{ED_{50}\ (1064)}$$

Three cases are possible:

R = 1 Unit additivity

This condition would exist if both wavelengths interact with ocular tissue at the same physical site and via the same mechanism, as for example, the thermal conversion of absorbed energy in the melanin granules of the pigment epithelium.

R < 1 Super additivity

This condition implies a sensitization of tissue to one wavelength as a consequence of the presence of the other. This would result for example, if absorption of 532 nm radiation in melanin produced an excited state which strongly absorbed 1064 nm radiation.

R > 1 Sub-additivity

This condition implies non-cooperative interaction of the two wavelengths with ocular tissue. This would occur, for instance, if the retinal absorption sites were not totally overlapping or if the damaged mechanisms were dissimilar and non-interactive.

CONCLUSIONS

Compute R for the data of this experiment.

$$R = \frac{2.23}{2.85} + \frac{60}{128} = 1.25$$

R > 1 therefore sub-additive

This result is consistent with the hypothesis that the retinal absorption sites at 532 nm and 1064 nm are different.

RECOMMENDATIONS

This experiment should be continued with different mixtures of 532 nm and 1064 nm radiation, and with other wavelengths. Histopathic examination of damage sites are required to support the conclusions so far indicated.

PUBLICATIONS

1. LUND, D. J. and E. S. BEATRICE. Ocular hazard of short pulse argon laser irradiation. Health Physics (in press)

2. BEATRICE, E. S., H. ZWICK, D. I. RANDOLPH, B. S. STUCK, and D. J. LUND. Laser hazards: Biomedical threshold level investigations. Milit Med 141: 889-891, 1977

3. STUCK, B. E., D. J. LUND and E. S. BEATRICE. Repetitive Pulse Laser Data and Permissible Exposure Limits. Report No. 58. San Francisco, California: Letterman Army Institute of Research, April 1978

PRESENTATIONS

1. BEATRICE, E. S., D. J. LUND, J. D. COURS, P. WAMPNER and D. H. SLINEY. (Project MILES). Biomedical research and coordination in safe field exercises. Presented at 1978 Army Science Conference (West Point, New York, 20-22 June 1978)

2. LUND, D. J., B. E. STUCK and E. S. BEATRICE. Ocular effects of repetitive pulse exposures limits. 1978 Gordon Research Conference on Lasers in Medicine and Biology (Meridan, New Hampshire, 26-30 June 1978)

3. STUCK, B. E. and D. J. LUND. Extrapolation of pulsed light data in scanned displays. Presented at Society of Photo Instrumentation Engineers 22nd Annual Technical Symposium. (San Diego, California, 28-31 August 1978)

STUDY NO. 4 — Electrophysiological evaluations of retinal functions

PROBLEM

The retinal appearance following laser irradiation has been well-documented. What has remained difficult to ascertain, however, is the effect upon vision following low level (below the threshold for an ophthalmoscopically visible lesion) laser exposures at wavelengths currently being used in the field. Gallium arsenide (GaAs) lasers which operate at 850 nm have previously been considered "eye-safe" systems. However, recent findings of this laboratory have indicated a subtle change in the appearance of the retina following exposure to this laser.

The purpose of study was to evaluate further the observed changes in the electrophysiological characteristics of the visual system following exposure to a prototype GaAs diode which may be incorporated into the Project MILES system.

RESULTS AND DISCUSSION OF RESULTS

The integrity of the visual system can be inferred in four ways. Opthalmoscopic techniques in which changes are seen but their significance is not directly apparent. Behavioral techniques in which the animal is required to perform certain vision-related tasks generally will not allow a quick response to the question of whether or not the animal can "see." The training is long and costly. Electroretinography (ERG) in which the integrity of the retina as a whole is evaluated is a good quick technique. However, the plethora of retinal elements unaffected by the laser exposure tends to mask the effects of a small sized retinal change. The evoked occipital potential (EOP), which has not been widely used to determine retinal dysfunction, may yield clues as to changes in the visual system following retinal exposures.

In the present study, one animal pre-anesthesized with Ketamine and maintained under pancuronium-Br, was presented with a large image of a grating. The grating was inserted into the optics of a Ziess Fundus camera and projected onto the retina of the dilated eye. In the first run, EOPs were recorded from each occipital lobe when alternately, the left, right, left and right eyes were stimulated. An exposure to the right eye of 7.4 X 10^{-3} J of total intraocular energy (TIE) for a 16 msec argon laser pulse (514.5 nm) was made and several thousand evoked potentials were then obtained. The averaged (N=64) potentials showed no immediate change from its pre-exposure amplitudes or latencies. Changes in the shape of the EOP were observed 15 to 20 min after exposure in the contralateral cortex. None were seen ipsilaterally, nor were any retinal changes observed during this period. The changes persisted until the end of the run (2 h).

Examination of the right eye one week later showed a small (500μ) irregular lesion superior and nasal to the fovea in the macula. Following the same format as above, the left eye was then exposed to GaAs radiation for 60 sec. Average intraocular power was 260 mW with pulse repetition rate of 1600 Hz. As before, waveform changes were seen after approximately 20 min after exposure, however, this time they occurred bilaterally. Examination showed no visible retinal changes. A follow-up evaluation two weeks later showed that the EOP had returned to normal.

In order to determine whether the observed changes were due in fact to laser irradiation or to retinal or central nervous system changes occurring over time, a second animal was placed into the system and sham-exposed. No changes in the EOP were seen over a 2-h period.

A second experiment in this series was designed to determine the effects of multiple pulses delivered for varying periods of time. A naive animal was tranquilized and its pupils dilated. Pancuronium-Br was administered and pre-exposure grating EOPs were obtained. The right eye was then irradiated with the same diode as in the previous experiment.

Exposure durations of 10, 30, 60, 120 and 300 sec were used with 15 min between exposures. The results showed definite bilateral changes in the EOP almost immediately following the 300 sec exposure. No retinal changes were observed.

The meaning and implications of the observed changes in terms of the visual system are difficult to determine at this time. The appearance of waveform alterations in the contralateral cortex, together with bilateral changes during the second run, indicates that we have a possible extremely sensitive tool to investigate suspected retinal dysfunction due to laser irradiation.

CONCLUSIONS

An apparently sensitive, relatively easy technique can be used to determine changes in a small retinal area induced by laser radiation.

RECOMMENDATIONS

After thresholds have been obtained for some visible, and near IR EOP changes, we will be able to evaluate quickly functional disturbances of the visual system. Correlative studies, utilizing animal behavior, ERG, and EOP should be conducted so that a more direct relationship can be established between EOP waveform changes and actual vision changes.

PUBLICATIONS

None

STUDY NO. 5 Electrophysiological evaluations of retinal functions

PROBLEM

In support of Project A813, data on the biological effects of neodymium (1160 nm), frequency doubled neodymium (530 nm), and combinations of these laser outputs are required to determine what protective devices will be necessary for air crew and ground crew operations. The characteristics of each laser wavelength and their differential biological effects upon retinal tissue are known. The combination of the two wavelengths has not been studied up to this time.

RESULTS AND DISCUSSION OF RESULTS

Data have been collected from the first frequency doubled neodymium laser-exposed animal. An oscillating grating within the optical path of a Zeiss fundus camera was used to elicit evoked occipital potentials (EOPs) from a rhesus monkey. The animal was rendered immobile with

pancuronium-Br, the pupil of the left eye was dilated and the upper lid was retracted. A series of EOPs was obtained at a rate of one per sec and placed on analogue tape before and after exposure of the animal's retina to a single pulse 530 nm frequency doubled neodymium laser radiation. The pulse had a duration of 1.40×10^{-7} seconds at 1×10^{-6} J/cm^2. Groups of 8 occipital potentials immediately before and after exposure were signal averaged. A decrease in the amplitude and distortion of the shape of the waveform resulted and continued for approximately 32 sec. The waveform regained its former amplitude and shape within 40 sec.

These initial results are consistent with results of other investigators of flashblindness phenomena who utilize a flash stimulus to produce electroretinograms. In the present study, the use of evoked cortical potentials to trace the decay and recovery of visual function is unique. The major problem of this technique is the usually low amplitude of the evoked response which necessitates signal averaging procedures. The procedure of signal averaging limits the perception of quick recovery phenomena. This has been obviated in the present study by the use of only 8 responses, averaged, to yield relatively stable and consistent EOPs.

A technique has recently been devised in which the evoked occipital potential can be obtained from stimulation of the retina with low level argon (514.5 nm) laser radiation. The spot size on the retina is approximately 50 to 75 μm. By signal averaging the EOPs, a series of evoked potentials both ipsilateral and contralateral have been obtained for various small retinal areas. When perfected, this technique could become valuable as a tool in assessing the viability of various retinal areas following laser exposures.

CONCLUSIONS

Evoked occipital potentials can be used to determine the short-term effects of frequency doubled neodymium upon visual function measured electrophysiologically.

RECOMMENDATIONS

Of primary importance is the evaluation of single pulses, multiple successive pulses, and combinations of pulses at 1060 and 530 nm. In addition, other wavelengths must be examined both alone and in combination to determine the additivity or non-additivity of laser retinal exposures.

PUBLICATIONS

1. RANDOLPH, D. I., D. J. LUND and E. BEATRICE. Changes in the evoked cortical potential following low level laser irradiation. (Abstract) In: Proceedings of the Association for Research in Vision and Ophthalmology (Sarasota, Florida, May 1978)

PRESENTATIONS

1. RANDOLPH, D. I., H. ZWICK, and B. STUCK. Laser bioeffects. Presented at NASA/Ames (Moffett Field, California, August 1978)

2. RANDOLPH, D.I., E. BEATRICE, and B. E. STUCK. Laser protection for helicopter aircrews. Presented at Aviation Research and Development Command (St. Louis, Missouri, August 1978)

3. RANDOLPH, D.I., E. BEATRICE, B. E. STUCK, H. ZWICK and D. J. LUND. Laser bioeffects. Presented at U. S. Army Electronics Command (Ft. Monmouth, New Jersey, June 1978)

4. RANDOLPH, D. I., D. J. LUND, and E. BEATRICE. Changes in the evoked cortical potential following low level laser irradiation. Poster Session presentation for the Association for Research in Vision and Ophthalmology (Sarasota, Florida, May 1978)

5. RANDOLPH, D. I., H. ZWICK, D. J. LUND and B. STUCK. Low light level radiation effects. Presented in panel at Gordon Research Conference on Lasers in Medicine and Biology. (Meridan, New Hampshire, June 1978)

6. RANDOLPH, D. I. Laser eye protection requirements. Presented to personnel protection group, NARADCOM. (Natick, Massachusetts, December 1977)

7. RANDOLPH, D. I. Electrophysiological evaluation of gallium arsenide laser radiation. Presented at MILES meeting (San Francisco, California, March 1978)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6078	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORDR INSTR	8b. SPECIFIC DATA-REACTOR ACCESS	9. LEVEL OF SUM
77 10 01	D. Change	U	U	NA	NL	YES ☐ NO	4. WORK UNIT

10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS01	00	201
b. SECONDARY	62772A	3E162772A813	00	025
c. CONTRACT/GRANT	CARDS 1141			

11. TITLE (Protect with Security Classification Code):

(U) Biological Investigations in Prediction and Protection Against Coherent Radiation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

009600 Masers and Lasers; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 12	Cont	DA	C. In-House

17. CONTRACT/GRANT:

a. DATE/EFFECTIVE: — EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: — d. AMOUNT:
e. KIND OF AWARD: — f. CUM. AMT:

18. RESOURCES ESTIMATE

FISCAL YEAR	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
78	3.0	224
79	4.0	238

19a. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS
NAME: Zwick, H., DAC
NAME: Randolph, D. I., DAC POC:DA

20. GENERAL USE:

Foreign Intelligence Not Applicable

21. KEYWORDS (Provide Each with Security Classification Code):

(U) Laser Systems Safety; (U) Laser Hazard; (U) Eye Damage; (U) Vision

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide individual paragraphs identified by the proper Security Classification Code):

23. (U) The objectives are to determine the effects on vision of repetitive low level laser radiation at and below "safe" exposure levels and to provide these data to USAEHA, the DARCOM developer and the Army user for their guidance in the safe use of lasers.
24. (U) Behavioral and neurophysiological techniques, adapted for rhesus, are used for visual testing. Temporary and/or permanent effects correlated with morphological changes most realistically represent the actual hazards of laser radiation. These measures provide the data base for safety standards ,revision.
25. (U)(7710 7809). Behavioral and neurophysiological techniques have been used to demonstrate that 1) no physiological effects can be demonstrated in the trained rhesus monkey after several thousand 10 second CO_2 laser exposures to the skin and cornea of the rhesus monkey for irradiances of up to 200 mw/cm^2, 2) repeated exposures of rhesus monkeys to low level argon, radiation has shown visual acuity and spectral sensitivity to be impaired at levels more than 100 times below the "safe" levels for viewing a diffuse, extended source 3) Changes in the amplitude and latency of components of the evoked occipital potential have been observed following a low level gallium arsenide laser diode exposure to the rhesus retina; 4) Further measures utilizing extracellular recording techniques indicate changes in the receptive fields and spectral sensitivity at levels below those of gross burn criteria; 5) Direct comparison of coherent and incoherent sources indicate that coherent light is more effective in altering visual mechanisms than incoherent light.

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

ABSTRACT

PROJECT NO. 3E162772A813 — Health Effects of Military Lasers

WORK UNIT NO. 025 — Biological Investigations in Prediction and Protection against Coherent Radiation

The following investigation has been conducted under this work unit:

STUDY NO. 1 Effects of laser irradiation on visual function

Low level laser exposure in non-human primates (rhesus) produced changes in visual function 1000 times below extended source permissible exposure criteria. These behavioral effects have been followed over 30 months with evidence of slow decline in remaining central vision. Two rhesus monkeys have so far been used in this study. Parallel electrophysiological investigations in other rhesus monkeys showed similar results at higher retinal irradiance levels but still below minimal permissible exposure criteria. In vertebrate subjects, electrophysiological experiments were done which indicate that coherency and associated physical phenomena are involved in producing low level laser light effects. More refined behavioral and neurophysiological assessment techniques have been developed this year to resolve the nature of the damage mechanisms involved. Collaboration on relevance of these findings to laser safety, as well as some work in developing more suitable visual assessment criteria for humans is in progress.

BODY OF REPORT

WORK UNIT NO. 025 — Biological Investigations in Prediction and Protection Against Coherent Radiation

STUDY NO. 1 — Effects of Laser Irradiation on Visual Function

PROBLEM

Changes in non-human primate vision produced by levels of laser irradiation at or below present permissible exposure limits must be evaluated. Present standards are based primarily on "gross" morphological criteria. Criteria that involve loss in process of "seeing" have not been extensively utilized. Animal research performed in this work unit will provide such information which in turn will be utilized to reevaluate present laser safety standards.

RESULTS AND DISCUSSION OF RESULTS

Low Level, Long-term Follow-up Measurements

Long-term follow-up measurements for more than two years post-exposure to coherent low level irradiation (514 nm) showed progressive change in spectral sensitivity long after irradiation ceased. Two rhesus monkeys were exposed to 2.1 log trolands for 2 h sessions for a total cumulative dose of 38 h. Retinal irradiation was 0.2 $\mu W/cm^2$. Post-exposure changes during the first 6 mo revealed loss in sensitivity at the finest Landolt acuity criterion in the intermediate spectral region. Rod intrusion was evident at lower but still photopic acuity criteria. Extended measurements made out to 2 yr post-exposure showed considerable change in spectral sensitivity occuring long after cessation of exposure. Periods of "waxing" and "waning" in sensitivity, progressive decline in sensitivity beginning at the finest acuity criteria, and continued evidence of rod intrusion as cone sensitivity declined were observed on both animals.

Electroretinographic (ERG) spectral sensitivity measurements made for single 2 h exposures at slightly higher levels of 514 nm coherent light showed comparable effects. Loss in photopic sensitivity and rod intrusion were obtained more than one year post-exposure. Corresponding electrophysiological studies in vertebrates in which coherent and incoherent exposure were equated suggest that the coherency of the laser source is a critical factor in producing these low level light effects.

Coherency Effects (Pseudemys)

In pseudemys, the effects of prolonged monochromatic coherent and incoherent light were compared on the adaptation of the long wavelength receptor system of pseudemys. Measurements of ERG spectral

sensitivity were made by using a lock-in amplifier. Coherent and incoherent backgrounds of 620 nm were equated in quantal flux and spatial extent. Bandwidths for coherent light were less than 0.05 nm compared to 10 nm bandwidths of incoherent light. Coherent light was more effective in reducing spectral sensitivity than incoherent light from 10^{8} to 10^{14} quanta/sec. Permanent effects of coherent light on spectral sensitivity were obtained at 10^{13} to 10^{14} quanta/sec for exposures of an hour or more. Such effects were manifested under conditions of chromatic adaptation by a gradual reduction in the short wavelength sensitivity and a shift in the peak of the chromatically adapted sensitivity function from 440 to 560 nm. Similar exposure to incoherent backgrounds up to 10^{14} quanta/sec produced a peak in the short wavelengths that was not altered with prolonged exposure. Alteration of spatial coherency attenuated the coherent effect which suggest that retinal processes may be specially sensitive to coherent light input.

Receptor Field Low Level Exposure Effects

We have investigated the effects of low-level exposures to long wavelength coherent light (633 nm) on single optic tectal cells in pseudemys. Significant changes in receptive field size and spectral sensitivities were obtained following both brief and prolonged exposures. Transient expansion and permanent constriction of receptive field size, changes in response pattern and responsiveness, and reduction in spectral sensitivity in the long and short wavelengths were characteristic over an extended range of retinal irradiances. Delayed losses in sensitivity and receptive field size were frequently obtained but were more characteristic at low exposure levels for exposure durations of an hour or more. When 633 nm coherent light was time-averaged to reduce the coherent speckle pattern, the effects obtained were greatly attenuated. These data are consistent with our ERG experiments in which coherent and incoherent light were found to have dissimilar effects on spectral sensitivity.

Low Level 20 nsec Transient Effects in Rhesus

These experiments involve measurement of threshold Landolt ring contrast for various gap sizes (spatial frequency). Immediately transient changes in contrast threshold for fine gaps have been obtained at energy levels less than 50 picojoules. These preliminary effects will be further evaluated for coarse gap sizes so that effects can be assessed for both very fine and coarser spatial frequencies. The behavioral technique being used allows for assessment of both stationary and moving Landolt ring targets. Investigation of transient effects for moving as well as stationary targets are planned.

CONCLUSIONS

Evidence for the possibilities of low level permanent effects on vision has been obtained. Permanency of these effects is evident across

various tests and animal species. Data strongly suggest that coherency or physical phenomena associated with coherency are responsible for the sensitivity of retinal processes to laser light.

The results of these experiments need to be carefully considered in defining ocular risks associated with possible prolonged exposure to various low level military laser systems. Supplementary experiments will further clarify the impact of these data on present or future laser systems.

RECOMMENDATIONS

Follow-up of low level animals should continue until evidence of visual stabilization is obtained. Further funduscopic evaluation of such animals will be made at this time.

Two animals will be exposed simultaneously to comparable levels of coherent and incoherent irradiation and sacrificed at various times post-exposure to obtain morphological correlates. Detailed analysis of coherency and speckle pattern at retinal surface will be made with Fourier techniques to determine those physical characteristics of laser light which cause disorientation of visual processes.

Other behavioral and electrophysiological techniques will be used to assess peripheral as well as central visual function (dynamic visual acuity criteria) and new animals will be exposed.

Visual test concepts will be integrated into visual surveillance test battery to aid in annual evaluation of visual function of laser personnel.

Data obtained in these experiments should be used in establishing permissible exposure criteria for prolonged low level viewing situations.

New concepts in optical protection for low level laser hazards must be explored. A laser dosimeter (badge), which would indicate amount of cumulative daily exposure to various levels and wavelengths, is highly recommended for wear by those who are in the area.

PUBLICATIONS

1. ZWICK, H. and D. JENKINS. Effects of coherent light on retinal receptor processes of pseudemys. (Abstract) Invest Ophthal (Suppl) April 1978 p 155.

2. ZWICK, H., E. S. BEATRICE, and T. GARCIA Effects of prolonged exposure to low level coherent light (514 nm). (Abstract) Invest Ophthal (Suppl) April 1978 p 172.

3. ZWICK, H., E. S. BEATRICE, and J. E. CANHAM. Laser bioeffects; low level effects; impact on Army laser systems. In: Proceedings of the 10th Army Science Conference, 1978.

4. ZWICK, H. Low level laser light effects. Society of Photo-optical Instrumentation Engineers 162: 112-118, 1978.

5. ZWICK, H. and E. S. BEATIRCE. Long-term changes in spectral sensitivity after low-level laser (514 nm) exposure. Mod Probl Ophthalmol 19: 319-325, 1978.

6. ZWICK, H. and ROBBINS, D. O. Is the rhesus protanomalous? Mod Prob Ophthalmol 19: 238-242, 1978.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6070	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. METHOD	8a. GRADE	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
77 10 01	A.Termination	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	001
b. CONTRIBUTING				
c. [illegible]	CARDS 1141			

11. TITLE (Procede with Security Classification Code)

(U) In Vitro Metabolism of RBCs Stored in CPD-with Increased Glucose and Adenine

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 11	78 09	DA	C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE
b. NUMBER: Not Applicable
c. TYPE
d. KIND OF AWARD
e. AMOUNT:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR 78	2.0	115
FISCAL YEAR 79	0.0	00

19a. RESPONSIBLE DOD ORGANIZATION

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20. GENERAL USE

Foreign Intelligence Not Applicable

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
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NAME: POC:DA

21. KEYWORDS (Provide Key Technical Phrases in Descending Order of Importance)

(U) CPD-Adenine; (U) Blood Storage; (U) ATP; (U) Glucose

22. TECHNICAL OBJECTIVE, APPROACH, AND PROGRESS (Provide brief paragraphs identified by number. Precede text of each with security classification code.)

23. (U) Glucose levels of 161.1 mM in citrate phosphate dextrose (CPD) adenine (Ad) will not maintain adenosine triphosphate (ATP) at levels greater than 2.0 uM/gHb (or 50% of the starting value) in HCT=85 packed erythrocytes (RBC) for 35 days of storage at 4 C. By increasing the glucose to 226 mM in CPD Ad, ATP was maintained at greater than 2.0 uM/gHb for 5 weeks. It is important to determine if a smaller concentration of glucose, i.e., 194 mM (1.5X standard CPD) or less in CPD, supplemented with 2.03 mM Ad will have sufficient glucose for maintenance of ATP at greater than 2.0 uM/gHb after 35 days of storage. This work is important to the Army's continuing effort to extend the shelf life of stored RBC.

24. (U) The levels of glucose, ATP, 2,3-diphosphoglycerate (DPG) and pH will be determined in blood stored in the CPD with various quantities of adenine and glucose.

25. (U) 77 10 - 78 09. Five anticoagulants with increased concentrations of adenine and/or glucose were evaluated in vitro at HCT's 40, 70, and 85. Although investigations are not complete, one of these anticoagulants (A2) produced acceptable maintenance of glucose, ATP, DPG, and pH in packed cells stored at HCT=85 for 35 or 42 days. All anticoagulants except A1 were adequate at HCT=70; all performed well with whole blood. Further in vitro optimization of adenine and glucose in CPD-adenine will be carried out under a new work unit.

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

ARSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 001 In Vitro Metabolism of RBCs Stored in CPD with Increased Glucose and Increased Adenine

The following investigations have been conducted under this work unit:

STUDY NO. 1 In vitro metabolism of packed erythrocytes stored in various glucose concentrations in CPD-adenine

CPD-A1 anticoagulant (161 mM glucose and 2.03 mM adenine) was licensed for national use by the Food and Drug Administration in June 1978. This anticoagulant allows for whole blood or packed cell storage of up to 35 days. However, the viability of the packed cell units at 35 days is marginal, with 30% falling below the 70% minimum survivability standard. To improve the storage characteristics of packed cells, modified formulations of CPD-adenine are being evaluated in conjunction with the blood bag manufacturers and one of the MRDC blood contractors. In vitro analysis of red cells stored as whole blood (Hct=40) or as packed cells (Hct=70 or 85) are in progress. Preliminary analysis indicates that at Hct=40 all modified formulations are adequate. However, at Hct=70, A1 is marginal and the rest adequate. At the Hct=85 level (which is frequently found in blood banks) A1, A5, and A6 are inadequate, while A2 and A3 appear efficacious.

BODY OF REPORT

WORK UNIT NO. 001 — In Vitro Metabolism of RBCs Stored in CPD with Increased Glucose and Increased Adenine

STUDY NO. 1 — In vitro metabolism of packed erythrocytes stored at various glucose concentrations in CPD-adenine

PROBLEM

Citrate-phosphate-dextrose (CPD) has been established as a useful anticoagulant for liquid blood storage up to 21 days, and, with adenine supplementation, CPD-adenine will permit storage of red blood cells for 35 days. European formulas for CPD-adenine have contained 4.06 mM adenine (0.50 mM in blood). Since adenine may cause renal toxicity, it is desirable to develop a formula for CPD which would contain the least possible amount of adenine that will still maintain blood storage in the liquid state for 35 days. Studies of packed cells stored in CPD-adenine have indicated that glucose in excess of that in standard CPD 129 mM or 1X may be necessary for adequate maintenance of erythrocyte ATP (>2 µm/gm Hb) through 35 days of storage. Thus, a systematic search for the optimal glucose and adenine content of CPD preservative is necessary.

RESULTS AND DISCUSSION OF RESULTS

Survival studies of CPD-A1 (1.25X glucose and 2.03 mM of adenine) indicate marginal survivals for packed cells on Day 35, with 30% below the mean of 70% survivability. Modified CPD-adenine formulations have been made up and tested in vitro as follows:

Anticoagulant	[glucose]	[Adenine]	Hct Tested
A1	161 mM (1.25X)	2.03 mM	70 and 85
A2	276 mM (1.75X)	4.06 mM	40, 70, and 85
A3	258 mM (2.0X)	4.06 mM	40, 70, and 85
A5	194 mM (1.5X)	2.03 mM	85
A6	194 mM (1.5X)	3.05 mM	85

At hematocrits of 40 or 70, A2 and A3 are in excess of the required glucose and adenine needs of the red cells during 42 days of 4 C storage. Experiments are now in progress to evaluate all 5 anticoagulants at Hct=85, which has been proposed as the upper limit of packing for red cell concentrates. In vitro evaluation of the platelets from these units were done under Work Unit No. 042. "Red cell" parameters measured include ATP, 2,3-DPG, pH, plasma hemoglobin, and blood glucose.

CONCLUSIONS

In order to maintain adequate red cell ATP levels (≥50% of initial ATP, T_0 ATP) for 35 or 42 days of packed cell storage (Hct=70), glucose and adenine concentrations need to be increased from that used in CPD-A1. At Hct=70 the anticoagulants A2 and A3 appear to be in excess, while A5 and A6 are adequate. When the system is stressed by packing the cells to Hct=85, the A5, A6 systems were not adequate. At this Hct the A2 and A3 systems were effective in ATP maintenance, with the A2 system showing the best results: 64% of the T_0 ATP on Day 42 of storage.

RECOMMENDATIONS

1. The effort to improve CPD-A1 should continue with the goal of effecting 42-day storage of packed red cells at a prescribed upper Hct, so that adequate ATP levels are maintained to assure acceptable in vivo survivability.

2. Optimization of CPD-adenine for 42-day storage should be continued in a new work unit designed to integrate the Army liquid blood preservation R&D effort into a cost-effective targeted program.

PUBLICATIONS

None

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 003 Preservation of 2,3-DPG in Red Cells Subjected to Extended Liquid Storage

The following investigations have been conducted under this work unit:

STUDY NO. 2 Stabilization of ascorbic acid as a red cell preservative

STUDY NO. 5 The stabilization of dihydroxyacetone (DHA) for use in maintaining red cell 2,3-DPG during liquid storage

Currently available techniques for the liquid preservation of red cells do not maintain the red cell 2,3 diphosphoglycerate (2,3-DPG) concentration sufficiently so that it can modulate hemoglobin to release oxygen optimally. This defect of stored red cells may be a critical factor in hemorrhagic shock, and thus, in resuscitation of the massively wounded soldier.

STUDY NO. 2 Solutions of ascorbate-2-phosphate (AsP) at a concentration of 41 mM were stable for 6 months in the presence of CPD, glucose, adenine, and saline. AsP at this concentration, but not in more dilute solutions, was also stable to autoclaving. AsP was evaluated as part of a packed cell preservative solution and was effective in 2,3-DPG maintenance.

STUDY NO. 5 The solution stability of DHA at 25 C was extended to 20 months, and proved to be stable in H_2O, saline, adenine, glucose, and low phosphate solutions. It was unstable in CPD, citrate, AsP, or high phosphate (160 mM) solutions. DHA was unstable in solutions of H_2O or saline if stored at 50 C for longer than 2 months, but was stable to autoclaving at 121 C for 20 minutes. Optimizing of the DHA concentration was done by a combination of experimentally testing several concentrations in whole blood, and the use of computer analyzed mathematical optimization techniques.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA OE 6091	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636					
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: B.Termination	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. CRYPTO SECTY: NL	8a. HANDLING DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT
10. NO./CODES: a. PRIMARY	PROGRAM ELEMENT: 62772A	PROJECT NUMBER: 3S162772A814	TASK AREA NUMBER: 00	WORK UNIT NUMBER: 003			
b. CONTRIBUTING							
c. CONTRIBUTING							

11. TITLE (Procede with security classification code): (U) Preservation of 2,3-DPG in Red Cells Subjected to Extended Liquid Storage

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 008800 Life Support; 003500 Clinical Medicine; 002300 Biochemistry; 002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMING ORGANIZATION
75 03	78 09	DA			C. In-House

17. CONTRACT/GRANT: a. DATE EFFECTIVE: b. NUMBER: Not Applicable c. TYPE: d. AMOUNT: e. KIND OF AGENCY: f. CUM. AMT.

18. RESOURCES ESTIMATE:

FISCAL YEAR	PROFESSIONAL MAN YRS	PAYMENTS ($ thousands)
78	0.2	36
79	0.0	00

19. PERFORMING ORGANIZATION: NAME: Letterman Army Institute of Research; ADDRESS: Presidio of San Francisco, CA 94129

20. PRINCIPAL INVESTIGATOR: NAME: Moore, Gerald L., PhD, DAC; TELEPHONE: (415) 561-5875; SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS: NAME: Marshall, J.D., Jr., COL, MS; TELEPHONE: (415) 561-3600

NAME:

NAME: POC:DA

21. GENERAL USE: Foreign Intelligence Not Applicable

22. KEYWORDS (Procede each with security classification code): (U) Blood Storage; (U) 2,3-DPG: (U) Hemoglobin Function

23. TECHNICAL OBJECTIVE: 24. APPROACH: 25. PROGRESS (Procede with security classification code):

23. (U) Although the significance at the tissue level of a left-shifted oxygen dissociation curve exhibited by 2,3 diphosphoglycerate (DPG) deficient red cells is not completely clear, it is highly probable that superior red cell function would improve organ function in the massively transfused, acutely wounded soldier. Currently used red cell storage anticoagulant-preservative solutions (APS) do not permit the red cell to maintain DPG concentrations. Normal oxygen off-loading characteristics of hemoglobin are dependent, at least in part, on the allosteric regulation of DPG within the red cell. These studies will develop and evaluate both additive and storage systems which will enable red cells to better maintain DPG during liquid storage, without impairing other glycolytic intermediates such as adenosine triphosphate.

24. (U) Most APS additives which currently appear to hold promise for improving red cell DPG during storage are unstable in the anticoagulant mix. Among these are dihydroxyacetone (DHA), ascorbic acid, and bicarbonate. Stable forms or systems employing these substances will be developed and tested. The stable forms must be inert to the plastic blood container, freely convertible to the active form upon contact with whole blood, and nontoxic both to the blood in storage and the recipient on infusion.

25. (U) 77 10 -78 09. Ascorbate phosphate is stable in CPD or Optional Additive Systems (OAS). DHA is stable in OAS solutions and both are stable to autoclaving at high concentrations. Both are effective in whole blood or packed cells to maintain 2,3-DPG levels during storage. This work will continue under a new work unit.

26. (Applicable to contractors and contractor efforts)

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

BODY OF REPORT

WORK UNIT NO.	003	Preservation of 2,3-DPG in Red Cells Subjected to Extended Liquid Storage
STUDY NO.	2	Stabilization of ascorbic acid as a red cell preservative

PROBLEM

After 14 days of liquid storage as whole blood or red cell concentrates, red cell 2,3-DPG is not maintained at levels permitting normal oxygen transfer to tissues. Preliminary data suggest that decreased oxygen transfer may be particularly deleterious to soldiers requiring massive transfusions to correct hemorrhagic shock. Recently, it has been found that ascorbic acid, especially in combination with dihydroxyacetone (DHA) will maintain 2,3-DPG levels above 50% during extended liquid storage (35 days). However, ascorbate, either as the acid or as the sodium salt, is not stable in solution and decomposes rapidly when exposed to the autoclaving necessary to sterilize blood bags. It is important, therefore, to develop ways of stabilizing this compound. The stability and efficacy of derivatives of ascorbic acid such as ascorbate-2-phosphate (AsP) will be evaluated.

RESULTS AND DISCUSSION OF RESULTS

Solutions of 42 mM AsP were stored in plastic transfer packs at 25 C for 7 months in the presence of CPD, saline, glucose, or glucose and adenine. Sodium ascorbate (NaAs) in saline served as the control. The contents of each bag was assayed at 30- or 60-day intervals for ascorbate phosphate, pH, and checked for chemical changes by ultraviolet spectroscopy and thin layer chromatography. AsP appeared stable through 7 months (experiment still in progress) in all of the above solutions, but the control sodium ascorbate was 50% decomposed after one month and 90% decomposed after 4 months. The stability of AsP to autoclaving was concentration dependent. 41 mM AsP and NaAs were both stable to autoclaving, but 1:10 dilutions of each were only 84 and 68% stable, respectively. A 1:100 dilution of each compound gave 60 and 0% stabilities, respectively.

AsP was added to packed cells for 42 days of 4 C storage. Final blood concentrations of 3.5 to 5.0 mM resulted in maintenance of red cell 2,3-DPG to near fresh blood levels for 6 weeks of storage. These studies are reported in more detail as optional additive studies in Work Unit No. 006.

CONCLUSIONS

AsP and NaAs are stable to autoclaving at a 41 mM concentration, but only AsP is stable for extended periods in solution in a blood bag. The stability is not affected by CPD, making it possible to be included as a component in a CPD preservative solution. AsP is also stable in solutions of adenine, glucose, and saline, and thus could be used as a component of an optional additive solution (see Work Unit No. 006).

RECOMMENDATIONS

Further investigations should be performed to evaluate the efficacy and potential toxicity of AsP when used as an additive to maintain red cell 2,3-DPG. The optimal concentration should be determined.

PUBLICATIONS

1. BENSINGER, T.A., T.F. ZUCK, B. TOLBERT, S. McLAUGHLIN, C.C. PECK, and M. KNIGHT. An enzymatic method for measurement of ascorbate-2-phosphate. Biochem Med 19:118, 1978

STUDY NO. 5

The stabilization of dihydroxyacetone (DHA) for use in maintaining red cell 2,3-DPG during liquid storage

PROBLEM

After 14 days of liquid storage as whole blood or red cell concentrates, red cell 2,3-DPG is not maintained at levels permitting normal oxygen transfer to tissues. Preliminary data suggest that decreased oxygen transfer may be particularly deleterious to soldiers requiring massive transfusions to correct hemorrhagic shock. It has recently been found that ascorbic acid, especially in combination with dihydroxyacetone (DHA) will maintain 2,3-DPG levels above 50% during extended liquid storage (35 days). The additive DHA appears to be unstable in solution with anticoagulants or when autoclaved.

It is important, therefore, to develop ways of either stabilizing this compound, or to develop modified compounds which have the same potential effect as DHA in preserving red cell 2,3-DPG. It is necessary to study the rate and mechanism of DHA decomposition in various aqueous solutions and develop a method for its stabilization. Stabilization of DHA may be achieved by development of a complex or derivative.

RESULTS AND DISCUSSION OF RESULTS

Solutions of 150 mM DHA in sterile blood transfer packs were stored at 25 C for 20 months. DHA was stable in water, saline, saline plus

adenine or dextrose, or 16 mM phosphate. DHA was unstable in CPD, citrate, 160 mM phosphate, or ascorbate-2-phosphate (AsP). DHA was added to CPD-A1 whole blood units in concentrations of 0-80 mM to find its optimal concentration, defined as the maximum maintenance of red cell 2,3-DPG with the minimum loss of ATP after 35 or 42 days of storage. Data were gathered from 3 experiments and were analyzed with a multiple linear regression program that produced equations from which the partial derivatives with respect to DHA were obtained. With these derivatives, it was possible to obtain optimal concentration predictions for usage of DHA. The predicted optimum was about 30 mM, which has been born out by experimental measurements. DHA was added to packed cells at 30 mM and shown to help maintain 2,3-DPG, but the effects were less than when whole blood was stored.

CONCLUSIONS

DHA is stable in liquid storage in H_2O, saline, or H_2O with adenine or glucose, at 25 C. It could be used in blood storage if added to the blood via an optional additive system (OAS) contained in a satellite bag attached to the primary bag.

RECOMMENDATIONS

The use of DHA to help maintain 2,3-DPG in stored blood should be further evaluated using the OAS approach. Optimization of 2,3-DPG maintenance should be continued in a new work unit designed to integrate the Army liquid blood preservation R&D effort into a cost-effective, targeted program.

PUBLICATIONS

1. LEDFORD, M.E., G.L. MOORE, and T.A. BENSINGER. Comparison of two 2,3-diphosphoglycerate assays. Clin Chem 24:517, 1978

2. MOORE, G.L., M.E. LEDFORD, M.R. BRUMMELL, and D.E. BROOKS. The potential use of dihydroxyacetone: Solution stability and use in packed cell storage. (Submitted for publication)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

				DA OE 6087	78 10 01		DD-DR&E(AR)636
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT
	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3S162772A814		00	004		
b. CONTRIBUTING							
c. CONTRIBUTING	CARDS 11cf						

(U) CPD-Adenine Clinical Trials

003500 Clinical Medicine; 008800 Life Support

75 01	CONT	DA	C. In-House

	PREVIOUS		
FISCAL YEAR	78	2.0	20
	CURRENT		
	79	2.0	10

a. CONTRACT/GRANT

a. DATE(S) EFFECTIVE: — EXPIRATION:

b. NUMBER(S): Not Applicable

c. TYPE: — d. AMOUNT:

e. KIND OF AGENCY — f. CUM. AMT.:

14. RESPONSIBLE DOD ORGANIZATION

a. NAME: Letterman Army Institute of Research

b. ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Marshall, J.D., Jr., COL, MS

TELEPHONE: (415) 561-3600

16. PERFORMING ORGANIZATION

a. NAME: Letterman Army Institute of Research
Division of Blood Research

b. ADDRESS: Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Provide Name, Title, Address, and Telephone)

NAME: Peck, Carl C., LTC, MC

TELEPHONE: (415) 561-5875

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Moore, Gerald L., PhD, DAC

NAME: Bolin, Robert B., LTC, MC POC:DA

20. GENERAL USE

Foreign Intelligence Not Applicable

19. KEYWORDS (Provide at least five keywords or phrases that describe the work)

(U) Blood Storage; (U) Military Blood Banking

21. TECHNICAL OBJECTIVE; 22. APPROACH; 23. PROGRESS (Provide individual paragraphs identified by number. Provide date of work and progress/completion date.)

23. (U) The final objective of this study, clinical trials of an improved anticoagulant, is Food and Drug Administration licensure, which would permit clinical use of red cells after prolonged liquid storage. Shipment of blood into combat areas necessitates delays between drawing and infusion; the impact of these delays on the quality of red cells infused will be minimized through use of an improved anticoagulant-preservative solution.

24. (U) Currently, red cell liquid storage in CPD-A1 anticoagulant-preservative is limited to 35 days and is not approved for component storage. Survivability of packed red cells (PC) stored in CPD-A1 for 35 days is marginally acceptable. In vitro studies of metabolism in red cells and platelets stored in modified CPD-adenine suggest that increased adenine and glucose in the preservative will improve survivability. Such improvements may allow extension of red cell storage time to 42 days or beyond. The Division of Blood Research, LAIR, will coordinate efforts with civilian and container-solution manufacturers in the execution of clinical trials of promising improved CPD-adenine formulations.

25. (U) 77 10 - 78 09. In compliance with FDA recommendations, 14 more human in vivo autologous red cell survivability studies were performed on blood stored for 35 days in CPD-A1. These data contributed to the successful licensure of CPD-A1 by FDA in June 1978. Human in vivo survival studies on platelet concentrate harvested from blood drawn in CPD-A1 are being planned.

* Indicate if contractor was originator of the work.

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ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 004 CPD-Adenine Clinical Trials

In compliance with Food and Drug Administration recommendations that the CPD-adenine clinical studies reported last year be supplemented by further investigations, 14 more human in vivo autologous red cell survivability studies were performed on packed cells stored in containers from 2 different manufacturers. Red cell survival (24 hour ^{51}Cr-recovery) was not materially different from those reported in the first series of CPD-A1 red cell survivability studies: manufacturer I -- packed cell survivability = 81.3±5.8% ($\bar{X}$±SD, n=6), whole blood survivability 68.3±16.8% (n=2); manufacturer II -- packed cell survivability 71.3±7% (n=4), whole blood survivability 83.0±1.2% (n=2). These data contributed to those of several cooperating laboratories and were summarized and submitted to FDA in support of licensure for CPD-A1. In June 1978, FDA granted a license to one manufacturer to make available CPD-A1 for the storage of whole blood and packed cells for up to 35 days.

Licensure for the harvesting of components, however, has been withheld pending the results of survival and function studies of platelets stored in CPD-A1. The Department of Blood Research, LAIR in cooperation with several civilian laboratories is currently planning survival and function studies of platelets stored in CPD-A1 to support licensure extension for the storage of blood components in CPD-A1.

In all studies of red cell survival of packed cells stored in CPD-A1, it has been noticed that survivability is marginal after 35 days storage (grand mean ±SD: 72.8±9.5%, n=41). In vitro studies of red cell metabolism on erythrocytes stored in modified CPD-A1 suggest that increased adenine and glucose in the preservative will improve survivability. Such improvements may allow extension of red cell storage time to 42 days with the assurance that packed red cells will survive in an acceptable fashion. When the modification of CPD-A1 is specified to be near optimal on the basis of in vitro studies, this work unit will support clinical studies of red cell and platelet survivability/function in the new preservative in support of a licensure effort.

BODY OF REPORT

WORK UNIT NO. 004 CPD-Adenine Clinical Trials

PROBLEM

The original objective of this work unit was to execute clinical trials of blood stored in CPD-adenine in support of Food and Drug Administration licensure which would permit clinical use of red cells in military field situations. This objective has been partially realized with the licensure of CPD-A1 by FDA in June 1978. With the execution of these studies, however, two problems have emerged which require further effort. One is the recognition that red cell survival of packed cells stored for 35 days in CPD-A1 is marginal. Thus, while whole blood preserved in CPD-A1 exhibits acceptable survivability following 35 days storage, packed red cells are acceptable for only 28 days storage. The other problem is the necessity to evaluate the survival and function of blood components stored in CPD-A1. FDA has licensed CPD-A1 for the storage of whole blood and packed red cells only, and does not sanction the storage of platelet concentrates in CPD-A1. The utility of CPD-A1 is thus severely limited under current civilian and military blood banking practices as harvesting of components and component therapy is the rule. Thus, evaluation of platelet survival and function in platelet concentrates stored in CPD-A1 emerges as an important secondary requirement for full utility of CPD-A1 for extended storage for military field use. Moreover, it is apparent from in vitro studies of red cell metabolism, that the storability of red cells in CPD-A1 can be extended to 42 days with the proper adjustment in the adenine and glucose content of a modified CPD-A1 formulation.

RESULTS AND DISCUSSION OF RESULTS

Fourteen additional human in vivo autologous red cell survivability studies were performed in this laboratory on blood stored in CPD-A1 for 35 days. These studies were performed on the recommendation of the Food and Drug Administration following the revelation that some of the original CPD-A1 red cell survival studies were performed in blood that had been stored in bags which were mislabelled as to the plastic type. Whole blood and packed red cells stored in CPD-A1 within containers from 2 different manufacturers were performed. Red cell survival was similar to that reported in the first series of CPD-A1 red cell survivability studies: manufacturer I -- packed cell survivability = 81.3±5.8% ($\bar{X}$±SD, n=6), whole blood survivability = 68.3±16.8% (n=2); manufacturer II -- packed cell survivability = 71.3±7% (n=4), whole blood survivability = 83.0±1.2% (n=2). These data contributed to those of several cooperating laboratories

and were summarized and submitted to FDA in support of licensure of CPD-A1. In June 1978, FDA granted a license to one manufacturer to make CPD-A1 commercially available for the storage of whole blood and packed cells for up to 35 days.

Licensure for the harvesting of components, however, has been withheld pending the results of survival and function studies of platelets stored in CPD-A1. Widespread use of any new preservative system is contingent upon licensing for blood component preparation. Hence, immediate availability to the military of the CPD-A1 blood preservation system must await licensure for component preparation. For this reason, we, in cooperation with several civilian laboratories, are planning survival and function studies of platelets stored in CPD-A1 to support licensure extension to the storage of blood components in CPD-A1.

In all studies of red cell survival of packed cells stored in CPD-A1, it has been noticed that survivability is marginal after 35 days storage. Although the mean red cell survival of packed cells (hematocrit 70±5%) is greater than 70% (72.7±9.6%, n=40), it is apparent that greater than 30% of packed cell units stored in CPD-adenine for 35 days will exhibit survivabilities of less than 70%. In vitro metabolic studies on erythrocytes stored in modified CPD-A1 suggest that increased adenine and glucose in the preservative will improve survivability. Such improvements may allow extension of red cell storage time to 42 days with the assurance that packed red cells will survive in an acceptable fashion. When the modification of CPD--A1 is specified to be near optimal on the basis of in vitro studies, this work unit will support clinical studies of red cell and platelet survivability/function in the new preservative in support of a new licensure effort.

CONCLUSIONS

The original objective of this work unit has been partially achieved with the licensure of CPD-A1 for 35 days storage of whole blood and packed cells in June 1978. This laboratory, in cooperation with several civilian laboratories, is currently planning survival and function studies of platelets stored in CPD-A1 to support licensure extension for the study of blood components in CPD-A1. An improved CPD adenine formulation is being developed. This should eventually result in 42 days storage life for whole blood and red cell concentrates with acceptable survivabilities.

RECOMMENDATIONS

1. Studies of platelet survival/function stored in CPD-A1 should be performed in support of licensure extension for the storage of blood components in CPD-A1.

2. In vitro studies of a modified CPD-A1 with increased glucose and adenine concentrations should be performed as a prelude to in vivo evaluation of this improved CPD adenine preservative. The goal of these investigations should be the optimization of the improved CPD-A1 to provide unquestionably acceptable storability of packed erythrocytes for 42 day storage.

3. When these studies are completed and the appropriate modifications of adenine and glucose in the modified CPD-A1 formulation have been made, this laboratory should participate in cooperative clinical trials of red cells and platelets in the support of a licensure effort for the new preservative.

4. The department should participate with the manufacturers in applying for a new drug application and applying through the Surgeon General's Office for the required amendment to the Institutional Blood Bank License held by the Surgeon General to permit the Army, as dictated by military requirements, to use CPD-A1 or improved CPD-A1 formulations.

PUBLICATIONS

1. Anticoagulant Citrate-Phosphate-Dextrose-Adenine Solution, New Drug Application (NDA) No. 77-420, Section 3, II. Clinical Studies, Fenwal Laboratories, 1 Baxter Parkway, Deerfield, IL 60015, 1978.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6094	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. GRADE MTHD: NL	9a. SPECIFIC DATA: CONTRACTOR ACCESS ☒ YES ☐ NO	9b. LEVEL OF SUM: A. WORK UNIT

10 NO CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	005
b. CONTRIBUTING				
c. CONTRIBUTING	CARDS 1141			

11. TITLE (Procede with Security Classification Code)

(U) Evaluation of DEHP (di-2-ethylhexyl-phthalate)Disposition in Primates(Including Man)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016800 Toxicology; 012600 Pharmacology

13. STUDY DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 07	80 10	DA	C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE

	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
FISCAL YEAR (PREVIOUS) 78	1.0	45
FISCAL YEAR (CURRENT) 79	1.0	30

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
NAME: Marshall, J.D., Jr., COL, MC
TELEPHONE: (415) 561-3600

20. GENERAL USE

Foreign Intelligence Not Applicable

19b. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
ADDRESS: Division of Blood Research
Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Provide last name, first name, middle initial)
NAME: Peck, Carl C., LTC, MC
TELEPHONE: (415) 561-5875
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Jess, Donald A., Spec. 5
NAME

POC:DA

21. KEYWORDS (Provide key words and security classification code)

(U) Di-2-ethylhexyl-phthalate (DEHP); (U) Blood Storage Containers; (U) Plasticizer

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide individual paragraphs identified by number. Provide year of work and security classification code.)

23. (U) The objective is to determine the distribution, biotransformation, and elimination characteristics of di-2-ethylhexyl-phthalate (DEHP) in primates (including man), as a step towards assessment of the potential for human toxicity of this contaminant in blood stored in polyvinylchloride (PVC) bags. (PVC bags are currently the method of choice, in the military, for transport and storage of blood.)

24. (U) Following development of a sensitive and specific assay for DEHP and its metabolites, the dependence of DEHP leaching on hematocrit, plastic surface areas exposed, time, and temperature will be determined. The pharmacokinetics and metabolism of DEHP will be determined in the monkey and man.

25. (U) 77 10 - 78 09. The plasma enzyme responsible for conversion of DEHP to MEHP can be inactivated by heat. A protocol for the study of the pharmacokinetics and metabolism of 13-C-DEHP in man has been approved and initiated. Rhesus monkey plasma stored in PVC plastic bags accumulates DEHP and exhibits partial conversion of DEHP to mono-2-ethylhexyl phthalate (MEHP).

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

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 005 Evaluation of DEHP (di-2-ethylhexyl-phthalate) Disposition in Primates (including man)

The following investigations have been conducted under this work unit:

STUDY NO. 2 Factors affecting DEHP leaching

STUDY NO. 5 Pharmacokinetic evaluation of DEHP

STUDY NO. 8 Phthalic acid ester (PAE) loads in stored platelet rich plasma (PRP) and plasma used in simulated transfusion therapy

STUDY NO. 2 The transformation of DEHP into MEHP during cold storage appears to be due primarily to an enzymatic process. This process can be inhibited by heating plasma samples to 60 C for 30 min.

STUDY NO. 5 A protocol detailing a ^{13}C-DEHP pharmacokinetic and metabolism study in man has been prepared, submitted for review, and approved. A commercial source for ^{13}C-DEHP impregnated polyvinylchloride blood bags has been identified and has initiated preparation of prototype bags.

STUDY NO. 8 In order to evaluate reports of DEHP toxicity in monkeys exposed to autologous plasma stored in polyvinylchloride (PVC) bags, DEHP leaching and metabolism were studied in rhesus monkey plasma. Rhesus monkey platelet concentrate (PRP) stored in PVC plastic blood bags accumulates DEHP at approximately the same rate as human PRP under similar conditions. Transformation of a portion of leached DEHP to mono-2-ethylhexyl-phthalate (MEHP) occurs during storage of rhesus monkey platelet concentrate at a rate which is quantitatively less than that in human platelet concentrate. We conclude that studies of the toxicity of plasma stored in PVC bags should take into account both DEHP and MEHP exposure.

BODY OF REPORT

WORK UNIT NO.	005	Evaluation of DEHP (di-2-ethylhexyl-phthalate) Disposition in Primates (including Man)
STUDY NO.	2	Factors affecting DEHP leaching

PROBLEM

Increasing the storage duration for whole blood and packed red cells will improve the logistics of field transfusion therapy. The plasticizer di-2-ethylhexyl-phthalate (DEHP) leaches from polyvinyl-chloride (PVC) plastic blood bags into plasma during storage. Concern has been expressed about its potential toxicity in transfusion recipients. Since DEHP leaching from PVC increases approximately linear with time, prolonged storage may increase the potential toxicity. To assess properly the increased risk of exposure to DEHP, detailed studies of leaching characteristics, the factors affecting leaching rate, and pharmacokinetic evaluation are essential.

Developing ways to limit human exposure to DEHP (in plasma) requires identification of factors affecting DEHP leaching into plasma from PVC blood bags. This will permit selection of storage conditions which minimize leaching. During studies of ^{14}C-DEHP disposition in the African green monkey (Study No. 5, FY 76), small quantities of mono-ethylhexyl-phthalate (MEHP) were discovered in infusion plasmas. We have shown that MEHP arises de novo during cold storage of blood products, probably as a result of enzymatic conversion of leached DEHP to MEHP (Study No. 2, FY 7). Since MEHP is known to be at least as toxic as DEHP, more information regarding the origin of this blood storage contaminant is necessary to prevent inducing toxicity to military recipients.

RESULTS AND DISCUSSION OF RESULTS

The effect of heating plasma samples containing DEHP and MEHP has been studied with regard to heat effects on DEHP and MEHP conversion. Heating plasma samples in a water bath for up to 150 min at 60 C showed the conversion of DEHP to MEHP to be inhibited after 30 min. This observation has prompted the heating of all plasma samples from DEHP leaching experiments so that conversion of DEHP to MEHP is inhibited during sample storage. Analysis of timed heating experiments at incubation between 23, 53, and 60 C enabled a study of the relative contributions of enzymatic and nonenzymatic hydrolysis of DEHP to MEHP accumulation rate. Enzymatic hydrolysis of DEHP is largely responsible for MEHP accumulation during storage.

CONCLUSIONS

Heating plasma samples to 60 C for 30 min inhibits further hydrolysis of DEHP to MEHP and can be used to inhibit conversion of DEHP to MEHP during sample storage. Enzymatic hydrolysis of DEHP is largely responsible for MEHP accumulation during storage.

RECOMMENDATIONS

Rates of accumulation of DEHP and MEHP should be studied in new blood products developed in this laboratory. Factors which affect these phenomena should be further elucidated.

PUBLICATIONS

1. PECK, C.C., D.G. ODOM, H.I. FRIEDMAN, P.W. ALBRO, J.R. HASS, J.T. BRADY, and D.A. JESS. Di-2-ethylhexyl phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP) accumulation in whole blood and red cell concentrates. Transfusion (in press)

STUDY NO. 5 Pharmacokinetic evaluation of DEHP

PROBLEM

The pharmacokinetics and metabolic fate of DEHP in primates has been elucidated in a subhuman primate species (African green monkey; see annual report FY 76, this work unit) and preliminarily in man (see annual report FY 77, this work unit). In order to determine risks of toxicity from this blood storage contaminant during massive transfusion, the metabolic disposition of DEHP in man must be investigated.

RESULTS AND DISCUSSION OF RESULTS

A protocol has been prepared which details a plan for the elucidation of the human pharmacokinetics and metabolism of ^{13}C-DEHP which has been incorporated into standard PL-146 blood bags and leached into plasma. Plasma from volunteers will be stored in these bags for 72 h at room temperature, during which time ^{13}C-DEHP will accumulate in the plasma. Following reinfusion, serial blood and urine samples will be taken for up to 96 h for the study of the disposition and kinetics of DEHP and MEHP. The protocol has been reviewed by local and Washington-based human use committees and was approved in August 1978. A commercial firm has been contacted and agrees to incorporate ^{13}C-DEHP into standard PL-146 plastic blood bags for the purpose of this study.

CONCLUSIONS

The protocol for the study of the pharmacokinetics and metabolism of ^{13}C-DEHP in man has been prepared and approved. It is in the initial stages of execution.

RECOMMENDATIONS

A definitive investigation of the disposition of DEHP in man should be undertaken using ^{13}C-DEHP.

PUBLICATIONS

1. PECK, C.C. International Forum: What is the toxicological importance of the liberation of phthalates from plastic containers into blood, its components, and derivatives? Vox Sang 34:244, 1978

2. PECK, C.C., P.W. ALBRO, D.G. ODOM, and J.R. HASS. Plasticizers in stored blood. In: Microaggregates, edited by L. Kozloff. St. Louis: Warren H. Green, Inc. (in press)

3. PECK, C.C., P.W. ALBRO, J.R. HASS, D.G. ODOM, B.B. BARRETT, and F.J. BAILEY. Metabolism and excretion of the plasticizer di-2-ethylhexyl phthalate in man. (Abstract) Clin Res 26:101A, 1978

STUDY NO. 8 — Phthalic acid ester (PAE) loads in stored platelet rich plasma (PRP) and plasma used in simulated transfusion therapy

PROBLEM

Hepatotoxicity ascribed to DEHP in stored monkey plasma has been reported (J Lab Clin Med 89:1066, 1977). In appraising such reports, it is necessary to know whether or not such plasmas contain MEHP in addition to DEHP since MEHP is at least as toxic as DEHP.

RESULTS AND DISCUSSION OF RESULTS

Platelet rich plasma (PRP) and platelet poor plasma (PPP) was harvested from 2 rhesus monkeys and stored in PVC blood bags for 3 days at 22 C. Results of these studies and values for human PRP stored under identical conditions are detailed below:

Source	[DEHP] n moles/ml	[MEHP] n moles/ml
PPP-rhesus monkey (n=1)	651.9	19.9
PRP-rhesus monkey (n=1)	963.4	9.4
PRP-human (n=3)	916±57.3	32.0±8.6 (S.D.)

CONCLUSIONS

While DEHP accumulation in human and the one rhesus monkey PRP studied are similar, MEHP concentrations in stored human PRP is 3 to 4 times that of the one rhesus monkey PRP studied. More studies of stored rhesus monkey PRP are needed before definitive conclusions can be made.

RECOMMENDATIONS

Studies of toxicity of DEHP in blood products should also take into account toxicity due to exposure from MEHP.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

DA OE 6098	78 10 01	DD-DR&E(AR)636					
77 10 01	E.Termination	U	U	NA	NL	YES	A

	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	006
b. CONTRIBUTING				
CARDS	114f			

(U) The Toxicity of Adenine When Used in Blood Anticoagulant Solution

016800 Toxicology; 012600 Pharmacology; 008800 Life Support

75 07	78 06	DA		C. In-House

	FISCAL YEAR 78	0.8	66
	FISCAL YEAR 79	0.0	00

a. NAME: Letterman Army Institute of Research
a. ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL
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GENERAL USE
Foreign Intelligence Not Applicable

a. NAME: Letterman Army Institute of Research
Division of Blood Research
a. ADDRESS: Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR
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ASSOCIATE INVESTIGATORS
NAME: Peck, Carl C., LTC, MC

POC:DA

(U) Adenine; (U) Blood Preservation; (U) 2,8 Dioxyadenine; (U) Nephrotoxicity

23. (U) The 21 day storage limit of blood used to transfuse the wounded soldier can be increased 66 to 100% by the addition of adenine to the preservative. Metabolic and kinetic data will be gathered to define the efficacy and toxicity of adenine when used as part of an anticoagulant solution for the storage of human blood. Investigations will be conducted to determine if 2,8 dioxyadenine, a catabolic metabolite of adenine, could accumulate in sufficient quantities in the massively transfused individual to cause toxicity problems.

24. (U) The rate of transport of adenine into red cells during cold storage (whole blood and red cell concentrates) and the rate of conversion to adenine nucleotides will be investigated. The kinetics of adenine catabolic metabolism by xanthine oxidase will be evaluated.

25. (U) 77 10 - 78 09. Adenine was used along with other chemicals as part of optional additive systems (OAS) to provide improved long term storage for packed red cells. Examination of several formulations showed that one containing adenine, glucose, and ascorbate-2-phosphate holds promise for long term storage (at least 42 days) with acceptable viability and good oxygen transport properties. The OAS studies will continue under a new work unit.

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

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 006 The Toxicity of Adenine When Used in a Blood Anticoagulant Solution

The following investigations have been conducted under this work unit:

STUDY NO. 2 Diffusion of adenine into and out of red blood cells during cold storage

STUDY NO. 3 Adenine metabolism and kinetics in CPD-adenine stored blood

STUDY NOS. 2 and 3 Experiments in this work unit during FY 78 were directed toward evaluating the effect of adenine and other chemical additives on the preservation of packed cells. An optional additive system (OAS) was used by which preservative chemicals were added to packed cells that had been drawn in CPD. Exploration of several systems showed one of exceptional merit. This one contained glucose, adenine, and ascorbate-2-phosphate (AsP). While the system is not yet optimized, it appears to provide for red cell survival and normal oxygen transport as measured by 2,3-DPG and P_{50} for at least 42 days of storage.

BODY OF REPORT

WORK UNIT NO.	006	The Toxicity of Adenine When Used in a Blood Anticoagulant Solution
STUDY NO.	2	Diffusion of adenine into and out of red blood cells during storage
STUDY NO.	3	Adenine metabolism and kinetics in CPD-adenine stored blood

PROBLEM

Our goal is (1) to gather data on the efficacy and potential toxicity of adenine when used as part of an anticoagulant solution for the storage of human blood and (2) to maximize the beneficial effects of adenine in both whole blood and red cell concentrates (RCC) when part of the anticoagulant mix, or when used in an optional additive system (OAS). In addition, we will determine the kinetics of adenine uptake, egress, and metabolism by red cells in the above situations, and determine the kinetic limitations of conversion of adenine to its insoluble oxides by xanthine oxidase.

RESULTS AND DISCUSSION OF RESULTS

The utilization of adenine in packed red cells, when the OAS approach is used, has been studied in 4 experiments. The OAS storage method involves drawing blood in CPD and harvesting plasma components from it. The packed cells remain in the primary bag then are mixed with a small volume of OAS solution that is contained in a small satellite bag attached to the main bag. This allows for extended liquid storage of the red cells in the most desirable combination of chemical additives without exposing the plasma components to these additives.

In the first study, cells were treated with 0.5 mM adenine, 16 mM glucose, and 30 mM dihydroxyacetone (all concentrations listed after dilution in blood). The addition of solution was at one of three time intervals, Day 0, 7, or 14. Addition of OAS solution at any of the time intervals maintained ATP levels above 50% of the starting values for 42 days of storage. The use of OAS solution caused significant improvement of 2,3-DPG maintenance, but only when it was added at Days 0 or 7.

In the second OAS experiment, pyruvate, mannitol, and sodium phosphate were added to the OAS formulation in an attempt to achieve further increases in ATP and 2,3-DPG while reducing red cell hemolysis. The OAS was added to the cells on Day 0 and 7. With this formulation, red cell ATP levels were less well maintained than in the first experiment, while 2,3-DPG levels were the same in both experiments. The OAS solution had increased osmolarity (>2000 milliosmols) which causes noticeable shrinkage of the cells during storage, but measurements of mean corpuscular volume indicated that the cells returned to normal size when diluted in normal isotonic solutions.

The third OAS formulation tested consisted of 16 mM glucose and 5 mM ascorbate-2-phosphate (AsP) with or without 0.25 mM adenine. Glucose-adenine controls were also run, with all OAS addition to red cells done on Day 0. The OAS formulations containing AsP all held 2,3-DPG levels in excess of 80% of the starting values for 42 days of storage. AsP does, however, cause a drop in red cell ATP during storage. In the fourth OAS experiment, correction of the latter defect was attempted by lowering the AsP concentration to 3.5 mM and using increasing doses of adenine (0.25 to 0.50 mM). This increase in adenine (with less AsP) results in 42-day ATP levels of about 40% of initial levels. The 2,3-DPG levels were well maintained as before, and measurements of P_{50} from Day 21 to Day 42 showed excellent maintenance of hemoglobin function through Day 42. The Day 42 P_{50} values range from 21 to 25 mm Hg; fresh blood is 25 mm, and CPD stored blood is usually down to 16 mm by Day 21.

CONCLUSIONS

The optional additive system appears advantageous for 42 day storage of packed red cells. The cells should remain viable, as measured by ATP levels, and have excellent oxygen transport properties, as shown by maintenance of 2,3-DPG at fresh blood concentrations, as well as normal P_{50} values. The OAS solution with AsP has yielded cells with superior oxygen transport properties to those seen in any other system. The OAS approach has great flexibility in that the plasma components are not exposed to the OAS chemicals and, in addition, the use of OAS may be delayed up to 7 days after drawing. Thus, if the blood is used during the first week of storage, the OAS chemicals would not be in it.

RECOMMENDATIONS

Studies should continue to evaluate the OAS system, containing AsP, glucose, and adenine, for use in long term storage of packed red cells. The toxicity and mechanism of action of AsP

needs to be determined. Studies should be instituted to determine concentrations of additives which simultaneously maximize ATP and 2,3-DPG. This will be done in a new work unit designed to integrate the Army liquid blood preservation R&D effort into a cost-effective targeted program.

PUBLICATIONS

1. MOORE, G.L., M.E. LEDFORD, and D.E. BROOKS. The distribution and utilization of adenine in red blood cells during 42 days of 4 C storage. Transfusion 18:538, 1978

2. MOORE, G.L., M.E. LEDFORD, and D.E. BROOKS. Plasma adenine and cellular ATP in red cell concentrates collected and stored in modified CPD at 4 C. Transfusion (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6099	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY CITY: U	6. WORK SECURITY: U	7. DOWNGRADING: NA	8. GROUP MARK: NL	9a. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	9b. LEVEL OF SUM: A. WORK UNIT
10 NO /CODES: PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3S162772A814		00	007		
b. CONTRIBUTING							
c. [illegible]	CARDS 114f						

11. TITLE (Protect with Security Classification Code): (U) Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 002600 Biology; 003500 Clin Medicine; 009800 Medical and Hosp Equip; 012900 Physiology

13. EVALY DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY		16. PERFORMANCE METHOD
75 07	CONT	DA		C. In-House

17. CONTRACT/GRANT
a. DATE/EFFECTIVE: Not Applicable
b. NUMBER:
c. TYPE:
d. AMOUNT:
e. END OF AGRMT:
f. CUML. AMT.

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
78	0.5	27
79	0.5	18

19. RESPONSIBLE DOD ORGANIZATION
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20. PERFORMING ORGANIZATION
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Division of Surgery
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d. ASSOCIATE INVESTIGATORS
NAME:
DATE:

21. GENERAL USE: Foreign Literature Reviewed

POC: DA

22. KEYWORDS (Precede Each with Security Classification Code): (U) Rapid Screening Techniques; (U) Blood Preservation; (U) Temperature; (U) Blood Respiratory Function

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

23. (U) The objectives are to devise an accelerated method for testing anticoagulant-nutrient preservatives by using elevated temperatures; to determine if rates of change of the observed parameters during storage are predictable on a chemical-kinetic basis; to apply such techniques to rapid screening of substances and techniques potentially beneficial to preservation of blood; and to evaluate the therapeutic effectiveness of blood so preserved for the treatment of battlefield injuries.

24. (U) Results have shown that the rates of change of functionally significant substances, such as H^+, 2,3-diphosphoglycerate (2,3-DPG), and adenosine triphosphate (ATP), in blood stored at 4 C can be predicted from observations at 37 C. As rates of change are often 30-fold greater than at 4 C, observations at 37 C of the effects of experimental anticoagulant-nutrient preservatives on blood storage can be condensed to about 24 hours compared to 35 or so days required at 4 C. This high temperature approach to such screening will be continued and extended to (a) optimize the composition of preservative media, (b) identify factors altering the collection and storage lesion, and (c) understand differences in individual donors with respect to blood storageability.

25. (U) 77 10 - 78 09 CPD preserved blood accumulates less fixed acid at 0 than at 4 C. Rate of loss of organic phosphates at 0 C is essentially the same as at 4 C, except during prolonged storage. There is an increase of blood ATP during the first week of storage at 8 C, but subsequent losses are more rapid than with storage at 0 or 4 C. Erythrocytes density segregated by centrifugation display large differences in viscosity. DPG is not well correlated with P_{50} when the molar ratio of DPG/Hgb is above 0.5. Under some experimental conditions, negative correlations between DPG and P_{50} are observed.

* Available in connection with contractor's request.

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

ABSTRACT

PROJECT NO.	3S762772A814	Military Trauma and Resuscitation
WORK UNIT NO.	007	Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives

The rates of loss of 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP), and the rate of accumulation of H^+ ions during long-term storage (6 weeks) at temperatures between 37 and 4 C agree with the thermokinetic (Arrhenius) equation. Thus, when these factors are screened at 37 C, a rapid assessment of the value of proposed blood preservatives is possible. The optimal liquid storage temperature for citrate-phosphate-dextrose (CPD) preserved blood appears to be near 0 C, although initial storage at 8 C for several days after collection may improve ATP retention. The erythrocytic properties of whole blood reflect the integrated effects of the individual red cells; thus, individual variability of donor blood during storage possibly reflects in part the age distribution of the donor's red cells. Observations of red cells kept at conditions (excess CPD, low pH, elevated temperature) similar to those occurring during the initial phase of blood collection revealed an unexpected and large decrease in hemoglobin oxygen affinity with a concurrent rapid fall in DPG. This negative correlation between P_{50} and DPG is being examined with particular regard to the collection lesion (i.e., the apparent damage to the first fraction of cells to be collected from a donor).

BODY OF REPORT

WORK UNIT NO. 007 Development of a Rapid System for Assessing Blood Anticoagulant-Nutrient Preservatives

PROBLEM

The supply and quality of liquid stored blood used for transfusion purposes in wounded soldiers can be seriously limited by the exigencies of warfare. Therefore, there is a unique military requirement for prolonging the present storage limit for preserved blood and improving the quality of this product. Most experiments designed to improve preserved liquid blood generally are performed at 4 C, presumably on the pragmatic ground that this temperature normally is used for liquid blood storage. For several reasons, however, this pervasive experimental approach may be undesirable. In the first place, the practice tends to perpetuate the notion that 4 C is the optimum temperature for blood storage; actually, this idea has not received extensive experimental inquiry. Furthermore, individual experiments at 4 C usually require prolonged periods of time to complete (up to 6 weeks or more). Because of the variability found in the blood of individual donors and the multiple permutations in storage conditions and preservation media that may require testing, satisfactory confidence in a new media or preservation idea can be slow in developing. Finally, phenomena important to the full understanding of the collection and storage lesions may be suppressed or imperfectly developed at 4 C. Therefore, the objective of this work unit is to devise accelerated means for evaluating blood preservatives and other variables affecting red cell storage in order (1) to economize on valuable technical resources, (2) to provide alternative theoretical approaches to understanding the deterioration of red cells during collection and storage, and (3) to develop a scientific basis for determining the optimal liquid storage temperature of blood.

RESULTS AND DISCUSSION OF RESULTS

Rates of change, expressed as the time in days required to double (H^+) or halve (DPG) and (ATP) the original concentrations of the constituents observed, are predictably affected by the storage temperature between 37 and 4 C. When the logarithm of the rate is plotted against the reciprocal of the absolute temperature (Arrhenius relation), a linear relationship is obtained. The rationale of this finding and the regularity with which it was obtained suggested, first, that the effects of experimental blood preservatives on stored blood could be advantageously examined at 37 C, where changes that require several weeks to develop at 4 C become manifest within several hours; second, that because there had been no suggestion of a change in "activation energy" (i.e., the slope of the Arrhenius plot) over the temperature range studied, the results might be extrapolatable to the freezing point of

blood (about -0.5 C). The latter speculation implies that the optimal storage temperature of blood, at least in terms of organic phosphate retention and suppression of H^+ ion accumulation, was lower than that in common use (4 C). An experimental test of this hypothesis in the temperature range between 0 and 8 C confirmed that H^+ ion accumulates less rapidly at 0 C (doubling time approximately 40 days) than at higher temperatures (doubling time approximately 27 days at 4 C). In the case of DPG and ATP, however, the loss of these compounds appeared to proceed at the same rate at 0 C as at 4 C (ATP halved in 30 days; DPG in 15 days). Although the rate of change (as described above) for each of these constituents at 8 C was consistent with previous findings, the concentration of ATP at this temperature increased markedly during the first week of storage. Increases of ATP in blood stored in CPD are sometimes noted at other temperatures. The consistency and degree to which this tendency was expressed at 8 C may explain earlier findings that had indicated that 7-8 C was an optimal temperature for blood storage. It should be noted, however, that after approximately one week of storage at this temperature, the rate of loss of ATP is considerably faster than at 0 or 4 C. Because ATP is thought to be an important chemical intermediate in relation to cell deformability and 24-hour in vivo survivability, retention of this constituent might be favored by initial storage of blood at 8 C for several days after collection, subsequent storage, if necessary, being continued at lower temperatures (0-4 C).

The collection lesion apparently results from the various physical and chemical manipulations of blood taken from donors for storage purposes. It has been suggested that this lesion may arise during the initial stages of blood collection when the preservative/blood ratio is far from its designed value. During the collection period and before blood is refrigerated, pH tends to be much lower and temperature much higher than during storage. There is contradictory evidence on whether or not ordinary variation in the time required for collecting and processing blood significantly alters the quality of the product; but it is generally conceded that inherent (usually unspecified) differences in blood from different donors does have an important effect in this regard. It is important to note that the collection lesion manifests itself most prominently after relatively prolonged storage. Consequently, the collection lesion problem is of much greater concern in situations such as those encountered by the military where logistic requirements extend required storage times. When erythrocytes are incubated (37 C) in an excess of CPD to simulate the conditions to which the first fraction of cells collected from a donor are exposed, pH rapidly increases (to about 6.3) from a low starting pH of about 6.0. This unusual pH change, opposite that observed with incubated blood having normal amounts of CPD, is associated with a rapid decrease in DPG and a pronounced increase in the P_{50} of oxyhemoglobin. While unconfirmed as yet, it appears reasonable that these changes are a result of an efflux of K^+ ion from the erythrocyte and an uptake of both Na^+ and H^+ ion. When these cells are returned to plasma or buffers in the

alkaline pH range (7.2-7.3), they have an enhanced transmembrane pH. The lower intracellular pH apparently accounts for the increased P_{50} that is observed in spite of the extremely low DPG values. Under conventional blood storage conditions (normal CPD and 4 C), a similar lack of correspondence between DPG values and oxygen affinity has been observed also, particularly in the first week or two of storage. Possibly, this experimentally produced "lesion" also occurs when standard blood collection and storage procedures are used.

There is some evidence to suggest that erythrocyte age may play a role in the deterioration process that occurs during the collection and liquid storage of blood. Although the idea has been challenged, it would appear that the matter has not actually received much experimental attention. It is generally agreed, however, that the deterioration or aging of erythrocytes during storage is different from the normal aging that occurs in vivo. For these reasons, an attempt is being made to investigate this problem by segregating cells according to age with high speed centrifugation and subsequently following the effects in these segregated cells of various storage conditions at elevated temperatures. There are numerous physical, chemical, and functional differences between young and old cells, and it seems hypothetically reasonable that they may respond differently to various storage environments. Our data indicate, for instance, that oxygen affinity (P_{50} and n), deformability, and viscosity vary greatly between young and old cells. The functional attributes of mixtures of young and old cells, moreover, appear to reflect the averaged attributes of the individual cells, but these "averages" can be insensitive to gross functional and chemical changes in the various age groups. A similar problem can exist in attempting to optimize the quality and extend the storage period of preserved blood by measuring "average" biochemical changes when the important alterations actually may be restricted to erythrocytes of a certain age group.

CONCLUSIONS

1. Storage of CPD-preserved blood at 0 C diminishes the rate of accumulation of fixed acid and for this reason, 0 C storage should improve the quality of transfused blood, particularly when this blood is stored for long periods.

2. High temperatures can be used to screen potentially useful preservative mixtures, thereby affording great economy of experimental resources and accentuating phenomena possibly related to the collection and storage lesions.

3. Large changes in blood quality can occur as a result of its exposure to excess CPD preservative during collection.

RECOMMENDATIONS

In vivo survivability of blood stored at 0 C should be tested and compared with 4 C blood. Because of the departure from LAIR of the co-investigator, this aspect of the work unit has not been accomplished. Efforts are being made to find a replacement medical officer able to perform such measurements.

A modified means of collecting blood that avoids exposure to excess preservative should be explored.

PUBLICATIONS

1. NEVILLE, J.R., T.A. BENSINGER, and T.F. ZUCK. Effect of temperature on blood storage. (Abstract) Transfusion 16:517, 1977

2. NEVILLE, J.R. Erythrocyte age and shape of the oxygen dissociation curve. (Abstract) In: Proc of the International Union of Physiologic Science XIII, 1977. p 548

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA OE 6305	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636					
3. DATE PREV SUMMARY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY ACTY: t	6. WORK SECURITY: U	7. REGRADING: NA	8. WORK MVMT: NL	9. 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	62772A	3S162772A814		00		008	
b. CONTRIBUTING							
c. EXTENSION	CARDS 114f						

11. TITLE (PRIMARY AND SECONDARY CLASSIFICATION CODE)

(U) Studies in Anesthesia for Combat Injured Soldiers

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support; 009800 Medical and Hospital Equipment; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: — b. NUMBER: Not Applicable — c. TYPE: — d. KIND OF AWARD: — e. AMOUNT: f. CUM AMT.: —

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
78	0.6	115
79	1.5	112

19a. PERFORMING ORGANIZATION

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19b. RESPONSIBLE INDIVIDUAL

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TELEPHONE: (415) 561-3600

20. GENERAL USE

Foreign Literature Reviewed;
Foreign Intelligence Considered

19a. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Division of Surgery
ADDRESS: Presidio of San Francisco, CA 94129

19b. PRINCIPAL INVESTIGATOR (PERSON DOING WORK REPORTED)

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Townsley, Mary I., DAC
NAME: POC: DA

21. KEYWORDS (U) Anesthesia; (U) Combat Injury; (U) Respiratory Physiology; (U) Cardiovascular Physiology; (U) Anesthesia-related Equipment

22. TECHNICAL OBJECTIVE, APPROACH, PROGRESS (PAST AND PLANNED)

23. (U) Because their administration is extremely hazardous in subjects with acute hypovolemia, anesthetic agents are avoided in injured civilians prior to replacement of blood volume. However, timely combat injury care in field settings frequently requires anesthetic administration to soldiers with incompletely replaced blood loss. The objective of this work unit is to establish physiologic data on the effects of anesthesia during acute hypovolemia. These data will permit appropriate selection of premedications, anesthetic agents and adjuncts, and anesthetic procedures for field use. Requirements for anesthetic-related equipment for field use also must be identified, and available equipment evaluated.

24. (U) The effects of premedications, anesthetic agents, anesthetic adjuncts during hypovolemia, with and without volume replacement, and their interactions on cardiopulmonary function will be studied in dogs. The influence of these factors on the cardiovascular system will also be studied in dogs and swine. Attempts will be made to determine the applicability of the data from these studies to the combat-injured soldier.

25. (U) 77 10 - 78 09 Several anesthetic agents have been investigated for their comparative ability to support cardiopulmonary function during hypovolemia. Although ketamine stimulates the cardiovascular system during normovolemia, there are no cardiovascular differences between halothane and ketamine with loss of 30% of total blood volume. At 30% blood loss, ketamine resulted in substantial metabolic acidosis, while there was no detectable acidosis with halothane. In the swine cardiopulmonary bypass model, morphine failed to alter left ventricular function or myocardial metabolism; halothane depressed left ventricular function and myocardial oxygen consumption equally without resultant anaerobic metabolism. The name of the work unit has been changed to more clearly reflect the nature of the research.

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

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 008 Anesthetic Management and Perioperative Care of the Acutely Wounded Soldier

The following investigations have been conducted under this work unit:

STUDY NO. 1 Selection of anesthetic agents for administration to the acutely wounded soldier

STUDY NO. 2 Evaluation of myocardial performance during anesthesia

STUDY NO. 3 Evaluation of tri-service experimental portable volume respirator

STUDY NO. 4 Basic studies in acid-base balance of human blood and laboratory animal blood

STUDY NO. 1 To provide the anesthesiologist with information regarding the comparative cardiorespiratory effects of anesthetic agents during moderate hemorrhage, we have initiated a series of experiments in chronically maintained dogs. We have, thus far, studied halothane 1.0% and ketamine at the minimal anesthetic dose. During normovolemia, ketamine produced a relative hyperdynamic cardiovascular state. With 30% blood loss, there were no cardiovascular differences between halothane and ketamine anesthetized dogs; however, with ketamine, oxygen consumption was increased and a metabolic acidosis resulted.

STUDY NO. 2 To provide comparative data for anesthetic agent effect on heart function and metabolism, we have utilized the swine right heart pulmonary by-pass model. The model allows for controlled heart rate, pre-load, and after-load. Morphine sulfate did not change myocardial function or metabolism. Halothane resulted in a 45% decrease in stroke volume and oxygen consumption, without alterations in lactate extraction or arterial coronary sinus base-excess.

STUDY NO. 3 We are testing a tri-service portable volume controller ventilator received from the U.S. Naval Medical Research and Development Command. Bench tests have indicated that the ventilator meets its major specifications. Completion of bench testing and animal testing awaits replacement of components.

STUDY NO. 4 To evaluate properly the data accumulated in Studies Nos. 1 and 2, it has been necessary to provide data regarding the acid-base chemistry of swine, canine, and human blood. The base-excess correction coefficient (related to the Haldane coefficient) for canine blood was found to be approximately 34% higher than human blood. Swine log PCO_2-pH curve and base-excess alignment nomograms have been constructed. Similar human nomograms are being constructed.

BODY OF REPORT

WORK UNIT NO.	008	Anesthetic Management and Perioperative Care of the Acutely Wounded Soldier
STUDY NO.	1	Selection of anesthetic agents for administration to the acutely wounded soldier

PROBLEM

The physiological effects of many of the currently used anesthetic agents are known in great detail in both laboratory animals and humans. However, little information is available regarding the physiological effects of these agents during states of deranged physiology. This is especially true regarding the effect of anesthetic agents during the hypovolemic state. This lack of knowledge is most likely the result of usual civilian practice of restoring blood volume to normal prior to induction of anesthesia. In the vast majority of civilian injuries, this is not only desirable but possible. Unfortunately, the nature and extent of many military injuries require induction and maintenance of anesthesia in an acutely hypovolemic soldier in order that a surgical procedure may be performed to prevent further blood loss and allow restoration of blood volume. The choice of anesthetic agents in this situation, at the current time, can only be based upon anecdotal information, personal preferences, and experience of the anesthesiologist.

RESULTS AND DISCUSSION OF RESULTS

We have embarked upon a series of experiments to provide data comparing several anesthetic agents for their ability to preserve physiological function during moderate hypovolemia. In chronically maintained splenectomized dogs who have been provided with chronic tracheostomies and exteriorized carotid arteries, we have evaluated cardiorespiratory function during graded hypovolemia. The dogs were anesthetized with halothane on one occasion, which was maintained at an end-tidal concentration of 1.0%, and on a second occasion with ketamine, which was administered via an intravenous drip at the minimal dose required to maintain anesthesia. Step-wise reductions of 10, 20, and 30% of each dog's blood volume were successively made. During halothane anesthesia, graded hemorrhage to 30% caused a significant fall in mean arterial blood pressure, cardiac output, stroke work, minute work, and oxygen transport. The stimulus provided by ketamine during normovolemia produced significantly higher values for these parameters than were measured during halothane anesthesia. These values fell during graded hemorrhage so that, with ketamine at 30% blood loss, blood pressure, cardiac output, stroke volume, stroke work, minute work, and oxygen transport were not different from the values at 30% blood loss with halothane, despite a significantly higher heart rate that had not changed with hemorrhage. Mean pulmonary arterial pressure decreased

progressively with hemorrhage, but was significantly different between ketamine and halothane. Oxygen consumption was significantly higher with ketamine anesthesia than with halothane anesthesia during normovolemia and during all levels of hypovolemia. Hemorrhage to 30% did not alter oxygen consumption from the normovolemic values with either anesthetic agent. Despite the fall in oxygen transport and the sustained oxygen consumption with hemorrhage, no change in base-excess was detected during halothane anesthesia. During normovolemia, base-excess with ketamine was not different from halothane. Base-excess decreased progressively with hemorrhage during ketamine and was significantly less at all levels of oligemia than during halothane. We conclude that the cardiorespiratory stimulation provided by ketamine during normovolemia lessens with 30% blood loss. This resulted in apparently inadequate compensation for the increased oxygen consumption and created a progressive metabolic acidosis. The latter was reflected in a progressively decreasing base-excess, which we did not detect during equivalent hypovolemia with halothane.

CONCLUSIONS

Ketamine appears to have adverse metabolic effects when used for maintenance of anesthesia during moderate hypovolemia. We did not detect such adverse effects with the use of halothane.

RECOMMENDATIONS

Recommendation for choice of anesthetic agent given to an injured soldier cannot be made until the effects of other anesthetic agents are compared with the effects of halothane. Therefore, it is recommended that (1) further physiological studies be carried out to assess physiological effects of halothane during graded hypovolemia; (2) other anesthetic agents be similarly investigated so that their effects may be compared with the effects of halothane, and (3) offer information to enable anesthesiologists to make a more rational choice regarding the proper anesthetic agent for use in the anesthetization of the acutely wounded soldier.

PUBLICATIONS

1. WEISKOPF, R.B. Editorial: Regulation of respiration and halothane. Anesthesiology, in press.

2. WEISKOPF, R.B., M.I. TOWNSLEY, K.K. RIORDAY, K.R. CHADWICK, and H.A. BRINKS. Cardiovascular responses to moderate hemorrhage during halothane and ketamine anesthesia. (Abstract) American Society of Anesthesiology, Abstract Papers, 1978.

3. TOWNSLEY, M.I., H.A. BRINKS, and R.B. WEISKOPF. Measurement of enflurane and isoflurane by mass spectrometry. (Abstract) American Society of Anesthesiology, Abstract Papers, 1978.

STUDY NO. 2

Evaluation of myocardial performance during anesthesia

PROBLEM

Although anesthetic agents are generally considered myocardial depressants, firm physiological data, with all appropriate variables controlled, are not available. We have therefore undertaken a series of experiments to provide such data.

RESULTS AND DISCUSSION OF RESULTS

Myocardial effects of anesthetic agents are being evaluated in the right heart pulmonary bypass swine model (see LAIR Work Unit No. 012). Fifteen swine were divided into three equal groups. All swine were anesthetized by conduction anesthesia. During the test periods in one group, conduction anesthesia was continual; to the second group, 10 mg/kg morphine was administered; to the third group, 0.5% halothane was administered. At constant after-load (mean arterial pressure 65 torr) and constant pre-load (left ventricular end diastolic pressure 14 torr), there was no significant change in left ventricular stroke volume during the experimental period, in comparison to the control period when no anesthetics were added to conduction anesthesia. The addition of morphine (10 mg/kg added to the extracorporeal circulation circuit) did not significantly alter myocardial performance. Addition of halothane 0.5% depressed stroke volume by 43%. This halothane-induced depression was significantly different from the lack of change in either the conduction anesthesia group or the group to which morphine had been administered. Coronary blood flow during the working period as measured directly by coronary sinus drainage did not change in any of the 3 groups. Myocardial oxygen consumption did not change in either the control group or in the group to which morphine had been administered. Administration of halothane caused a 45% reduction in myocardial oxygen consumption. This decrease in oxygen consumption was significantly different from the response of the other 2 groups. Values for myocardial lactate extraction did not change in any of the 3 groups, nor were there statistically significant differences among the 3 groups. Similarly, arterial-coronary sinus base-excess differences did not change between the control and test periods in any of the 3 groups.

CONCLUSIONS

We conclude that in the swine left ventricular preparation, with controlled heart rate, pre-load, after-load, arterial blood gases, and pH, that (a) morphine sulfate 10 mg/kg does not alter coronary blood flow, myocardial oxygen consumption, or myocardial performance (stroke volume); (b) halothane 0.5% does not alter myocardial blood flow but reduces equally both myocardial oxygen consumption and stroke volume by approximately one-half; (c) lack of alteration of myocardial lactate extraction tends to indicate that neither morphine nor halothane,

within the limits of this experimental model, result in detectable myocardial anaerobic metabolism.

RECOMMENDATIONS

It is recommended that further physiological studies be carried out to assess the relative influences of other anesthetic agents upon myocardial performance and metabolism.

PUBLICATIONS

1. WEISKOPF, R.B., W.Y. MOORES, K.K. RIORDAN, M.I. TOWNSLEY, D. WILLFORD, W.P. DEMBITSKY, K. CHADWICK, and J. CRUM. Depression of swine left ventricular function by halothane. (Abstract) Clin Res 26:98A, 1978.

2. WEISKOPF, R.B., W.Y. MOORES, K.K. RIORDAN, M.I. TOWNSLEY, J.D. CRUM, D.C. WILLFORD, and W. DEMBITSKY. Left ventricular dynamics: A comparison of morphine and halothane during normoxia. (Abstract) American Society of Anesthesiology, Abstract Papers, 1978.

STUDY NO. 3 Evaluation of tri-service experimental portable volume respirator

PROBLEM

All 3 uniformed military services have recognized the need for a portable volume controlled ventilator capable of ventilating injured military personnel while in the field, during any one of the many modes of transportation and evacuation, as well as in the hospital environment. Currently, commercially available ventilators cannot meet these requirements. As a result, primarily through the Office of Naval Research, General Electric Re-entry and Environmental Systems Division has produced an experimental ventilator designed to meet these needs. Twelve prototypes have been produced. This prototype now requires full laboratory and clinical testing prior to acceptance by the 3 services for production and deployment.

RESULTS AND DISCUSSION OF RESULTS

Through the U.S. Naval Medical Research and Development Command, this laboratory has received for evaluation for the U.S. Army Medical Research and Development Command a prototype model of the tri-service experimental portable volume controlled respirator. We have completed the majority of required bench testing, and the ventilator meets its specifications in all major aspects. There have been several major failures of the ventilator which have required long delay periods for repair. Both oxygen sensors in the ventilator have failed, and additional studies are suspended awaiting receipt from USNMRDC of replacement oxygen sensors.

CONCLUSIONS

None

RECOMMENDATIONS

Bench testing should be completed and animal testing should be initiated upon the receipt of replacement oxygen sensors.

PUBLICATIONS

None

STUDY NO. 4 — Basic studies in acid-base balance of human blood and laboratory animal blood

PROBLEM

In order to assess appropriately the data accumulated in Studies Nos. 1 and 2, the acid-base relationships of the laboratory animals used must be known. Such information is not available. This study will provide the basic physiochemical data for dog, swine, and human blood.

RESULTS AND DISCUSSION OF RESULTS

To support the animal research indicated in Studies Nos. 1 and 2, we have examined basic acid-base parameters of canine and swine blood. We have determined that the oxygen-linked hydrogen ion binding of canine hemoglobin is different from that of human hemoglobin, the base-excess correction factor being approximately 34% greater than that for human blood (0.26 mEq/l per gram desaturated hemoglobin). We have constructed an acid-base nomogram for pig blood (hemoglobin independent curve plot of log PCO_2 versus pH), and have found this plot to be significantly different from the accepted standard for human blood. We are currently developing acid-base alignment nomograms for both swine and human blood.

CONCLUSIONS

None

RECOMMENDATIONS

The study should be continued until completion.

PUBLICATIONS

1. RIORDAN, K.K., M.I. TOWNSLEY, K.R. CHADWICK, and R.B. WEISKOPF. Oxygen-linked hydrogen ion binding of canine hemoglobin. (Abstract) Clin Res 453A, 1978.

2. RIORDAN, K.K., M.I. TOWNSLEY, K.R. CHADWICK, H.A. BRINKS, and R.B. WEISKOPF. Acid-base nomogram for pig blood. Physiologist 21:99, 1978.

3. RIORDAN, K.K., R.B. WEISKOPF, M.I. TOWNSLEY, and K.R. CHADWICK. Oxygen-linked hydrogen ion binding of canine hemoglobin. J Appl Physiol, in press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6306	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. HANDLING	9. SPECIFIC DATA-CONTRACTOR ACCESS	10. LEVEL OF SUM
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

11. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	009
b. CONTRIBUTING				
c. REIMBURSABLE	CARDS 114f			

12. TITLE (Procede with Security Classification Code)

(U) Metabolic Support Following Combat Injury

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 008800 Life Support; 016200 Stress Physiology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY			17. PERFORMANCE METHOD
76 10	CONT	DA			C. In-House

18. CONTRACT/GRANT

a. DATE/EFFECTIVE — EXPIRATION

b. NUMBER* Not Applicable

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM AMT

19. RESOURCES ESTIMATE

	FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PREVIOUS	78	2.5	158
CURRENT	79	2.5	125

20. PERFORMING ORGANIZATION

a. NAME* Letterman Army Institute of Research

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c. RESPONSIBLE INDIVIDUAL

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TELEPHONE (415) 561-3600

d. GENERAL USE

Foreign Intelligence Not Applicable

a. NAME* Letterman Army Institute of Research
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e. PRINCIPAL INVESTIGATOR (Provide last name, first name, middle initial)

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SOCIAL SECURITY ACCOUNT NUMBER

f. ASSOCIATE INVESTIGATORS

NAME Scott, Rhonda, 1LT, MSC

NAME POC: DA

21. KEYWORDS (Provide Security Classification Code) (U) Body Composition Change; (U) Wound Healing; (U) Military Trauma; (U) Parenteral Nutrition; (U) Animal Model

22. TECHNICAL OBJECTIVE, APPROACH, PROGRESS (Provide brief paragraphs identified by number. Provide text at same security classification level.)

23. (U) Combat injuries produce a uniquely severe form of stress to normal metabolism. Within days following significant combat injury, up to 21% of body muscle mass may be lost. Improved techniques of metabolic support may minimize this deleterious body compositional change, maximize wound healing, and reduce morbidity. The objective of these studies is to develop optimal post-traumatic metabolic support techniques and formulae to facilitate rapid return to duty.

24. (U) An in vitro perfused rodent hind limb, wounded to simulate combat injury, has been prepared. Using this preparation, we can study the effects of the post-traumatic hormonal milieu on normal and wounded muscle substrate utilization, and can design a post-traumatic regimen which can be deployed in field settings. It is planned to test the metabolic support formulae in higher animals and in traumatized man.

25. (U) 77 10 - 78 09 Carrageenan injections into the muscle of the rat hind limb result in a severe inflammatory response progressing to fibrosis that simulates histological and metabolic changes following combat injury. A stroma-free hemoglobin solution has been evaluated as a proposed substitute for a standard perfusate by examining tissue concentrations of adenine nucleotides, lactate/pyruvate and β-hydroxybutyrate/acetoacetate ratios. This solution is not an acceptable substitute for the standard perfusate. Efficiency of protein utilization has been shown to be improved by increasing the ratio of nonprotein calories to nitrogen in parenteral nutrition solutions above 325/1. The Nutritional Support Service has now provided over 6000 days of care for more than 300 patients. These critically ill patients have provided a valuable base of information regarding the practicality of safe implementation and monitoring of intravenous nutritional support under combat conditions.

* Contains no information useful to abstract section.

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

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 009 Metabolic Support Following Combat Injury

The following investigations are being conducted under this work unit:

STUDY NO. 1 Metabolic support following combat injury

STUDY NO. 2 Effects of the ratio of carbohydrate calories to protein supplied intravenously in minimizing body compositional loss following combat injury

STUDY NO. 3 Effects of surgical dressings and procedures on skin bacteria surrounding venous catheters

STUDY NO. 1 A model has been developed in 180 to 200 g male rats which permits isolation of skeletal muscle metabolism apart from the remainder of the animal. As a consequence, the influence of neurohumoral control and substrate supply on skeletal muscle metabolism can be carefully controlled. Initial evaluation of the preparation involved determination of the presence of adequate perfusion and normal hormonal sensitivity.

Subsequently, an isolated perfused muscle wound model has been developed by using carrageenan injections into muscle. This preparation appears to be histologically and metabolically similar to a healing wound; thus, a setting has been created whereby a study of hormone substrate interrelationships in wound healing in combination with the effects on normal muscle can be made.

Stroma-free hemoglobin solutions (SFHS) have been evaluated as a substitute for erythrocytes in isolated perfused organ systems. These solutions have been unable to maintain tissue oxygenation at SFHS concentrations low enough to prevent hemoglobin precipitation in the perfusion apparatus.

STUDY NO. 2 Four rhesus monkeys (Macaca mulatta) were chaired to allow continuous intravenous infusion of nutrient solutions designed to replace the monkey's daily dietary regimen. Initial problems with catheter placement and dietary requirements were encountered; hence extensive investigations of caloric, protein, and micronutrient requirements of chaired versus unrestrained monkeys, and modifications of catheter placement have become necessary. These problems were followed by the development of severe pulmonary infections necessitating the abandonment of this model. However, data derived from isolated liver perfusions demonstrated a specific effect of carbohydrate to improve efficiency of nitrogen utilization (which further supports the basic concept).

STUDY NO. 3 A study is now underway to determine the optimal techniques for central venous catheter dressing care. A specially designed dressing tray that can be easily adapted to combat conditions is being evaluated.

ADDENDUM. Tasks under the protocol "Studies in Combat Wound Healing" (Project No. 3S762772A814, work unit No. 010, terminated 30 September 1977) of LTC Stuart Gourlay have been incorporated to prevent waste of substantial supplies ordered prior to his departure. These studies have demonstrated a possible role of essential fatty acids in drug metabolism and an apparent role in membrane proliferation. In addition, it has been found that the type of fat-free intravenous substrates supplied affects essential fatty acid metabolism or distribution.

BODY OF REPORT

WORK UNIT NO.	009	Metabolic Support Following Combat Injury
STUDY NO.	1	Metabolic support following combat injury

PROBLEM

Combat injuries present the most pronounced example of stress metabolism. The response to this type of trauma is one of extreme catabolism which often results in body compositional losses of over 1 kg (2.2 lb) daily. These compositional changes reflect losses of stored carbohydrate (glycogen), triglycerides (subcutaneous fat), and muscle mass, which are rapidly utilized for energy by the altered post-trauma metabolism.

Body fat is considered an efficient form of stored calories, and its utilization for this end during periods of high caloric requirements, is not generally considered detrimental. However, muscle is an important structural element and, as a consequence, relatively small losses of muscle mass result in significant alterations in total body function. With severe injury, the loss of body nitrogen is accelerated from 3 to 5 g per day (the loss during times of simple starvation) to a range of 15 to 30 g per day for several days. The loss of nitrogen is reflected by loss of muscle mass; therefore, with severe injury, the loss of nitrogen represents 450 to 900 g loss of muscle mass. Caloric supply during this period will spare body fat but is thought to have little effect on loss of body nitrogen. Loss of limb muscle mass leads to marked weakness and impaired mobility. Loss of intercostal and diaphragmatic muscle mass leads to inability to clear bronchial secretions and may result in pneumonia and death.

The interrelationship between the general biochemistry of injury and convalescence and the local changes of wound healing has not been investigated in detail. It is known that early wound healing will occur during a period of negative energy balance, i.e., general catabolism. There is clearly a high biological priority of the wound in the early days and weeks after injury. This favored biological priority, however, is transient. One to two weeks following trauma, there is of necessity a prolonged phase of protein synthesis and lipogenesis to restore tissue mass to normal. At this point, if intake of foodstuffs can be resumed, wound healing moves normally toward completion. If, however, there is prolonged starvation and continued catabolism, the wound must begin to compete with other tissues for substrate, and wound healing begins to suffer severely.

The objective of this investigation is to develop a specific therapeutic regimen for post-traumatic metabolic support that will facilitate the rate of wound healing while minimizing body compositional changes. The

steps necessary to accomplish this objective are as follows: (1) investigation of the effect of the post-trauma hormonal milieu on substrate utilization by normal muscle; (2) investigation of substrate utilization by wounded muscle and the alterations in this utilization by the post-trauma hormonal abnormalities; (3) design of a post-trauma therapeutic regimen based on steps 1 and 2 that will maximize the rate of wound healing while minimizing muscle compositional changes; (4) investigation of this formula in traumatized dogs and nonhuman primates; (5) investigation of this formula in traumatized man; and (6) modification of this formula as necessary for feasibility of use under combat conditions.

RESULTS AND DISCUSSION OF RESULTS

The first phase of this study involved development of the experimental model to study muscle metabolism in situ. The isolated perfused rat hindquarter technique was evaluated for viability and effect of insulin on glucose clearance.

The second phase of this study involved the development of an isolated perfused wounded rodent hindlimb model that would simulate both metabolically and histologically a healing combat injury. The objective was to replace as much muscle mass as possible with the wound without interfering appreciably with blood flow. Three types of experimental wounds were evaluated as to their effect on animal weight change following wounding and gross anatomical replacement of muscle mass with fibrous tissue. All three techniques produced an obvious inflammatory reaction. Although the Ivalon sponge and stainless steel wound chamber techniques produced a localized response, it was technically difficult to place sufficient numbers of these devices in the hindlimb because placement of large numbers of these devices required removal of muscle mass to the point of vascular impairment. Carageenan injections, however, allowed uniform distribution of the wounding agent without vascular injury. Carrageenan injections caused marked weight loss which was dose dependent and abated with wound healing. Food consumption did not appear to be impaired in these animals. Histologically, the postwound effects of carrageenan showed marked inflammatory responses that slowly progressed to fibrosis.

Since substrate utilization in any perfused system is dependent to a significant degree upon perfusate flow rate, it was necessary to demonstrate that the wound did not interfere with flow rate. Evidence indicates that the wound did not interfere with the flow rate (1% carrageenan: 9.75±0.2 ($\bar{X}$±SEM) versus control 9.94±0.1 ml/min and 1.5% carrageenan 10.1±0.2 versus control 9.4±0.25 ml/min). No edema or hematoma formation occurred during perfusion.

Preliminary perfusion data demonstrate a definite increase in glucose clearance by the wounded hindlimb in the absence of insulin, from 0.775 ml/min in a 5-day old wound to 1.349 ml/min in a 14-day old wound.

CONCLUSIONS

Preliminary data indicate the feasibility of a perfused wound preparation that has the characteristics of (1) replacement of a major portion of the hindlimb with a metabolically active wound, (2) no interference with perfusate flow rate, (3) a significant injury to the animal. With further development, this preparation should obviate many of the difficulties encountered by other investigators in this area and allow hormone-substrate interrelationships to be studied for the first time with ease in a healing wound.

RECOMMENDATIONS

1. The concentration of carrageenan should be altered slightly to reduce the inflammatory response, thus proliferation of fibrous tissue could occur more rapidly. This should allow the time sequence for wound healing to approach the intervals found by other investigations.

2. In vivo hormone-substrate interrelationships in these carrageenan wounded animals should be characterizeds.

3. The flow distribution in the perfused wound preparation and the substrate utilization by the preparation should be characterized.

4. After baseline determinations are made the experiments as originally outlined under this project protocol should be initiated.

The third phase of this project involved the evaluation of a stroma-free hemoglobin solution (SFHS) as a substitute for the standard perfusate.

Carbohydrate metabolism is altered in metabolically active wounds so that anaerobic metabolism with resultant lactate production is favored. As a consequence, it will be necessary to separate lactate formation from the isolated perfused wound model from that of the eryt' rocytes of the perfusate. One method to obviate this problem would be to utilize a cell-free oxygen-carrying source.

Due to the number of animals required for these experiments, the previous experience in our department with electron microscopy of livers from animals exchanged with SFHS and the greater technical ease of liver perfusion, the isolated perfused rat liver was chosen as a model to evaluate SFHS as a substitute for the standard perfusate. Hepatic concentrations of ATP, ADP, AMP, lactate, pyruvate, β-hydroxybutyrate, and acetoacetate were determined and combined with electron microscopy to evaluate the ability of SFHS to maintain adequate tissue oxygenation. Prior to these experiments, the validity of the analyses were evaluated by comparing alterations produced in the liver following

2 min of nitrogen inhalation versus inhalation of room air in phenobarbital anesthetized rats. Lactate/pyruvate concentration ratios, ATP, ADP, and AMP concentrations, and adenylate energy charge correlated well with tissue hypoxia.

Following this preliminary experiment, 24-h fasted rats were perfused with standard techniques and the 8 perfusates (4 red blood cell perfusates with varying hematocrits, 42% or 21%, and P_{50}s, 28 or 13 mm Hg; and 4 SFHS, 7% human and 7% bovine, and 14% human and 14% bovine hemoglobin). Perfusate containing erythrocytes was superior to that containing 7% SFHS (H-Hb 7%, B-Hb 7%) in its ability to maintain adequate tissue oxygenation. These biochemical data were supported by electron microscopic evaluation which showed swollen mitochondria and centrolobular cytoplasmic vacuolization, and necrotic changes consistent with hypoxia in the 7% SFHS groups which were not seen in the other groups. Although 14% SFHS (H-Hb 14%, B-Hb 14%) appeared promising as an oxygen carrier in the isolated liver perfusion system, it repeatedly precipitated in the perfusion apparatus and rendered the solution in its present useless for organ perfusion via our techniques.

CONCLUSIONS

Stroma-free hemoglobin solution does not appear to be a viable substitute for erythrocyte-containing perfusate for isolated organ perfusion studies.

RECOMMENDATIONS

Erythrocyte-containing perfusate must be used for isolated organ perfusions awaiting improvement in stroma-free hemoglobin solutions. Flow through perfusions with radiolabeled glucose should be able to separate wounded muscle lactate production from that of erythrocytes.

PUBLICATIONS

1. CALDWELL, M.D., W.W. LACY, and J.H. EXTON. Effects of adrenalectomy on substrate utilization in muscle. J Biol Chem, in press.

STUDY NO. 2 — Effects of the ratio of carbohydrate calories to protein supplied intravenously in minimizing body compositional loss following combat injury

PROBLEM

Tissue catabolism following the stress of trauma, sepsis, and major surgery is a common and well established phenomenon. The consequences stemming from this catabolic state are protean and include generalized muscle wasting, impaired wound healing, and impaired immune response. Deterioration in any of these critical factors compounds any clinical

problem leading to further complications and potentiation of the catabolic state.

The catabolic response to trauma with resultant changes in body composition is thought to be secondary to a complex interrelationship between the post-trauma hormonal milieu and exogenous substrate supply. In recent years, due to the advent of total parenteral nutrition, it has become possible to reduce and in some cases reverse this catabolic cycle by vigorous intravenous nutritional support. It has been proposed that an optimal relationship exists between endogenous hormonal flux and exogenous substrate provision that will minimize body compositional change following trauma. This has yet to be determined. In addition, when intravenous nutrition is used under combat conditions, a decrease in the amount of intravenous protein necessary for each individual would result in substantial savings in transport space and weight. The decrease in bulk will also facilitate our logistic capabilities for providing this type of therapy.

RESULTS AND DISCUSSION OF RESULTS

The first phase of this study involved establishment of the experimental model. This involved chronically maintained male rhesus monkeys with indwelling central venous catheters. Two specific problems were encountered. (1) Available data regarding the nutrient requirements for 7 kg monkeys were found to be in error for chaired monkeys. When nutrients were supplied intravenously to meet reported requirements, the monkeys uniformly lost 0.5 to 1.0 kg body weight and had a marked loss of subcutaneous fat. (2) Broviac silastic catheters were placed in the internal jugular vein and guided through a subcutaneous tunnel to exit over the occipital region. This site of exit provoked chronic head-shaking with chronic catheter-cutaneous junction irritation and eventual catheter sepsis in 3 monkeys.

As a consequence of the problems, a new method for catheter placement was devised and a detailed study of nutrient intake in chaired monkeys was completed. The latter study has revealed that chaired monkeys require a 75 to 80% increase in caloric intake to maintain stable body weight and nitrogen equilibrium. As a consequence, maintenance intravenous nutrient solutions were designed for chaired monkeys to allow progression of this protocol.

A new complication was encountered shortly after the problems had been obviated. The chaired monkeys appeared to be far more susceptible to pulmonary infections. This resulted in the eventual loss of 2 more chaired monkeys, and the other 2 were removed from their chairs to salvage them. As a consequence of these severe problems in chronically catheterized chaired monkeys, it was decided to investigate another chronic model for these studies.

In the interim, the effect of carbohydrate calories on protein metabolism was evaluated by using the isolated perfused liver preparation, where the addition of carbohydrate to the perfusate decreased urea production from amino acids at all concentrations of amino acids used. This is apparently a specific effect of carbohydrate. Increased concentrations of triglycerides furnished to the liver did not affect urea production from amino acids. The increased glucose production in these latter experiments is apparently due to the glycerol contained in the triglyceride emulsion (Intralipid) used.

CONCLUSIONS

These data support the concept that elevated carbohyd[illegible] calorie/nitrogen ratios in intravenous solutions should limit amino acid mediated gluconeogenesis and improve efficiency of protein utilization. If further experiments bear out these conclusions in wounded animals, supplies necessary to provide metabolic support for combat wounded individuals could be markediy decreased.

RECOMMENDATIONS

1. Another chronic animal model for these studies is necessary; other possible models should be evaluated as soon as possible.

2. These studies should be expanded to evaluate methods for improving the efficiency of protein utilization following injury.

PUBLICATIONS

1. CALDWELL, M.D. Effect of altered calorie/nitrogen ratio on urea production. (Abstract No. 988) Fed Proc 37:400, 1978.

STUDY NO. 3 — Effect of surgical dressings and procedures on skin bacteria surrounding venous catheters

PROBLEM

In a recent report, the Center for Disease Control has suggested that the incidence of infectious complications from central venous catheters is directly related to the type of skin preparation prior to catheter insertion, the type of catheter care following insertion, and the duration of central venous catheterization. An additional factor is the occurrence of simultaneous sepsis from other sources in the patient (i.e., a patient with wound sepsis and concomitant bacteremia will have a higher incidence of catheter sepsis due to the foreign body of the catheter in this patient's blood stream).

The Department of Surgery, LAIR, has proposed guidelines for the optimal care of central venous catheters. The rationale for some of the

procedures stems from empirical knowledge, extrapolation of in vitro studies, and an old body of published in vivo investigations. Their particular procedures have not been prospectively examined from a microbial and ecologic viewpoint. To date, the experience reveals a 0% incidence of central venous catheter induced sepsis in over 3,500 patient days. Although the guidelines are probably responsible in part for the low incidence of nosocomial infections on the wards, supportive experimental data are lacking.

The Vietnam conflict saw the first wide-spread use of subclavian catheterization techniques for rapid fluid administration and monitoring of central venous pressures. The use of the subclavian vein as an easily accessible route for intravenous fluid administration and for central venous pressure monitoring was stressed by the fact that a detailed description of this technique was given in the NATO Handbook, Emergency War Surgery. In addition, Hardaway (NATO Handbook) reported that supraclavicular puncture of a subclavian vein was a routine method for administering intravenous fluids, obtaining venous blood samples, and measuring central venous pressure in the Vietnam conflict.

The combat injured soldier is at a particularly high risk for the development of a central venous catheter infectious complications for the following reasons: (1) skin preparation prior to catheter insertion is often inadequate due to time constraints; (2) since no organized method of central venous catheter care has been established for the combat injured, it may be that care of the catheter entry site and care of the intravenous line to prevent infection is absent; and (3) the principal cause of postoperative death in combat injured individuals is sepsis. The combat wound is generally the origin of sepsis. The presence of this invariably infected wound in a patient with a chronically indwelling foreign body (catheter) in his venous system significantly increases the chances of catheter-induced sepsis as well.

To highlight the concern over catheter sepsis under combat conditions, the CINCPAC 5th War Conference Manual has recommended: (1) changing central venous catheters every 48 to 72 hours, and (2) meticulous dressing changes, to include defatting the skin and application of antibiotic (Neosporin^{R}) ointment. This approach increases the risk of mechanical catheter complications (e.g., tension pneumothorax, hydro- or hemothorax, subclavian arterial injury, brachial plexus injury) due to repeated percutaneous subclavian catheterization. In addition, Neosporin^{R} ointment is not fungicidal, and in current practice of central venous catheter care, routine use of Betadine^{R} rather than Neosporin^{R} ointment is recommended. (There is recent evidence to suggest that the manner of skin cleansing is more important than the type of antibiotic ointment used.)

Therefore, studies to investigate a method of skin preparation that is effective and can be performed rapidly, in conjunction with studies to evaluate types of dressing techniques for maintenance of central venous

catheters should be extremely advantageous in preventing an accelerated incidence of catheter-induced sepsis in combat injured soldiers.

The goals of this study are to optimize dressing care of central venous catheters inserted under combat conditions: (1) to improve or modify dressings and central venous catheter care and procedures which minimize risk of infection; and (2) to design a simple kit for combat conditions that will facilitate central venous catheter care.

RESULTS AND DISCUSSION OF RESULTS

This protocol has been approved and has awaited the production of a specially designed dressing care kit that can be easily adapted to field use. To date, the experience with the proposed dressing care technique has yielded a 0.33% catheter sepsis rate in over 6000 patient days.

CONCLUSIONS

A highly effective dressing care technique has been developed that prevents catheter sepsis from chronically indwelling central venous lines.

RECOMMENDATIONS

None

ADDENDUM

PROBLEM

Work Unit No. 010, Project No. 3S762772A814, "Studies in Combat Wound Healing," under the direction of LTC Stuart Gourlay was terminated at the end of FY 76 because of the departure of the principal investigator. Following LTC Gourlay's departure, these studies (to investigate the requirements for essential fatty acids in combat injured individuals) were incorporated into our ongoing research to prevent waste of substantial supplies that had been previously ordered. The co-investigator has been assigned to LAIR during this subsequent period.

The need for essential fatty acids (EFA) has been recognized in animals for a long time; without EFA, a syndrome characterized by caudal necrosis of the tail, scaly skin lesions, impaired growth, renal degeneration, and premature death develops. The occurrence of essential fatty acid deficiency (EFAD) in humans has been documented only recently, and the only clearly proven effect of EFAD in adult humans so far is a characteristic scaly dermatopathy. Biochemical serum fatty acid patterns of EFAD predate the appearance of cutaneous lesions, and their onset is markedly accelerated by severe trauma. There is evidence of a significant delay in human wound healing with EFAD, but no studies to define the role of EFA in wound healing have been undertaken, although the importance of EFA in maintenance of plasma membrane

integrity suggests that EFAD may interfere with fibroplasia. Delivery of adequate EFA to prevent EFAD in combat casualties in forward hospitals may be necessary if EFAD significantly retards the rate of healing.

RESULTS AND DISCUSSION OF RESULTS

Two specific experiments were undertaken: (1) evaluation of the effect of essential fatty acid deficiency on membrane formation and ability to metabolize injected medications; (2) effect of the intravenous supply of various substrates on EFA mobilization and utilization.

Experiment 1. Essential fatty acid deficiency markedly alters the biochemical composition of all membranes. It has been postulated that these biochemical alterations would interfere with membrane function and possibly membrane synthesis. This should become extremely important for membrane repair following tissue injury and may account for the delayed wound healing found in EFAD animals. Initial experiments to evaluate the role of EFA in membrane synthesis involved the administration of phenobarbital (which stimulates microsomal membrane synthesis) to EFAD and rat chow fed animals. It was found that the EFAD animals did not have the normal smooth endoplasmic reticulum proliferation in response to phenobarbital injection. As a consequence, studies are underway to evaluate the effect of EFAD on mixed function oxidoses. These studies should give an indication of the role of EFA supply on an animal's ability to metabolize various medications.

Experiment 2. It has been suggested that an alteration in fat-free intravenous substrate supply can markedly change the requirements for essential fatty acids. As a consequence, EFAD, EFAD + linoleic acid supplement to meet requirement, and chow-fed control animals were placed on fat-free intravenous regimens. Data seem to indicate a marked difference in EFA metabolism; this difference probably occurs at the tissue level. That possibility is being investigated.

CONCLUSIONS

Essential fatty acid deficiency influences the composition of microsomal membranes. The supply of fat-free substrates administered intravenously appears to affect metabolism or distribution of essential fatty acids.

RECOMMENDATIONS

These investigations should be continued as outlined, and the studies incorporated under this work unit.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6105	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADE	8. GROUP INDEX	9a. SPECIAL DATA CONTRACTUAL ACCESS	9. LEVEL OF SUM
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	011
b. CONTRIBUTING				
c. [redacted]	CARDS 114f			

11. (U) Problems in Abdominal Trauma

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMING METHOD
76 01	79 06	DA			C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE	EXPIRATION
b. NUMBER: Not Applicable	
c. TYPE	d. AMOUNT
e. END OF AGENCY	f. CUM. AMT

18. RESOURCES ESTIMATE

	PROFESSIONAL MAN YRS	FUNDS ($ Thousands)
FISCAL YEAR 78	0.8	31
FISCAL YEAR 79	0.5	32

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research
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20. GENERAL USE
Foreign Literature Reviewed

19b. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR: Funded R&D & Author Participation
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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Griffin, Donald G., CPT, VC
NAME: POC: DA

21. KEYWORDS: (U) Combat Injuries; (U) Abdominal Trauma; (U) Small Bowel Physiology; (U) Short Bowel Syndrome

22. TECHNICAL OBJECTIVE: 23. APPROACH: 24. PROGRESS (Previous and current paragraphs identified by number. Provide year of each entry.)

23. (U) Significant resection of the small bowel (greater than 75%) necessitated by extensive missile wounds usually result in prolonged hospitalization and frequently leads to postoperative death because of decreased mucosal absorptive surface area. The objective of this work unit is to develop a functional substitute for small bowel.

24. (U) In dogs, artificial conduits will be fashioned from homologous colonic segments stripped of mucosa, or from synthetic materials such as the GORE-TEX vascular graft. The morphology of the artificial conduits will be studied by light and/or scanning electron microscopy.

25. (U) 77 10 - 78 09 The colonic segments stripped of mucosa appear to regenerate neo colonic mucosa with goblet cells rather than small bowel neomucosa. Preliminary data indicate that small bowel mucosa will migrate across the internal surface of the GORE-TEX graft. Technical problems with strictures and early bowel obstruction are now being addressed.

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

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 011 Problems in Abdominal Trauma

The following investigations have been conducted under this work unit:

STUDY NO. 1 The short bowel syndrome

Extensive gunshot and missile wounds to the abdomen often necessitate massive resection of the small intestine. If an insufficient functional absorptive area (less than 25%) remains, severe postoperative complications, including death, result. Previous experiments under this work unit attempted to convert segments of mucosa-denuded colon into small intestine. Colonic segments (10 cm long) were stripped of mucosa and then treated with collagenase, glutaraldehyde, ischemia, hyaluronidase, bile salts, or no treatment. The segments were removed 6 months to one year later, and morphologic studies of the endothelial lining were performed. Growth at the anastomosis was small bowel epithelium; however, the majority of the colonic segment itself was covered with residual colonic cells. Studies have been initiated in which artificial conduits are used in small bowel as a site for possible growth of small bowel mucosa.

BODY OF REPORT

WORK UNIT NO. 011 Problems in Abdominal Trauma

STUDY NO. 1 The short bowel syndrome

PROBLEM

Severe complications occur after removing large lengths of small intestine because of gunshot or shrapnel wounds. The primary defect is an inadequate length of absorptive mucosa to provide enough nutrient material to allow healing or to sustain health. A potential solution to this problem involves increasing the small bowel surface area.

RESULTS AND DISCUSSION OF RESULTS

Six mongrel dogs underwent mobilization of a 10 cm segment of colon with an intact vascular supply. The mucosa was removed by blunt dissection. One of the colonic segments had no treatment. The other segments were treated in one of the following ways: with one hour of ischemia, or with hyaluronidase, gluteraldehyde, collagenase, or bile salts. Colonic continuity was restored with a double layer anastomosis; the mobilized colonic segment was placed in continuity with the small bowel in the ileum. The segments were removed at timed intervals from 6 months to 12 months later, and morphologic studies were performed. The epithelium near the anastomosis was small bowel mucosa; the next centimeter of epithelium was a mixture of small bowel and colonic mucosa; and the remainder of the colonic segment was covered with mucosa that could not be differentiated from colonic. These observations are significantly different from those reported in the literature.

CONCLUSIONS

Colon segments are not reasonable substitutes for extending the length of small bowel absorptive surface after massive resection.

RECOMMENDATIONS

Studies are now in progress to evaluate the growth of small bowel epithelium on artificial conduits placed in continuity with small bowel. If these artificial conduits can be covered internally with mucosa, the ability of this neomucosa to transport nutrient material will be evaluated. Additional studies should be conducted which will test the effect of massive small bowel resection on the rate of growth of small bowel mucosa in those artificial conduits.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OF 6077	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMRY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. ORIG'G AGENCY: NL	9. SPECIFIC DATA-CONTRACTOR ACCESS: ☒ YES ☐ NO	10. LEVEL OF SUM: A. WORK UNIT
11. NO./CODES: PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62772A	3S162772A814		00	012		
b. CONTRIBUTING							
c. [redacted]	CARDS 114f						

12. TITLE (Procede with security classification code)

(U) Swine Model for Evaluation of Therapeutic Modalities for the Combat Injured Soldier

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support; 016200 Stress Physiology; 009800 Medical and Hospital Equipment

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY			17. PERFORMING METHOD
74 11	CONT	DA			C. In-House

18. CONTRACT/GRANT

a. DATE(S) EFFECTIVE: [redacted] EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

19. RESOURCES ESTIMATE

	PROFESSIONAL MAN YRS	FUNDS ($K)
FISCAL YEAR PREVIOUS	78 2.0	76
FISCAL YEAR CURRENT	79 0.25	5

20. RESPONSIBLE DOD ORGANIZATION

a. NAME: Letterman Army Institute of Research

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21. GENERAL USE

Foreign Literature Reviewed

22. PERFORMING ORGANIZATION

a. NAME: Letterman Army Institute of Research
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c. PRINCIPAL INVESTIGATOR (Name first MI last, Authority, Discipline)

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SOCIAL SECURITY ACCOUNT NUMBER

d. ASSOCIATE INVESTIGATORS

NAME: Weiskopf, Richard B., LTC, MC

NAME: FOC: DA

23. KEYWORDS (Procede each with (U)) (U)Pulse Pressure; (U)Combat Surgery; (U)Trauma; (U)Wet Lung Syndrome;(U)Pulsatile Perfusion;(U)Left Ventricular Function;(U)Oxyhemoglobin Diss

24. TECHNICAL OBJECTIVE

23. (U) Newly developed artificial blood substitutes and whole blood stored using new techniques must be physiologically evaluated to insure their ability to support tissue function. The objective of this work unit is to develop an appropriate subprimate model which will permit total extracorporeal circulation and precise measurements of ventricular hemodynamic and metabolic function. This model has been used to investigate the importance of pulsatile flow and to test the effectiveness of newly developed blood substitutes and blood having altered oxyhemoglobin dissociation characteristics.

24. (U) An extracorporeal circulation system capable of supplying whole body perfusions with pulsatile flow at various pulse pressures has been employed. Left heart performance and myocardial oxygen transport dynamics will be assessed to determine the ability of newly developed blood substitutes to support normal tissue function.

25. (U) 77 10 - 78 09 A cardiovascular investigational laboratory is functioning for accurate measurements of stroke volume, dp/dt, ejection fraction, myocardial metabolism, and coronary flow distribution. Investigations using hemoglobins with altered affinity for oxygen revealed decreased ventricular function with a decrease in P_{50}. A right shifted dissociation curve was also associated with increased coronary blood flow, and decreased myocardial oxygen extraction. The title of the work unit has been changed to more closely reflect the nature of the research. New investigations have been temporarily suspended pending assignment of a new investigator. We are continuing to analyze copious data thus far accumulated.

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

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 012 Studies in Pulsatile Extracorporeal Circulation

The following investigation is being conducted under this work unit:

STUDY NO. 2 The effect of variation in the oxyhemoglobin dissociation curve on left ventricular function in swine

STUDY NO. 2 The relationships between preservation of myocardial performance and the oxyhemoglobin dissociation curve of priming solutions have been investigated in the isolated swine heart preparation described in previous reports. These studies were designed to determine whether or not the P_{50} of resuscitation fluids, including whole blood, is a significant determinant of recovery from hemorrhagic shock secondary to massive combat wounds. Completed studies have indicated that variations in P_{50} have a significant effect on left ventricular function at normal arterial oxygen tensions and hemoglobin concentrations. These findings contrast with the findings of preliminary studies which were reported last year. In further work, some of which is nearing completion, we are attempting to determine whether or not changes in the oxyhemoglobin dissociation curve are important in preserving heart performance during situations which mimic those seen in combat injury, e.g., hypoxia, hypovolemia, and/or anemia.

BODY OF REPORT

WORK UNIT NO.	012	Studies in Pulsatile Extracorporeal Circulation
STUDY NO.	2	The effect of variation in the oxyhemoglobin dissociation curve on left ventricular function in swine

PROBLEM

Recently, with the understanding that the oxyhemoglobin dissociation curve is affected by concentrations of 2,3-DPG and that stored blood has a low 2,3-DPG level, there has been concern that massive transfusions with blood which has been stored for prolonged periods may have a detrimental effect on oxygen delivery to critical tissues. Myocardial function is intimately tied to adequate oxygen transport which, if less than optimal, may depress heart performance in the combat injured soldier. Some studies have suggested that there is a relationship between P_{50} and left ventricular performance. If an adequate P_{50} is crucial to preserving heart performance during periods of combat injury, then aged blood with a low P_{50} and low 2,3-DPG may have limited usefulness, and fresh blood or blood with enriched 2,3-DPG must be made available. If P_{50} is not a major determinant of left ventricular function, aged blood could be employed, especially during combat situations which would require massive transfusions and maximal utilization of blood bank resources.

RESULTS AND DISCUSSION OF RESULTS

Our in situ perfused swine heart model has been used for this study. Left ventricular function and metabolic responses have been evaluated and, in addition, myocardial tissue PO_2 and PCO_2 have been directly measured with a medical mass spectrometer.

This study is a secondary stage, following the establishment of an in vitro capability for enriching or depleting 2,3-DPG and subsequently altering the oxyhemoglobin dissociation curve and the measured P_{50}. The technique of performing an exchange transfusion during cardiopulmonary by-pass has now been well-established. Preliminary results reported last year indicated that with a normal hematocrit and arterial oxygen tension, P_{50} changes in the range of 24-50 torr did not directly affect heart performance, although myocardial oxygen extraction was higher with the higher P_{50}.

Two phases of this study have been completed. The initial attempts to evaluate the effect of P_{50} changes on myocardial function and metabolism have demonstrated that a decrease in P_{50} decreases myocardial performance. When this initial group of 10 animals was examined, a decrease in P_{50} was associated with decreases in both stroke volume and oxygen extraction across the myocardium. These findings are in contrast

to the results of preliminary studies which were reported last year, in which it appeared that with normoxia and a normal hemoglobin concentration P_{50} had little or no effect on myocardial performance. The second phase, just nearing completion and not yet completely analyzed, seems to indicate that with a decrease in the hemoglobin concentration there may possibly be an even greater decrease in left ventricular function with 2,3-DPG depletion and a lowered P_{50}.

CONCLUSIONS

Our basic conclusion at this stage is that P_{50} is indeed an important determinant of left ventricular function, and that this effect is apparent even at a normal hemoglobin concentration and during normoxia. The second phase now entering completion may also lead us to the conclusion that this effect is possibly augmented when the hemoglobin concentration is decreased, as would be the case in an acutely injured soldier. The crucial question of the role of oxyhemoglobin dissociation curve changes in other abnormal hemodynamic situations, such as hypoxia and hypotension, that might be encountered in a combat injury situation has not been adequately addressed in this study and will require additional work before conclusions can be reached.

RECOMMENDATIONS

The question of the role of P_{50} changes in blood and oxygen transporting resuscitation fluids should be addressed because oxygen transport is critical in providing optimal resuscitation for soldiers injured in combat. Our initial results indicate that, even during normoxia and adequate arterial blood pressure and oxygen tensions, P_{50} is a factor in determining left ventricular function and that care should be used when administering blood with low 2,3-DPG concentrations during resuscitation if massive transfusions are needed; therefore, further work in this field is needed. At present there are no immediate plans for continuation of this project beyond its present expiration (after the departure of the primary investigator). Recommendations at this stage include the publication of detailed manuscripts representing investigations performed under this work unit. These publications will be written by the departing principal investigator. Material from these studies should prove valuable in the Army's attempt to determine the proper significance of preserving 2,3-DPG concentration levels in blood for transfusion in combat situations.

PUBLICATIONS

1. MOORES, W.Y., J.P. HANNON, J.D. CRUM, and D.C. WILLFORD. Continuous and pulsatile extracorporeal coronary perfusion in the beating and fibrillating swine myocardium: Effects on left ventricular function. Surg Forum 28:262, 1977

2. GLANTZ, S.A., G.A. MISBACH, W.Y. MOORES, D. MATHEY, J. LEKVEN, D. STOWE, W.W. PARMLEY, and J.V. TYBERG. The pericardium substantially affects the dog ventricular diastolic pressure-volume relationship. (Abstract) Circulation 5?, Suppl 4: 52, 1977

3. WEISKOPF, R.B., W.Y. MOORES, K.K. RIORDAN, M.I. TOWNSLEY, D.C. WILLFORD, W.P DEMBITSKY, Y. CHADWICK, and J.D. CRUM. Depression of swine left ventricular function by halothane (Abstract) Clin Res 26:98A, 1978

4. MOORES, W.Y., J.P. HANNON, J.D. CRUM, D.C. WILLFORD, W.G. RODKEY, and J.W. GEASLING. Coronary flow distribution and dynamics during continuous and pulsatile extracorporeal circulation in the pig. Ann Thorac Surg 24:582, 1977

5. GLANTZ, S.A., G.A. MISBACH, W.Y. MOORES, D.G. MATHEY, J. LEKVEN, D.F. STOWE, W.W. PARMLEY, and J.V. TYBERG. The percardium substantially affects the dog left ventricular diastolic pressure-volume relationship. Circ Res 42:433, 1977

6. STOWE, D.F., D.G. MATHEY, W.Y. MOORES, S.A. GLANTZ, R.M. TOWNSEND, P. KABRA, K. CHATTERJEE, W.W. PARMLEY, and J.V. TYBERG. Segment stroke work and metabolism depend on coronary blood flow in the pig. Am J Physiol (in press)

7. HEYDORN, W.H., J.W. GEASLING, W.Y. MOORES, and L.O. LOLLINI. The behavior of expanded polytetrafluoroethylene as a large vein replacement in the dog. Ann Thorac Surg (in press)

8. MOORES, W.Y., J.P. HANNON, J.V. TYBERG, J.D. CRUM, W.G. RODKEY, and D.C. WILLFORD. Synchronized pulsatile extracorporeal coronary perfusion: its effects on preservation of left ventricular function in the beating nonworking canine heart. Report No. 57. San Francisco, California: Letterman Army Institute of Research, November 1978

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6100	2. DATE OF SUMMARY: 78 10 01		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMMARY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. ORGN MATCH: NL	9. SPECIFIC DATA FOR FOREIGN ACCESS: ☒ YES ☐ NO	10. LEVEL OF SUM: A. WORK UNIT

11. CD CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	013
b. CONTRIBUTING				
c. [redacted]	CARDS 1141			

12. TITLE (Precede with security classification code)

(U) Effect of Blood-Oxygen Affinity During Experimental Hemorrhagic Shock and Hypoxemia

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 012900 Physiology; 016200 Stress Physiology

14. START DATE	15. ESTIMATED COMPLETION DATE	16. PERFORMANCE METHOD
75 07	CONT	C. In-House

17. CONTRACT/GRANT

a. DATE(S) EFFECTIVE: — EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: — d. AMOUNT:
e. KIND OF AWARD: — f. CUM. AMT.:

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR 78	0.5	43
FISCAL YEAR 79	0.5	34

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: Letterman Army Institute of Research
b. ADDRESS: Presidio of San Francisco, CA 94129

c. RESPONSIBLE INDIVIDUAL
NAME: Marshall, J.D., CCL, MSC
TELEPHONE: (415) 561-3600

20. GENERAL USE

Foreign Literature Reviewed

20. PERFORMING ORGANIZATION

a. NAME: Letterman Army Institute of Research
Division of Surgery
b. ADDRESS: Presidio of San Francisco, CA 94129

c. PRINCIPAL INVESTIGATOR (Provide last name, first name, middle initial)
NAME: Neville, J. Ryan, Ph.D., DAC
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SOCIAL SECURITY ACCOUNT NUMBER:

d. ASSOCIATE INVESTIGATORS
NAME:
NAME:

POC: DA

21. KEYWORDS (Precede each with security classification code)

(U) Resuscitation Solutions; (U) Experimental Hemorrhagic Shock; (U) Trauma; (U) Blood-gas Transport

22. TECHNICAL OBJECTIVE, APPROACH, PROGRESS (Precede paragraphs with security classification code)

23. (U) The objective is to evaluate the oxygen transport function of blood and resuscitation solutions, particularly with reference to the role of hemoglobin-oxygen affinity in modifying physiologic responses of military personnel to trauma and environmental stress.

24. (U) The approach to this problem incorporates three areas of effort: (a) design and improvement of techniques and equipment for performing unusual or difficult biomedical measurements related to oxygen transport function, (b) theoretical analysis of the oxygen transport function of blood and related physiologic systems, and (c) use of experimental animals, particularly specialized animal preparations such as the swine heart by-pass model (see LAIR work unit 012), to test and evaluate relevant questions regarding the implications of hemoglobin-oxygen affinity to tissue oxygen transport in the combat wounded soldier.

25. (U) 77 10 - 78 09 To improve field and bedside gas transport measurements required following combat injury (see work unit 015), tests were performed to validate a new gas-dilution method for determining oxygen consumption, carbon dioxide production, and respiratory quotient in humans. This approach yielded accurate estimates of these metabolic parameters and simplified the equipment and procedural requirements for such measurements. Preliminary analyses of oxygen transport in stroma-free hemoglobin indicate the potential need to modify this blood substitute in order to increase both P_{50} and intravascular retention. Aged erythrocytes having high oxygen affinity decreased myocardial function in swine, but sustained metabolism of perfused rat liver as well as fresh blood; these apparently contradictory results may be explained by the altered rheological properties of aged erythrocytes.

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

ABSTRACT

PROJECT NO.	3S762772A814	Military Trauma and Resuscitation
WORK UNIT NO.	013	Effect of Blood Oxygen Affinity During Experimental Hemorrhagic Shock and Hypoxemia

Tests have been performed to validate a gas-dilution method for measuring oxygen consumption (V_{O_2}), carbon dioxide production ($\dot{V}_{CO_2}$), and respiratory quotient (R.Q.) in humans. This approach simplifies the equipment requirements for making these observations and appears to give results at least as good as those using more elaborate methods. The intravascular retention and the P_{50} of stroma-free hemoglobin must be increased before this product can be considered an effective blood substitute. Theoretically, these requirements can be met, in part, by modification of the dissociation of tetramer hemoglobin into dimers. Erythrocytes having high oxygen affinity produced a decreased myocardial function in swine but sustained metabolism of perfused rat liver as well as erythrocytes with normal oxygen affinity.

BODY OF REPORT

WORK UNIT NO. 013 Effect of Blood-Oxygen Affinity During Experimental Hemorrhage Shock and Hypoxemia

PROBLEM

A continuous supply of oxygen is a major requirement for maintenance of vital functions in man and animals. Compared to food and/or water deprivation, which may be tolerated in man for prolonged periods, oxygen deprivation can be fatal in minutes. Although some tissues withstand the effects of oxygen deprivation better than others, the body as a whole tolerates a deficit oxygen economy only to the extent that it can activate a variety of compensatory mechanisms designed to sustain the minimum metabolic needs of the tissues during emergencies. Trauma associated with bleeding produces an immediate threat to the oxygen economy of the body, and the effectiveness of compensatory adjustments designed to cope with this situation is a decisive factor in the clinical consequences of such trauma. Apart from the potentially lethal effects of oxygen deprivation, tissue hypoxic episodes can lead to permanent functional damage and prolong recovery from the basic injury.

The occurrence of trauma and hemorrhage in a remote and hostile battlefield setting presents several unique problems not ordinarily encountered. Loss of consciousness, for instance, is sometimes considered to be beneficial after trauma because it tends to place the body in a horizontal position favoring cardiac output and maintenance of circulation to the brain. Such a response may be hazardous even under normal circumstances, but its occurrence under battle conditions definitely jeopardizes the individual's already precarious safety and his potential for self-preservation. Medical assistance, furthermore, may be delayed by the exigencies of warfare, thus extending the stress upon compensatory reserves. Finally, blood replacement therapy, once available, may be compromised by the quality of blood products available in forward areas. Either separately or in combination, these factors make it significantly more difficult for the military physician to normalize the oxygen economy prior to permanent functional damage or in time to prevent prolonged recovery times from the basic injury.

Thus, there are unique circumstances justifying the military need to understand the complex compensatory mechanisms that sustain the oxygen economy of the body. The objective of this work unit is to evaluate the oxygen transport function of blood and resuscitative solutions, particularly with reference to the role of hemoglobin-oxygen affinity in modifying the compensatory reserves of combat personnel to trauma and environmental stress.

RESULTS AND DISCUSSION OF RESULTS

The estimation of total body $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and R.Q. can provide valuable information concerning both the experimental study and the clinical treatment of combat trauma. Unfortunately, there are many circumstances in which the usual clinical methods for making these estimates are awkward or impractical to use, and potential opportunities for improving the medical care and understanding of trauma and wound healing are often forfeited. The usual approach to making these measurements involves measuring the volume, composition, temperature, and pressure of respired gases. Such measurements are relatively simple, as well as noninvasive, and during steady-state conditions these respiratory values will reflect overall body metabolism.

A common method for obtaining these gas exchange values utilizes the open circuit technique in which the subject inspires air through a breathing valve that permits the separation and collection of expired gas into a large Tissot spirometer or Douglas gas collection bag for a measured time interval. Because they are large, water-filled devices, Tissot spirometers require considerable effort to transport, set up, and calibrate. Better mobility can be realized by use of the Douglas bag, but a separate means of making volume determinations with some form of gas meter is required. Many commonly used gas meters do not accurately measure small volumes of gas, and fairly large expired gas collections must be obtained in order to secure suitable accuracy.

Techniques for measuring gas composition have been greatly simplified and improved in recent years. Relatively compact rugged instruments are available that are easy to transport and calibrate. To take advantage of this improved technology, a technique was improvised that made use of composition measurements to obtain estimates of volume; direct volume measurement of the collected and expired gas using this approach is not necessary and the drawbacks described above are avoided.

In essence, this method makes use of either a standard dilution or enrichment of one of the measured gases (for instance, oxygen) to estimate the original volume of expired gas. This is accomplished by first measuring the composition of oxygen and carbon dioxide in the mixed original volume of expired gas, adding a known preset amount of oxygen or nitrogen, and repeating the estimation of oxygen in the enriched or diluted expired mixture. In its least complex form, the mathematical expression for determining the unknown expired volume (V_x) after dilution with a known amount of nitrogen (V_k) is:

$$P_b V_x = P_a(V_x + V_k)$$

$$\text{and } V_x = \frac{P_a V_k}{P_b - P_a}$$

where P_b is the partial pressure of oxygen in the expired mixture before dilution and P_a is the partial pressure after dilution. V_x is reduced to standard conditions in the usual manner. In the laboratory evaluation of this procedure, use has been made of a large syringe for introducing the diluting or enriching gas into the expired mixture. A preferred embodiment for this purpose, however, would incorporate a small pressured cannister containing a known amount of gas. The contents of the cannister would be released into the collected expired gas and the volume (V_k) at ambient temperature and pressure would be estimated from Boyle's law. Cannisters of the type required are widely available commercially and should pose no problems from the standpoint of size, weight, or cost. Filling these cannisters with a known amount of gas is a fairly simple manufacturing process, and storage for long periods without significant alteration or leakage is feasible.

Using this approach on 2 human subjects, we have demonstrated the general feasibility and convenience of the method. Expected values for expired volume, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and R.Q. are realized. The measurements can be obtained by using fairly short collection periods and small expired volumes. Direct comparison with conventional techniques of the estimates made with the method are presently in progress.

Simplification of the equipment and procedural requirements are the primary advantages of this approach. Only gas composition analysis is required rather than gas composition and volume measurements as with more conventional techniques.

Further analysis of oxygen transport with stroma-free hemoglobin solution has emphasized the fact, previously reported, that significant improvement in tissue oxygen transport (compared with asanguineous solutions) is obtained with stroma-free hemoglobin solution (SFHS) only under extenuating circumstances (i.e., with hematocrits of 10% or below), primarily because of the high oxygen affinity of this product. Available information indicates that only a few emergency resuscitation cases require the large volumes of fluid necessary to decrease hematocrits to this level, and thus realize an oxygen transport advantage with SFHS. Because of the ability of the organism to withstand hemodilution, failure to supply additional oxygen transport with quantities of SFHS smaller than those producing a 10% hematocrit may be assumed to be immaterial. The use of SFHS would at least provide protection where the required emergency transfusion necessitated more extreme hemodilution. However, current evidence indicates that SFHS in its present form is not an effective volume replacement fluid, regardless of the merits of its oxygen carrying potential. A relatively rapid loss of SFHS from the vascular space has been observed when this product is transfused. This loss is associated with diuresis, reduced blood volume, and lowered cardiac output, responses that are obviously inconsistent with therapeutic objectives following hemorrhage. Both of these deficiencies (of tissue oxygen transport and intravascular retention) appear to be related in large measure to the breakdown of the tetramer form of

hemoglobin to dimers, the latter being able to cross the capillary barrier. Thus, the use of SFHS as a blood substitute appears to depend on preventing or decreasing this molecular dissociation. Future research in this area should emphasize this goal.

During experiments in which rat livers were perfused with SFHS, the metabolic function of these preparations was not sustained at normal levels (compared with erythrocyte-containing perfusates) when SFHS concentrations of 7 g/dl were used. A 14 g/dl solution of SFHS did, however, maintain liver metabolism, as reflected by the cellular level of high energy organic phosphates, almost as well as erythrocyte-containing perfusates. At high hemoglobin concentrations, the tendency of tetramers to dissociate is suppressed, although it is not clear whether or not such suppression played a decisive role in the outcome of these experiments. The oncotic pressure of a 14 g/dl solution of hemoglobin is much higher than normal plasma, and in vivo such a solution would rapidly sequester large quantities of extravascular fluid, diluting the hemoglobin concentration in the process. This diluting effect was not observed in the liver perfusion studies, probably because of the large perfusate to tissue volume ratio.

In contrast to the hemoglobin concentration effect, the oxygen affinity of the perfusate appeared to have little influence on the maintenance of high energy organic phosphates in rat livers. This was true with both erythrocytes and SFHS. Oxygen consumption, however, was maintained more vigorously at reduced total oxygen delivery with bovine SFHS than with human SFHS. Bovine SFHS has a reduced oxygen affinity (increased P_{50}) compared to human SFHS.

With the use of the swine cardiopulmonary by-pass model, we found that myocardial function was depressed when aged erythrocytes with high oxygen affinity were substituted for fresh erythrocytes having normal affinity. This effect might be attributable to changes in red cell deformability and blood viscosity, however, as these latter properties have been observed (see Work Unit No. 007) to change radically with erythrocyte age. Further investigation is necessary to reconcile the disparate conclusions regarding oxygen affinity that are obtained from the liver and cardiopulmonary by-pass experiments. Conceivably, the circulation in the working heart is more responsive to change in the rheological properties of blood than is the perfused liver. The depressed myocardial function is an expression of this responsiveness rather than of increased oxygen affinity.

CONCLUSIONS

With the use of a gas dilution or enrichment method for measuring expired gas volumes, it is possible to avoid some of the problems related to estimation of $\dot{V}_E$, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and R.Q., particularly in cases requiring a greater degree of flexibility and mobility than is normally afforded by conventional means.

In its current form, SFHS has some undesirable physiologic effects that limit its usefulness as a blood substitute. The fundamental cause of these effects appears to be grounded in the propensity of the hemoglobin tetramer molecule to dissociate into dimers with subsequent loss from the vascular space.

Experiments to date have not convincingly categorized the physiologic or clinical significance of changes in oxygen affinity. Purported effects of oxygen affinity, as obtained by using blood aged to increase oxygen affinity, could be caused by changes in red cell deformability and blood viscosity. Bovine SFHS (with its lower oxygen affinity) supports oxygen consumption in liver better than human SFHS when total oxygen delivery is limited (for instance, in anemia, shock, and other low perfusion states), but this apparent advantage is not obvious with regard to tissue high energy organic phosphates. Human SFHS, however, has an extraordinarily high oxygen affinity and reaches values not normally encountered in vivo.

RECOMMENDATIONS

1. $\dot{V}_E$ values obtained with the gas dilution or enrichment method should be directly compared with conventional techniques.

2. Research with SFHS should be mainly directed toward achieving a hemoglobin tetramer molecule less subject to dissociation into dimers.

3. More precise information is needed to distinguish between the possible effects of hemoglobin-oxygen affinity and red cell deformability.

PUBLICATIONS

1. NEVILLE, J.R. Altered heme-heme interaction and tissue oxygen supply: a theoretical analysis. Br J Haematol 35:385, 1977.

2. NEVILLE, J.R. Theoretical analysis of altitude tolerance and hemoglobin function. Aviat Space Environ Med 48:409, 1977.

3. NEVILLE, J.R. and T. CLEMMER. Hemoglobin-oxygen affinity in organic heart disease. (Abstract) In: Proceedings of the Third Symposium of the International Society of Oxygen Transport to Tissue, 4-7 Jul 77, Cambridge, England, p 65.

4. ZUCK, T.F., F. DEVENUTO, J.R. NEVILLE, and H.I. FRIEDMAN. Oncotic and oxygen transport effects of hemoglobin solutions. In: Blood Substitutes and Plasma Expanders, G.A. Jamieson and T.J. Greenwalt, eds. New York: Alan R. Liss, Inc., 1978.

5. MOORES, W.Y., D.C. WILLFORD, J.D. CRUM, J.R. NEVILLE, R.B. WEISKOPF, and W.P. DEMBITSKY. Alteration of myocardial function resulting from changes in hemoglobin oxygen affinity. (Abstract) In program of 51st Scientific Session, American Heart Association, (Dallas, Texas, 1978).

6. WILLFORD, D.C., P.K. BARNES, W.T. MOORES, T.A. BENSINGER, and J.R. NEVILLE. The porcine oxyhemoglobin dissociation curve. (Abstract) In: Proceedings of the 58th Annual Meeting (Pacific Division). American Association for the Advancement of Science, San Francisco, February 1978.

7. NEVILLE, J.R. and T. CLEMMER. Hemoglobin oxygen affinity in organic heart disease. Advan Exp Med Biol 94:443, 1978.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

				DA OE 6103	78 10 01		
78 06 26	D. Change	U	U	NA	NL	☑ YES ☐ NO	
	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61102A	3M161102BS01		00	204		
b. SECONDARY	62772A	3S162772A814		00	014		
c. CONTRIBUTING	CARDS 114f						

11. TITLE (Enter title in unclassified unless classification is necessary.)

(U) Care of the Combat-Injured Eye

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 05	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE(S) EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. FROM AGY.:

18. RESOURCES ESTIMATE

	PROFESSIONAL MAN YEARS	b. PROFESSIONAL MAN YEARS	c. MANHOURS IN THOUSANDS
FISCAL YEAR	PRECEDING	0.2	28
	CURRENT	1.5	10
	79		

19. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Marshall, J. B., COL, MS

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20. GENERAL USE

Foreign Intelligence Not Applicable

20a. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Division of Biorheology

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PRINCIPAL INVESTIGATOR (Provide name and telephone number.)

NAME: Beatrice, Edwin S., COL, MC

TELEPHONE: (415) 561-3344

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Randolph, David I., DAC

NAME: Zwick, Harry, DAC POC: DA

21. KEYWORDS (Provide at least three keywords or phrases separated by semicolons.)

(U) Dark Adaptometer; (U) Night Vision; (U) Personnel Selection; (U) Ocular Physiology

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide brief but factual statements of the technical objective, approach, and progress in that order.)

23. (U) The Army has no screening device to measure an individual's ability to function in very dimly lighted environments. Sufficient biological variation in dark vision exists to warrant such an instrument in order to select those individuals best able to function during nocturnal warfare and to protect those individuals who function poorly in low ambient lighting conditions.

24. (U) Emphasis was placed on development of a portable dark adaptometer for the determination of normal function in Army ground crew and air crew personnel. Studies of the effectiveness of systemic anti-inflammatory drugs in the treatment of laser injury would provide effective medical interim therapy.

25. (U) 78 06 - 78 09 During this period, transfer of this work unit was authorized by Commander, LAIR (from LTC Silmon Biggs to COL Edwin Beatrice). Further development of a smaller prototype adaptometer was initiated using direct readout and a hand-held device. This work has been temporarily delayed until further support from MRDC is received for continuation of this effort in FY 79. Additional vision testing, including contrast sensitivity functions and Farnsworth-Munsell 100 Hue testing, has been accomplished. Initial experimental results for a pilot study on the use of indomethacin after laser exposure indicate that this systemic drug considerably reduces the edema associated with suprathreshold laser exposure.

* Applicable to contracting agency and performing agency.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. AR 70-11 AND 1498-1 WILL BE USED FOR ARMY USE AFTER OBSOLETE.

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 014 Care of the Combat-Injured Eye

The following investigation has been conducted under this work unit:

STUDY NO. 2 Dark adaptometer prototype

STUDY NO. 2 A prototype screening dark adaptometer has been developed and tested. This device allows dark vision testing without the labor commitment of technicians required by other systems. Results of testing indicate that retinal sensitivity threshold responses of normal subjects are distributed in a Gaussian fashion with a large standard deviation. Thus, there is a 10-fold difference in light sensitivities within the central 95% of the population. Two and a half percent of the population have either extremely good or extremely poor night vision. The device successfully separates degrees of normalcy, separates out malingerers from those with organic problems, and allows identification of those individuals with disease processes which affect night vision. The patenting process is underway and a prototype field device is near completion. This device is to be used in screening aviation personnel and combat troops and for evaluating the ocular effects of chronic exposure to low level coherent light sources.

BODY OF REPORT

WORK UNIT NO. 014 Care of the Combat-Injured Eye

STUDY NO. 2 Dark adaptometer prototype

PROBLEM

The U.S. Army has no efficient means of screening the night vision of combatants, even though intensive and sustained conflict may occur during nighttime. Analysis of existing data indicates that on an overcast night before moonrise, 30% of normal, fully dark adapted ground forces cannot see anything without external sources of illumination or electronic night vision aids. There is a portion of the normal population (≈15%) with very sensitive night vision who can function adequately under the same ambient light conditions with only partial dark adaptation. A need exists to screen personnel for critical military tasks in order to assign those individuals with superior night vision and to protect those with poor night vision so that they may perform optimally in effort to accomplish the mission of the Army. In addition, those with poor night vision may jeopardize essential night missions because of their inability to see at night and because of their inability to comply with the discipline required for maintenance of the dark-adapted state.

RESULTS AND DISCUSSION OF RESULTS

A prototype, laboratory model, dark adaptometer has been developed which uses light emitting diodes (LED) as the stimulus source. The apparent intensity of emitted light is controlled by varying the duty cycle of the LED by a microcomputer. A real time data display of the dark adaptation curve is achieved on either a cathode ray tube or on an X-Y recorder for permanent records. No technician time is required during the testing period since the test subject signals the computer when the threshold stimulus is seen; thus with a battery of adaptometers, one technician could screen 100 subjects daily. Both rod and cone function are tested during the same session by alternating stimuli of deep red LED (for cones) and green LED (for rod function).

Appropriate human use committee approval was granted and a normative data base was obtained from volunteers. Additional data were obtained from subjects with known or suspected organic problems or because of documented poor performance during nocturnal military exercises; this group of subjects was referred by various military ophthalmologists. The LED dark adaptometer yields population data with a distribution identical to that of the classic, labor-intensive adaptometers at energy levels identical to those reported by others in the literature. Within the population of 60 volunteers, two individuals were discovered who had unsuspected congenital stationary night blindness. The diagnosis was confirmed by electroretinography. Thus, this disease entity may be

more prevalent in the military population than was previously suspected. The study of physician-referred subjects documents the fact that LED adaptometry can distinguish those individuals with disease processes affecting night vision. Furthermore, it can distinguish those individuals who are malingering from those with genuine organic problems.

CONCLUSIONS

The results from the laboratory prototype device are extremely promising, and a prototype field device is nearing completion. Plans have been developed for joint laboratory cooperation to develop and test the device further.

RECOMMENDATIONS

The development and testing of an inexpensive reliable screening dark adaptometer for military selection should be continued. The project should be given the highest priority, as requested from the Surgeon General by TRADOC.

PUBLICATIONS

None. Open publication has been constrained until the patent application process has been completed. The project was presented as a paper to military ophthalmologists at the 1978 Walter Reed Biannual Course in Ophthalmology and as a poster session at the 1978 AGARD-NATO meeting. Reaction by the uniformed forces to these presentations was favorable.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA OE 6108	2. DATE OF SUMMARY: 78 10 01	REPORT CONTROL SYMBOL: DD-DRAE(AR)636						
3. DATE PREV SUMMARY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING: NA	8. DOD MOD'S: NL	8b. SPECIFIC DATA CONTRACTOR ACCESS: ☒ YES ☐ NO	9. LEVEL OF SUM: A. WORK UNIT	
10. NO CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER		
a. PRIMARY	62772A	3S162772A814		00		015		
b. CONTRIBUTING								
c. CONTRIBUTING	CARDS 11-1							

11. TITLE (Protect with Security Classification Code)

(U) Animal Models for Surgical Repair of Musculoskeletal Structures

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology; 003500 Clinical Medicine; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY			16. PERFORMANCE METHOD
76 05	CONT	DA			C. In-House

17. CONTRACT GRANT

a. DATE EFFECTIVE: EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: d. AMOUNT:
e. KIND OF GRANT: f. CUM AMT.

18. RESOURCES ESTIMATE

	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
FISCAL YEAR 78	2.5	26
FISCAL YEAR 79	0.2	6

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: Letterman Army Institute of Research
b. ADDRESS: Presidio of San Francisco, CA 94129

c. RESPONSIBLE INDIVIDUAL
NAME: Marshall, J.D., COL, MSC
TELEPHONE: (415) 561-3600

d. GENERAL USE

Foreign Intelligence Not Applicable

20. PERFORMING ORGANIZATION

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Division of Surgery
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TELEPHONE: (415) 561-3385
SOCIAL SECURITY ACCOUNT NUMBER

d. ASSOCIATE INVESTIGATORS
NAME
NAME

POC: DA

21. KEYWORDS (Provide keywords that describe this project)

(U) Surgical Repair; (U) Extensor Tendon; (U) Nerve; (U) Muscle Transplantation; (U) Trauma; (U) Nerve Graft; (U) Microsurgical Technique

22. TECHNICAL OBJECTIVE, APPROACH, AND PROGRESS (Insert after last paragraph)

23. (U) Extremity injuries in military personnel are extremely costly. To minimize the resulting lost duty days, permanent disability, and the expenditure of medical resources, efforts are being made to improve current surgical therapeutic injuries in order to return personnel to duty with maximum function in the minimum time.

24. (U) Segmental damage to the ulnar nerves in cats was repaired either by interfascicular graft or an epineurial technique under tension. Critical evaluations of the neurorrhaphies were made 6 months after repair. To determine the rate and morphometric pattern of axon regrowth across an anastomosis, ulnar nerves of 6 rhesus monkeys were severed, repaired, and then biopsied at either 1, 2, 3, 4, 5, or 6 weeks. Severed extensor tendons in rhesus monkey hands were repaired and immobilized for various periods of time to evaluate optimum immobilization for extensor tendon healing. Numerous techniques were used to freegraft partial or entire skeletal muscles in cats which were evaluated by electromyography

25. (U) 77 10 - 78 09 There was no statistical difference between the 2 techniques in overcoming segmental nerve defects in cats. Neither technique was as good as end-to-end repair when no segmental defect occurred. Nerve biopsies from all 6 rhesus monkeys have been obtained, and light and electron microscopic examinations are pending. All attempts to produce tenodesis in rhesus monkeys failed; however, excellent histologic studies of extensor tendon healing have been completed. Histologic study of the specimens will continue. Attempts to freegraft entire muscles have been totally unsuccessful, and this portion of the work unit has been terminated.

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 MAY 68 AND 498-1 1 MAR 69 FOR ARMY USE ARE OBSOLETE

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 015 Animal Models for Surgical Repair of Musculoskeletal Structures

The following investigations have been conducted under this work unit:

STUDY NO. 1 Nerve repair in cats: grafts vs tension

STUDY NO. 2 Digital extensor tendon repairs in monkeys

STUDY NO. 3 Study of autogenous graft techniques to restore muscle functions after traumatic injury

STUDY NO. 1 The ulnar nerve of the domestic cat was used as a model for repair of lacerated peripheral nerves. Sixteen cats underwent bilateral ulnar neurorrhaphy after a 2 cm segment of the nerve was removed. By using microsurgical methods, one side was repaired by an epineurial technique under tension, and the other side was repaired by using multiple aural nerve grafts. All cats were evaluated for return of function 6 months following nerve repairs. There was no statistical difference between these 2 techniques in overcoming segmental nerve defects in cats, which suggests that moderate tension is neither worse, nor better, than inserting avascular grafts. The results of either technique were not as good as those seen with end-to-end repair of a nerve when no segment existed. The rate and morphometric pattern of axon regrowth is being examined by segmental biopsies of the ulnar nerves of rhesus monkeys at weekly intervals following neurorrhaphy.

STUDY NO. 2 Twenty-four adolescent and three skeletally mature rhesus monkeys underwent common digital extensor tendon transection and repair in the mid-metacarpal region of the index and little fingers. Regardless of the period or method of immobilization, tenodesis occurred in none of the repaired tendons. Consequently, it has not been possible to determine the optimal period of immobilization when this animal model is used. Regardless of the period of immobilization, tensile strength did not start to increase appreciably until the 21st postoperative day. An excellent histologic study of the healing processes of repaired extensor tendons has now been completed.

STUDY NO. 3 Several methods were used to autotransplant and free-graft partial or entire skeletal muscles in cats. Electromyographic studies and later histologic studies were completed on the grafted muscles. Regardless of the method used, no successful technique was found to maintain viability of the grafted muscles. This study has been terminated.

BODY OF REPORT

WORK UNIT NO.	015	Animal Models for Surgical Repair of Musculoskeletal Structures
STUDY NO.	1	Nerve repair in cats: grafts vs tension

PROBLEM

Peripheral nerve injuries are common in both combat and noncombat military accidents. Many of the war injuries from the Vietnam conflict included severe damage to the peripheral nerves of the upper and lower extremities. During one 24-month period, 54% of all casualties in military hospitals had such injuries. Although our technical capabilities in the surgical repair of peripheral nerves have progressed greatly during the last several years, we still do not have a good method of managing segmental nerve defects. Tension at the repair site is considered detrimental to nerve regeneration and healing. Consequently, the use of a multiple nerve graft has been advocated. Problems of repairing a nerve under tension, where joints must be flexed, nerves must be mobilized, and vascularity is diminished, are not completely overcome by the use of multiple nerve grafting procedures in which an avascular unmatched segment is used to bridge the defect and relieve tension. Intrafascicular grafting not only results in the interposition of an avascular segment which loses all endoneurial elements and structure, but this technique also requires 2 separate neurorrhaphies which regenerating neurites must cross. We know of no evaluation comparing nerve repairs with tension to those neurorrhaphies which have been done with the use of multiple grafts. This study critically compares, by objective evaluation, epineurial end-to-end repairs with tension to interfascicular grafts done without tension following loss of a nerve segment.

RESULTS AND DISCUSSION OF RESULTS

We have previously described an experimental model for peripheral nerve repair by using both ulnar nerves of domestic cats. In the present study, 16 house cats underwent bilateral resection of a 2 cm length of the ulnar nerve proximal to the medial humoral epicondyle. One nerve was sutured under tension with size 8-0 nylon by using an epineurial technique. The other nerve was repaired by using a multiple caudal cutaneous sural graft that eliminated all tension at both suture lines. Size 10-0 nylon was used to suture the grafts. Microsurgical technique was used for all nerve repairs. Six months after the nerve sutures, cats were evaluated for comparison of return of function. Subjective evaluation included observation of gait, ability to fan claws (intrinsic function), and withdrawal from pin prick (sensation). Objective evaluation included efficiency and maximum strength of the ulnar innervated flexor muscles, weight of the flexor carpi ulnaris muscle, and regrowth

of myelinated nerve fibers by total axon counts proximal and distal to the repairs.

Evaluations have been completed and statistically analyzed. There was no statistical difference between these 2 techniques in overcoming segmental nerve defects in cats; these findings suggest that moderate tension is no worse, nor better, than inserting avascular grafts. When compared to an initial study where nerves were repaired primarily without tension, it was found that all animals with segmental defects had less return of function than those animals which had no segmental defect but merely an acute laceration and end-to-end repair. To determine the rate and morphometric pattern of axon regrowth following nerve laceration and repair, the ulnar nerves of 6 rhesus monkeys were severed and repaired primarily. At one-week intervals beginning 7 days after the neurorrhaphies, the nerves were biopsied and prepared for light and electron microscopic examination. Analysis of these sections has just begun, but it appears that neurite sprouting occurs almost immediately with little lag time after transection and repair. These histologic examinations will continue in great detail as the biopsy material is available.

CONCLUSIONS

Although we do not yet have a satisfactory answer to the management of segmental defects of peripheral nerves, we have demonstrated that nerves repaired without tension, when compared to those nerves with segmental defects, have a greater return of function. We now also have preliminary information indicating that neurite sprouting occurs almost immediately after transection and repair. From these findings we conclude that the most ideal nerve repair is one performed as soon as possible after the injury without tension, without grafts, with atraumatic technique, and with appropriate alignment of the fascicular nerve ends.

RECOMMENDATIONS

The final portion of this study should be continued to determine in greater detail the progressive ultrastructural changes which occur following peripheral nerve transection and repair.

PUBLICATIONS

None

STUDY NO. 2 Digital extensor tendon repairs in monkeys

PROBLEM

Study of tendon injuries and their repair has centered around flexor tendons. Lack of attention to extensor tendon injuries may be

attributed to the fact that extensor tendons are the positioners of the hand and fingers, and they are the muscles which allow the flexors to perform the primary functions of pinch, grasp, and hook. Consequently, there is a paucity of published data regarding healing of extensor tendons, even though extensor tendon injuries are common in both combat and noncombat situations. When dealing with a clean, sharp transection of an extensor tendon, it is general practice to reapproximate the severed ends surgically and immobilize the injured part during healing. The optimal duration of immobilization, however, is subject to great controversy. Prolonged immobilization of a repaired extensor tendon may result in increased scar formation, tenodesis, and contracture. This may result in a temporary or permanent disability of the part served by the tendon, and additional surgical procedures such as tenolysis may be required to restore mobility to the tendon. Conversely, use of a repaired extensor tendon too soon after repair may result in failure. Again, this leads to additional surgical procedures to correct the problems. This study has been designed to determine the optimal period of immobilization of a repaired extensor tendon, and to help identify and describe the cellular processes involved in healing of extensor tendons repaired after transection.

RESULTS AND DISCUSSION OF RESULTS

As previously discussed, we have described the histologic appearance of the healing extensor tendon. We have also found that the healing extensor tendon of the rhesus monkey does not develop appreciable strength until after 21 days following surgery. However, we have not been able to determine accurately the optimal period of immobilization following extensor tendon repair because all experimental groups healed in the same satisfactory manner, and no tenodesis developed in any of the repaired tendons regardless of the length and method of immobilization. Consequently, we attempted to produce a tenodesis by using animals in an older age group, but our attempts were still unsuccessful.

CONCLUSIONS

We feel that our model is not adequate to evaluate the optimal period of immobilization of a repaired extensor tendon. Consequently, further attempts to make such determinations will not be continued in this model.

RECOMMENDATIONS

The final report of the histologic appearance of the healing extensor tendons is now in preparation and will be completed. No further studies will be conducted using this animal model.

PUBLICATIONS

None

STUDY NO. 3

Study of autogenous graft techniques to restore muscle functions after traumatic injury

PROBLEM

Limb and skeletal muscle viability may be impaired or often destroyed following severe trauma or limb swelling and compartmental syndrome. Subsequent surgical treatment, which out of necessity includes extensive debridement of all devitalized tissues, further adds to the functional loss and disfigurement of the part. Currently, there is no technique to restore or augment severely traumatized skeletal muscles following such functional loss. Small muscles have been used successfully to restore function to sphincters and small areas of the face in human patients, but there are no reports of successfully replacing large muscle masses. This study is designed to develop techniques in animal models which will permit large muscle masses to be autotransplanted to another area of the same limb or a different limb, restoring function to a severely traumatized part.

RESULTS AND DISCUSSION OF RESULTS

Several different techniques were tried in efforts to effect free muscle grafts. The transplanted muscles were evaluated electromyographically and histologically. Regardless of the technique used, we were unsuccessful in maintaining viability of our free muscle grafts. Only those muscles which were transplanted by microneurovascular anastomoses returned to a satisfactory degree of function.

CONCLUSIONS

We have been unable to develop a technique of free muscle grafting which will provide an adequate blood and nerve supply to the grafts to survive. Only those muscles which are revascularized by direct vascular anastomoses appeared to survive and regain function.

RECOMMENDATIONS

Based on our findings and our unsuccessful attempts to free-graft partial or entire skeletal muscles, we recommend that this study should be discontinued. Further attempts at such free muscle grafts are not currently justifiable.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

				DA OE 6309	78 10 01			
2. DATE PREV SUMRY	3. KIND OF SUMMARY	4. SUMMARY CCTY	5. WORK SECURITY	6. REGRADING	7a. GROUP HISTORY	7b. DOD/DOE DATA/CONTRACTOR ACCESS	8. LEVEL OF SUM	
77 10 01	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT	
10a. NO./CODES: PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER		
a. PRIMARY	62772A	3S162772A814		00		016		
b. CONTRIBUTING								
c. CONTRIBUTING	CARDS 114f							

11. TITLE (Procedure and Security Classification Code)

(U) Studies in Combat Fracture Healing

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine; 012600 Pharmacology; 012900 Physiology

13. START DATE	13. ESTIMATED COMPLETION DATE	14. FUNDING AGENCY			15. PERFORMANCE METHOD
77 08	CONT	DA			C. In-House

16. CONTRACT/GRANT

a. DATE/EFFECTIVE — EXPIRATION

b. NUMBER* Not Applicable

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

17. RESOURCES ESTIMATE

	FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
CURRENT	78	1.0	54
BUDGET	79	0.8	55

18. RESPONSIBLE DOD ORGANIZATION

NAME* Letterman Army Institute of Research

ADDRESS* Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME Marshall, J.D., COL, MSC

TELEPHONE (415) 561-3600

19. GENERAL USE

Foreign Intelligence Not Applicable

20. PERFORMING ORGANIZATION

NAME* Letterman Army Institute of Research
Division of Surgery

ADDRESS* Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Provide name and work telephone number)

NAME* Cabaud, H. Edward, MAJ, MC

TELEPHONE (415) 561-3385

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME

NAME POC: DA

21. RESEARCH TOPICAL AREAS AND KEYWORDS (Provide Classification Code)

(U) Combat Injuries; (U) Fractures: (U) Ligamentous Injuries; (U) Trauma; (U) Surgery

22. TECHNICAL OBJECTIVE; 23. APPROACH; 24. PROGRESS (Provide individual paragraphs identified by number. Provide text of each and provide Classification Code.)

23. (U) Fractures and ligamentous injuries due to combat frequently result in delayed healing and permanent disability. Prolonged hospitalization and multiple surgical procedures delayed return to duty, and eventual medical separations are common sequelae to such injuries. Multiple systemic and mechanical factors are known to retard fracture and ligament healing, but considerable controversy still exists about how fracture and ligament healing can be accelerated. Under this work unit biochemical alterations and various surgical modalities will be investigated. The results will be transferred into management principles and techniques for combat fracture healing.

24. (U) Transverse bilateral mid-shaft ulnar fractures were created in 36 domestic house cats. The cats were divided into 6 experimental groups to evaluate the effects of vitamin D metabolites, fluoride, calcitonin, and excess phosphate in accelerating or altering fracture healing. Ten dogs and six rhesus monkeys underwent bilateral knee joint arthrotomies with transection and repair of one anterior cruciate ligament in each animal. Evaluation was carried out 4 months after the cruciate ligament repairs to determine the restoration of tensile strength following ligamentous repair.

25. (U) 77 10 - 78 09 In the fracture healing study, no specific effects or differences could be detected in any of the experimental groups using mechanical, radiologic, or histologic criteria. Significant differences were noted in serum chemistry and bone mineral content. Healing of the anterior cruciate ligament in dogs was unsatisfactory due to inadequate immobilization and early stress; however, excellent healing was accomplished in the monkey cruciate ligaments with as much as 60% return of maximum strength following repair.

*Available to contractors only (optional)

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

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 016 Studies in Combat Fracture Healing

The following investigations have been conducted under this work unit:

STUDY NO. 1 Effects of electrical stimulation on experimental ununited fractures and the establishment of nutritional supplemental controls in accelerating fracture healing

STUDY NO. 2 Evaluation of potential combined effects of electrical stimulation and nutritional attitudes in accelerating fracture healing

STUDY NO. 3 Evaluation of repair techniques in treating avulsion fractures and injuries of the anterior cruciate ligament

STUDY NO. 1 Electrical stimulation has been shown to stimulate hydroxyapetite formation and to heal experimentally produced fractures. Clinically, electrical stimulation has been used to heal delayed or ununited fractures. An experimental nonunion model utilizing the canine tibia has been developed to allow controlled investigation of the role of electrical stimulation on ununited fractures. Similarly, active metabolites of vitamin D, calcitonin, and fluorides are known to affect bone formation, and therefore have a potential effect on fracture healing. The effects of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, calcitonin, fluorides, and excess phosphate have been evaluated for their effects in accelerating or altering fracture healing in feline fracture models.

STUDY NO. 2 Since electrical stimulation stimulates the healing of experimental ununited fractures and osteotrophic agents accelerates fracture healing, the combined effects of electrical stimulation and such agents are being evaluated. The selection of appropriate agents will be based on the results of Study No. 1 of this protocol.

STUDY NO. 3 Surgically produced avulsion fractures and injuries of the canine anterior cruciate ligament have been repaired with standard surgical techniques. After satisfactory healing, the knee has been evaluated for clinical stability and restoration of tensile strength of the repaired ligament. Gross and microscopic pathologic studies have been done on the tissues.

BODY OF REPORT

WORK UNIT NO.	016	Studies in Combat Fracture Healing
STUDY NO.	1	Effects of electrical stimulation on experimental ununited fractures and the establishment of nutritional supplemental controls in accelerating fracture healing

PROBLEM:

A significant percentage of combat and noncombat injuries are fractures. These injuries not only require front line treatment but often require rear area surgical procedures and prolonged hospitalizations. Due to the adverse environments of combat zones and the unique nature of such combat-incurred fractures, delayed healing and permanent disability often result. Since electrical stimulation has been shown to stimulate fracture healing and the healing of delayed or ununited fractures, it is the purpose of this investigation to evaluate the role of electrical stimulation in accelerating fracture healing and preventing ununited fractures. Various osteotrophic agents have been implicated in stimulating fracture healing, and deficiencies of these agents have resulted in delayed fracture healing. This study also will evaluate the potential roles of such agents, including vitamin D, calcitonin, fluorides, and excess phosphate (reversed calcium-phosphate ratio) in accelerating or altering fracture healing and preventing ununited fractures.

RESULTS AND DISCUSSION OF RESULTS

Prototypical studies with canine tibiae have resulted in the development of a bilateral experimental nonunion model. Other techniques for developing nonunion models have been described, have been utilized in similar studies, and have shown that electrical stimulation does accelerate or produce fracture healing in nonunions.

Thirty-six domestic house cats underwent bilateral midshaft ulnar osteotomies and were divided into 6 test groups for evaluation of osteotrophic agents. The study groups included a control group, a pathologic group with excess phosphate, and the following experimental groups: $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, fluoride, and calcitonin. Twelve weeks after the osteotomies, the animals were evaluated for fracture healing, bone mineral content, serum chemistry, and alterations in calcium phosphorus metabolism.

CONCLUSIONS

Evaluation of data obtained in the cat fracture study has indicated that none of the osteotrophic agents accelerated or altered the rate of fracture healing in any of the experimental groups. Statistically significant differences were noted in the serum chemistries and bone mineral content between the various groups.

RECOMMENDATIONS

Evaluation of data should continue in order to determine if any subtle statistical differences existed between the experimental groups, and if any deleterious effects were produced by the osteotrophic agents.

PUBLICATIONS

None

STUDY NO. 2 — Evaluation of potential combined effects of electrical stimulation and nutritional attitudes in accelerating fracture healing

PROBLEM

Since it has been shown that electrical stimulation accelerates fracture healing with mechanical strength increased approximately 30%, it is possible that substrates and osteotrophic agents may become the rate-limiting factor if they are not available in adequate amounts. In Study No. 1, the potential roles of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, calcitonin, fluorides, and excess phosphate in accelerating or altering fracture healing have been evaluated. If it can be shown statistically that one of these agents accelerates fracture healing, then the combination of that agent with electrical stimulation may further accelerate fracture healing so as to provide earlier return to duty for the combat injured soldier and to reduce the incidence of delayed or ununited fractures.

RESULTS AND DISCUSSION OF RESULTS

Since the results of Study No. 1 are incomplete, no work has been done on this study.

CONCLUSIONS

None

RECOMMENDATIONS

None

PUBLICATIONS

None

STUDY NO. 3

Evaluation of repair techniques in treating avulsion fractures and injuries of the anterior cruciate ligament

PROBLEM

Incompetence of the anterior cruciate ligament and the resulting rotatory instability of the knee is a militarily devastating handicap. A significant percentage of soldiers who sustain anterior cruciate ligament injures in training or combat develop knee instability and require medical separation regardless of methods of treatment. Although excellent functional, anatomical, and biomechanical studies of the anterior cruciate ligament have been reported, there is still considerable disagreement as to whether a ruptured or avulsed anterior cruciate ligament should be repaired, discarded, replaced, or ignored. This study will investigate the results of repairs in the proximal and distal portions of the anterior cruciate ligament and the potential role of supplemental autogenous or synthetic materials in repairing or replacing injured anterior cruciate ligaments.

RESULTS AND DISCUSSION OF RESULTS

Ten dogs and six rhesus monkeys underwent transection and repair of the anterior cruciate ligament at either the femoral or tibial end. Seven of the ten canine and all of the monkey ligaments that had been transected and repaired did heal. Functional and clinical instability was demonstrable in all knees, but the monkeys developed less degenerative changes and had grossly more normal appearing ligaments after the repairs. Failure testing on an Instron Materials Testing Machine revealed maximum strength of the repaired ligaments to be only 10.4% for the femoral repairs and 2.0% for the tibial repairs in the dogs compared to the control knees. In monkeys, the femoral repairs were 46.8% and the tibial repairs 62.6% of the control knees.

CONCLUSIONS

Under appropriate conditions, acutely injured anterior cruciate ligaments that are appropriately repaired do have the potential of healing as shown in this study. Therefore, injured anterior cruciate ligaments that can be repaired should be repaired. Perhaps supplemental dynamic and static supporting procedures should be used to augment the acute anterior cruciate ligament repair. Adequate immobilization for a sufficient period of time seems to be essential in protecting and allowing satisfactory healing of the repaired anterior cruciate ligament. Following adequate immobilization, avoidance of early stress is crucial.

RECOMMENDATIONS

Based on the results of this study where inadequate immobilization and early stress produced unsatisfactory healing of repaired anterior cruciate ligaments, an experimental model has been developed to supplement the repaired anterior cruciate ligament by utilizing a portion of the patellar tendon. Supplementing the repaired anterior cruciate ligament will increase the blood supply, increase the actual strength, and act as an internal splint for the healing of the repaired anterior cruciate ligament. This experimental model will be evaluated, by similar criteria, in an effort to improve long-term results from repaired anterior cruciate ligaments.

PUBLICATIONS

1. CABAUD, H.E., W.G. RODKEY, and J.A. FEAGIN. Experimental studies of acute anterior cruciate ligament injury and repair. Am J Sports Med 1979 (in press).

2. CABAUD, H.E. and W.G. RODKEY. Use of a durable, light-weight, thermoplastic cast material. Am J Sports Med 1978 (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6314	78 10 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY GRADE	6. WORK SECURITY	7. REGRADING	8. GROUP INDEX	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
78 01 31	D. Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A814	00	017
b. CONTRIBUTING				
c. OTHER(S)	CARDS 114f			

11. TITLE (Protect with Security Classification Code)

(U) The Role of Humoral Agents in the Pathogenesis of Stress Ulceration

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008800 Life Support; 012900 Physiology; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 01	79 06	DA	C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE — NUMBER

b. NUMBER: Not Applicable

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS (In Thousands)
FISCAL YEAR 78	0.9	37
FISCAL YEAR 79	0.75	35

19a. RESPONSIBLE DOD ORGANIZATION

NAME: Letterman Army Institute of Research

ADDRESS: Presidio of San Francisco, CA 94129

RESPONSIBLE INDIVIDUAL

NAME: Marshall, J.D., COL, MSC

TELEPHONE: (415) 561-3600

19b. PERFORMING ORGANIZATION

NAME: Letterman Army Institute of Research
Division of Surgery

ADDRESS: Presidio of San Francisco, CA 94129

PRINCIPAL INVESTIGATOR (Provide name if U.S. employee preferred)

NAME: Watson, Larry C., MAJ, MC

TELEPHONE: (415) 561-3385

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME

NAME

20. GENERAL USE

Foreign Literature Reviewed

POC: DA

21. KEYWORDS (Provide Key Technical Phrases and Geographic Classification Code)

(U) Acute Gastric Erosions; (U) Stress Ulcers; (U) Hypovolemic Shock; (U) Humoral Agents

22. TECHNICAL OBJECTIVE; 23. APPROACH; 24. PROGRESS (Provide brief but factual paragraphs numbered to match items. Insert page breaks as needed.)

23. (U) During modern warfare, 3-6% of all combat injuries are further complicated by upper gastrointestinal tract hemorrhage. This complication results in a prolonged absence from duty and a 50% mortality rate. The overall objectives of this work unit are to prevent or improve therapy of stress ulceration. The specific objectives are to determine if a humoral agent is responsible for the stress ulceration occurring in pigs after hypovolemic shock, and to determine if stress ulceration will occur in hypovolemic pigs if normal gastric perfusion is maintained.

24. (U) Cross circulation will be established between the femoral vessels of pairs of anesthetized pigs. One pig will be hypovolemic (mean arterial blood pressure 50 mm Hg) and the second pig will be normovolemic. Control experiments will be performed with normovolemia in both animals. Gastric lesions will be compared at sacrifice 24 hours later. In control studies the effects of gastric perfusion at normal pressure and flow rate will be evaluated in normovolemic animals.

25. (U) 78 01 - 78 09 No stress ulcers were seen in the normovolemic partner while all hypotensive partners had typical fundic stress ulcers and hemorrhage. Technical difficulties, including massive gastric wall edema and mucosal ulcerations, after pump-perfusion in normotensive pigs are being investigated.

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

ABSTRACT

PROJECT NO. 3S762772A814 Military Trauma and Resuscitation

WORK UNIT NO. 017 The Role of Humoral Agents in the Pathogenesis of Stress Ulceration

The following investigations have been conducted under this work unit:

STUDY NO. 1 Identification of the humoral agents associated with stress ulceration

EX-1 Search for a humoral agent responsible for ulceration during acute hypovolemia

EX-2 The effect of adequate gastric vascular perfusion during acute hypovolemia on ulceration formation

STUDY NO. 1 This study is designed to determine the etiology of stress ulceration so that modification of its occurrence by rational preventive therapy can be attempted.

Attempts to indicate a humoral agent as the etiologic agent have been conducted by cross-circulation between the femoral vessels of pairs of anesthetized piglets. One piglet was hypovolemic with a mean arterial blood pressure of 50 mm Hg, while the second piglet was normovolemic. Control experiments were performed with both animals normovolemic. Both animals were sacrificed 24 hours later and gastric lesions were compared.

Normovolemic animals did not demonstrate stress ulcerations, while the hypotensive partners all had typical fundic stress ulcerations and hemorrhage. This would indicate that the etiologic agent for stress ulceration is not transferable by cross-circulation.

If a humoral agent were responsible for stress ulceration after hypovolemia, then it should be active even though the stomach is adequately perfused with a normal flow rate, a normal pressure, and adequately oxygenated blood. Attempts to answer this question have been thwarted because of technical difficulties, including massive gastric wall edema and mucosal ulcerations in the normotensive control pigs. Further investigation into eliminating these difficulties is being conducted.

BODY OF REPORT

WORK UNIT NO. 017 — The Role of Humoral Agents in the Pathogenesis of Stress Ulceration

STUDY NO. 1 — Identification of the humoral agents associated with stress ulceration

EX-1 Search for a humoral agent responsible for ulceration during acute hypovolemia

PROBLEM

Gastrointestinal tract hemorrhage requiring transfusion occurred in 3% of soldiers with major combat trauma during a Vietnam study period. In a group of soldiers who suffered intra-abdominal trauma treated at the 24th Evac Hospital, Long Binh, Vietnam, 6% required transfusion because of intestinal bleeding. Hemorrhage because of stress ulceration is accompanied by a 50% mortality rate. Many common battlefield injuries that would not prohibit return to combat within a 30-day period are complicated by upper gastrointestinal hemorrhage because of stress ulceration. Many soft tissue injuries from missile injury, as well as arterial disruptions and perhaps simple amputations, would not in themselves require evacuation out of the corps area for treatment. Substantial hypovolemia that accompanies these injuries frequently induces stress ulceration and this stress ulceration requires additional hospitalization time, additional blood transfusions, and additional operations; all of which preclude return to combat within 30 days.

The etiology of stress ulceration is not known. Stress ulceration will occur in parabiotic rats when only one rat is restrained, indicating that a humoral agent may partially be responsible for restraint-induced ulceration in rats. The hypovolemic piglet is a well known model for stress ulceration. The objective of Study No. 1 is to determine if a humoral agent is responsible for ulceration after acute hypovolemia in pigs.

RESULTS AND DISCUSSION OF RESULTS

Five pairs of piglets underwent cross-circulation between the femoral vessels. One pig was made hypovolemic with a mean arterial blood pressure of 50 mm Hg by bloodletting while the second pig was maintained normovolemic by adequate transfusion and fluid replacement. The hypotension was maintained for 3 hours. The serum lactate levels demonstrated that the hypotensive pigs were in a moderate to severe state of hypovolemia, while the normotensive pigs had normal lactate levels. Cardiac output was also reduced by approximately 50% in the hypotensive pigs, while the cardiac output did not change in the normotensive pigs. At sacrifice 24 hours later, the normotensive pigs had normal appearing stomachs with no hemorrhage and no ulceration. The hypotensive pigs

demonstrated typical 1-2 cm linear ulcerations in the fundus with multiple areas of ecchymosis and frank hemorrhage. Control experiments, with normovolemic pigs being cross-transfused as in the experimental study, demonstrated no ulceration in either pig at 24 hours sacrifice later.

CONCLUSIONS

There is no evidence for a humoral agent associated with the stress ulceration that occurs after acute hypovolemia in pigs.

RECOMMENDATIONS

Further studies are now in progress to cross-circulate total blood flow from the thoracic aorta of the 2 pigs rather than the femoral vessels, so that complete transfer of cardiac output is possible. Preliminary data in control pigs demonstrate that thoracotomy and cannulation of the thoracic aorta is enough stress to cause mild areas of hemorrhage in the stomach. Work should be continued until studies can be performed with no ulceration.

PUBLICATIONS

None

EX-2 The effect of adequate gastric vascular perfusion during acute hypovolemia on ulceration formation

PROBLEM

If a humoral agent is responsible for stress ulceration, then stress ulceration would still occur even though the stomach was adequately perfused during hypovolemic hypotension. The objective of this study was to determine if a humoral agent is active when gastric ischemia during acute hypovolemia is prevented.

RESULTS AND DISCUSSION OF RESULTS

Five pigs were anesthetized and the abdomen opened in the midline. The celiac axis was isolated and cannulated for perfusion. The first 5 animals were perfused with homologous blood with the aid of a roller pump for 3 hours. The animals were maintained at normal blood pressure. All 5 control animals demonstrated severe gastric wall edema and mucosal ulcerations at sacrifice 24 hours later.

CONCLUSIONS

At the present time, definite conclusions concerning the objective of the study have not been made.

RECOMMENDATIONS

Since technical difficulties of perfusing the gastric vasculature exist, we are attempting modifications of operative procedures and use of different pumps. These studies should be continued until gastric ischemia can be prevented when hypovolemia occurs secondary to massive trauma.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA OE 6090	2. DATE OF SUMMARY: 78 10 01		REPORT CONTROL SYMBOL: DD-DR&E(AR)636
3. DATE PREV SUMMARY: 77 10 01	4. KIND OF SUMMARY: D. Change	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. PROGRAM: NA	8. DUPN METHOD: NL	9A. SPECIFIC DATA: CONTRACTOR ACCESS: ☒ YES ☐ NO	9B. LEVEL OF SUM: A. WORK UNIT
10. NO./CODES: PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
A. PRIMARY	62772A	3S162772A814		00		019	
B. CONTRIBUTING							
XXCONTRIBUTINGX	CARDS 114f						

11. TITLE (Procede with Security Classification Code):

(U) Investigation of Cell-Free Resuscitating Solutions

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:

008800 Life Support; 003500 Clinical Medicine; 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMING METHOD
75 03	CONT	DA	C. In-House

17. CONTRACT/GRANT

a. DATE/EFFECTIVE: — b. NUMBER: Not Applicable — c. TYPE: — d. AMOUNT: — e. KIND OF AWARD: — f. CUM. AMT.: — g. EXPIRATION:

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
78	1.3	129
79	1.5	152

19. RESPONSIBLE DOD ORGANIZATION

a. NAME: Letterman Army Institute of Research

b. ADDRESS: Presidio of San Francisco, CA 94129

c. RESPONSIBLE INDIVIDUAL: NAME: Marshall, J.D., COL, MS; TELEPHONE: (415) 561-3600

20. GENERAL USE:

Foreign Intelligence Not Applicable

21. PERFORMING ORGANIZATION

a. NAME: Letterman Army Institute of Research

b. ADDRESS: Division of Blood Research, Presidio of San Francisco, CA 94129

c. PRINCIPAL INVESTIGATOR: NAME: DeVenuto, Frank, PhD, DAC; TELEPHONE: (415) 561-5875

d. ASSOCIATE INVESTIGATOR: NAME: Peck, Carl, C., LTC, MC

e. NAME: — POC:DA

22. KEYWORDS: (U) Acute Resuscitation; (U) Stroma-Free Hemoglobin; (U) Blood Substitute Solutions, (U) Hemorrhagic Shock

23. TECHNICAL OBJECTIVE: 24. APPROACH: 25. PROGRESS (Furnish with each annual report referenced by number from previous year and with terminal report):

23. (U) Hemoglobin, free of cell constituents, can provide the basis for an ideal resuscitating fluid for the severely wounded soldier. It has several advantages as compared to other blood substitutes or plasma expanders. It is capable of in vivo on-loading and off-loading oxygen with sufficient efficiency to maintain oxygen consumption in experimental animals rendered virtually free of circulating red cells. Hemoglobin can be stored for extended time thus alleviating logistic problems in fluid therapy of mass casualties in combat situations. The object of these studies is to evaluate the effectiveness of the hemoglobin solution as a resuscitating fluid for military use.

24. (U) Hemoglobin, prepared by crystallization from outdated human red cells is being evaluated as a cell-free resuscitation solution in animal models for its effect on critical organ function and maintenance of morphological integrity. Formulations of solutions optimal with regard to concentration and physical configuration of the hemoglobin molecules are being investigated.

25. (U) 77 10 -78 09. Hemoglobin solutions, lyophilized in the presence of a stabilizer, can be stored for at least 6 months at 25 C or for a longer period at 4 C without deterioration. Lyophilized hemoglobin reconstituted 7 months after storage was used for transfusions in rats and was effective as a blood substitute. A 14 g/dl hemoglobin solution, used as a perfusate in an isolated liver model, maintained the respiratory metabolism of the cells. Studies on the encapsulation of hemoglobin have been initiated.

26. RESERVED FOR CONTRACTOR'S REMARKS:

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

ABSTRACT

PROJECT NO. 3S162172A814 Basic Mechanisms of Recovery from Injury

WORK UNIT NO. 019 Investigation of Cell-Free Resuscitating Solutions

Crystalline hemoglobin solution, developed in our laboratory, has been evaluated further and the results demonstrate that hemoglobin, because of its ability to transport oxygen and to maintain oncotic pressure, can provide the basis for a useful resuscitating solution for the severely wounded soldier. Perfusion studies of hemoglobin solution in the isolated rat liver indicate that cellular respiratory metabolism can be maintained with the same efficiency as that of whole blood. Biochemical and ultrastructural studies of several rat organ tissues after massive transfusions with a crystalline hemoglobin solution demonstrate that the structural integrity of the liver, kidney, and brain is maintained just after hemoglobin infusion. However, as the hemoglobin disappears from plasma, reversible changes are observed in the morphology of the liver and also in blood volume. This temporary loss of blood volume could be prevented by addition of a small quantity of albumin to the hemoglobin solution infused. The storage stability of hemoglobin in solution represents an important characteristic for stockpiling this blood substitute. Solutions of hemoglobin can be lyophilized (freeze-dried) in the presence of glucose as a stabilizer and maintained as a powder for at least 6 months at room temperature or for longer periods of time at lower temperatures. Thus, at the time of transfusion, addition of sterile water is required to obtain a solution ready for fluid therapy. The possibility of producing sterile water in field situations, by using available water pools, has been investigated. Lyophilized hemoglobin, reconstituted 7 months after storage at room temperature was used for transfusion in rats and proved to be beneficial in restoring and/or maintaining vital functions. The storage stability of hemoglobin in liquid or dry form is essential for stockpiling and fulfilling the logistic requirements for supply, storage, and transport when massive fluid support is needed in the transfusion of military casualties as well as civilian casualties in accidents and mass disasters.

BODY OF REPORT

WORK UNIT NO. 019 Investigation of Cell-Free Resuscitating Solutions

PROBLEM

It has long been evident that significant advantages can be gained by the development of a resuscitating solution capable of transporting oxygen, maintaining oncotic pressure, and being readily available when massive clinical transfusions are required. Stringent requirements must be met by any resuscitating solution in order to be effective. As a blood substitute, this solution not only must be capable of restoring vital functions, but also must not elicit permanent adverse effects when administered to mass casualty victims. Furthermore, it must be uniquely suited to fulfill the supply, storage, and transportation requirements for field use in combat situations. Plasma, dextran, albumin, and other preparations have been used, and although they appear to be effective as plasma expanders, they do not transport oxygen. As a resuscitating fluid, blood has a limited storage life, must be stored in bulky energy-requiring refrigerators, and requires typing and cross-matching prior to use.

In most civilian settings in this country, the transfusion requirements associated with massive trauma can be met with conventionally stored blood and its components. However, military field requirements frequently demand massive fluid support in areas remote from supply sources. The inability to predict when modest transfusion requirements may suddenly increase complicates fluid therapy logistics. The ability to stockpile a stable protein solution capable of carrying and exchanging oxygen would minimize many of these difficulties.

Hemoglobin is a protein which has such potential. A solution of hemoglobin presents numerous advantages as compared to other blood substitutes or plasma expanders. Hemoglobin is a component of normal blood, can be prepared from outdated human erythrocytes, does not require typing or cross-matching prior to use, is capable of transporting oxygen to the tissues, has oncotic activity, has low viscosity, does not cause microaggregates, and may not induce significant immunologic reaction. Furthermore, hemoglobin is highly soluble in physiological solutions and can be stored for extended periods of time.

The potential value of hemoglobin solution as an oxygen-carrying blood substitute has been recognized also in some special situations. (1) This solution could be used in the treatment of hemorrhagic shock in circumstances where compatible blood is not available or in cases in which constriction of the capillary vessels in the microcirculation would dictate the use of a fluid with a lower viscosity than blood for normovolemic hemodilution. (2) It could

be helpful in the military field operating room when prolonged and continued blood loss occurs; until bleeding is under control, hemoglobin solution could be used, thus saving a large volume of donor blood which could be more properly utilized later. (3) In open heart surgery, hemoglobin solution could be of great advantage in priming the pump and/or maintaining circulation during surgery, again saving the patient blood intact, without any mechanical stress, for better utilization at the end of surgery. (4) Hemoglobin solution can be used as a perfusate to preserve various organs for long periods of time in a normothermic environment, maintaining the normal oxygen tension and oncotic pressure necessary during preservation. (5) In metabolic studies, solutions of hemoglobin can be formulated with the required components and used in organ perfusion allowing results which are unaffected by background compounds which are present when blood is used.

However, it is imperative that if hemoglobin is used as a blood substitute, it must be free of any stromal particle, stromal lipid, or other soluble and insoluble cell components which have been implicated in adverse effects on kidney function and on coagulation factors. Hemoglobin has the potential to become an important blood substitute and could provide the basis for an ideal resuscitating solution for the severely wounded soldier. The problem of developing an effective blood substitute is pertinent not only to military combat casualties, but also to civilian casualties such as in accidents and mass disasters.

RESULTS AND DISCUSSION OF RESULTS

Using the crystallization procedure developed in our laboratory for the isolation of hemoglobin from outdated lysed human red cells, investigators at Hyland Division, Travenol Laboratories at Costa Mesa, California have proceeded with large scale preparation of hemoglobin. Several preparations were obtained from Hyland and they were assayed in our laboratory to ascertain that our specifications were met. The hemoglobin solutions received were found suitable to be used as a blood substitute. These preparations were used by LTC William Moores, MC, Department of Surgery, LAIR, to study oxygen transport by hemoglobin solutions across an isolated swine heart. Although, after exchange of blood with a hemoglobin solution, a small quantity of the animal's own erythrocytes were still present, provisions were made for evaluating separately the oxygen transport provided by free hemoglobin and that of the residual red cells. We participated in these studies by monitoring several biochemical and physiological parameters essential in the evaluation of the ability of an isolated heart to perform useful work which is dependent upon adequate oxygen transport. The model also provided for the examination of anaerobic versus aerobic metabolism and for information regarding coronary flow distribution and adequacy of tissue oxygen and carbon dioxide

tensions. The results of these investigations indicate that although hemoglobin solution is not as efficient as whole blood, it provides adequate oxygen delivery to permit the isolated heart to perform mechanical work.

Considerable progress has been made in the studies on the lyophilization of hemoglobin solutions in the presence of protective stabilizing agents. Glucose and sucrose were most active in protecting the hemoglobin molecule from transformation to methemoglobin during the lyophilization process. Hemoglobin lyophilized under sterile conditions in the presence of 3% glucose and maintained at 4 C did not show significant alteration in structure and function for a period of 9 months. Freeze-dried hemoglobin samples stored at 25 C were unchanged for 6 months, but after this time a progressive increase in methemoglobin content and decrease in P_{50} were observed; after 9 months of storage the methemoglobin increased from an initial value of 3 to 15 g/100g hemoglobin and the P_{50} decreased from 16 to 12 mm Hg. Transfusions in rats exchanged to blood replacements of 95 or 75% were performed with lyophilized hemoglobin reconstituted after 7 months of storage at 25 C. Animals transfused with hemoglobin solution to 95% blood replacement lived for about 9 hours, and, during this interval, physical activity appeared normal. Animals similarly transfused with albumin solution died within 15 minutes after transfusion. All animals transfused to 75% blood replacement survived. Hemoglobin, hematocrit, plasma oncotic pressure, P_{50}, and oxygen capacity were monitored, and although changes were observed after transfusion, their values returned to normal pretransfusion levels within 7 days. These studies suggest that lyophilized-reconstituted hemoglobin is effective in maintaining vital functions. A scientific article reporting the results of these studies has been submitted and accepted for publication.

To reconstitute lyophilized hemoglobin to a solution suitable for transfusion, sterile water is needed. The possibility of producing sterile water utilizing water available in field situations such as sea, lake, pond water or even urine was investigated. Allied Water Corporation, San Francisco, California has developed a compact apparatus, weighing 100 pounds, which through a system of filtration and reverse osmosis can produce drinking water from polluted and/or salt water. We have analyzed the water produced by this apparatus and the results show that organic material, electrolytes, and other impurities are efficiently removed. Further studies are under way to insure that the water obtained is completely sterile and free of pyrogenic activity.

Stability studies of hemoglobin solutions at freezer (-20 C) or refrigerator (4 C) temperatures for extended periods of time and the investigation of the plasma oncotic pressure of the rat

during and after transfusion with a 7 g/dl hemoglobin or albumin solution have been completed. Two scientific articles reporting the results have been submitted and accepted for publication. The solutions of hemoglobin stored at -20 C demonstrated no alterations in methemoglobin content, P_{50}, n-values, osmolality, oxygen capacity, Na, K, and pH after 2 years of storage. Solutions kept at 4 C remained stable for 12 months; after 12 months and especially after 18 months, deterioration was evident in the refrigerator-stored solutions, as demonstrated by an increase in methemoglobin content and a decrease in the P_{50}. Investigations on the plasma oncotic pressure demonstrate that this pressure is maintained during infusion of hemoglobin solution; after transfusion, when free hemoglobin disappears from plasma, the oncotic pressure due to hemoglobin molecules decreases, but the rapid intravascular replenishment of plasma protein contributes significantly to the value of the plasma oncotic pressure.

Investigations on possible effects of hemoglobin solution on the histology and ultrastructure of liver, kidney, and brain cells of transfused rats have been expanded to include determinations of blood volume, analysis of urine output and body weight loss after transfusion. These studies have been done in cooperation with MAJ Harold Friedman, MC, Department of Surgery, LAIR. In animals transfused to 75% blood replacement, blood volume was reduced approximately 10% during the first 18 hours in the control group transfused with albumin, while hemoglobin treated rats experienced a 42% blood volume decrement in only 6 hours. Blood volumes returned to near normal levels in all animals by 24 hours. Urine volumes were three times greater in the hemoglobin-exchanged group than in the albumin-treated group and the urine was relatively hypo-osmolar in the former group (650±73 mOsm in the hemoglobin and 1,197±103 mOsm in the albumin groups). In regard to the effects on tissue cells, the results of these studies suggest that hemoglobin protects the liver from hypoxia immediately after exchange transfusion, presumably by its ability to transport and release oxygen. However, the eventual disappearance of hemoglobin from the intravascular space is associated not only with a reduction in blood volume but also with the appearance of hepatic ischemia and centrolobular necrosis. Such alterations are temporary and disappear within 2 weeks after transfusion. Consideration should be given to prevent these temporary effects by repeated infusions of hemoglobin solution or by prolonging the vascular retention time of hemoglobin. Hemoglobin, when transfused in massive quantities, does not appear to affect renal or brain ultrastructural morphology adversely. The results of these studies have been included in several scientific articles which have been submitted and accepted for publication.

In an attempt to control the loss of blood volume after transfusion with hemoglobin solution as described above, rats were transfused to 75% blood replacement with a solution containing a mixture of hemoglobin (7%) and albumin (5%). Although no significant differences were observed in the plasma half-time disappearance time of hemoglobin when compared with animals transfused with a solution containing only hemoglobin, the loss of blood volume was similar to that observed in the animals transfused with a solution containing only albumin; that is, approximately 10% blood volume loss was noted during the first 18 hours with a return to normal levels within 24 hours. Earlier experiments had shown that the animals appear to benefit from the hemoglobin-albumin mixture since all the animals transfused to 90% blood replacement survived without additional transfusion, whereas control animals died at 5 hours following blood replacement with hemoglobin solution not supplemented with albumin. As expected, after transfusion with hemoglobin-albumin mixtures the plasma oncotic pressure showed higher values (34 torr) than normal (18 torr, in the rat); however, these values returned to normal levels at 24 hours after transfusion.

In cooperation with LTC Michael D. Caldwell, MC, and MAJ Harold I. Friedman, MC, of the Department of Surgery, LAIR, preliminary studies have been initiated on the perfusion of isolated animal organs with hemoglobin solutions. These studies were designed to evaluate the effect of the hemoglobin-oxygen affinity (P_{50}) and of the hemoglobin concentration on the metabolic and respiratory activity of the cells. Appropriate controls, with whole blood as a perfusate, have been included in these studies. Physiological, hematological, and biochemical tests in the perfusate before, during, and after perfusion, and electron microscopic analysis of the tissue after perfusion have provided important information in regard to the respiratory metabolism of the cells. In these preliminary studies, the liver of the rat was isolated in situ in the animal. Under these experimental conditions, the respiratory metabolism of the cells is greatly influenced by the concentration of the hemoglobin in the perfusate; at higher hemoglobin concentrations (14 g/dl) the respiratory metabolic activity of the liver cells is comparable to that observed in the control experiments in which whole blood was used as a perfusate. These studies will be continued in the future; the organ perfusion model appears appropriate to study not only the effects of variations in the concentration of hemoglobin, but also the effects obtained by different formulations of hemoglobin solution containing addition of different biological compounds such as sugars, vitamins, and/or hormones. These investigations could be extended to study the function of organs other than liver, such as kidney, muscle, heart, and other organs.

When hemoglobin solution is transfused in a living organism,

The proportion of hemoglobin solution and residual blood will vary according to the extent of transfusion. It was considered important to have knowledge of physical and physiological parameters of different mixtures of blood and hemoglobin solution. Human blood and hemoglobin solution were mixed in different proportions and the relative viscosity, the density, the oxygen dissociation curves, and oxygen content of the mixtures were determined. A progressive decrease of relative viscosity, density, and hemoglobin-oxygen affinity (decrease in P_{50}) ensues as the proportion of hemoglobin solution increases in the mixture. These results may be important in regard to circulation through microcapillaries which in conditions of shock may be constricted. A fluid of lower viscosity and density may be able to pass through and deliver needed oxygen to the tissue with greater facility than a fluid with higher viscosity and density. It is possible that oxygen transport by free hemoglobin in the plasma may not require the same P_{50} as intra-erythrocytic hemoglobin. It is conceivable that oxygen delivery may not be dependent on the P_{50}, but upon the relative proximity of the oxyhemoglobin molecule to the capillary membrane. These possibilities will be further explored with the use of hemoglobin solutions at different concentrations and determining the contributions to the oxygen dissociation curves by the free and erythrocytic hemoglobin in the mixtures.

Hemoglobin, as prepared by the crystallization procedure, contains small quantities of several glycolytic enzymes which are tightly bound to the hemoglobin molecule. By treatment with CM--Sephadex or DEAE--cellulose, the enzymatic proteins were quantitatively separated from the hemoglobin molecule and spotted on polyacrylimide gel--electrophoresis. The remaining hemoglobin, stripped of glycolytic enzymes as described, appears to be affected adversely; the results demonstrate a decrease in P_{50} (higher oxygen affinity) and a marked increase in methemoglobin content. Samples of hemoglobin solution, separated enzymes and stripped hemoglobin have recently been sent to E. Sarcione, Ph.D. at Roswell Park Memorial Institute in Buffalo, New York, for determination of possible antigenic activity.

In normal blood, hemoglobin molecules are confined inside the red cell which may be conceived as a bag of lipid and protein material containing a solution of hemoglobin, enzymes, and other biological compounds essential for glucose metabolism. We are exploring the possibility of encapsulating the hemoglobin molecules, free in a solution, in an artificial membrane which simulates the red blood cell. The formation of such artificial cells (microencapsulation) can be achieved by treatment of the hemoglobin solution with lipid substances, or gel, and/or cellulose material. The hemoglobin molecules would remain enclosed in an artificial membrane or in the matrix of the gel. It is hoped that by this process the retention of the hemoglobin in the

plasma, when infused, would be prolonged so that the effect of hemoglobin on oxygen transport and delivery to the tissue would be maintained for longer periods of time. It is possible that encapsulation will allow the hemoglobin-oxygen affinity and the concentration of hemoglobin to be approximated to the normal values of whole blood. Preliminary results indicate that encapsulating hemoglobin molecules in gel matrix or lipid membranes is possible, but the feasibility of such processes from a clinical point of view will have to be investigated.

CONCLUSIONS

Crystalline hemoglobin solution, developed in our laboratory, has been further evaluated. Hemoglobin, because of its ability to transport oxygen and maintain oncotic pressure, could provide the basis for a useful resuscitating solution for the severely wounded soldier. As a blood substitute, hemoglobin appears beneficial in restoring and maintaining vital functions and does not directly cause adverse effects in the ultrastructure of liver, kidney, or brain cells, as demonstrated by the results of the morphology of these organs after massive transfusions with crystalline hemoglobin solution. The storage stability of hemoglobin in solution represents a decisive advantage. Solutions of hemoglobin can be lyophilized (freeze-dried) in the presence of a stabilizer and maintained as a powder for at least 6 months at room temperature or for a longer period of time in a refrigerator. At the time of transfusion addition of sterile water is required to obtain a solution ready for fluid therapy. The storage stability of hemoglobin in liquid or dry form is essential for stockpiling and fulfilling the logistic requirements for supply, storage, and transport where massive fluid support is needed in the transfusion of military casualties as well as civilian casualties of accidents and mass disasters. Investigations designed to improve the formulation of hemoglobin solution as a blood substitute, such as increasing the concentration of hemoglobin or encapsulating the molecule in an artificial membrane, are in progress.

RECOMMENDATIONS

Investigations aimed at prolonging the plasma retention of hemoglobin either by chemical manipulation of the hemoglobin molecule or by encapsulation should be continued and intensified. The evaluation of the effectiveness of hemoglobin solution in the isolated organ model should be focused on exploring different formulations of this blood substitute and the effect on the functional activity of the organ and on the respiratory metabolism of the cells.

A commercial company is now capable of providing large quantities of crystalline hemoglobin which should be used for clinical studies in higher animals not only in our laboratory at LAIR, but also by other interested investigators in other laboratories throughout the country. These studies will allow us to monitor, with a high degree of accuracy, a larger range of important physiological, biochemical, and hematological parameters. The amount of oxygen released by free hemoglobin at the tissue level should be determined in different clinical conditions. The question of a possible saturation or blockade of the reticuloendothelial system after infusion of free hemoglobin should be addressed.

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APPENDIX A

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APPENDIX B

DIRECTORY OF OFFICERS AND SENIOR PROFESSIONAL STAFF

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Commander and Director	John E. Canham, COL, MC M.D. (Col. of P&S, Columbia Univ.)
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Medical Audio/Visual Aid Division	
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	Marjorie A. Kadner, GS09 M.A. (San Francisco State)
Department of Nutrition	
Chief	Howerde E. Sauberlich, PL 313 Ph.D. (Univ. of Wisconsin)
Biochemistry Division	
Acting Chief	James H. Skala, GS14 Ph.D. (Univ. of Minnesota)
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Supply Division Chief	Robert H. Neuteboom, CPT, MSC B.S. (Brigham Young Univ.)
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	James R. Neville, GS14 Ph.D. (Stanford Univ.)
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M.D. (Johns Hopkins Univ.)

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William G. Reifenrath, CPT, MS
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	Barbara Brisentine, CPT, AN B.S. (Univ. of Maryland)
	Mary Jo Romano, CPT, AN B.S. (Villanova Univ.)
	Jacqueline Stewart, GS07 B.S. (Univ. of Colorado)
	Lucille D. Herger, GS07 B.S. (Univ. of S.F.)
Metabolic Laboratory Division Chief	Louis Hagler, COL, MC M.D. (Univ. of Colorado)
Department of Tropical Medicine	
Acting Chief	Ruth Lynn Hooper, CPT, MS Ph.D. (Kansas State Univ.)
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