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ANNUAL RESEARCH PROGRESS REPORT

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Army Medical Research and Development
Command

30 June 1974

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) During Fiscal Year 1974 progress was obtained 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; the development of techniques for the early management of complex maxillofacial wounds and injuries; the identification of diseases of the oral and maxillofacial tissues which are of special importance to the field soldier; basic nutritional biochemistry; basic biochemical processes of meta- bolism; basic and applied nutrition; clinical nutrition; basic (see reverse)		

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Block No. 20 (Cont)

and applied aspects of the influence of environment on man; the metabolism of normal man and as altered by disease; 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.

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FOREWORD

The research conducted at the Letterman Army Institute of Research, Presidio of San Francisco, California, was accomplished in Fiscal Year 1974 under the following projects and task areas:

3A161101A91C - In-House Laboratory Independent Research

3A161102B71P - Basic Research in Support of Military Medicine

01 - Biomedical Sciences

3A161102B71R - Research in Biomedical Sciences

02 - Internal Medicine

04 - Dentistry

05 - Environmental Medicine

3A162110A825 - Oral and Maxillofacial Sciences

3A162110A830 - Military Dog Improvement

3A762758A827 - Environmental Medicine

3A762759A831 - Other Tropical Medicine

3A762760A822 - Internal Medicine

Tasks are subdivided into work units, as appropriate, to accomplish the objects of the task.

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

Note that the list of Publications, Appendix A, is not combined, but will be combined in the next fiscal year.

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**U. S. Army Medical Research and
Development Technical Report**

FISCAL YEAR 1974

30 June 1974

**LETTERMAN ARMY INSTITUTE OF RESEARCH
Presidio of San Francisco, California 94129**

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		61101A		3A061101A91C		00	
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NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
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TELEPHONE: 415 561-3600				TELEPHONE: 415 561-4323			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME: Raica, N.			
				NAME: Wallace, D. L., CPT DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Vitamins; (U) Vitamin A; (U) Retinol; (U) Military Nutrition (U) Vitamin A Metabolism; (U) Vitamin A Functions; (U) Military Medicine							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The function of vitamin A, aside from its participation in vision remains unknown. Yet the effects of a deficiency of the vitamin are marked and diverse. Of military interest, is its apparent participation in resistance, infection and stress and its requirements in wound healing. Vitamin A is also responsible for normal keratinization in epithelial tissues. In order to provide a better rationale for the use of vitamin A and its requirements, investigations would be conducted on vitamin A functions at the molecular level. The studies would determine the specific tissue functions of vitamin A and its active form.</p> <p>24. (U) Studies will be conducted on the involvement of vitamin A on sulfur amino acid metabolism and sulfation, on membrane carrier lipids, on erythrocytes and membrane functions, on wound healing and infection, on amino acid incorporation and enzyme synthesis, and on DNA and RNA metabolism. Studies will use tissue culture techniques, cellular blocking agents, labeled substrates, electronmicroscopy, etc.</p> <p>25. (U) 73 07 - 74 06 Studies on vitamin A deficiency in the Carworth CFE strain of rat showed liver and serum vitamin A to decrease during vitamin A depletion while no significant changes occurred in kidney levels. Zinc levels in liver, serum and urine were unchanged. Leaching of vitamin A during tissue processing for EM was process dependent but can be held to insignificant levels. Carbon-14 caused tracking on the EM autoradiograph which indicates a need to use Tritium labelled vitamin A. Although HeLa cells could be maintained for several generations in the presence of vitamin A or citral morphologic changes were induced which made interpretation inconclusive. Screening procedures on other cell strains may determine the most responsive system for studying vitamin A metabolism.</p>							

^a Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3A061101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	040	The Molecular Basis of Vitamin A Activity

The following investigations have been conducted under this work unit:

STUDY NO. 1	Investigation of the Subcellular Distribution of Vitamin A in the Rat
STUDY NO. 2	Glycoprotein Synthesis in Cell Culture Systems

Study No. 1. Cell fractionation by differential centrifugation and electron microscopic autoradiography have been developed as useful tools for tracing radioactive label in the cell for further studies on molecular function of vitamin A. Results show that in the rat, serum vitamin A decreases as the liver depletes and kidney vitamin A increases initially followed by a decrease to values below control values as depletion progresses. Additional data are being collected to confirm and establish the statistical significance of these trends.

Study No. 2. Preliminary studies indicated that although HeLa cell tissue cultures could be maintained for several generations in the presence of either vitamin A or citral, morphological changes were induced in both systems which made interpretation inconclusive. Similar screening procedures will be done on other cell strains to determine the most responsive system in which to study vitamin A metabolism.

BODY OF REPORT

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

STUDY NO. 1 Investigation of the Subcellular Distribution of Vitamin A in the Rat

PROBLEM: To investigate the molecular function of vitamin A at the cellular level by determining its location and its influence on metabolism of other cellular components.

RESULTS AND DISCUSSION OF THE RESULTS: Cell fractionation by differential centrifugation has been refined to yield, based on electron microscopy, the following fractions: nuclei (95% pure), intact mitochondria (60-70% pure) and microsomes (95% pure). Definition of the progression of vitamin A depletion in the Carworth CFE strain of rat is still in progress. Results have indicated that this strain depletes at a slower rate (12-14 weeks) than other strains (i.e., Holtzman) of rats. The question of whether diet or strain difference is causing this slower rate is now being investigated. Chemical analysis of the diet has shown no vitamin A, but the possibility exists that a small amount of biologically active material is present.

Tissue levels of vitamin A have also been followed during vitamin A depletion. Preliminary results show the liver to deplete in an exponential manner. The serum vitamin A level decrease as the liver depletes, while the kidney vitamin A level increases initially and then decreases to values below control values as depletion progresses. In an effort to determine the significance of the trends in the data, a repeat experiment was performed and the tissue are currently being analyzed.

In light of the role of zinc in the release and transport of retinol binding protein and consequently of vitamin A from the liver, zinc determinations were performed on serum, urine and liver. No differences were noted in the zinc levels as vitamin A depletion progressed.

Experiments on tissue preparation for electron microscopic autoradiography indicate that water-soluble embedding media, or standard embedding media employed with techniques used for fatty acids retention, resulted in high retention of vitamin A in the processed tissue. Carbon-14 labelled vitamin A caused tracking on the autoradiographic emulsion which indicates that carbon-14 has too high an energy level and that tritium would be a better isotopic label to use.

The molecular Basis of Vitamin A Activity (Cont)

CONCLUSIONS: Electron microscopic autoradiography will be extremely useful in localization of isotopically labelled vitamin A in the cell, with cell fractionation by differential centrifugation procedures being an adequate backup. Vitamin A depletion does not cause a change in zinc metabolism based on excretion and tissue levels of zinc.

RECOMMENDATIONS: Studies should be continued that may shed light on the mechanism of function of vitamin A. Specifically, studies should be conducted in the whole animal and with cell culture systems with labelled precursors of nucleic acids and proteins to demonstrate the location of the label in the cell. The investigations should include the quantitation and identification of the labelled material present in the cell as well as the effect of metabolic inhibitors on these labelled compounds.

The Molecular Basis of Vitamin A Activity (Cont)

STUDY NO. 2

Glycoprotein Synthesis in Cell
Culture Systems

PROBLEM: To develop relatively simple in vitro model systems at the cellular level that will respond to various forms of vitamin A and its antagonist.

RESULTS AND DISCUSSION OF THE RESULTS: A preliminary study on vitamin A metabolism in HeLa cells was started with the purpose of finding a cell line which was responsive to the absence or excess of vitamin A. The cells were cultured in Minimum Essential Medium (Eagle) with Earle's Salts + 10% Fetal Calf Serum. Various concentrations of Vitamin A, citral (a vitamin A blocking agent), and combinations of both were added to the media in a small amount of carrier at the time of inoculation. Controls included untreated flasks and flasks with carrier only added. The flasks were examined twice a day over a period of a week or until cell death. The results showed that both vitamin A and citral induced a morphological effect on the cells and that it was hard to distinguish between the two treatments. All three treatments inhibited cell growth, but, the various combinations of citral and vitamin A showed a much lesser effect than either of the other treatments. After an initial shock period the HeLa cells would tolerate and eventually grow in the presence of vitamin A at the levels studied. Fluorescent microscopy of these cells was inconclusive. A stock culture of HeLa cells tolerant to vitamin A at a level of 5 µg vitamin A/ml media was grown for several generations and frozen and stored for future study. Although a morphological effect was noted, it was relatively undefined and further study is needed in this area. Similar screening procedures will be conducted on other cells to determine the most responsive system in which to study vitamin A metabolism. Future studies in vitamin A research will include analysis of the media and cell fractions for various forms and metabolites of vitamin A and also some radioactive labeling studies to determine the role of vitamin A in the cell. These studies will be extended to other human cells in which vitamin A is thought to be an active metabolite.

CONCLUSIONS: Cell culture systems appear to have promise as a model for replacement of the intact animal in investigations on the molecular functions of vitamin A.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
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19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research Presidio of San Francisco, CA 94129 ADDRESS:				NAME: Letterman Army Institute of Research Department of Nutrition ADDRESS: Food Hygiene Division Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish DDAN if U.S. Academic Institution) NAME: Fowler, James L., COL, VC TELEPHONE: 415 561-2878 SOCIAL SECURITY ACCOUNT NUMBER: 259-34-3058			
RESPONSIBLE INDIVIDUAL NAME: Canham, J. E., COL, MC TELEPHONE: 415 561-3600				ASSOCIATE INVESTIGATORS NAME: Ladiges, Warren C., CPT, VC NAME: DA			
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Foreign Intelligence Not Considered				(U) Public Health; (U) Food Poisoning; (U) Microbiology; (U) Bacteriological Techniques; (U) Food Microbiological Data Bank			
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The development of microbiological standards for foods is a major concern of numerous governmental regulatory agencies, and much effort is being directed toward accomplishing this goal. DOD has a direct interest, from a public health standpoint, in developing microbiological guidelines for its internal use, since microbiological standards are not available. DOD has unique requirements and opportunities for research in food microbiology, since it operates a variety of food handling establishments (i.e., central messes, hospital messes, PX cafeterias, vending machines, in-flight kitchens, and Central Food Preparation Facilities). Problems concerning microbiological guidelines, laboratory methodology, food safety, and public health will be investigated under this work unit.</p> <p>24. (U) The Centralized Microbiological Data Collection program previously reported will continue, thus providing a basis for defining problem areas and in developing microbiological guidelines. Analysis of data from in-house analyses of pre-cooked frozen meals will be completed and reported along with suitable recommendations for guidelines. Analyses of green salads for microbiological flora will continue, and when statistically valid numbers are available, will be reported to OTSG as a basis for establishing guidelines. The potential microbiological and chemical hazards from fresh seafoods will be investigated.</p> <p>25. (U) 73 07 - 74 06 Data from the microbiological data collection program for CY 72 have been analyzed and published; data for CY 73 are ready for analyses, and the CY 74 file begun. Problem areas in military subsistence have been tentatively identified. The delicatessen salad study has been published, and data partially developed on which to recommend microbiological guidelines. Tentative protocols for analyses of seafoods purchased by DOD for military consumption are being formulated.</p>							

* Available to contractors upon originator's approval.

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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.

Wholesomeness Aspects of Military Subsistence (Cont)

detected rapidly died off; however, yeasts and molds, as well as other microorganisms, reflected by the Standard Plate Count, proliferated to extremely high levels in shrimp, macaroni, carrot and raisin, and egg salads. Sixty-four salads and specialty items were surveyed for compliance with Army and Air Force Exchange Service (AAFES) microbiological limits. Standard Plate Count violations occurred in 15.6% of the samples, coliform violations in 21.8% of the samples, and yeast and mold violations in 45.3% of the samples. On a combined basis, 56.3% of the samples failed to meet AAFES microbiological limits. Suitable recommendations were made to adjust the AAFES microbiological limits for these products.

Experiment 2: Microflora of Fresh Salads Prepared by a Military Food Service Establishment

Microbiological guidelines for fresh green salads, prepared in a DOD Central Food Preparation Facility for serving in satellite dining halls, are not available. In order to establish criteria for these products, samples of green salad, cole slaw, and mixed green salads were selected from two military food service establishments. Microbiological analyses, including analyses for selected pathogens, are being performed. After analyses of 100 samples of each type are completed, statistical analyses will be performed, and proper recommendations for guidelines will be made to OTSG.

BODY OF REPORT

WORK UNIT NO. 041

Wholesomeness Aspects of Military
Subsistence

STUDY NO. 1

Microflora of Meals, Precooked,
Frozen, and Non-Specification
Convenience Foods Procured by DOD

PROBLEM:

The purchase and use of "convenience" food by DOD has continued at a high level during FY 74, with many problems in microbiological safety occurring during the year. Although military specifications for meals, precooked, frozen, have been used for many years by DOD, adverse criticisms are often voiced by producers concerning the acceptable microbiological limits. During the fiscal year, at least one court dispute concerning rejection by the government of precooked frozen meals was settled in favor of government; the decision was partially influenced by data generated in this study. The microbiological quality of meals, precooked, frozen, both specification and commercial, purchased by DOD have been investigated under this work unit. Emphasis was placed on the detection of food-borne pathogens.

RESULTS AND DISCUSSION OF THE RESULTS:

Laboratory analyses have been completed on 690 samples of precooked frozen meals, representing 17 different kinds of meals. These lots with 5 samples per lot (15 total samples) were selected as being representative of the meal. Thirteen kinds of commercial meals and 4 menus of specification meals were utilized, with 8 analyses per sample (5520 analyses).

The general trend of these results shows two striking observations: (a) the generally low microbial counts and (b) considerable variability of meals within a lot and between lots. Of the 690 samples, 26 (or 3.77%) exceed the military specification limits (Standard Plate Count) of not more than 100,000/g. Seventy-four (10.41%) contained coliform organisms, with 0.9% exceeding the specification limits. Escherichia coli was isolated from 14 (or 2%) of the samples; yeasts and molds were found in 223 (or 33.3%) of the samples, fecal streptococci in 270 (or 38.8%), while Staphylococcus aureus was found in 46 (or 6.6%) of the samples. Clostridium perfringens was isolated from only 2 samples, while all samples gave negative Salmonella results.

In comparing the microbiological quality of commercial versus specification meals, little difference could be observed except for the geometric mean of the Standard Plate Counts. The arithmetic average of the

Wholesomeness Aspects of Military Subsistence (Cont)

commercial meals was 19,000/g, while that of specification meals was 23,000/g. Geometric mean (Standard Plate Count) of the commercial meals was 794/g, while that of the specification meals was 151/g. The latter data indicates the wide variability between lots and kinds of commercial meals, while indicating conformity in specification meals.

CONCLUSIONS AND RECOMMENDATIONS:

The microbiological quality of meals analyzed was found to be quite good. The present limits of Military Specification Mil-M-0013966D, Meal, Precooked, Frozen, appear quite generous to the consumer and could conceivably be lowered. Adoption of the geometric mean in lieu of arithmetic mean for the Standard Plate Count might possibly remove the reasons for criticism of the specification by producers; this, however, will require more study. Meals analyzed appeared to offer little hazard to the consumer from food-borne pathogens.

PUBLICATIONS:

None.

STUDY NO. 2

Computerized Data Collection
Program in Food Microbiology

PROBLEM:

An adequate data base in food microbiology is essential on which to base microbial guidelines for food products. The DOD operates several laboratories which perform food microbiological testing under rigidly controlled conditions using official testing methods. Valuable food microbiological data is generated on a continuing basis from these laboratories which, if properly tabulated, could serve important functions in military food hygiene. A Computerized Microbiological Data Collection program was designed in 1971, tested, and expanded in 1972. This is intended as a continuing and expanding program, with 1973 data having been entered. Retrievals and analysis of the 1972 file is completed and action is being taken to analyze 1973 data.

RESULTS AND DISCUSSION OF THE RESULTS:

Preliminary analysis of data was partially reported in the 1973 report. Completed analysis has indicated several areas of food hygiene which need intensive research in food microbiology; such areas identified by this program are precooked frozen meals, beef and pork products, and seafoods.

Twenty-one types of foods (195 samples) were tested by DOD official testing laboratories as being suspected of having caused food poisoning;

Wholesomeness Aspects of Military Subsistence (Cont)

24 isolates of pathogenic organisms were found. Organisms isolated were Vibrio parahemolyticus, Staphylococcus aureus, salmonella, fecal streptococci, Escherichia coli, Enterobacter, and Escherichia cloacae. In addition, analyses of 1335 samples (14 food type.) were reported; these were submitted for testing for other reasons. In addition to the organisms previously listed, Clostridium perfringens was isolated from 4 samples (2 from vegetable products and 2 from seafoods).

Tabulation of data from procurement testing was tabulated; this data has been utilized when comments on proposed or revised food specifications were necessary. In addition, sufficient data for a variety of ground beef products, prepared sandwiches, and delicatessen salads is available from the 1972 file on which to base recommendations.

RECOMMENDATIONS AND CONCLUSIONS:

The data accumulated in this file should be used as a basis for establishing microbiological guidelines for DOD use. Problem areas identified should be investigated through research projects. Additional methods of analysis and presentation of data should be developed and utilized.

PUBLICATIONS:

Fowler, J.L., P.B. Ruckh, T.G. Murnane and W.F. Ganz. Report of Analyses of 1972 Microbiological Data Collection Program. USAMRNL Laboratory Report 339, 1973.

STUDY NO. 3

Experiment 1. Microflora of Prepared Salads and Specialty Items

PROBLEM:

The Army and Air Force Exchange Service (AAFES) officially requested assistance in establishing realistic microbiological guidelines for prepared delicatessen salads from the Food Hygiene Division, LAIR, in 1972. To accomplish this, a collaboration effort between AAFES (to arrange and deliver samples) and the Food Hygiene Division was developed and executed; preliminary results were reported in the 1973 report. Completed analysis of the data has been accomplished.

RESULTS AND DISCUSSION OF THE RESULTS:

Sixty-four salads and specialty items were surveyed for compliance with AAFES microbiological limits (Standard Plate Count not to exceed 100,000/g, coliform organisms not more than 10/g; yeast and mold not more than 20/g, and E. coli negative). Standard Plate Count violations

Wholesomeness Aspects of Military Subsistence (Cont)

occurred in 15.6% of the samples; coliform violations in 21.8% of the samples; and yeast and mold violations in 45.3% of the samples. On a combined basis, 56.3% of the samples failed to meet AAFES microbiological limits.

In addition to in-house analyses, data from 1057 salads reported by DOD Testing Laboratory (from Study No. 2 - Computerized Data Collection Program) were utilized in developing the final report on this project. Violations in Standard Plate Count limits occurred in 17.7% of the samples, coliform violations occurred in 10.0% of the samples, and yeast and mold violations occurred in 37.3% of the samples. E. coli was detected in 3.8% of the samples.

RECOMMENDATIONS:

1. The present microbiological limits should be investigated for adequacy.
2. Consideration should be given to increasing the yeast and mold limits.
3. On the basis of analyses of individual types of salads, storage of shrimp, egg, carrot and raisin, and macaroni salads should be limited to 2 weeks.
4. Investigation of these items should continue.

CONCLUSIONS:

On the basis of this report and comments from other sources, the AAFES has increased the yeast and mold limits to not more than 100/g. With this increase, statistics developed in this study should be re-calculated to determine the percentage of samples which now comply with limits. Consideration is being given by AAFES to include limits for Staphylococcus aureus in the specification.

PUBLICATIONS:

Fowler, J.L., R.E. Thomas, J.J. Jorgensen and D. Stutzman. Microflora of Prepared Salads and Specialty Items Procured for Use by DOD Installations. USAMRNL Laboratory Report No. 338, 1973.

STUDY NO. 3

Experiment 2. Microflora of Fresh Salads Prepared by a Military Food Service Establishment

PROBLEM:

Department of Army is in the process of developing a Central Food Preparation Facility for troop feeding at Fort Lee, VA. The concept

Wholesomeness Aspects of Military Subsistence (Cont)

of operation is to centrally prepare most food items, including fresh salads, with subsequent delivery to satellite dining halls. Microbiological guidelines are not available for these products; the Food Hygiene Division was requested to make recommendations to OTSG concerning acceptable microbial flora. Two military dining facilities were selected as the sample source and frequent samplings of these fresh salads were accomplished. It is anticipated that 100 samples of each type will be analyzed and statistically analyzed. Recommendations will be formulated from the results.

RESULTS AND DISCUSSION OF THE RESULTS:

Preliminary analysis of 30 samples of each type have yielded the following results:

TYPE OF SALAD	SPC*/g	COLIFORMS/g (plate method)	COLIFORMS/g (MPN** method)
Mixed Green	13,000,000	9,717	40,282
Cole Slaw	13,000	1,410	36,042
Green	810,000	769	3,249

* Standard Plate Count

** Most Probable Number

CONCLUSIONS:

None reached since the selected number of samples have not yet been analyzed.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^b	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6362	74 07 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^c	6. WORK SECURITY ^d	7. REGRADING ^e	8A. DES'N INST'N	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
73 07 01	Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ^f		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
		61101A		3A161101A91C		00	
a. PRIMARY		61101A		3A061101A91C		054	
b. XXXXXX		61101A		3A061101A91C			
c. XXXXXX							
11. TITLE (Proceed with Security Classification Code) ^g							
(U) Ultrastructure of Normal and Diseased Animal Tissue (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^h							
002300 Biochemistry; 002600 Biol.; 006500 Food; 010100 Microbiology; 016800 Toxic							
13. START DATE		14. ESTIMATE COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
68 01		74 06		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	20. FUNDS (in thousands)
a. DATES/EFFECTIVE:				PRECEDING			
b. NUMBER: ⁱ				FISCAL		74	3.0
c. TYPE: Not Applicable				YEAR		75	0
d. KIND OF AWARD:				CURRENT		0	0
e. AMOUNT: ^j							
f. CUM. AMT.							
21. RESPONSIBLE DOD ORGANIZATION				22. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: Plopper, C.G., CPT			
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-4714			
				SOCIAL SECURITY ACCOUNT NUMBER: XXXXXXXXXX			
23. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence Not Considered				NAME: Bucci, T.J., LTC, VC			
				NAME: Emson, R.N., CPT, VC			
				DA			
23. KEYWORDS (Proceed EACH with Security Classification Code)							
(U) Microscopy; - Electron; (U) Cytology; (U) Nutrition							
(U) Infection; (U) Disease; (U) Cellular Injury; (U) Animal; (U) Tissue; (U) Pathology							
24. TECHNICAL OBJECTIVE, ^k 25. APPROACH, 26. PROGRESS (Provide individual paragraphs identified by number. Proceed to 11 of each with Security Classification Code.)							
23. (U) Tissues from animals subjected to physiologic stresses similar to those encountered in military operations, nutritional deficiencies, etc., undergo a variety of structural changes. Many of these are undetectable or poorly defined with light microscopy but may be visualized by electron microscopy (EM). The broad objectives of this work unit were: (1) characterize the changes occurring under natural or induced conditions (2) compare the changes with normal cellular morphology, and (3) correlate structural changes with functional changes and with the alterations visualized by light microscopy.							
24. (U) Tissues subjected to the above kinds of stresses were studied by EM and the ultrastructural morphology were correlated with routine histopathology on the same tissue; this approach revealed changes not clearly visualized by light microscopy since the identity and significance of structures poorly visualized could be confirmed with the electron microscope. Sequential studies revealed processes or mechanisms and help relate structure to functional changes which were observed. Considerable experimentation with fixation, embedding and staining for EM examination was required.							
25. (U) 73 07-74 06 Study 4: Perfusion-fixed tissues from rats on eight different diets comprising two levels of calcium (high and low) and two levels of phosphorus (adequate and low) each with or without a Vitamin D supplement, were evaluated for a) bone ash and bone development, and b) parathyroid and thyroid "C" cell ultrastructure. Only two diets produced severe rickets: high calcium, low phosphorus, Vitamin D and low calcium, low phosphorus, no Vitamin D. One reduced mild rickets: low calcium, low phosphorus, with Vitamin D. Tentative electron microscopic results indicate little change in parathyroid chief cell activity with diet. More marked changes were observed in thyroid "C" cells. This work unit is being terminated.							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A061101A91C **In-House Laboratory Independent
Research**

WORK UNIT NO. 054 **Ultrastructure of Normal and
Diseased Animal Tissue**

**The following investigations have been conducted under this work
unit:**

**STUDY NO. 4 Light and electron microscopic analysis of dietary
influence on the thyroid and parathyroid glands of
bones of the rat.**

**To test the influence of dietary calcium (Ca) and phosphorus (P) on
the cytological state of the parathyroid and thyroid parafollicular
cells (C-cells), weanling rats were fed one of four diet combinations
of 1.2% or 0.02% Ca and 0.3% or 0.02% P. One half of each diet group
received oral supplements of Vitamin D. Despite widely varying levels
of serum P and serum Ca/P ratios, parathyroid activity, as judged by
cellular ultrastructure, was most closely correlated with the level of
serum Ca. The C-cells showed depressed activity only in the group with
the highest serum Ca level, which was fed a P-deficient diet. This
work unit is being terminated.**

BODY OF REPORT

WORK UNIT 054

Ultrastructure of Normal and
Diseased Animal Tissue

STUDY NO. 4

Light and electron microscopic
analysis of dietary influence on
the thyroid and parathyroid glands
and bones of the rat.

PROBLEM:

The maintenance of calcium homeostasis in the mammalian body is the primary function of two endocrine glands: the parathyroid and the parafollicular cells of the thyroid. Their secretory mechanisms respond to serum levels of calcium. Parathyroid hormone synthesis and secretion is stimulated by a drop in serum calcium levels, while calcitonin is stimulated by high levels of serum calcium.

Since the initial description of the ultrastructural characteristics of parathyroid chief cells, numerous studies have evaluated the functional state of this cell type at various levels of serum calcium. The degree of synthetic activity is inversely related to serum calcium level. Ultrastructural studies of thyroid C-cells (parafollicular cells), in contrast, indicate a state of cellular activity corresponding to the level of serum calcium. Relatively few studies have focused on both glands from the same animal under experimental conditions.

In the absence of pathological conditions in the parathyroid glands and C-cells, dietary levels of calcium determine the concentration of calcium in the blood. The intestinal absorption of the calcium is increased by Vitamin D. While considerable work has been conducted regarding the physiological and morphological responses to blood calcium levels, some of it correlated with dietary calcium, little effort has been directed toward the relationship of the ratios of this mineral and phosphorus in the diet or the effect of the presence of Vitamin D.

This study examined the effect of dietary calcium and phosphorus, or the ratio of these minerals in the diet, with or without Vitamin D, on the chronic cytological changes in the parathyroid and thyroid C-cells. The morphological changes were correlated with blood Ca and P levels, as well as mineral content in bones.

Ultrastructure of Normal and Diseased Animal Tissue (Cont)

RESULTS AND DISCUSSION OF THE RESULTS:

The tentative results of this study are presented in Table 1. Weanling rats were fed one of four diets for eighteen days: 1.2% Ca and 0.02 P (ratio 60 to 1); 0.02% Ca and .3% P (ratio 0.06 to 1); .02% Ca and 0.02% P (ratio 1 to 1); 1.2% Ca and 0.3% P (ratio 4 to 1). One half of each diet group received vitamin D (31 IU) by oral supplement three times weekly. Only three diet groups (low Ca, low P, + and - D; high Ca, low P, -D) had morphological signs of rickets (i.e., elongated, poorly developed epiphyseal plates in the radii). These were also the groups with extremely low bone ash values. The groups which had morphologically normal bones exhibited two degrees of calcification, one normal and the other significantly less than normal, but intermediate between the ricketic and normal animals.

On the bases of cytological criteria, three degrees of activity were present in the parathyroid glands. Highly active glands, with the majority of the cells characterized by tortuous plasmalemal indentations, a very large golgi apparatus, dense cytoplasmic matrix and prosecretory granules, were observed only in animals of one diet group (low Ca, adequate P, no Vit. D) which was the only group with subnormal serum Ca levels. Only two dietary groups (high Ca, low P, + and - D) had inactive parathyroids, characterized by cells with few plasmalemal indentations, pale cytoplasm, a small golgi apparatus, and no prosecretory granules. Serum Ca levels were highest in these two groups, and one of them (high Ca, low P, -D) had severe rickets, the other groups, despite a wide variation in bone development (two had rickets), had moderately active essentially normal, parathyroids and normal levels of serum Ca.

Two degrees of activity were present in the parafollicular C-cells of the rat thyroid. All the cells in one diet group (high Ca, low P, -D) had very pale cytoplasm, few secretory granules, little granular endoplasmic reticulum (GER) and very small golgi apparatus. These animals also had the highest levels of serum Ca, but relatively normal bones. Active C-cells, with a large dense cytoplasm, massive concentrations of secretory granules, arrays of GER and very large golgi, were characteristic of the thyroids of all other diet groups.

CONCLUSIONS:

Low levels of dietary and/or serum phosphorus are necessary for the production of rickets in the growing rat. Vitamin D and calcium appeared to have no influence on bone development and a moderate influence on mineralization in the presence of adequate levels of dietary and serum phosphorus. Adequate or high levels of both Vitamin D and calcium must be present to counteract the influence of inadequate phosphorus.

Ultrastructure of Normal and Diseased Animal Tissue (Cont)

Parathyroid gland activity is directly related to serum Ca level. Phosphorus, either in the diet or serum, must have its influence, if any, on the parathyroid indirectly through serum calcium by influencing bone development and mineralization or via renal excretion and resorption. Alternately, the ratio of serum calcium and phosphorus may be a regulating factor; however, in this study Ca/P is a direct reflection of serum calcium. Thyroid C-cell activity appears not to be stimulated by high calcium levels in the presence of Vitamin D and low phosphorus. Stimulated thyroid C-cells are normal during growth, a time of high mineral flux. Their activity as reflected morphologically did not correlate well with the dietary supply of these minerals or Vitamin D available to the animal.

RECOMMENDATIONS:

The results of this work should be refined to publishable form.

PUBLICATIONS:

NONE

Ultrastructure of Normal and Diseased Animal Tissue (Cont)

Table 1 Preliminary Results of Study 04

	With Vitamin D				Without Vitamin D			
	High Ca	Lo P(7)	Ad P(7)	Low Ca	High Ca	Lo P(7)	Ad P(7)	Low Ca
Serum ca ^b	9.5	11.7	9.1	9.2	9.7	10.0	4.3	8.6
Serum P ^b	7.2	3.3	9.9	3.9	6.4	21.3	9.3	3.1
Serum ca/p	1.3	3.6	.9	2.4	1.6	7.9	0.5	2.8
Plate Depth, u	288.0	250.0	263.0	738.0	283.0	1046.0	242.0	1428.0
Head, Ash, %	41.9	31.8	33.9	23.1	45.0	18.4	31.2	17.0
Parathyroid c	+	0	+	+	+	0	++	+
C-cell d	+	++	+	+	+	+	+	+

a - number in () refer to group size

b - mg/100 ml

c - activity: 0 = inactive, + = moderately active, ++ = highly active

d - activity: 0 = inactive, + = moderately active

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ²	2. DATE OF SUMMARY ³	REPORT CONTROL SYMBOL DD-DR&E(AK)636	
				DA OA 6372	74 07 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCY ⁴	6. WORK SECURITY ⁵	7. REGRADING ⁶	8A. DISB ⁷ INST ⁸	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
73 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ⁹		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
		61101A		3A161101A91C		00	
a. PRIMARY						WORK UNIT NUMBER	
						059	
b. XXXXXXXX							
c. XXXXXXXX							
11. TITLE (Precede with Security Classification Code) ¹⁰							
Performance, Fatigue and Exhaustion (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ¹¹							
00230 Biochemistry; Physiology; Pharmacology; Environmental Biology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
70 08		72 06		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				FISCAL YEAR		b. FUNDS (In thousands)	
b. NUMBER ¹²				74		1.5	
c. TYPE: Not Applicable				75		0	
d. KIND OF AWARD:				CUM. AMT.		0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ¹³ : Letterman Army Institute of Research				NAME ¹⁴ : Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME ¹⁵ : Weiser, P.C., MAJ, MSC			
TELEPHONE: 415 561-3600				TELEPHONE:			
				SOCIAL SECURITY ACCOUNT NUMBER: 5 [REDACTED]			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME: Sterner, R.T., CPT, MSC			
				NAME: Sullivan, F.J. DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Dog Performance; (U) Fatigue (U) Exhaustion; (U) Military Performance; (U) Antifatigue Measures							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To describe the biochemical, physiological, and psychological factors that contribute to effective military performance and those that are responsible for fatigue and exhaustion. To study the interactions of fatigue, chemical agents, nutrition, and military performance effectiveness and for ameliorating fatigue and exhaustion.</p> <p>24. (U) The studies will fall into three general categories: 1) organismic studies will focus upon the performance characteristics of the individual; 2) systemic studies will be directed at the contributions of the various organs and organ systems to the performance of different tasks; 3) cellular studies will concentrate on those aspects of cell function which limit overall organ and hence body functions.</p> <p>25. (U) Due to the departure of the principal investigator during the first quarter of the fiscal year and the subsequent departure of the associate investigators in the 3rd and 4th quarters minimal research on this work unit was accomplished. Since their departures, however, they have been reducing data and writing manuscripts from data acquired during the preceding fiscal year. This work unit is being terminated.</p>							

*Available to contractors upon originator's approval

BODY OF REPORT

WORK UNIT NO.	059	Performance, Fatigue, and Exhaustion
STUDY NO.	6	Rehearsal and Psychomotor Performance

Because of the transfer of function from the Medical Research and Nutrition Laboratory, Denver, to Letterman Army Institute of Research, Presidio of San Francisco, and the associated dissolution of the Physiology Division this work unit is being terminated. Even though the principal and associate investigators have resigned from the Army, they have indicated a desire to publish work accomplished under this work unit, once they have completed data analysis and manuscript preparation. Such reports as they are received will be processed through normal review and clearance channels.

RESULTS AND DISCUSSION OF THE RESULTS:

Subsequent to the initial study described in the FY 73 annual progress report, additional data were collected from 23 subject volunteers to assess the efficacy of rehearsal for maintaining performance of a psychomotor skill under conditions of distributed practice. Again, mental rehearsal interfered with the retention of a target-tracking task.

CONCLUSIONS AND RECOMMENDATIONS: The data evaluation and manuscript preparation should be completed.

PUBLICATIONS:

The following manuscripts were prepared and submitted for review and clearance:

1. Sterner, R.T.: Interpretation of analysis of variance effects in designs yielding significant subjects X treatment interactions. (To be submitted to scientific journal)
2. Sterner, R.T.: An inexpensive periodic scanner for obtaining multiple analog measurements with a single channel output device. (Laboratory Report)
3. Sterner, R.T., J.T. Wheeler and L.F. Krabill.: Program post-hoc: one-way analysis of variance with post-hoc Dunnett, Newman-Keuls or Scheffe' mean comparisons. (Laboratory Report)
4. Weiser, P.C., Kinsmen, R.A., and Stamper, D.A.: Task Specific symptomatology changes resulting from prolonged submaximal bicycle riding. Med. Sci. Sports 5: 79, 1973.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8. DISEM INSTR ⁶	9. SPECIFIC DATA- CONTRACTOR ACCESS	
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ⁷		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		61101A	3A161101A91C	00	U60		
XXXXXXXXXX		61101A	3A061101A91C				
XXXXXXXXXX		CARDS 144(f)					
11. TITLE (Precede with Security Classification Code) ⁸							
(U) Vitamin D, Calcium and Phosphorus Metabolism (U6)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁹							
002300 Biochemistry; 002600 Biology; 0016800 Toxicology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 04		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER: ¹⁰ Not Applicable				FISCAL YEAR		c. FUNDS (In thousands)	
c. TYPE:				74		.7	
d. KIND OF AWARD:				75		35.0	
e. AMOUNT:				75		.7	
f. CUM. AMT.							
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ¹¹ Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ¹² Letterman Army Institute of Research Radioisotope Division			
ADDRESS: ¹³				ADDRESS: ¹⁴ Department of Nutrition Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: ¹⁵ Morrissey, R. L., CPT, VC			
TELEPHONE: (415) 561-3600				TELEPHONE: (415) 561-4770			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence Not Considered				NAME: Bucci, T. J., LTC, VC			
				NAME: Empson, R. N., Jr., CPT, VC DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Combat Bone Injuries; (U) Vitamin D; (U) Calcium; (U) Phosphorus; (U) Mineral Metabolism							
23. TECHNICAL OBJECTIVE, ¹⁶ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) It is hypothesized that Vitamin D₃ is converted to 25-hydroxycholecalciferol (25-HCC) in the liver, which is then converted to 1, 25-dihydroxycholecalciferol (1,25-DHCC) in the kidney prior to its metabolic effects on intestine, which include induction of calcium binding protein (CaBP) and increased calcium absorption. Volunteers or inductees entering military service have a need for 8.5 tooth restorations per man. At the current cost of dental procedures in military facilities and at an entrance rate of 5,000 men/month cost of dental care equals \$2,363,400 per year to treat inductees or new enlistees. From January 1965 through September 1970, 2,347,464 man days were lost due to hospitalization of 59,472 soldiers in Vietnam with bone and joint injuries. It is anticipated that results of this research may improve these statistics through reduction of the prolonged convalescence following bone and joint injury and improvement in periodontal and dental health.</p> <p>24. (U) An assay for human calcium binding protein (CaBP) is being developed so that studies can be more readily performed. These studies will include the evaluation of the impact of dietary calcium and phosphorus, other nutritional factors and various disease conditions on the intestinal absorption of calcium in man. The antibody to CaBP (Anti-CaBP) used in the above assay will also be used to localized CaBP in tissues and to study the influence of nutritional factors on such localization.</p> <p>25. (U) 73 07 - 74 06 Rabbit anti-CaBP has been prepared and used to demonstrate CaBP in the intercellular and basal region of human jejunum, in pancreatic islets of cat, mouse and rat, and in a limited (approximately 20-20%) subpopulation of renal tubular cells in several species. Within stained renal cells, some nuclei stain while others do not, which is an observation that may be of critical importance to an understanding of the mechanism of regulating vitamin D and calcium metabolism.</p>							

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

supernatant at 0, 7 and 14 days respectively. CNBr-activated Sepharose 4B coupled Ab absorbed CaBP from a crude renal homogenate of post mortem tissue, confirming the presence of CaBP specific Ab in the Ab preparation. Based on the above successful results of the CaBP assay, another collaborative study with the Department of Medicine, LAIR, was initiated. The study was designed to measure the CaBP concentration in jejunal biopsies of 5 obese human subjects who were fasting or receiving a 400 calorie diet. Samples have been collected and are being stored at -70°C until the assay can be re-established subsequent to the laboratory move. New ^{125}I labeled CaBP will have to be prepared before the assay can be reestablished.

A portion of the purified CaBP has also been used to determine its amino acid composition. This was accomplished by collaboration with Dr. David Cohn and co-workers at the Veterans Administration hospital in Kansas City, Mo. The results are presented in table 1 along with the published composition of the vitamin D dependent CaBP from chick intestine. A marked similarity of the two proteins is apparent. Composition studies of this nature are considered to be of considerable importance to provide data relative to the possible vitamin D dependency of CaBP in humans. It is not ethically feasible to induce vitamin D deficient rickets in man, which would be the only conclusive way to demonstrate such a dependency.

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

TABLE 1.

Amino Acid Composition of Human Renal CaBP and Chick Intestinal CaBP.

Amino Acid	Residues/mole	
	Human Renal CaBP	Chick Intestinal CaBP
Alanine	15	17
Arginine	6	5
Aspartate	30	34
Cystine	3	3
Glutamate	37	44
Glycine	15	13
Histidine	5	3
Isoleucine	9	11
Leucine	28	31
Lysine	17	24
Methionine	4	3
Phenylalanine	11	13
Proline	8	3
Serine	11	0
Threonine	12	9
Tryptophan	not determined	2
Tyrosine	5	8
Valine	7	5

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

CONCLUSIONS:

A radioimmunoassay for determining relative concentrations of CaBP in human intestinal biopsies is feasible.

RECOMMENDATIONS:

1. Continued application of the CaBP radioimmunoassay to the study of the role of CaBP in nutritional and/or metabolic disease processes.
2. Continued efforts to prepare a more intestinal CaBP specific antibody which would improve the sensitivity and specificity of the assay.
3. Initiate studies to determine the amino acid sequence and chemical characteristics of CaBP as a means of gaining insight into the basic mechanism whereby CaBP alters the efficiency of calcium absorption.

PUBLICATIONS:

1. Morrissey, R. L. and D. F. Rath. Purification of Human Renal Calcium Binding Protein from Necropsy Specimens. Proc. Soc. Expt. Biol. Med. 145: 699-703, 1974.
2. Morrissey, R. L., E. G. Lufkin, T. J. Bucci and R. H. Herman. Radioimmunoassay for and Cellular Localization of Human Calcium Binding Protein. Fed. Proc. 33(3): 714, 1974 (Abstract).

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

STUDY NO. 4

Electron Microscopic Localization of
of CaBP in Human Intestinal Mucosa

PROBLEM:

It is anticipated that the problem outlined above (ST-2) can be more readily dealt with if the mechanism and route of intestinal calcium transport are known. Precise tissue localization of CaBP is of critical importance to this objective. Taylor and Wasserman have reported the localization of CaBP on the intestinal brush border and PAS positive cells of the intestinal mucosa, using the fluorescent antibody technique in chicks. However, the resolution of the technique was not adequate to demonstrate the presence of CaBP in or between intestinal absorptive cells. More recently, Wasserman has reported that lanthanum complexes with CaBP avidly. Also, electron dense particles can be demonstrated in the intercellular space of intestinal mucosa predosed with lanthanum. It was not known whether CaBP was involved in this route of transport or whether calcium was transported via this route. When Ab to human renal CaBP became available as a consequence of ST-2 of this work unit it became possible to localize CaBP in human tissues by the peroxidase-labeled antibody technique and thus determine the possible merit of the hypothesis that calcium is absorbed by passing between intestinal cells rather than through them.

RESULTS AND DISCUSSION OF RESULTS:

The laboratory methodology for conjugating horse radish peroxidase to antibodies has been mastered and peroxidase conjugates of sheep anti-rabbit gamma globulin and rabbit anti-CaBP gamma globulin have been prepared.

Biopsy specimens of normal human jejunum were obtained with a Crosby-Kugeler capsule and fixed in paraformaldehyde. Upon staining, the presence of CaBP was indicated by brown reaction product in the intercellular space around absorptive cells of the villus tips. It was associated with the lateral and basal plasma membranes, but there was little in the microvillar region of the epithelial cells. Reaction product was also present in the basement membrane region beneath absorptive cells. There was no reaction product within the cytoplasm of absorptive cells.

The same technique was employed to localize CaBP in kidney and pancreas. The pattern of localization of CaBP in the kidney was strikingly similar in man (autopsy), monkey, dog, cat, rat, mouse, and chick. In the outer cortex, only certain regularly-spaced proximal and distal tubules contained CaBP, suggesting a non-random distribution. CaBP was also present inconsistently in cells of straight segments, collecting ducts, and in thin loops deep in the papilla. Sections

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

of positive tubules contained cells with reaction product in cytoplasm or nucleus, or both. Cell membranes and brush borders were consistently stained in positive cells. Completely unstained cells were present immediately adjacent to positive ones. No reaction product was present in blood vessel walls, in cells of the glomerulus, or in basement membrane of glomerulus or tubules. The selective distribution of CaBP among segments of particular nephrons was consistent and present in the several species studied. Whether the CaBP-positive regions are constant or shift with changing functional states remains to be shown. Nevertheless, the distribution implies a degree of heterogeneity among renal tubules which has not been demonstrated previously. The variable nuclear staining in renal tubule cells raises the possibility that CaBP might function as a regulator of gene expression in those cells.

CaBP was present in the pancreatic islets of the cat, dog, rat, mouse and chick. Human pancreas was not examined. In the islets, the reaction product was distributed intracellularly in a majority of the islet cells and the nuclei were unstained. The distribution of the labeled cells and the coarse granular pattern of the reaction product within cells suggests that the CaBP is associated with Beta cells. Exocrine tissue of the pancreas did not contain CaBP. A relation between calcium homostasis and pancreatic function has been suggested by earlier reports. Previously reported methods had not demonstrated CaBP in pancreas, but a relatively high concentration of calcium in pancreatic islets has been reported.

CONCLUSIONS:

1. The presence of CaBP in intercellular spaces adds support to Wasserman's proposal of a "paracellular" component for Ca^{++} transport. The apparent contradiction between our results in human intestine and the results of Taylor and Wasserman in chick intestine could be due to either species differences or technique differences.
2. Based on the above immunologic evidence, CaBP is quite probably a component of pancreatic tissue in spite of earlier negative reports.
3. CaBP is only present in a limited subpopulation of renal tubules, rather than in a portion of all tubules as was previously supposed.

RECOMMENDATIONS:

As investigator and technical support personnel became available, studies with the following objectives should be initiated:

1. Chemical characterization of purified human renal CaBP; including amino acid sequencing, determination of the calcium binding constant and number of calcium binding sites per molecule, S-S bridge potential, and possibilities for subunit organization.

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

2. The influence of nutritional and metabolic factors on the nuclear staining pattern of renal cells should be determined in order to assess the possibility that CaBP is involved in the regulation of gene expression in those cells. The factors should include high strontium diet, low calcium diet, vitamin D deficient diet, vitamin D toxicity, renal perfusion with varying concentrations of EDTA, renal perfusion with varying concentration of calcium, renal perfusion with strontium, parathyroid hormone injection, parathyroidectomy, thyroidectomy, renal perfusion with varying phosphate concentrations both pre and with fixation solution, insulin injection, insulin antibody injection, streptozotocin injection and alloxan injection.
3. CaBP should be isolated from pancreas and bone in order to chemically demonstrate its presence in these tissues.
4. Electron microscopic studies should be conducted to more precisely localize CaBP within cells.
5. Studies should be initiated to determine the role and function of CaBP in pancreatic islets and find the connection, if any, between osteoporosis in diabetics and CaBP function.
6. Studies should be initiated to determine where CaBP is formed within the intestinal mucosa (i.e. in mucosal cells or goblet cells).

PUBLICATIONS:

1. Bucci, T. J., R. L. Morrissey, R. W. Empson, Jr., and C. G. Plopper. Immunohistochemical Localization of Calcium-Binding Protein in Jejunum, Pancreatic Islets and Kidney. Am. J. Path. 74: 83a, 1974 (Abstract).
2. Morrissey, R. L., T. J. Bucci, R. N. Empson, Jr., and E. G. Lufkin. Cellular Localization of Calcium-Binding Protein. Proceedings of the 1974 Army Science Conference. In Press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ⁸	2. DATE OF SUMMARY ⁸	REPORT CONTROL SYMBOL	
				DA OC 6813	74-07-01	DD-DR&E(AR)636	
3. DATE OF REV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ⁹	6. WORK SECURITY ⁹	7. REGRADING ⁸	8. DISSEM INSTR ⁸	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73-07-01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ⁸		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		61101A	3A161101A91C	00	386		
b. SECONDARY		61101A	3A061101A91C	00			
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ⁸ (U) Development of a Rapid Clinical Procedure for Assessing Blood-Oxygen Affinity Curves (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁸ 003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
07 09		CONT		DA		C. In-house	
17. CONTRACT/GRANT				19. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
a. DATES/EFFECTIVE:				PRECEDING			
b. NUMBER: ⁸				FISCAL YEAR		74	.8
c. TYPE: NA				CURRENT		75	.5
d. AMOUNT:							40.3
e. KIND OF AWARD:							32.0
f. CUM. AMT.							
18. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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NAME: Canham, J.E., COL MC				NAME: ⁸ Neville, J.R.			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4714			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: <input type="checkbox"/> 6			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U)Blood oxygen affinity; (U)Blood-gas transport; (U)Arteriosclerotic heart disease							
23. TECHNICAL OBJECTIVE, ⁸ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The objective is to evaluate the oxygen transport function of blood, particularly oxygen affinity, and define its role in modifying physiologic responses of military personnel to trauma, environmental stress, and disease. Related objectives: (1) develop and evaluate a simple and reliable means to measure blood oxygen affinity; (2) discover practical agents for manipulating oxygen affinity during hemorrhagic shock and physical performance.</p> <p>24. (U) Because available techniques are generally slow, laborious and, in retrospect, poorly designed to account for dynamic relationships between red cell nutritional status, metabolic integrity and functional capability, this study necessarily involves a major effort to develop improved methodology. Using human and experimental animal blood, oxygen affinity data is compared with existing information, where available, or analyzed in relation to present theory, clinical diagnosis (humans), or experimental manipulation (laboratory animal). Drugs and physical agents are tested <u>in vitro</u> to assess potential <u>in vivo</u> modifiers of oxygen transport.</p> <p>25. (U) 130 additional patients from LAMC have been tested with the oxygen affinity technique previously devised. These results continue to show numerous patients with elevated oxygen affinity, suggesting compromised oxygen transport to tissues, particularly the heart. Elevated oxygen affinity is statistically associated with change in shape of the dissociation curve; these results (and those of others) suggest that oxygen affinity is altered with changes <u>in vivo</u> mean cell age. High oxygen affinity was prevalent in arteriosclerotic heart disease and patients with angina.</p>							

*Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

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BODY OF REPORT

WORK UNIT NO. 386

Development of a Rapid Clinical Procedure for Assessing Blood Oxygen Affinity

STUDY NO. 1

Development of Blood Oxygen Affinity Technique (Biotometry)

PROBLEM:

Available techniques for measuring blood oxygen affinity often are difficult to use, especially for routine screening of numerous blood samples. Also, practically all techniques utilize equilibration procedures that are inconsistent with present knowledge of the relation between erythrocyte metabolism and oxygen affinity. Recognition of the dynamic role of such constituents as 2, 3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP) in modifying erythrocyte functional attributes has stimulated widespread clinical interest in this matter. This study seeks to develop improved means of measuring blood oxygen affinity.

RESULTS AND DISCUSSION OF RESULTS:

Continued experience with the biotometry technique developed during this study supports previous impressions regarding the method's advantages. It is simple, rapid and inexpensive. Repeat trials show good reproducibility. Equilibration procedures required with earlier techniques are unnecessary. Because alterations of erythrocyte DPG levels have been shown to occur rapidly under conditions similar to those used for such equilibrations, results with the biotometry method may reflect more accurately the in vivo status of blood oxygen affinity.

CONCLUSIONS:

The biotometry method can be successfully applied to problems requiring blood oxygen affinity information and will be used in connection with related studies in this research.

RECOMMENDATIONS:

None.

PUBLICATIONS:

Neville, J. Ryan. Hemoglobin Oxygen Affinity Measurement Using Biotometry. J. Appl. Physiol., 1974 (Accepted for Publication)

Development of a Rapid Clinical Procedure for Assessing Blood Oxygen Affinity (Cont)

STUDY NO. 2

Oxygen Affinity Changes in Response to Trauma, Stress and Disease

PROBLEM:

Until recently, scientific consideration of the manner in which oxygen combines with hemoglobin was confined almost exclusively to textbook accounts or specialized publications dealing with the molecular aspects of the heme-proteins. When considered at all from a practical standpoint, for instance in determining cardiac output using the Fick principle, it was widely assumed that blood oxygen affinity for any species was an invariant relation that could be normalized by simply correcting for pH and temperature. Although other factors were known to affect this equilibrium, such influence was felt to be negligible under most circumstances, notable exceptions being carbon monoxide inhalation and the possible presence of abnormal hemoglobin. This established dogma has been put to rest only recently following intensive investigation of the effect of high energy phosphates (ATP, DPG, etc.) on the gas transport function of blood. The practical significance of these important findings, however, has not been demonstrated, despite the compelling theoretical arguments that have been advanced. Using a new technical approach (see Study No. 1) this effort is designed to evaluate the effect of oxygen affinity change on overall oxygen transport and define its actual role during trauma, stress and disease. For this purpose, use is made of both experimental animals and blood samples from patients confined at the Letterman Army Medical Center (LAMC). In view of the obvious experimental as well as potential therapeutic advantages afforded by controlled manipulation of oxygen affinity, the study includes a search for agents capable of modifying this function.

RESULTS AND DISCUSSION OF RESULTS:

One of the more interesting observations on reviewing the accumulated data now available (on about 275 LAMC patients) concerns the prevalent association of high oxygen affinity with arteriosclerotic heart disease (ASHD) and angina. This finding adds factual support to what has heretofore been a speculative theory regarding the etiology of these conditions. Previous attempts to correlate oxygen affinity with ASHD and/or angina had either been inconclusive or yielded lower than normal oxygen affinity. In the latter instance, it was assumed that low oxygen affinity was a compensatory response favoring tissue oxygen delivery rather than an etiologic

Development of a Rapid Clinical Procedure for Assessing Blood Oxygen Affinity (Cont)

factor. The implications of high oxygen affinity in these patients are being pursued in collaboration with LANC clinicians.

As previously reported, the shape of the curve is a significant variable affecting oxygen affinity. As a group, patients have Hill shape factors (n) ranging from 2.0 to 3.0, normals most commonly having values close to 2.5. Low values of the shape are regularly associated with high oxygen affinity. As a consequence, even minor increases on oxygen affinity will theoretically have a significant effect on the efficiency of oxygen extraction from blood by an organ such as the heart, since much of its oxygen is delivered from the low end of the dissociation curve. The previously reported effect of the tranquilizers Valium and Librium on oxygen affinity has not been confirmed by direct test in experimental animals (rats). In re-evaluating the original finding in humans, it was established that in practically every case where high oxygen affinity was associated with these drugs, the patients had sustained a recent myocardial infarct or were being evaluated for this condition. Thus the apparent relation between these drugs and high oxygen affinity may have been an artifact. When previously analyzed, patient clinical information was not available in all cases, and the possible overriding influence of the pathology involved was not considered.

CONCLUSIONS:

Definite proof of the tentative findings reported above may provide useful insight into prevention and treatment of arteriosclerotic heart disease. Such proof would also considerably strengthen the case, now advocated mostly on theoretical grounds, for oxygen affinity being an important variable in other diseases, in trauma, and during environmental stress.

RECOMMENDATIONS:

Further analysis of present data should be pursued, particularly after review of patient records for more complete clinical information. Accumulation of oxygen affinity profiles should be continued on a more selective basis.

PUBLICATIONS:

Neville, J. Ryan and J.P. Hannon. Altitude Tolerance and Oxygen Affinity. Science, 1974 (Submitted).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OC 6926	74 07 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8. UNCLAS INSTN ⁶	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM A. WORK UNIT
73 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10 NO./CODES: ⁹		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61101A		3A161101A91C		06	
b. STAFFWORK		61101A		3A061101A91C		387	
c. STAFFWORK							
11 TITLE (Precede with Security Classification Code) ⁸							
(U) Pathogenesis of Coliform Induced Colitis in Mice (05)							
12 SCIENTIFIC AND TECHNOLOGICAL AREAS ⁷							
003500 Clinical Medicine; 010100 Microbiology							
13 START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
72 03		74 06		DA		C In-House	
17 CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PREVIOUS		b. FUNDS (in thousands)	
b. NUMBER: ¹⁰				FISCAL YEAR		21.7	
c. TYPE: Not Applicable				CURRENT		0	
d. KIND OF AWARD:				75		0	
e. AMOUNT:				0		0	
f. CUM. AMT.							
13 RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ¹¹ Letterman Army Institute of Research				NAME: ¹² Letterman Army Institute of Research			
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: King, R.D., CPT, MSC			
				NAME: DA			
22 KEYWORDS (Precede each with Security Classification Code)							
(U) Pathology (U) Experimental Animal; (U) Model; (U) Colitis in Mice; (U) Bacterial Colitis							
23 TECHNICAL OBJECTIVE, ¹⁶ 24. APPROACH, 25. PROGRESS (Punish individual paragraphs identified by number. Precede rest of each with Security Classification Code.)							
<p>23. (U) To further characterize the mouse model of chronic ileitis and colitis caused by <u>Citrobacter freundii</u> (ANL). Diarrheal diseases, especially in tropical areas, rank second in cause of man-days lost among soldiers. This study further characterizes a mouse model of chronic ileitis and colitis caused by <u>Citrobacter freundii</u> (ANL), for applicability to study similar diseases in man.</p> <p>24. (U) To eliminate virus contamination of culture of <u>Citrobacter freundii</u> (ANL), freeze-thawed disrupted cells will be passed to weanling mice. To define the host range of <u>Citrobacter freundii</u> (ANL) using standard laboratory animals species. To study one-micron sections of affected mouse colons to better evaluate light microscopic changes and to determine requirement for electron microscopic studies, using material collected during FY 73.</p> <p>25. (U) 73 07-74 06 Hamsters and rabbits exposed to high levels of washed saline suspensions of <u>Citrobacter freundii</u> (ANL) did not demonstrate clinical or pathological evidence of infection. Disrupted bacterial cells were millipore-filtered and passed to weanling mice without the development of colitis. The disease thus does not appear to be viral, and is of limited host range. Electron microscopic studies were not initiated because of the delay in completion of facilities in Phase I of the new research facility. This work unit is being terminated due to the departure of the principal investigator.</p>							

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ABSTRACT

PROJECT NO. 3A161101A91C In-House Laboratory Independent
Research

WORK UNIT NO. 387 Pathogenesis of Coliform
Induced Colitis in Mice

Investigations of coliform-induced murine chronic colitis described in FY 73 continued. To determine whether infectious cultures of *Citrobacter freundii* (ANL) were contaminated with filterable agents (viral), and these were the causative agent rather than the coliform, cultures were disrupted by sonication and freeze-thawing followed by ultrafiltration. The filtrate, which contained no viable bacteria, was not infectious.

To explore the host range of the coliform, it was administered to weanling rabbits and hamsters in doses highly infectious to mice. No evidence of clinical or pathological changes were produced. These studies indicate that intact *Citrobacter freundii* are necessary to produce infection in mice, and that the organism has a limited host range. This system could be a useful model for study of mechanisms involved in human bacterial colitis.

This work unit is being terminated, due to transfer of the principal investigator.

BODY OF REPORT

WORK UNIT NO. 387

Pathogenesis of Coliform
Induced in Colitis in Mice

PROBLEM:

The study of chronic colitis in mice using *Citrobacter freundii* (ANL) as the initiating agent may represent a convenient tool to study chronic colitis of man. Studies to date have included a description of the sequential development and clearance of lesions in mice. Additional parameters that require elucidation include the host response in other laboratory animal species when exposed to the organism, elimination of the possibility of viral contamination of bacterial cells used in exposures, and completion of the electron microscopic examination of material collected during FY 73.

RESULTS AND DISCUSSION OF THE RESULTS:

Cultures of *Citrobacter freundii* (ANL) were washed in saline and disrupted using sonication and freeze-thaw methods followed by filtration through a 0.45 micron HA millipore filter. Weanling mice were exposed by gavage. No clinical, gross, or microscopic evidence of colitis were found in animals treated with disrupted cells. These data militate against viral contamination of the bacterial culture as the cause of the induced disease in mice. Weanling rabbits and hamsters were exposed to large numbers of saline washed *Citrobacter freundii* (ANL) organisms. No evidence of clinical infection or pathological changes were found in treated animals. Although preliminary, these data suggest a limited host range for *Citrobacter freundii* (ANL).

CONCLUSIONS:

Cultures of *Citrobacter freundii* (ANL) cause severe hyperplastic colitis of mice when the intact organisms are given by gavage. Because of the limited host range of the agent and the lengthy delay in clearance of the lesions (see Annual Report, FY 73) this system may be useful as a comparative model in the study of bacterial colitis of man.

Pathogenesis of Coliform Induced in Colitis in Mice (cont)

RECOMMENDATIONS:

Recommend this study be terminated following critical examination of 1-micron sections to evaluate light microscopic changes and to determine requirement for electron microscopic study of tissues already collected.

PUBLICATIONS:

Ediger, R.D., Kovatch, R.M. and Rabstein, M.M.: Colitis in mice with a high incidence of rectal prolapse. LAB ANML. SCI. 24:488-494, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL	
				DA OC 6928	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8A. DES'N INSTR ⁶	8B. SPECIFIC DATA- CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
73 07 01	H Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ⁷		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		61101A		3A161101A91C		00	
B. CONTRIBUTION		61101A		3A061101A91C		00	
C. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ⁸ (U) The Relationship of Polymorphonuclear Neutrophil (PMN) Chemotactic Activity to Inflammatory Periodontal Diseases in Military Personnel (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁹							
003500 Clinical Medicine							
13. START DATE			14. ESTIMATED COMPLETION DATE		15. FINDING AGENCY		16. PERFORMANCE METHOD
72 03			74 06		DA		C In-House
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		A. PROFESSIONAL MAN YRS	B. FUNDS (in thousands)
A. DATES/EFFECTIVE. EXPIRATION:				FISCAL YEAR		74	1
B. NUMBER: ¹⁰ Not Applicable				FURNERY		75	0
C. TYPE				AMOUNT:		0	0
E. KIND OF AWARD:				F. CUM. AMT.			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ¹¹ Letterman Army Institute of Research				NAME: ¹² Letterman Army Institute of Research			
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21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME: Cutcher, J. L., COL, DC			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ¹⁶ (U) Periodontal Disease; (U) Inflammation; (U) Parotid Fluid; (U) Chemotaxis; (U) Polymorphonuclear Neutrophil; (U) White Blood Cell							
23. TECHNICAL OBJECTIVE, ¹⁷ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) a. Determine the white blood cell (WBC) chemotactic activity of serum, whole saliva and parotid fluid; b. Determine the WBC chemotactic activity of a reaction mixture of saliva and parotid fluid with fresh serum.							
24. (U) White blood cell migration (chemotaxis) will be measured in the laboratory using Boyden chambers. The chemotactic response of WBCs to fresh serum and saliva will be compared to a reaction mixture of serum with saliva or parotid fluid. Endotoxin activated serum will be used as a positive control and Gey's medium will be the negative control.							
25. (U) 73 07 - 74 06 Parotid fluid and whole saliva both react with fresh serum to produce WBC chemotactic activity. While parotid fluid - serum interaction produces mild chemotactic activity, whole saliva - serum interaction produces very marked activity. Heating the serum at 56 degree Centigrade for 30 minutes prior to the reaction with saliva causes a marked reduction in WBC chemotactic activity. This phenomenon, if it occurs in the human oral cavity following trauma or surgery, may be an important defense mechanism in the highly infected oral environment and therefore enhance wound healing. Study terminated due to discontinuation of the department.							

*Available to contractors upon originator's approval.

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BODY OF REPORT

WORK UNIT NO. 389

The Relationship of Polymorphonuclear Neutrophil (PMN) Chemotactic Activity to Inflammatory Periodontal Diseases in Military Personnel

PROBLEM:

The objective of this investigation was to evaluate in vitro neutrophil (PMN) migration (chemotactic activity) resulting from the interaction of parotid fluid and whole saliva with fresh serum. A great deal of knowledge is available on the physiologic function of parotid fluid, saliva and serum, however, there is no information on biological activity resulting from the interaction of these components. If biological mediators of inflammation occur following the interaction of serum and saliva, they are of possible significance to host defense and, therefore, wound healing in the highly infected oral environment. Our hypothesis is that PMN chemotactic activity is enhanced when whole saliva reacts with fresh serum and that the biological activity is dependent upon a heat labile constituent in fresh serum.

This hypothesis was tested by comparing the level of PMN chemotactic activity of fresh serum, whole human saliva, parotid fluid and various combinations thereof. Five healthy individuals served as experimental subjects for this study. The experimental methods and controls are outlined as follows:

1. Collection of parotid fluid and saliva.

Parotid fluid was collected with a Currey cup placed over Stenson's duct. Whole saliva was collected in a 50 ml plastic tube.

2. PMN Chemotaxis.

- a. Twenty (20) ml of blood was drawn from the antecubital vein into a heparinized tube. Ten (10) additional ml of blood was used to prepare fresh serum.

- b. The heparinized blood was sedimented in a 2 percent Dextran solution. The plasma was separated and PMNs diluted in Gey's solution. Cells were washed twice and concentration adjusted to 2.2×10^6 cells per ml. The final suspension of PMNs was placed in the upper part of the Boyden chamber after the chemotactic substance was placed in the lower part.

- c. The serum was removed from the clotted blood and frozen if not used immediately.

The Relationship of Polymorphonuclear Neutrophil (PMN) Chemotactic Activity to Inflammatory Periodontal Diseases in Military Personnel (Cont)

d. Millipore filters were numbered and placed in the Boyden chamber.

e. The chemotactic stimuli or control were placed in Gey's solution in the lower part of the Boyden chamber; the PMN suspension was placed in the upper part.

f. The following test substances were used to evaluate WBC chemotactic activity of various reaction mixtures.

Chemotactic Test Substances

- (1) Bacterial CTX (whole sterilized saliva)
- (2) Endotoxin activated serum
- (3) Heat inactivated serum plus endotoxin (control)
- (4) Whole saliva plus serum
- (5) Heat inactivated serum plus whole saliva (control)
- (6) Parotid fluid plus serum
- (7) Parotid fluid plus heat inactivated serum (control)
- (8) Gey's medium control

g. The chambers were incubated for three hours at 37°C, 5 percent CO₂, in high humidity.

h. Filters were washed, stained with hematoxylin, cleared and mounted on microscopic slides with a cover glass.

i. Cells were counted on the bottom of the filter discs using 450X magnification and a grid. Ten random fields were averaged. The average number of PMNs per high power field was the chemotactic activity. The chemotactic activity minus the background cell migration was the chemotaxis index. Reading of slides was done in a single blind manner on coded slides. When the chemotaxis values were established, the values were decoded.

RESULTS AND DISCUSSION OF THE RESULTS:

Text mixtures with associated PMN chemotactic activity are as follows: (Chemotactic activity is the mean PMNs per high power field \pm standard error for five test subjects.)

The Relationship of Polymorphonuclear Neutrophil (PMN) Chemotactic Activity to Inflammatory Periodontal Diseases in Military Personnel (Cont)

<u>Test Substance</u>	<u>PMN Chemotactic Activity</u>
Serum	41 + 5
Parotid fluid	6 + 2
Serum + parotid fluid	142 + 30
Heated serum + parotid fluid	71 + 23
Saliva	239 + 54
Serum + saliva	359 + 51
Heated serum + saliva	311 + 52
Serum + endotoxin	341 + 43
Gey's medium	8 + 2

Previous studies have established that products from bacteria in saliva are chemotactic for PMNs and that parotid fluid does not exhibit chemotactic activity. Fresh serum activated by antigen antibody complex (IgG and IgM), endotoxin, zymosan, casein or cobra venom is strongly chemotactic for PMNs. The chemotactic activity develops from heat labile serum complement. In the present study, parotid fluid produced a mild PMN chemotactic activity following reaction with serum, while whole saliva produced marked activity. The PMN chemotactic activity of serum activated by whole saliva was equivalent to the chemotactic activity produced by reaction of endotoxin with serum. Heat inactivation of serum resulted in a reduced PMN chemotactic activity following reaction with either endotoxin or whole saliva. The minimal activity produced by parotid fluid is not considered biologically significant and is perhaps due to contamination. Whole saliva reacts with serum to produce marked PMN chemotactic activity. This reaction is of possible significance in wound healing of oral tissues following trauma or surgery in the highly infected oral environment. The PMN chemotactic response would provide an important first line of defense and allow wound healing to proceed without overt infection.

CONCLUSIONS:

Parotid fluid reacts with fresh serum producing PMN chemotactic activity. This phenomenon is of possible significance in the early migration of PMNs in oral wounds exposed to various oral secretions.

RECOMMENDATIONS:

This study is terminated because of the closure of this department.

The Relationship of Polymorphonuclear Neutrophil (PMN) Chemotactic Activity to Inflammatory Periodontal Diseases in Military Personnel (Cont)

PUBLICATIONS:

Tempel, T. R., H. L. Lazarus, B. Cheney and J. L. Cutcher. Parotid fluid-serum interaction: Generation of PMN chemotactic activity. J. Dent. Res. 53: 176, 1974 (Abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OC 6929	73 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY ACT ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. OBS'D INSTR ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM A. WORK UNIT
72 07 01	II Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10 NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61101A	3A061101A01C		00	391		
b. SECONDARY							
c. THIRDARY							
11 TITLE (Precede with Security Classification Code) ^a							
(U) Some Effects of Hypertonic Medium on Cultured Mammalian Cells (05)							
12 SCIENTIFIC AND TECHNOLOGICAL AREA ^a							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 04		NA		DA		C In-House	
17. CON/TRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	20. FUNDS (in thousands)
a. DATES/EFFECTIVE.				EXPIRATION:			
b. NUMBER ^a Not Applicable				f. AMOUNT:		2	0
c. TYPE.				g. CUM. AMT.			0
d. KIND OF AWARD				20. PERFORMING ORGANIZATION			
19. RESPONSIBLE DOD ORGANIZATION				NAME ^a Letterman Army Institute of Research			
NAME ^a Letterman Army Institute of Research				ADDRESS ^a Presidio of San Francisco, CA 94129			
ADDRESS ^a Presidio of San Francisco, CA 94129				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
RESPONSIBLE INDIVIDUAL				NAME ^a Dettor, C. M., LTC MSC			
NAME. Canham, J. E., COL MC				TELEPHONE: 415 561-3600			
TELEPHONE: 415 561-3600				SOCIAL SECURITY ACCOUNT NUMBER:			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME:			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Cell Growth; (U) Cell Division; (U) Stimulation of Cell Growth							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) Stimulate cell division for application to faster wound healing. A cell culture system will be used to investigate the effect of nontoxic additives to the culture medium. Quantitate changes effected by hypertonic treatment in mammalian cells relating to: sulhdryl-disulfide ratios; mitotic index; macromolecular synthesis; ultrastructure; and induction of division as an approach to enhancement of wound healing.							
24. (U) Cell culture experiments using biochemical, light microscopy and electron micrographic technique.							
25. (U) 73 07 - 74 06 Shaking experiments to produce cells in the mitotic state only gave yields of up to 30 percent. Transfer of the principal investigator precluded further experiments to improve cell yield and the work unit was terminated.							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

BODY OF REPORT

WORK UNIT. 391

Some Effects of Hypertonic Medium
On Cultured Mammalian Cells

STUDY NO. 1

Changes in Mitotic Index of Mammalian
Cells Effected by Hypertonic Treatment

PROBLEM: The ability to control cell proliferation is especially important in wound healing. Wounds made in the skin of Rhesus monkeys respond by showing an increase in the number of epidermal cells undergoing mitosis. The mitotic rate is not uniform, but proceeds in waves as if the cells were partially synchronized. This study was designed to first establish a method of synchronization to yield large numbers of cells in one phase of the cell cycle, then to quantitate changes in cell size and mitotic index effected by hypertonic treatment.

RESULTS AND DISCUSSION OF THE RESULTS: A cell line, Chinese hamster ovary, was established under laboratory conditions. Shake experiments gave yields of only up to 30% mitotic cells. Further experiments to improve cell yield ended with the loss of the principal investigator.

CONCLUSIONS: The study ended in the developmental stage, no conclusions can be drawn and the work unit was terminated.

PUBLICATIONS : None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ⁶	2. DATE OF SUMMARY ⁶	REPORT CONTROL SYMBOL	
				DA OC 6930	74-07-01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCY ⁷	6. WORK SECURITY ⁷	7. REGRADING ⁸	8A. DR&E INSTR ⁸	8B. SPECIFIC DATA - CONTRACTOR ACCESS ⁸	8C. LEVEL OF SUM A. WORK UNIT
73-09-25	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO. CODES ⁹	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	61101A	3A161101A91C	00	392			
b. SECONDARY	61101A	3A061101A91C	00				
c. THIRDARY							
11. TITLE (Precede with Security Classification Code) ⁹ (U) Chemotherapeutics of Ocular Metallosis (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁹ 003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 09		75 06		DA		C. In-house	
17. CONTRACT DATA				18. RESOURCE ESTIMATE		19. PROFESSIONAL MAN TIME	
A. DATE EFFECTIVE				PERIOD		PERIOD	
B. NUMBER				74		.3	
C. TYPE				75		.5	
D. KIND OF AWARD				F. CUM. AMT.		40.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL MC				NAME: Gardner, H.B., LTC, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4714			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: 527-50-0914			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Ocular foreign bodies; (U) Deferoxamine; (U) Penicillamine; (U) Metallosis; (U) Siderosis; (U) Chalcosis							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Precede individual paragraphs identified by number. Precede text of each with Security Classification Code)							
<p>23. (U) Ocular trauma and foreign bodies are a tragic accompaniment of warfare. Two widely used metals, iron and copper, cause damage from their chemical reactivity within the eye. Treatment by surgical removal requires major equipment and personnel considerations and is only partially effective. The possible use of non-surgical methods to delay or prevent the toxic effects of these metals would greatly enhance the care of these injuries, since this care could be administered even at the first level of medical care.</p> <p>24. (U) Rabbits will receive chemical challenges with copper or iron. The response of treated versus non-treated eyes will be determined using clinical and electrophysiological criteria. Removal of copper from the eye will be determined using radio-active copper. Development of a simple treatment regimen will be based upon the rabbit model and confirmed in sheep and possibly monkey models.</p> <p>25. (U) Excretion of intra-ocular iron and its enhancement by Desferal treatment has been documented in rabbits. Since maximum removal rates of 2 micrograms per day were found, the removal of iron foreign bodies by this method appears untenable. However, prevention of further siderotic changes until definitive care is available still appears feasible. Currently, electroretinogram facility has been developed and its use as a criteria of ocular damage from iron is being investigated. A more significant criteria the visual evoked response (which assesses the entire visual system), should be developed for ocular damage assessment.</p>							

* Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498

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

ABSTRACT

PROJECT NO. 3A161101A91C In-House Laboratory Independent
Research

WORK UNIT NO. 392 Deferoxamine-Efficacy by Various
Routes of Administration

The following investigations have been conducted under this work unit:

- STUDY NO. 1 Intra-ocular Iron Removal by Desferal
- STUDY NO. 2 Comparison of BID IM Desferal, Single Injection
IM Desferal, and Retrobulbar Desferal

Treatment of iron intra-ocular foreign bodies requires prevention of siderosis and removal of the iron. Desferal has been suggested for both of these purposes.

Using a rabbit model, radioactive intra-ocular iron removal was assessed by excreta collection and counting. Initial solid iron foreign bodies showed less than one PPM removal and were below the sensitivity of the method. Ionic ferrous and ferric iron showed a 25 fold increase in urinary excretion under the influence of Desferal. Retrobulbar injection was shown to be similar to single dose IM administration, although slightly more effective. Maximum removal rate was below 2 micrograms per day, indicating limited usefulness of Desferal for iron removal. Therefore, IM administration will be utilized in future work, and experiments in protection of the eye from siderosis will replace iron removal studies.

BODY OF REPORT

WORK UNIT NO. 392 Deferoxamine-Efficacy by various routes of administration.

STUDY NO. 1 Intra-ocular iron removal by Desferal

PROBLEM:

Iron intra-ocular foreign bodies are a tragic accompaniment of warfare. Surgical removal is only partially effective and requires major equipment and personnel considerations. Desferal has been suggested as a removal method. A "Desferal test" has been described showing a 0.5mg increase in urinary iron in patients with iron foreign bodies treated with Desferal. The origin of this iron is uncertain, however.

RESULTS AND DISCUSSION OF RESULTS:

Radioactive iron foreign bodies placed in the eyes of rabbits showed no detectable radioactivity in the excreta, either with or without Desferal treatment. The detection was good to one part per million of the foreign body per day. Ionic iron did show a 25 fold increase in urinary radioactivity in treated animals over controls, but this total amount was less than 2 micrograms per day. Also, fecal excretion was unaffected, and accounted for over 20% of the total in the treated animals. Thus, a 1-3 mg foreign body would take over three years to be excreted, even if it were totally ionized.

CONCLUSIONS:

Desferal would be ineffective in removing significant amounts of intraocular iron, especially in the metallic form.

RECOMMENDATIONS:

Desferal should not be considered as a removal mode for intra-ocular iron. Its use as a preventive agent in siderosis should be considered.

PUBLICATIONS:

None.

Deferoxamine-Efficacy by Various Routes of Administration (Cont)

STUDY NO. 2

Comparison of BID IM Desferal,
Single Injection IM Desferal, and
Retrolbulbar Desferal

PROBLEM:

Desferal has received use as an injection (BID IM), as a local salve, and as a local injection (sub-conjunctivally or retrolbulbar). No one route has been shown more beneficial.

RESULTS AND DISCUSSION OF RESULTS:

BID IM injections, single dose IM injections, and retrolbulbar routes were compared in rabbits with intra-ocular ionic iron. Single dose IM and retrolbulbar routes both gave a transient effect, lasting no more than 48 hours. Although a slightly higher peak excretion was obtained with the retrolbulbar route, BID IM injections can be accomplished more readily and give a greater total response.

CONCLUSIONS:

The IM route is adequate, convenient, and reproducible. The retrolbulbar route is too hazardous to suggest its routine use, despite a slight increase in effectivity.

RECOMMENDATIONS:

Due to its ease and reproducibility, the IM route should be utilized as a standard when comparing other parameters. Since removal of iron is minimal at best, the preventive aspect of Desferal on siderosis should receive the major consideration.

This work unit should be expanded to look at the broader problem of metallosis. A parallel study involving Penicillamine and its effect on intra-ocular copper, another commonly used metal which causes toxic effects by nature of its chemical reactivity, should be undertaken.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OC 6931	74-07-01	DD-DR&E(AR)636	
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^b	6. WORK SECURITY ^b	7. REGRADING ^c	8A. DISSEM INSTR ^d	8B. SPECIFIC DATA - CONTRACTOR ACCESS	
73-09-25	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
9. LEVEL OF SUM		A. WORK UNIT					
10. NO./CODES: ^e		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
						WORK UNIT NUMBER	
a. PRIMARY		61101A		3A161101A91C		00	
b. SECONDARY		61101A		3A061101A91C		00	
c. THIRDARY		CARDS 114 (f)					
11. TITLE (Precede with Security Classification Code) ^g							
(U) Lacrimal gland uptake and discharge of gallium (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^h							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 09		74 09		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER: ⁱ Not Applicable				FISCAL YEAR		.3	
c. TYPE:				CURRENT		40.5	
d. KIND OF AWARD:				75		.5	
e. AMOUNT:				f. CLM. AMT.			
10. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^j				NAME: ^j			
Letterman Army Institute of Research				Letterman Army Institute of Research			
ADDRESS: ^k Presidio of San Francisco, CA 94129				ADDRESS: ^k Department of Surgery			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Publish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL MC				NAME: ^l Gardner, H.B., LTC MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4714			
				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME:			
				NAME:			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Gallium-67; (U) eye; (U) lacrimal gland; (U) trauma							
23. TECHNICAL OBJECTIVE, ^m 24. APPROACH, 25. PROGRESS (Publish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To determine normal lacrimal gland dynamics with gallium. A reproducible uptake of gallium in the lacrimal gland will allow (1) the assessment of the affects of lacrimating agents (CS, CN) and (2) scanning of the gland in military trauma cases. Both of these possibilities will be useful in future work.</p> <p>24. (U) Rabbits will be injected with gallium 67 (70 microcuries/kg-avg. human dose) and lacrimal gland activity monitored on the LAIR camera. Mechanical corneal abrasions will be utilized to induce tearing in some animals. Animals exhibiting lacrimal gland gallium uptake will have gallium concentration in the tears measured by collection of tears on filter paper and well counting.</p> <p>25. (U) Reproducible differential lacrimal output by the two eyes of a single rabbit appears feasible by mechanical or chemical irritation. The study has not proceeded beyond this point due to the LAIR gamma camera dysfunction. With the move completed, at least a pilot study is hoped for even if the computer portion of the camera remains inoperative.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

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

WORK UNIT NO. **393** **Lacrimal Gland Uptake and Discharge
of Gallium**

The following investigations have been conducted under this work unit:

**STUDY NO. 1 Differential lacrimal Gland Output Induction
and Collection.**

A rabbit model for differential lacrimal output in the two eyes has been established. Chemical irritation and collection of secretion on modified filter paper strips allows analysis of lacrimal gland function. Further developemtn awaits repair of the gamma camera.

BODY OR REPORT

WORK UNIT NO. 393

Lacrimal Gland Uptake and Discharge
of Gallium

STUDY NO. 1

Differential Lacrimal Gland Output
Induction and Collection

PROBLEM:

Lacrimal gland secretion is normally symmetrical in the two eyes. this would require comparison of lacrimal gland function in two separate animals to view gallium dynamics as a function of gland secretion. In humans, unilateral ocular irritation may result in unilateral increased lacrimal gland secretion. The production and measurement of this phenomenon has not been recorded in animals.

RESULTS AND DISCUSSION OF RESULTS:

Mechanical irritation of one eye of a rabbit produces only a very slight and transient increase in tear production. Chemical irritants produce a somewhat higher and more prolonged unilateral increase. Tear collection with filter paper strips is both quantitative and readily accomplished.

CONCLUSIONS:

Unequal lacrimal secretion from the two eyes of a single rabbit may be produced and will allow assessment of gallium dynamics as a function of tear production in a single animal.

RECOMMENDATIONS:

With the gamma camera move completed, repair of the strip chart recorder would allow pilot experiments to be completed. If the computerized data system is also made operational, the original protocol could be started.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OC 6934	74-07-01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8A. DISEM INSTR ^a	8B. SPECIFIC DATA - CONTRACTOR ACCESS	8. LEVEL OF SUM
73-04-25	A. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61101A	3A161101A91C		00	394		
b. NONCONTRIBUTOR	61101A	3A061101A91C		00			
c. CONTRIBUTING							
11. TITLE (Precede with Security Classification Code) ^a							
(U) Metabolic and microcirculatory factors in hemorrhagic shock (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 09		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER: ^a NA				FISCAL YEAR		74 .5 4.7	
c. TYPE:				CURRENT		75 .5 40.0	
d. KIND OF AWARD:				f. CUM. AMT.			
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ^a Letterman Army Institute of Research Exp. Surg. Div. ADDRESS: ^a Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL MC				NAME: ^a Neville, J.R.			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4714			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: NAME: DA			
22. KEYWORDS (Precede SSAN with Security Classification Code)							
(U) shock; (U) erythrocyte deformability; (U) hypertensive rats							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Despite improvements in medical care and evacuation procedures, combat injuries frequently lead to overwhelming cardiovascular collapse refractory to known therapy. This effort is designed to investigate the phenomenon of red cell deformability and to establish the role such deformability may play in modulating tissue perfusion during shock. Agents modifying deformability will be studied to assess the potential therapeutic value of such agents during experimental hemorrhagic shock.</p> <p>24. (U) Blood from experimental rats and human subjects is tested for deformability by comparing the time required for a constant volume (at constant pressure and temperature) to flow through polycarbonate filters of known porosity. With this technique surveys are being conducted to determine base-line values in blood from groups of rats and human volunteers. Blood from LAMC patients has also been tested to assess possible pathologic changes. <u>In vitro</u> and <u>in vivo</u> tests of the effect of drugs on deformability have been carried out. Normal and spontaneously hypertensive rats have been tested for changes in deformability during hemorrhage.</p> <p>25. (U) Rat and human erythrocytes with a mean diameter of approximately 7 or 8 microns will rapidly pass through polycarbonate filters having porosities of 3 microns. This unusual behavior is related to the plasticity of the normal erythrocyte and little, if any, hemolysis occurs, bank blood (stored 3 weeks) appears unable to pass through such small bore filters. Hypertensive rats display prolonged filtration times compared to normotensive rats; in both groups deformability decreased in response to hemorrhage. Further work is required to assess sources of variability in the deformability test.</p>							

ABSTRACT

PROJECT NO. 3A161101A91C In-House Laboratory Independent
Research
WORK UNIT NO. 394 Metabolic and Microcirculatory
Factors in Hemorrhagic Shock

The following investigations have been conducted under this work unit:

STUDY NO. 1 Blood Deformability: its Measurement and Significance

This study was initiated only recently and preliminary efforts have been devoted mainly to devising a suitable technique for observing red cell deformability. Preliminary observations show that normal red cells from both rats and humans can pass through filters with porosities 3 microns in diameter. Three week old blood bank blood displays altered deformability and is often unable to pass through such small sized pores. Spontaneously hypertensive rats display decreased deformability compared to normal rats. Further work is needed to assess the physiologic significance of these changes.

BODY OF REPORT

WORK UNIT NO. 394

Metabolic and Microcirculatory
Factors in Hemorrhagic Shock

STUDY NO. 1

Blood Deformability: its Measurement
and Significance

PROBLEM:

Cardiovascular collapse leading to irreversible shock remains a prevalent hazard in the treatment of combat injuries and other trauma despite many improvements in evacuation procedures, fluid and electrolyte replacement, and surgical techniques. Of the numerous ramifications of this condition, perhaps the most prominent feature common to all forms of shock is the widely noted hypoperfusion of tissues. If prolonged, such hypoperfusion inevitably results in a pyramiding of, first, compromised and, secondly, permanently deranged physiologic and biochemical functions. Consequently, measures aimed at restoring perfusion, including fluid replacement, increased venous return and cardiac output, as well as lowering peripheral resistance, are widely used to treat this condition and maintenance of adequate perfusion to tissues is a well-regarded factor in the management of shock-prone medical emergencies. Present concepts of perfusion, however, are neither well quantified nor understood in great detail, and these deficiencies are handicaps in the treatment of shock. The present effort is concerned with perfusion decrements during shock and is specifically directed toward understanding the role that the erythrocyte may play in altering perfusion. It is postulated that: 1) a metabolically determined deterioration of mechanical adaptiveness (deformability) or perfusion effectiveness; 2) this deterioration plays a part in events leading to irreversible shock; and 3) measures designed to reverse or moderate this deterioration would favorably influence recovery from and resistance to shock.

RESULTS AND DISCUSSION OF RESULTS:

This is a recently initiated study and efforts have been primarily directed toward perfecting a suitable means to measure red cell deformability. Polycarbonate filters (General Electric) of known porosities have been used in the preliminary observations. With a suitable pressure differential (about 15 mm Hg) fresh citrated blood with buffy coat removed will flow at measurable rates through filters having pores down to 3.0 microns in diameter. The order of magnitude of the flow is about lcc/min for filters of 13 mm diameter. Larger porosity filters have been used to remove clots, this usually leaving a "clean" blood that displays less variability in flow through the small-pored filter than before such treatment.

With the above approach, it has been possible to gain some preliminary

Metabolic and Microcirculatory Factors in Hemorrhagic Shock (Cont)

insight into sources of variability in this technique and recent trials have yielded fairly uniform results. There are still sources of variability not completely understood, however, and further work is needed to bring such variability under control. It is a remarkable fact that erythrocytes with a mean diameter of 7 or 8 microns can rapidly pass through a 3 micron pore without hemolysis or permanent change and, in view of similar dimensional relationships in the microcirculation, it is surprising that this plasticity has not received wider attention. Under comparable conditions, it is found that 3 week old bank blood is slowed compared to fresh blood and in some cases is unable to pass through such filters, its deformability apparently being greatly altered by the metabolic changes that occur with storage. Preliminary observations of normal rats of approximately 200-300 grams compared to a special breed of spontaneously hypertensive rats has shown a consistent difference in erythrocyte deformability in the two groups, normal erythrocytes being more deformable than those of the hypertensive rat.

Tests of red cell deformability in human patients confined at LAMC has shown great variability in deformability---much more than with normal blood. However, the number of tests performed is too small to allow conclusions at this time. There is presently no valid way of inferring the quantitative significance of the differences in deformability that have been observed, much less the clinical implications of such changes. This problem will be addressed in future experiments.

CONCLUSIONS:

The method for measuring deformability appears capable of showing differences in deformability but needs further work to ascertain the quantitative and physiologic significance of these changes. Preliminary results encourage the belief that changes in deformability may play an important role in perfusion.

RECOMMENDATIONS:

None.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION #	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
				DA OA 6335	74 07 01	DD-DR&E(AR)636	
3. DATE PREV. SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DES'N INST. N	9. SPECIFIC DATA - CONTRACTOR ACCESS	
74 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO. CODES		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		61102A		3A161102B71P		01	
B. SECONDARY		61102A		3A061102B71P		01	
C. TERTIARY		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code)							
(U) Basic Studies in Lipids (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS							
002300 Biochemistry; 012900 Physiology; 002600 Biology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 04		CONT		DA		C-In-house	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE				FISCAL YEAR		B. FUNDS (in thousands)	
D. NUMBER				74		0.5	
E. TYPE				75		1.0	
F. KIND OF AWARD						34.1	
G. AMOUNT						50.0	
H. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: Herman, R. H., COL, MC			
TELEPHONE: 415 561 3600				TELEPHONE: 415 561 4147			
21. GENERAL ISE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME: Hagler, L., LTC, MC			
				NAME: DA			
22. KEY WORDS (Precede EACH with Security Classification Code)							
(U) Lipid Utilization by Muscle; (U) Carbohydrate Utilization by Muscle; (U) Muscle Function, Combat Soldier; (U) Lipids; (U) Clofibrate; (U) Bile Steroids							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Maximum muscular activity is essential for the effective performance of the combat soldier. The energy sources for muscle function are dietary lipid and carbohydrate. Dietary carbohydrate is transformed by the liver into lipid and stored in adipose tissue until needed. It is essential to determine the precise amounts and types of dietary lipid and carbohydrate that serve for optimum muscle function. The absorption of carbohydrate occurs as monosaccharides which are water soluble. Lipid is absorbed only when emulsified with bile steroids. The regulation of bile steroid production and secretion is not clearly known. Defects in absorption or metabolism of monosaccharides and/or lipids will lead to impairment of effectiveness of the combat soldier.</p> <p>24. (U) Hypertriglyceridemia and obesity may occur alone or together and are very common metabolic disorders. Both are chronic conditions which are resistant to therapy and result in prolonged periods of therapy and disabling complications which are important causes of military ineffectiveness. Clofibrate is an effective agent in reducing elevated triglyceride levels. Its mechanism of action is unknown. Clofibrate inhibits certain glycolytic enzymes in the rat jejunum, liver and fat and the membrane enzyme, adenylyl cyclase. Some of these enzymes are acutely regulated by various hormones. It was shown that the administration of clofibrate to obese men blocked the action of these enzymes to insulin and epinephrine. A water soluble form of clofibrate is now available and will be used to test its in vitro and in vivo effect in animals with regard to hormone-responsive enzyme systems.</p> <p>25. (U) 73-07 - 74-06 Work on this project was carried through the planning stage but performance was deferred due to the cessation of research in anticipation of the movement of the Dept. of Medicine from Denver, Colorado to San Francisco, California in April 1974.</p>							

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6344	74 07 01		
3. DATE PREV SUMRY ³	4. KIND OF SUMMARY ⁴	5. SUMMARY SCY ⁵	6. WORK SECURITY ⁶	7. REGRADING ⁷	8. DUSE ⁸ INSTR ⁸	9. SPECIFIC DATA- CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
72 07 01	D Change	U	U	NA	NL		
10. NO./CODES ⁹		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61102A		3A161102B71P		01	
b. XXXXXXXXXX		61145011		3A014501B71P		01	
c. XXXXXXXXXX		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ¹⁰							
(U) Basic Studies of Nutrition and Metabolism (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ¹¹							
002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. FUNDS (In thousands)	
a. DATES/EFFECTIVE:				EXPIRATION:			
b. NUMBER: Not Applicable				FISCAL YEAR		20. PROFESSIONAL MAN YRS	
c. TYPE:				74		1	
d. KIND OF AWARD:				75		3	
e. AMOUNT:				CURRENCY		21.3	
f. CUM. AMT.						105.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL MC				NAME: Milne, D. B.			
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-4305			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Kuhl, G. L., 1LT, MSC			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ¹² (U) Military Nutrition; (U) Military Rations (Nutrients); (U) Mineral Metab; (U) Proteins; (U) Lipids; (U) Carbohydrates; (U) Nutrition							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) ¹³							
<p>23. (U) Fundamental research is directed towards present or potential military problems concerning nutrition and metabolism and the role of diet in health and disease. Environmental factors, such as altitude and cold, have a marked effect on nutrition and metabolism. Because of the military implications of such adverse effects, an understanding of the effect and means of avoiding or correcting the conditions is a major objective of the investigations. Techniques will be developed and investigations conducted that will provide knowledge as to the metabolism, utilization or functions of dietary nutrients that may be utilized in applied studies in military ration development and troop feeding.</p> <p>24. (U) The significance of dietary and environmental interactions and their relevancy to human health and the adequacy of military rations will be studied. The effect of diet, altitude, cold, exercise and stress on protein metabolism will be determined through the use of defined diets and radioactively labeled amino acids. Metabolic products will be studied which may be responsible for decreased physical or mental performance at altitude. Alteration in subcellular components necessary for protein synthesis as related to dietary composition will be investigated. Experiments will be developed with laboratory animals to study the influence of minerals and their interaction with other dietary nutrients on promoting the healing of bone injuries as may be sustained in combat or on the prevention of renal calculi as occurs in troops in a hot climate.</p> <p>25. (U) 73 07 - 74 06 This work unit was inactive during the past year due to the loss of senior investigator. A replacement senior investigator has recently been employed and necessary equipment and supplies have been procured to initiate studies.</p>							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 66

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

ABSTRACT

PROJECT NO.	3A061102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biochemistry
WORK UNIT NO.	060	Basic Studies of Nutrition and Metabolism

This work unit has been inactive during the past year. With the pending transfer of the laboratory to San Francisco, investigators associated with these activities either accepted positions elsewhere or retired. Upon the availability of qualified investigators, aspects of this work unit will be considered for reactivation.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6341	74 07 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8A. DES'N INST'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input type="checkbox"/> YES <input type="checkbox"/> NO	
73 07 01	H Termination	U	U	NL			
10. NO./CODES ⁶		PROGRAM ELEMENT		TASK AREA NUMBER	WORK UNIT NUMBER		
		PROJECT NUMBER					
A. PRIMARY		61102A		01	061		
B. SECONDARY		61102A					
C. THIRDARY		CARDS 114 (f)					
11. TITLE (Precede with Security Classification Code) ⁷ (U) Mineral Metabolism - The requirements of Trace Minerals in Man Under Various Stresses (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁸ 002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07		Terminated		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				FISCAL YEAR		20. FUND (OF PROGRAM)	
B. NUMBER ⁹				74		2.5	
C. TYPE				75		0	
D. KIND OF AWARD:				0		0	
19. RESPONSIBLE OOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: ¹⁰ Letterman Army Inst of Rsch				NAME: ¹¹ Letterman Army Inst of Rsch			
ADDRESS: ¹² Presidio of San Francisco California 94129				ADDRESS: ¹³ Department of Nutrition Presidio of San Francisco, California 94129			
RESPONSIBLE INDIVIDUAL				NAME: ¹⁴ Johnson, H. L.			
NAME: Canham, J. E., COL, MC				TELEPHONE: 415 561 5066			
TELEPHONE: 415 561 3600				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
22. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME: Consolazio, C. F.			
				NAME: Burk, R.F., MAJ, MC DA			
23. KEYWORDS (Precede EACH with Security Classification Code) ¹⁵ (U) Human Mineral Balances, (U) trace mineral requirements; (U) Rations and Minerals; (U) Selenium and Vitamin E							
24. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To study the interaction between macro mineral metabolism and water balances as affected by nutritional and environmental stresses encountered during military maneuvers; (2) to determine the essentiality and requirements of trace minerals which may become limiting in rations; (3) to study the distribution, metabolism and requirement of selenium (Se).							
24. (U) Mineral balances, including sweat losses, are determined during human studies in which the effects of diet, environment and training upon physical performance, body composition and physiological function are evaluated. The effects of diet upon whole body retention, tissue distribution, plasma protein binding and excretion of injected ⁷⁵ Se and upon interactions of selenium and mercury when injected simultaneously have been investigated.							
25. (U) 73 07-74 06 This work unit will be terminated. Mercury and selenium (Se) appear to bind to each other and the complex is bound to a protein. Mercury excretion was slower initially in rats fed a Se adequate diet, but after about 3 days this excretion increased several fold above that of rats receiving a Se deficient diet. Se toxic diets produce liver damage within 6 days of feeding. This damage cannot be reversed, in the rat, by return to a normal diet. This toxic diet is fatal in 12 to 21 days. Dietary methionine supplementation did not alter whole body Se retention in either Se adequate or Se deficient animals. Retention of a single dose of radioactive Se was generally increased in older animals as compared to weanling rats when they were fed any of the diets for longer periods.							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO.	3A061102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biochemistry
WORK UNIT NO.	061	Mineral Metabolism - The Require- ment of Trace Minerals in Man Under Various Stresses
STUDY NO.	06	Selenium Metabolism Experiment 8. Pathogenesis of liver disease and anemia in selenium toxicity.
STUDY NO.	07	The Effects of Selenium and Mercury Upon Their Binding to Plasma Proteins
STUDY NO.	08	The Effects of Dietary Methionine Supplementation Upon Serum Selenoproteins and Whole-body Retention of Radioactive Selenium

The following investigations have been conducted under this work unit:

Study No. 06. Diets containing 30 ppm selenium fed to rats for 6 days initiated liver damage and jaundice. The toxic effects were only partially ameliorated by subsequent feeding of normal or selenium deficient diets. Furthermore, serum bilirubin values, serum glutamic oxalacetic transaminase activity and liver histology indicated that the Se deficient diet was less effective than the normal diet in ameliorating the toxic syndrome.

Study No. 07. A series of three experiments were conducted to examine the effects of mercury exposure on tissue and whole-body retention and excretion of ^{203}Hg in rats fed either selenium deficient or adequate diets. Mercury levels ($^{203}\text{HgCl}$) in all measured organs tended to be increased by selenium in the diet. Prior exposure to mercury (20 ppm) in drinking water generally decreased the organ retention of ^{203}Hg in rats fed both 0.5 ppm selenium and selenium deficient diets. Prior exposure to mercury caused an increased ^{203}Hg output in urine. Based upon whole body ^{203}Hg retention measurements, whole body loss of ^{203}Hg in the Hg exposed rats was not entirely accounted for by urinary and fecal losses, indicating an additional mode of Hg excretion.

**Mineral Metabolism - The Requirement of Trace Minerals in Man
Under Various Stresses (Cont)**

Study No. 08. Fifty male weanling rats were assigned to each of four groups: 1) Control - fed a vitamin E supplemented 25% torula yeast basal selenium deficient diet; 2) selenium supplemented - receiving the basal diet plus 0.2 ppm Se as sodium selenite; 3) methionine supplemented - consuming the basal diet plus 0.8% methionine, and 4) methionine-selenium supplemented - fed the basal plus the above levels of both supplements. After 4, 7, 10, 13 and 16 weeks, two rats from each group were injected with 50 μ ci of ^{75}Se . Twenty-four hours later, these animals were exsanguinated and the plasma used to determine protein binding of the selenium tracer. After the same feeding times, six rats were injected with 1 μ ci of ^{75}Se to measure urinary and fecal excretion of the tracer for 10 days, whole body retention for 35 days, and organ distribution of ^{75}Se at 35 days. The proteins and radioactive tracer distribution in plasma were very similar in the four dietary groups. Whole body retention was reduced to 15% of the injected dose in the animals receiving selenium supplemented diets, and was 70% of the dose after 35 days in the selenium deficient rats. Methionine did not affect excretion or whole body retention of the tracer. The older animals that had been fed diets for a longer period of time retained more ^{75}Se than the younger rats.

BODY OF REPORT

WORK UNIT NO. 061 Mineral Metabolism
STUDY NO. 06 Liver Pathogenesis

PROBLEM:

Selenium toxicity causes irreversible liver damage, jaundice and anemia. This study was designed to examine the time course of the toxicity syndrome and the effects of different dietary levels of selenium (Se) upon its' development, after the initial induction of 6 days on a 30 ppm Se diet.

RESULTS AND DISCUSSION OF RESULTS:

The selenium induced liver damage was examined in a study of 131 male Holtzman rats. The control group of 30 rats were fed a 30% torula yeast diet supplemented with 0.5 ppm selenium (control diet). Ninety rats received 30 ppm selenium in the yeast diet (toxic diet) for 6 days. Two control and 3 toxic animals were sacrificed daily for liver biopsy and examination during the initial 6 days. The rats consuming toxic diets were then divided into 3 groups: a) 19 rats continuing on the toxic diet; b) 31 rats consuming control yeast diet (supplemented with 0.5 ppm Se). Two control, 2 toxic, 3 toxic-deficient and 3 toxic-control rats were killed every other day for liver examination until the study was terminated on day 21. At time of necropsy, the rats were weighed, anesthetized with ether, exsanguinated from the aortic bifurcation and the liver removed and placed in formalin. Blood was pooled for each group and the serum was collected for bilirubin and glutamic oxalacetic transaminase (SGOT) activity determinations.

The control animals doubled their weights during the 25-day period, while the animals fed the toxic diets lost over 30% of their weight during the first week at the end of which the body weight plateaued. Rats fed the selenium deficient or control diet after 6 days of the toxic diet grew slowly with a 50% increase in body weight.

SGOT activity remained at about 200 milli-units per milliliter for 6 days in the toxic rats and throughout the study for the controls. By day 8, the rats continuing on the toxic diet had a 9-fold increase in enzyme activity, while the animals fed the selenium deficient and control diets had 7- and 3-fold increases, respectively. Enzyme activity in animals removed from the toxic diets returned to control levels within 8 days, while animals consuming toxic diets continued increasing thru 14 days and then decreased to about 6 times normal.

Mineral Metabolism - The Requirement of Trace Minerals in Man Under Various Stresses (Cont)

Serum bilirubins of the control rats varied, while the rats consuming toxic diets had an increase on day 5. By day 8, rats continuing on the toxic diet and those switched to the control diet had bilirubin levels between 3 and 4 mg/ml, while those switched to the selenium deficient diet increased to 8 mg/ml. Further increases were noted on day 10 for these 3 groups. Rats consuming the toxic diet had a plateau in bilirubin levels, while those switched to non-toxic diets began decreasing.

The histopathological examination of the livers of the rats maintained on the toxic diet disclosed 100% with disseminated hepatocellular necrosis and 56% with mid-zonal toxic hepatitis. Rats removed from the toxic diet to the deficient diet showed 85% with disseminated individual hepatocellular necrosis and 44% with random areas of hepatocellular necrosis. Animals placed on the control diet had 26% with disseminated individual hepatocellular necrosis and 34% with random areas of hepatocellular necrosis. The livers of the control group were normal.

STUDY NO. 07

The Effects of Selenium and Mercury Upon Their Binding to Plasma Proteins

PROBLEM:

Selenium and mercury have been shown to be antagonists in the development of their respective toxicities. This study was designed to investigate the interrelationships between Se and mercury on protein binding, excretion and whole body retention.

RESULTS AND DISCUSSION OF RESULTS:

Forty-four male Holtzman weanling rats (80-100 gm) were placed on either basal (0 ppm Se) or selenium (0.5 ppm Se) torula yeast diets and housed individually in stainless steel hanging cages. Thirty days later, half of each dietary group received 20 ppm HgCl_2 in the drinking water for an additional 14 days. Six rats from each treatment were injected subcutaneously with 1 μCi $^{203}\text{HgCl}_2$ in saline for experiment A. Urine and feces collection with daily whole body gamma counting was performed for 14 days. Tissue concentration of ^{203}Hg was determined in experiment B. Five rats from each of the 4 treatments were injected subcutaneously with 100 μCi $^{203}\text{HgCl}_2$ in saline. Rats were exsanguinated 14 days later and tissues removed and counted. In experiment C, 8 rats ingesting mercury (4 rats on basal and 4 rats on selenium diet) were injected intraperitoneally with 10 μCi of $\text{H}_2^{75}\text{SeO}_3$ in saline and subcutaneously with 10 μCi of $^{203}\text{HgCl}_2$ in saline. Urine and feces content

Mineral Metabolism - The Requirement of Trace Minerals in Man Under Various Stresses

of ^{75}Se and ^{203}Hg were determined by dual channel ratio counting. Hg ingestion in experiment A lowered whole body Hg retention to 10% of the injected dose in basal rats and 36.5% in selenium rats. Rats on the other two treatments retained approximately 40 to 50% of the dose. Basal rats ingesting Hg had large (20.2% of dose), rapidly declining amounts of ^{203}Hg excreted in the urine. Selenium ingesting rats had increasing ^{203}Hg excretion through day 9 (5.2% of dose on day 8) and then excretion began declining slightly. Some ^{203}Hg excreted in the two Hg ingesting groups was not accounted for by the urinary and fecal losses. Selenium treated rats in experiment B had a greater ^{203}Hg content in the tissues than basal rats. Hg ingestion tended to lower tissue retention of ^{203}Hg as compared to rats not receiving Hg. In experiment C, basal rats excreted 20.2% of the injected ^{203}Hg in the first collection of urine which declined each collection while selenium rats excreted ^{203}Hg and ^{75}Se at near constant rates (5.0% of dose) after a small decline between days 3 and 7. Fecal concentration of ^{203}Hg in both groups were similar but ^{75}Se concentration was greater in the selenium group. Possibly, Hg ingestion stimulated existing or established new elimination routes for Hg. Se-Hg binding may have caused tissues to retain the Hg with resultant decreased excretion rates.

STUDY NO.

08

The effects of Dietary Methionine Supplementation Upon Serum Selenoproteins and Whole-body Retention of Radioactive Selenium

PROBLEM:

An earlier study in this laboratory showed that increased dietary methionine increased blood Se levels. This study was designed to examine the effects of dietary methionine upon plasma protein binding of selenium, organ distribution of ^{75}Se and whole body retention of the tracer.

RESULTS AND DISCUSSION OF THE RESULTS:

One study was conducted on the effects of dietary methionine levels upon whole body and tissue retention of selenium. Two hundred male weanling rats were randomly assigned to four dietary groups: 1) Group I, basal diet consisting of 25% torula yeast, 5% stripped lard, 66.2% cerelose, plus minerals and vitamins (adequate in vitamin E); 2) Group II, basal plus 0.2 ppm Se as sodium selenite; 3) Group III, basal plus 0.8% methionine, and 4) Group IV, basal plus selenium and methionine. After four weeks on these diets, 2 animals from each group were injected intraperitoneally with 50 μCi of high specific activity ^{75}Se and 6 animals fed each of the diets received

Mineral Metabolism - The Requirement of Trace Minerals in Man Under Various Stresses

1 μCi ^{75}Se intraperitoneally. The eight 50 μCi rats were sacrificed at 24 hours by exsanguinating via the abdominal aorta. Plasma proteins were partitioned on a sephadex column and the column eluates, collected in one milliliter aliquots were counted for radioactivity. Radioisotope retention of the 1 μCi rats was counted daily for 10 days, and then 3 times per week for an additional 25 days in a small animal whole body counter. Urine and feces were collected separately for 10 days and the radioactivity was evaluated to determine excretory routes. Thirty-five days after injection, the rats were sacrificed and various organs were excised, weighed and the radioactivity counted.

Both methionine supplemented diets resulted in higher body weights of the animals from 4 thru 16 weeks of feeding. By 6 weeks the methionine-Se diets, increased growth by at least 7% over the other diets for the remainder of the study. The basal diet with selenium increased the body weights of rats by 10% or more at 13 and 16 weeks when compared to basal diet fed rats.

Whole body selenium retention showed significant effects of diet (dietary selenium supplementation reduced retention at 35 days from 70% to 15% of the injected dose); of time (the older and larger rats that had been fed the diets for longer times retained about 5-10% more selenium than the younger animals); and a significant diet time interaction. During the 10 days post-injection of radioactive selenium, most of the tracer excretion was urinary with less than 1.5% of the injected dose appearing in the feces. Most of the tissue data has been prepared for statistical analyses.

CONCLUSIONS:

(All studies under this work unit.) Liver damage induced by selenium toxic diets at 6 days was not reversible by feeding selenium deficient or normal diets. Dietary selenium increased the initial retention and organ contents of mercury but one week after injecting mercury, selenium also increased mercury excretion. Dietary mercury via the drinking water prior to injecting ^{203}Hg increased its excretion. Whole body retention of a single dose of ^{75}Se was: reduced by selenium supplementation of the torula yeast diet; not affected by dietary methionine levels; and increased as the animals become older and larger.

RECOMMENDATIONS:

(All studies on this work unit.)

1. Institute new work unit on mineral metabolism to encompass other minerals.

Mineral Metabolism - The Requirement of Trace Minerals in Man
Under Various Stresses

2. Continue to study the losses of trace elements via sweat and the impact of such losses on trace mineral requirements. Minimal work was initiated in this area during the past year due to limited manpower (loss of one principle investigator).

3. The interrelationships between selenium and mercury metabolism should receive further investigation.

PUBLICATIONS:

1. Kiker, K. W., R. F. Burk, and C. F. Consolazio. Influence of dietary selenium in urinary excretion of ^{75}Se in the rat. J. of Colo-Wyo Acad. of Sci. 7:10, 1973 (Abstract #36).
2. Burk, R. F., A. M. MacKinnon, and F. R. Simon. Selenium and hepatic microsomal hemoproteins. Biochem. and Biophys. Research Communication 56:431, 1974.
3. Burk, R. F., K. A. Foster, P. M. Greenfield, and K. W. Kiker. Binding of simultaneously administered inorganic selenium and mercury to a rat plasma protein. Proc. Soc. Exp. Biol. Med. 145:782-785, 1974.
4. Kiker, K. W. and R. F. Burk. Effect of dietary selenium on the production of urinary selenium metabolites in the rat following $^{75}\text{SeO}_3^{2-}$ administration. Am. J. Phys. (in press).
5. Burk, R. F. Effect of dietary selenium level on ^{75}Se binding to rat plasma protein. Proc. Soc. Exp. Biol. Med. 143:719-722, 1973.
6. Johnson, H. L. A selenium deficiency in the rat. Laboratory Report submitted to Publications Review Committee.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENT / ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8. DES'N INSTR' ⁶	9. SPECIFIC DATA - CONTRACTOR ACCESS ⁷	
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ⁸		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		61102A		3A161102B71P		01	
B. SECONDARY		61102A		3A061102B71P		01	
C. THIRDARY		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ⁹							
(U) Haemopoietic Metabolism as Related to Nutrition, Genetics and Metabolic Disease (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ¹⁰							
002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (in thousands)	
B. NUMBER: ¹¹				FISCAL YEAR		C. FUNDS (in thousands)	
C. TYPE:				74		0.5	
D. KIND OF AWARD:				75		40.0	
E. AMOUNT:				CURRENT		40.0	
F. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ¹²				NAME: ¹³			
Letterman Army Institute of Research				Letterman Army Institute of Research			
ADDRESS: ¹⁴				ADDRESS: ¹⁵			
Pre. idio of San Francisco, CA 94129				Dept. of Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: ¹⁶				NAME: ¹⁷			
Canham, J. E., COL, MC				Herman, R. H., COL, MC			
TELEPHONE: ¹⁸				TELEPHONE: ¹⁹			
415 561 3600				415 561 4147			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: ²⁰			
Foreign Intelligence not Considered				[REDACTED]			
				ASSOCIATE INVESTIGATORS			
				NAME: ²¹			
				Hagler, L., LTC, MC			
				NAME: ²²			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ²³							
(U) Vitamin Requirements of the Combat Soldier; (U) Red Blood Cell Enzymes; (U) Red Blood Cell Membrane; (U) Blood Cell Metabolism; (U) Folic Acid							
23. TECHNICAL OBJECTIVE, ²⁴ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The diet of the combat soldier must contain sufficient amounts and types of vitamins necessary for optimum performance. To establish the amounts and types of vitamins necessary for the diet a suitable assay system must be established. It is known that folic acid increases the activities of glycolytic enzymes and fructose diphosphatase in the jejunum and liver of man. Red blood cells contain an elaborate folic acid metabolizing system which corresponds to that found in the liver and jejunum. It is postulated that the action of folic acid on the various enzymes is, in part, mediated via its effect on purine biosynthesis where 2 different forms of folate are obligatory cofactors. There is evidence to show that the jejunum and red blood cells during hematopoiesis derive purines preformed from the liver. If so, the action of folic acid on jejunal enzymes cannot be via the stimulation of purine biosynthesis. The red blood cell is an ideal cell in which to study the mechanism of action of folic acid on glycolytic enzymes.</p> <p>24. (U) Red blood cells will be incubated <u>in vitro</u> with various amounts and types of folate derivatives and analysis of glycolytic enzymes will be performed. Analysis of purine biosynthetic enzymes will also be done to establish to what extent purine biosynthesis is possible in red blood cells. The incorporation of ¹⁴C-glycine into red blood cell purines in the presence of various amounts and types of folate cofactors will be investigated.</p> <p>25. (U) 73-07 - 74-06 Work on this project was carried through the planning stage but performance was deferred due to the cessation of research in anticipation of the movement of the Dept. of Medicine from Denver, Colorado to San Francisco, California in April 1974.</p>							

* Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A061102B71P Basic Research in Support of
Military Medicine

TASK NO. 01 Biochemistry

WORK UNIT NO. 063 Haemopoietic Metabolism as Related
to Nutrition, Genetics and
Metabolic Disease

The following investigations have been conducted under this work unit:

See Annual Research Progress Report USAMRNL, FY73. Because of the projected move of the Dept. of Medicine, Letterman Army Institute of Research, Denver, Fitzsimons Army Medical Center, Denver, Colorado (formerly Metabolic Division, U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons Army Medical Center, Denver, Colorado) from Denver, Colorado to San Francisco, California it was not possible to implement studies in FY74.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6321	74 07 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^b	6. WORK SECURITY ^b	7. REGRADING ^c	8a. DOW'N INSTR ^d	8b. SPECIFIC DATA - CONTRACTOR ACCESS ^e	9. LEVEL OF SUM A. WORK UNIT
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO / CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61102A	3A161102B71R		02	058		
b. SECONDARY	61145011	3A014501B71R		02			
c. THIRDARY	CARDS 114 (F)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Nutritional and Metabolic Adaptations and Interrelationships (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
002300 Biochemistry; 002600 Biology; 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
65 05		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PREVIOUS		FUNDS (In thousands)	
b. NUMBER: ^a Not Applicable				FISCAL YEAR		96.2	
c. TYPE:				74		3.5	
d. KIND OF AWARD:				75		4.0	
e. CUM. AMT						100.0	
19. RESPONSIBLE DOD ORGANIZATION ^a				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a Presidio of San Francisco, CA 94129				ADDRESS: ^a Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL MC				NAME: ^a Askew, E. W., CPT MSC			
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-4323			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Hecker, A. L., CPT MSC DA			
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Exercise; (U) Metabolism; (U) Diet; (U) Adaptation (U) Enzymes; (U) Fatigue; (U) Military Physical Performance							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To study adaptive changes in energy metabolism occurring in response to exercise and to identify potential rate-limiting steps in energy generation during exercise. To determine the interaction of environment and nutrition of these adaptations. Obtain basic information on aspects of biochemistry, physiology, and nutrition as related to exercise that will ultimately allow the recommendation of an improved program of physical training and nutrition in order to improve the ability of the soldier to cope with various environmental and military situations.							
24. (U) Rats physically conditioned by a standardized 12-week program of treadmill running were utilized to study the effect of physical training on: 1) the absorption of nutrients from the gut, 2) mineral metabolism, 3) adipose tissue turnover rate and 4) cellular response to hypoxia.							
25. (U) 73 07 - 74 06 Studies concerning the effect of exercise on muscle mineral metabolism and absorption of nutrients from the gut have been completed and the results are currently being analyzed. Twelve weeks of treadmill running increased the turnover rate of rat adipose tissue from a t _{1/2} of 27 days (untrained) to t _{1/2} of 16 days (trained). Supplementing the diet of trained rats with carnitine did not significantly alter the turnover rate of adipose tissue indicating that tissue carnitine levels do not limit fat utilization by the exercising animal. Treating hypoxic or exhausted rats with supplemental cortisol prior to altitude exposure or exhaustive exercise did not alter mitochondrial oxidative processes thus providing no support for the hypothesis that supplemental cortisol can influence hypoxia-induced aerobic metabolic alterations.							

* Available to contractors upon order/their approval

DD FORM 1498
1 MAR 66

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

ABSTRACT

PROJECT NO. 3A061102B71R Research in Biomedical Sciences
TASK NO. 02 Internal Medicine
WORK UNIT NO. 058 Nutritional and Metabolic
Adaptations and Interrelationships

The following investigations have been conducted under this work unit:

- STUDY NO. 5 Biochemical Adaptation to Exercise
- STUDY NO. 6 Dietary Control of Lipid Metabolism
- STUDY NO. 8 Studies on Mineral Metabolism and Interactions
- STUDY NO. 9 Effect of Stress on Nutrient Absorption and Metabolism

STUDY NO. 5: Rats were treated with the hormone cortisol prior to exposure to two experimental conditions designed to result in muscle hypoxia (altitude exposure and exhaustive exercise) in an attempt to prevent a predicted decrease in oxidative capacity due to hypoxia. No deleterious effects on muscle mitochondrial oxidative capability due to hypoxia were noted, thus precluding any conclusions relating to the role of cortisol in ameliorating such effects.

STUDY NO. 6: The technique of odd carbon medium chain fatty acid labelling of adipose tissue was employed to estimate the effect of exercise and dietary carnitine on adipose tissue turnover rate in the rat. Twelve weeks of treadmill running approximately doubled the rate at which fatty acids fluxed through adipose tissue. Supplementing the diet of trained rats with 0.5% L-carnitine did not significantly increase adipose tissue fatty acid turnover rate, indicating that carnitine does not limit fatty acid utilization by the exercising rat.

STUDY NO. 8: A study concerning the effect of exercise on mineral metabolism and tissue distribution in the rat has been completed. The data are currently being prepared for statistical analysis.

STUDY NO. 9: An investigation on the effect of physical training on carbohydrate and lipid absorption has been completed. The data from the initial experiment are currently in the final stages of statistical analysis.

BODY OF REPORT

WORK UNIT NO. 058

Nutritional and Metabolic
Adaptations and Interrelationships

STUDY NO. 5

Biochemical Adaptation to Exercise

PROBLEM:

Previous investigations conducted under this study have indicated that exhaustive exercise and acute altitude exposure both exert similar deleterious effects on mitochondrial respiration, perhaps through the common mechanism of hypoxia. Recent reports in the literature suggest that glucocorticoids play a crucial role in increasing the ability of rats to survive in a low oxygen tension atmosphere, perhaps through a facilitation of aerobic metabolism. To test this hypothesis, both cortisol injected and control rats were exposed to a simulated altitude of 25,000 ft (ASL) for 12 hours and sacrificed immediately upon return to 5,280 ft (Denver altitude). Skeletal muscle mitochondria were isolated and tested for their ability to oxidize pyruvate and palmitate.

RESULTS AND DISCUSSION OF THE RESULTS:

Neither altitude exposure nor exhaustive exercise was effective in this experiment in causing any significant decrease in pyruvate or palmitate oxidation, ADP/O ratio or respiratory quotient. Cortisol injections (5 mg/rat 6 days prior to sacrifice and 5 mg/rat 1 day prior to sacrifice) did not significantly change any of the above measurements of aerobic metabolism. Since hypoxia was apparently not accompanied by decreased mitochondrial function in this study, the question of whether cortisol would be effective in preventing such a decrease remains a moot point. The experiment should be repeated, but present facilities do not provide an adequate hypo-baric chamber for conducting studies of this nature.

CONCLUSIONS:

No conclusions can be drawn relating to the efficacy of cortisol in preventing hypoxia-induced depression of mitochondrial function. The dose level and duration of the cortisol treatment used in this study did not appear to influence muscle pyruvate oxidation, fatty acid oxidation, ADP/O ratios, respiratory control, or blood lactate concentrations under conditions of unaltered mitochondrial function. Recommend repeating the study if access can be attained to an adequate environmental chamber.

PUBLICATIONS:

None

Nutritional and Metabolic Adaptations and Interrelationships (cont)

STUDY NO. 6

Dietary Control of Lipid Metabolism

PROBLEM:

Glycogen and fat are both important energy sources during exercise, however glycogen stores are rapidly depleted whereas an abundance of fat is still available at the point of exhaustion. If a greater portion of the energy expended during exercise could be derived from fat, crucial glycogen stores could be prolonged, perhaps forestalling exhaustion. Carnitine is a transporting agent for long chain fatty acids across the mitochondrial membrane to the enzymes of fatty acid oxidation. If tissue carnitine levels limit fatty acid oxidation during exercise supplying supplemental dietary carnitine would be a convenient method of increasing fat utilization during exercise.

RESULTS AND DISCUSSION OF THE RESULTS:

Rats were trained by 12 weeks of treadmill running and fed either a control or a 0.5% L-carnitine supplemented diet. Untrained rats served as a sedentary control group. The turnover rate ($t_{1/2}$) of adipose tissue was found to be 26.7 days for the sedentary controls, 15.8 days for the trained controls and 14.0 days for the trained group fed carnitine.

CONCLUSIONS:

The results of this study confirm previous reports indicating that the smaller adipose cells of trained rats are metabolically more active than those of untrained rats. Feeding 0.5% dietary carnitine did not significantly increase utilization of adipose tissue fatty acids indicating that tissue levels of carnitine are adequate and probably do not limit fatty acid oxidation in the rat. Further studies are planned to investigate carnitine deficient diets in relation to fatty acid oxidation.

PUBLICATIONS:

1. Vacca, J. B., P. P. Waring, M. Nugent, R. M. Nims, and E. W. Askew. Disappearance of tri-, di-, and monoglycerides from the circulation of dogs. USAMRNL Report No. 340. Sept. 1973.
2. Askew, E. W., A. L. Hecker, W. R. Wise, Jr., and G. L. Kuhl. Adipose tissue metabolism and turnover rate: Response to exercise and dietary carnitine, Fed. Proc. 33: 677, 1974 (Abstract)
3. Askew, E. W., G. L. Dohm, W. H. Doub, Jr., R. L. Huston, and P. A. Van Natta. Lipogenesis and glyceride synthesis in the rat: Response to diet and exercise. Submitted to J. Nutrition, 1974.

Nutritional and Metabolic Adaptations and Interrelationships (cont)

4. Askew, E. W., H. Barakat, G. L. Kuhl, and G. L. Dohm. Response of lipogenesis and fatty acid synthetase to physical training and exhaustive exercise in rats. Submitted to publication review committee, 1974.

STUDY NO. 8

Studies on Mineral Metabolism and Interactions

PROBLEM:

Information on the role of minerals in exercise is rather limited when compared to the wealth of material available on fuel sources such as carbohydrates, fats and proteins. Although the literature is somewhat inconsistent in its description of the effect of exercise on mineral metabolism, it offers evidence that minerals play a more important role than is generally recognized. Several elements (Ca, P, K, Na, Fe, Cl, Mg, Mn, Zn, Cu and Cr) have been shown to alter muscle metabolism by their involvement in certain key enzyme reactions. It may be possible that the onset of fatigue and exhaustion may not be entirely controlled by energy-producing nutrients, but may also experience some control via insidious mineral deficiencies or excesses in body tissues. Information of this type will be utilized to design further studies to determine if alterations in tissue minerals has any influence on the dietary requirement for these elements.

RESULTS AND DISCUSSION OF THE RESULTS:

A study of the effect of exercise on mineral metabolism and tissue distribution in the rat has been completed. The basic protocol called for an assessment of the Ca, P, K, Na, Mg and Zn content of red and white muscle fibers of trained and untrained animals. The individual mineral assays have been completed and the data are currently being prepared for statistical analysis.

CONCLUSIONS:

None

PUBLICATIONS:

None

STUDY NO. 9

Effect of Stress on Nutrient Absorption and Metabolism

PROBLEM:

The effect of exercise upon the absorptive functions of the small and large intestine is essentially unknown. It has been shown that severe exercise causes a reduction in the absorptive capacity of the gastro-

Nutritional and Metabolic Adaptations and Interrelationships (cont)

intestinal tract (GIT), especially in reference to carbohydrates. From the limited literature available, it appears that the effect of exercise on absorption depends not only on the severity of the exercise, but also on the extent of prior physical training. The mechanism involved is not understood, but can tentatively be assumed to include such factors as blood supply to the GIT, alterations in delivery of food from the stomach to the intestine, rate of peripheral utilization of substances and increased nutrient uptake by intestinal cells. These factors may have an indirect regulatory effect on the ultimate performance of an individual by controlling the supply of energy. The possibility exists that dietary requirements for certain nutrients may be altered.

RESULTS AND DISCUSSION OF RESULTS:

A study concerning the effect of physical training on carbohydrate and lipid absorption has been completed. Individual intestinal cells, obtained via an everted vibration technique, were employed to monitor overall glucose uptake while homogenates of these cells were used to measure the activity of pyruvate kinase, lactase, maltase, sucrase, and monoglyceride acyltransferase enzyme systems. For purposes of data expression total cell number, viability, total protein, intestinal length and weight were determined. Conclusions and final results are pending complete statistical analysis.

CONCLUSIONS:

None

PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OA 6334	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUM ^b	4. KIND OF SUMMARY	5. SUMMARY SCTY ^b	6. WORK SECURITY ^b	7. REGRADING ^b	8. OMB'S INSTR ^b	9. SPECIFIC DATA - CONTRACTOR ACCESS	
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^c		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61102A		3A161102B71R		02	
b. SECONDARY		61102A		3A061102B71R		02	
c. THIRDARY		CARDS 114(1)					
11. TITLE (Precede with Security Classification Code) ^d							
(U) Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet, and Steroids in Normal Man and Disease (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^e							
003500 Clinical Medicine; 002300 Biochemistry							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
65 11		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PREVIOUS		b. FUNDS (in thousands)	
b. NUMBER: ^g Not Applicable				FISCAL YEAR		0.5	
c. TYPE:				CURRENT		39.6	
d. KIND OF AWARD:				75		1.5	
e. AMOUNT:						50.0	
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^h Letterman Army Institute of Research				NAME: ^h Letterman Army Institute of Research			
ADDRESS: ^h Presidio of San Francisco, CA 94129				ADDRESS: ^h Dept. of Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede with SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: ⁱ Herman, R. H., COL, MC			
TELEPHONE: 415 561 3600				TELEPHONE: 415 561 4147			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Hagler, L., LTC, MC			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Muscle Function of the Combat Soldier; (U) Muscle; (U) Metabolism; (U) Exercise; (U) Electrolytes; (U) Diet; (U) Steroids; (U) Myoglobin							
23. TECHNICAL OBJECTIVE, ^g 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code ^g)							
23. (U) Optimal muscle function of the combat soldier in terms of endurance and strength is essential for the successful outcome of military operations. In order to maximize muscle endurance and strength it is highly desirable to develop musculotropic agents for use in conjunction with physical training and intake of essential nutritional substances e.g. carbohydrate and lipid as energy sources, and vitamins. In order to design musculotropic agents one must study the metabolic processes of muscle and the mechanism of muscle contraction and relaxation.							
24. (U) Muscle tissue will be obtained from animals and patients with a variety of muscle diseases. Metmyoglobin reductase will be assayed in the muscle tissue and compared with values obtained from muscle of patients undergoing surgery. The various components of this enzyme system will be examined and compared.							
25. (U) 73-07 - 74-06 A specific metmyoglobin reductase (analogous to the methemoglobin reductase of red blood cells) has been discovered in cardiac muscle. Analytical techniques have been established which enable us to characterize this enzyme in various tissues and species. Myoglobin is an important constituent of cardiac and red muscle and exceptionally prone to oxidation to the metmyoglobin form. In the oxidized form it is incapable of binding oxygen. Function of muscle may be limited by the capacity of the metmyoglobin reductase system which reduces metmyoglobin to its oxygen binding state. It is postulated that certain muscular disorders may be related to deficiencies in the metmyoglobin reductase system. Preliminary evidence has suggested that the metmyoglobin reductase system is quite complex and is composed of a number of different components.							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A061102B71R **Research in Bio-Medical Sciences**

TASK NO. 02 **Internal Medicine**

WORK UNIT NO. 062 **Muscle Metabolism as Related to
Exercise, Serum Electrolytes,
Diet and Steroids in Normal Man
and Disease**

The following investigations have been conducted under this work unit:

- STUDY NO.** 1. **Studies Concerning the Mechanism which
Control the Redox State of Myoglobin.**

Studies have been carried out which for the first time clearly demonstrate the presence of metmyoglobin (MetMb) reducing activity in the soluble supernatant fraction of beef heart homogenate. Some of the optimum conditions for enzyme assay, and certain characteristics of the assay system have been evaluated. The two critical aspects of the assay system are proper preparation of myoglobin substrate, and ferrocyanide ion activation.

BODY OF REPORT

WORK UNIT NO. 062

Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet and Steroids in Normal Man and Disease

STUDY NO. 1.

Studies Concerning the Mechanism which Control the Redox State of Myoglobin.

PROBLEM:

Hemoglobin (Hb) and myoglobin (Mb) share a number of properties which include reversible oxygenation to form HbO_2 or 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 incompletely 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 which requires ferrocyanide ion activation has been described by Hegesh and Avron.

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 Rossi-Fanelli et al, however, a specific MetMb reductase activity was not found. Presumably enzymatic reducing activity has also been demonstrated in both intact and ground meat, but without clarification of the mechanism. Furthermore, Brown and Synder have shown efficient non-enzymatic MetMb reduction under suitable circumstances in vitro.

Despite the failure of past investigators to convincingly demonstrate specific enzymatic MetMb reduction, it is logical to conclude that if MetHb reductase exists in red blood cells, an analogous

Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet and Steroids in Normal Man and Disease (Cont)

enzyme for MetMb reduction should exist in muscle. In this study we attempted to determine whether metmyoglobin reductase activity could be detected, and if so, to establish the optimum conditions for its assay and some of its properties.

RESULTS AND DISCUSSION OF THE RESULTS:

Using Methb as a substrate in the ferrocyanide-activated assay system described by Hegesh and Avron (J. Lab. Clin. Med. 72: 339, 1968) high levels of reducing activity were found in the supernatant solution from heart muscle homogenate. Subsequently, initial attempts to demonstrate similar activity against MetMb substrate were hampered by low activity levels and marked turbidity. A number of methods of myoglobin preparation were attempted, until it was found that the method of Van den Oord et al. (Eur. J. Biochem. 10: 140, 1969) yielded chromatographically and electrophoretically pure myoglobin substrate. Using this myoglobin substrate the assay gave reproducibly high levels of activity without turbidity. Substrate preparation thus proved to be one of the key factors in elucidating the presence of enzymatic MetMb reductase activity.

Once substrate myoglobin had been purified, the enzymatic nature of the system and some of its characteristics were examined. The effect of enzyme concentration was determined, no activity being detected with enzyme boiled or omitted; and increased activity in proportion to the amount of enzyme added. The effect of varying substrate concentrations was determined, and all subsequent studies utilized non-limiting amounts of substrate. The enzymatic reaction occurred only in the presence of NADH, other pyridine nucleotides being without effect. Hegesh and Avron showed that the Methb reductase assay required ferrocyanide ion activation and ferrocyanide ion was found to similarly activate the MetMb reductase system. The pH optimum of the reaction was determined, and the course of these studies, the effects of buffer type and ionic strength were evaluated. Under certain conditions spontaneous, rapid reoxidation of the myoglobin was encountered, providing an interesting phenomenon worthy of further investigation. At this point the studies were interrupted for the movement of the laboratory to the Presidio of San Francisco.

CONCLUSIONS:

These studies have conclusively demonstrated for the first time the presence of a specific, NADH-dependent, met myoglobin reductase in the soluble supernatate fraction of homogenized beef heart. The enzymatic nature of the reaction has been clearly shown, and some

Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet and Steroids in Normal Man and Disease (Cont)

of the conditions required for optimum activity rates have been ascertained. The two key factors in demonstrating enzyme activity are substrate preparation and ferrocyanide ion activation.

RECOMMENDATIONS:

These studies represent a continuation of previous investigations of muscle function, the biochemistry of myoglobin, and of the primary and secondary myoglobinurias. Since myoglobin provides the only oxygen reservoir in muscle, defective oxygen uptake and diminished availability would result if MetMb were present in increased amounts. The unavailability of sufficient oxygen could lead to both functional and structural defects. Furthermore, there are analogous defects which when present in the red cell lead to biochemical abnormalities and presumably may do so in muscle. These studies are potentially of great importance and should be continued.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8A. DISB'N INSTR' ⁶	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input type="checkbox"/> YES <input type="checkbox"/> NO		9. LEVEL OF SUM A. WORK UNIT
73 07 01	DChange	U	U	NA	INTL			
10. NO./CODES ⁷	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	61102A	3A161102B71R		02	065			
B. SUPPORTING	61102A	3A061102B71R						
C. CONTRIBUTING	CARDS 114 (H)							
11. TITLE (Precede with Security Classification Code) ⁸ (U) The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Status (06)								
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁹ 005900 Environmental Biology; 012900 Physiology; 000350 Clinical Medicine, Food								
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD		
71 07		CONT		DA		C In-House		
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS		20. FUNDS (In thousands)
A. DATES/EFFECTIVE:				B. NUMBER: ¹⁰ Not Applicable		C. TYPE:		
D. KIND OF AWARD:				E. AMOUNT:		F. CUM. AMT.		
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION				
NAME: ¹¹ Letterman Army Inst of Rsch				NAME: ¹² Letterman Army Inst of Rsch				
ADDRESS: ¹³ Presidio of San Francisco California 94129				ADDRESS: ¹⁴ Bioenergetics Division Department of Nutrition Presidio of San Francisco, California 94129				
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)				
NAME: ¹⁵ Canham, J. E., COL, MC				NAME: ¹⁶ Krzywicki, H. J.				
TELEPHONE: ¹⁷ 415 561 3600				TELEPHONE: ¹⁸ 415 561 5066				
21. GENERAL USE				22. ASSOCIATE INVESTIGATORS				
Foreign Intelligence not Considered				NAME: ¹⁹ Consolazio, C. F.				
				NAME: ²⁰ Nelson, R. A. DA				
22. KEYWORDS (Precede EACH with Security Classification Code) ²¹ (U) Diet and Work Performance of Soldiers; (U) Heat, Cold, Altitude Stress; (U) Nutrients and Body Composition; (U) Pulmonary Function								
23. TECHNICAL OBJECTIVE, ²² 24. APPROACH, ²³ 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)								
<p>23. (U) Attainment and maintenance of physical fitness is of prime import to the Armed Forces and requires a constant search for new methods for improving and evaluating physical performance. Performance reflects nutriture, body hydration and training and may be affected by age, state of health, dietary constituents, vitamins, hormones, therapeutic agents, and varied environmental stresses all of which should be reflected in body composition changes and related physiological parameters.</p> <p>24. (U) Treadmill work and physical activity will be studied with respect to the following physiological functions: respiratory, cardiovascular, hormonal, temperature regulation and body composition changes. The effects of environment (heat and cold) and dietary components (fat, protein and CHO will be evaluated singly or combined).</p> <p>25. (U) 73 07-74 06 Anthropometric measurements were made on 223 males and 36 females. Data for males indicated that waist and buttocks circumferences, and body weight were highly correlated with fat estimates by densitometry ($r=0.70$ to 0.85). The female data showed skinfolds to be highly correlated with body fat ($r=0.66$ to 0.87). In this study, 3 techniques (densitometry, potassium⁴⁰ and D₂O dilution) involving independent body compartments were utilized to estimate body fat, protein and water. All estimates of body fat were significantly different. Choice of technique depends upon cost and mobility. Two groups of men consumed 2 protein levels (1.4 and 2.9g/kg body wt) for 40 days. Daily urinary nitrogen excretions remained essentially unchanged for the lower protein group during training. Nitrogen balances were positive for both groups. Blood Hb, Hct and serum proteins were unchanged during the entire period. Although both groups increased muscle mass, work performance was not enhanced by high protein diet. In this study, 100g of protein/day was adequate for men performing fairly heavy work.</p>								

*Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A061102B71R Research in Biomedical Sciences
TASK NO. 02 Internal Medicine
WORK UNIT NO. 065 The Effects of Nutrition and
Environmental Stress Upon Work
Capacity and Nutritional Status

The following investigations have been conducted under this work unit:

STUDY NO. 1 Body Composition Studies

- a. A comparison of methods for estimating human body composition.
- b. The relationship of anthropometric measurements to body fat.
- c. To determine the efficacy of dieldren as a method of determining total body fat.

STUDY NO. 2 Work Performance

- a. The effects of ingesting electrolyte and sugar upon physical training and performance in young adults under conditions of profuse sweating.
- b. The effects of two levels of dietary protein on physical conditioning.

STUDY NO. 4 Effects of Time of Sampling Upon Extracellular Water Volumes Determined With Thiocyanate

Study No. 1-a. Three methods were utilized to estimate body fat in humans, densitometry, potassium⁴⁰ counting and deuterium oxide dilution. Although each method was significantly different from the other, any method would provide estimates of body fat in a population. The choice of procedure would be decided on the basis of cost, convenience and cooperation of the subjects but none possess the desired accuracy.

Study No. 1-b. A variety of anthropometric measurements were made on 273 male and 36 female military personnel. Total body fat was estimated by density, ⁴⁰K counting and D₂O dilution. Simple correlations with body fat estimates indicate that for the male population, waist, weight and buttocks circumference were most highly

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Status (Cont)

correlated with fat estimates ($r=.70$ to $.85$). In the female population skinfold thickness was most highly correlated with body fat ($r=.66$ to $.87$). Stepwise multiple regression analysis showed that five of the variables could account for 60-70% of the variation in fat in males and up to 90% in females. Correlations of measurements were higher with fat estimated by density than with estimates derived from 40-potassium counting or D_2O dilution.

Study No. 1-c. An animal investigation using tracer levels of ^{14}C dieldrin as an organic diluent to estimate body fat has been completed. The data are currently being prepared for statistical analysis.

Study No. 2-a. The effects of oral electrolyte, sugar and vitamin E supplements on work performance were evaluated in 6 highly trained, heat acclimatized subjects in a $35^{\circ}C$ environment. The supplements were ingested during the 4-hour exercise period and each supplement was tested for 5 consecutive days.

The standard diet fed the subjects fulfilled the daily NRC allowances of calories, protein, vitamins and minerals. Work performance of subjects was measured daily at two levels of submaximal work after 3 and 1/2 hours of exercise. Maximal treadmill testing was performed weekly. Physiological body functions were monitored during ingestion of the supplement and compared to those obtained using water as the control. Electrolyte and glucose supplementation had no demonstrable beneficial effects upon the work performance parameters measured. Oxygen consumption, heart and ventilation rates were essentially unchanged as a result of supplementation. Time of maximal performance and body temperatures were also unaffected. Vitamin E did not alter any of the same measured physiological functions. Results again indicate that heat acclimated men working in hot environments need only replace water to maintain optimum performance.

Study No. 2-b. Three levels of physiologic work performance were evaluated in eight subjects (two groups of 4) who consumed either 197.3 or 100.7 gm of protein/day for a 45-day period. All subjects underwent maximal, submaximal and stamina work performance tests while participating in a heavy physical training regimen. A number of biochemical and physiologic body functions were monitored during exercise throughout the study. Pulmonary ventilation and oxygen uptakes were not significantly different from pre-treatment values in either protein intake group during the entire study. The higher protein intake did not produce a significant increase in performance or physical fitness during maximal or stamina exercise.

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Status (Cont)

Study No. 4. The influence of sampling time on thiocyanate space estimation of extracellular fluid volume was studied. Large variations in values calculated from sample-to-sample (taken 30-300 min. post infusion) suggested that 3 to 5 timed samples should be drawn for calculation of this volume. Even with these conditions, half of the values deviated by 10-16% for two determinations, on the same man, made one week apart. These large variations would suggest that any interpretations of changes in this space must be made very cautiously.

BODY OF REPORT

WORK UNIT NO. 065

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Stress

STUDY NO. 1-a.

A Comparison of Methods for Estimating Human Body Composition

PROBLEM:

There are a variety of methods for estimating the body composition of humans, however they involve uncertainties since only indirect analytical methods may be utilized. This means that the results of any method of study can only be compared with those of another or with tables of body composition for particular populations. A comparison of three independent techniques to estimate body fat and water was made in a total population of 223 male soldiers and 36 WAC's. Body density (D) and fat was estimated from body volume by water displacement, whole body burden of potassium (^{40}K) was measured by a sodium iodide crystal shadow shield counter, and total body water was obtained by analysis from the dilution of orally ingested deuterium oxide (D_2O) in body fluids.

RESULTS AND DISCUSSION OF RESULTS:

The average fat content of the 223 male subjects determined by the three techniques showed all estimates of body fat, D (17.8 kg), ^{40}K (21.6 kg), D_2O (15.6 kg) to differ significantly from each other. Densitometric estimates of body fat were virtually similar for the males as well as the females, however on a percent body weight basis, the female population differed. Correlation between group estimates of body fat ranged between 0.47 to 0.72.

CONCLUSIONS:

Three procedures were compared for estimating body fat in humans. Estimates of body fat by densitometry, potassium 40 counting and by deuterium oxide dilution differed significantly. The choice of techniques to be utilized are dependent on cost and mobility.

STUDY NO. 1-b.

The Relationship of Anthropometric Measurements to Body Fat

PROBLEM:

The principle objective was to identify anthropometric measurements that could be used routinely to estimate body fat by personnel conducting field nutrition studies.

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Stress (Cont)

RESULTS AND DISCUSSION OF RESULTS:

A variety of anthropometric measurements were made on 223 male and 36 female military personnel for whom total body fat was estimated by density, ^{40}K counting and D_2O dilution. The male population data indicate that weight, and the circumferences of waist and buttocks were most highly correlated with fat estimate ($r = .70$ to $.85$). In the female population skinfold thickness was most highly correlated with weight of body fat ($r = .66$ to $.87$).

Stepwise multiple regression analysis showed that five of the variables could account for 60-70% of the variation in fat in males, and 90% in females. Correlations of measurements were higher with fat as estimated by density than with estimates derived from 40-potassium counting or D_2O dilution.

CONCLUSIONS:

It can be concluded from the differences in simple and multiple correlation coefficients that certain measurements are superior to others.

STUDY NO. 1-c.

To Determine the Efficacy of Dieldrin as a Method of Determining Total Body Fat

PROBLEM:

The efficiency of military units depends to a degree upon the nutritional status of the soldier and is related to the body composition of individual soldiers. If accurate methods to estimate body fat and lean body mass were available the relationship between work performance and body composition could be better defined. At the present time, no such methods exist. Since the principle variant in body composition is fat, the ability to measure it accurately and simply would be a great asset in the study of body composition. Of the many methods employed in attempts to determine total body fat perhaps one of the most promising is that of tracer dilution. As it is currently used, this technique fails to provide the necessary accuracy and/or repeatability for widespread application. However, the precision of this approach might be improved simply by using a different type of tracer. Several reports in the literature indicate that the chlorinated hydrocarbons and their analogs possess the necessary characteristics to make them compatible with a technique of this type. These are: 1) fat soluble; 2) evenly distributed in body fat; 3) rapidly equilibrated throughout the body fat depots, and 4) slowly metabolized. The objective of this

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Stress (Cont)

experiment was to investigate the efficacy of dieldrin as a model of possible organic diluents that could be used for determining total body fat.

RESULTS AND DISCUSSION OF RESULTS:

Tracer levels of ^{14}C dieldrin were administered either intravenously or intraperitoneally to rats and sheep. The animals were sacrificed and the tissue distribution on the label was determined. The data are currently being prepared for statistical analysis.

ADDITIONAL COMMENT:

At a recent child health symposium, a review was presented and is now being published by NIHCD on the "Performance of the Adolescent." In summary, the data suggests that prior to puberty both boys and girls showed no significant differences in performance. During the adolescent years the boys become more efficient and this is probably due to the increased muscle mass of boys and the increase in body fat in the girls. During the adolescent period, the maximal oxygen uptakes in l/min are increased with age for both girls and boys, however when related to ml/kg/min, maximal oxygen uptakes are essentially unchanged for boys after age 9. After age 9, girls show a continued decrease during the adolescent years.

CONCLUSIONS:

Complete data analysis.

STUDY NO. 2-a.

The Effects of Ingesting Electrolyte and Sugar Upon Physical Training and Performance in Young Adults Under Conditions of Profuse Sweating

PROBLEM:

In recent years the soft drink industry has introduced a group of non-carbonated beverages designed for consumption in hot environments. These supplements are intended to replace body fluids and electrolytes lost through excessive perspiration during heavy physical activity. These products are basically composed of minerals and sugars in various concentrations and some contain vitamin C. Claims by manufacturers and athletes have been subjective and have not been substantiated under carefully controlled scientific conditions. Any supplement which may increase endurance and retard fatigue would be of great benefit to the soldier required to perform heavy work and merits investigation.

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Stress (Cont)

The primary objective was to evaluate oral electrolyte and sugar supplements for maintenance of work performance and water and mineral balances under conditions of profuse sweating and to compare their effects with water ingestion alone.

RESULTS AND DISCUSSION OF RESULTS:

Six healthy male subjects, between 21 and 25 years of age, were physically conditioned and heat acclimated for 10 days prior to the beginning of the study. Work assignments and energy expenditures were kept constant throughout the study and included sub-maximal, maximal and stamina tests of physical performance.

The supplements were ingested during the daily 4-hour work period in a 35°C environment and compared to water ingestion alone. Vitamin E, electrolyte and sugar supplements did not produce significant changes in heart rate, respiration rate, or oxygen uptake in liter/min or ml/kg/min (Tables 1 & 2). Electrolyte supplements did not significantly improve maximal performance walking times. In addition, there were no demonstrable beneficial effects upon the recovery parameters of physical fitness index or sum of recovery heart rates.

An adequate water and electrolyte supply are important to the maintenance of optimal physical performance and it is essential that fluid replacement rate be at the same level as water losses for maintenance of fluid balances. Ad lib water intakes resulted in slightly negative water balances during the work periods. This could be due in part to thirst not being a good indicator of fluid requirements.

CONCLUSIONS:

In a hot environment neither the commercial "anti fatigue drinks" nor mineral supplementation produced improvements in performance. Other experiments have also shown no immediate deleterious effects of mineral depletion under severe conditions in hot climates when heat acclimated subjects are provided adequate quantities of minerals in their normal diet.

RECOMMENDATIONS:

The commercial energy liquid supplements are not needed for maintaining electrolyte balances during periods of heavy physical activity. Normal dietary replacement of the electrolyte is sufficient. However, fluid replacement during periods of heavy physical activity and heat stress is essential and water should be consumed at a rate required for maintaining fluid balances.

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Stress (Cont)

Table 1. Mean heart rates/min at 3 levels of work.

Treatment No. †	Sub Max 4% Grade Mean*	Sub Max 10% Grade Mean*	Max Work Variable Grade Mean**
1 - Water	111.5	139.8	186.1
2 - Comm #1	114.3	136.7	174.2
3 - NaCl + sugar	110.3	141.0	183.1
4 - NaCl	109.6	136.6	189.3
5 - NaCl, K-gluconate	112.3	142.0	186.1
6 - High K-gluconate	113.3	143.2	186.1
7 - Comm #2	113.9	141.1	187.6
8 - Water	114.4	143.3	180.3
9 - Low K-gluconate	115.7	146.0	187.3
10 - Comm #3	110.7	139.3	191.9
11 - K-gluconate + sugar	112.6	138.3	182.1
12 - Water + Vit F	114.3	143.9	184.4

* Mean of 4 repetitions from 6 subjects.

** Mean values obtained once per week from 6 subjects.

† Six treatments used per week.

Commercial #1 (Gatorade), #2 (Sportade), and #3 (Olympade)

Table 2. Mean oxygen uptakes, ml/kg/min at 3 levels of work.

Treatment No.	Sub Max 4% Grade Mean*	Sub Max 10% Grade Mean*	Max Work Variable Grade Mean**
1	17.97	25.59	40.04
2	17.89	26.20	41.65
3	17.66	25.84	43.76
4	17.80	25.75	44.99
5	17.96	26.44	41.46
6	18.12	26.00	44.70
7	17.40	25.13	45.59
8	18.66	26.05	41.27
9	18.35	26.69	42.24
10	17.58	25.59	45.01
11	18.26	26.12	42.71
12	18.00	25.85	45.39

* Values of 4 repetitions from 6 subjects.

** Mean values obtained once per week from 6 subjects.

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Stress (Cont)

STUDY NO. 2-b.

The Effects of Two Levels of Dietary Protein on Physical Conditioning

PROBLEM:

Increasing physical activity, whether in military basic training, sports training or exercising to improve physical fitness, results in increased muscle protein in the body and an increase in normal biosynthesis of proteins from dietary protein. Increased dietary intakes of protein during sports training has been advocated, although only a limited number of reports of controlled, scientifically designed studies on the effect of protein levels during training or heavy physical activity have appeared in the literature. Some investigators have recommended that protein allowances be increased from 0.9 to 2.5 gm/kg body weight during strenuous physical conditioning since this would prevent protein catabolism at the expense of hemoglobin and serum proteins.

The physical performance of the individual soldier is of primary concern to the military. A considerable part of the basic training program is devoted to increasing physical fitness. An increase in either the rate of physical fitness attainment of the individual would be most beneficial to the military. Since protein is an expensive calorie source, it would not be economical to increase the protein intake if it did not have any beneficial effects. The objective of the study was to gather more information on this subject because of the disagreement as to whether or not protein requirements are increased under these conditions.

RESULTS AND DISCUSSION OF RESULTS:

Two groups of young adults consumed either 100.7 (I) or 197.3 (II) grams of protein/day during 10 days of intensive physical training and 30 additional days of continued heavy physical activity. Contrary to reports of other studies, the weekly hemoglobin, hematocrit and serum protein levels were essentially unchanged during the entire study for Group I. Daily urinary nitrogen excretions also remained fairly constant for Group I and both nitrogen and potassium balances were positive, inclusive or exclusive of the daily sweat losses.

Three levels of physiologic work performance were evaluated on the eight subjects (maximal, submaximal and stamina work performance tests). Pulmonary ventilation and oxygen uptakes in ml/kg/min were not significantly different from pre-treatment values in either protein intake group during the entire study.

The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Stress (Cont)

CONCLUSIONS:

Although the men did increase body protein stores and muscle mass (positive nitrogen balances) with the high protein diets in this study, the additional body protein did not enhance physiological work performance. In this study, 100 g of protein/day was found to be adequate for men performing fairly heavy work.

RECOMMENDATIONS:

Studies should be continued to evaluate the NRC daily protein allowances (0.8 g/kg body wt) during strenuous physical conditioning.

STUDY NO. 4.

Effects of Time of Sampling Upon Extracellular Water Volumes Determined With Thiocyanate

PROBLEM:

Body compositional changes that occur during periods of stress (environmental, heavy physical activity, etc.) continue to be of major interest in the evaluation of the physiological status of troops. The importance of obtaining accurate determinations are apparent since any errors in methodology will reflect changes in body compartments. Thiocyanate is one technique that has been used extensively to estimate extracellular fluid (ECF) volume. The purpose of this study was to determine the optimal times for blood sampling following a test dose of thiocyanate.

RESULTS AND DISCUSSION OF RESULTS:

The determination of the thiocyanate space from samples drawn between 30 and 300 minutes post infusion indicated that blood samples could be obtained at anytime during this interval. However, the variability in the results would strongly suggest that between 3 and 5 samples should be drawn for each determination of thiocyanate space. In applying these methods and obtaining means from 2 to 7 values for each determination, volumes obtained one week apart on the same man differed from each other by as much as 15.4% or almost 3 liters. The large variability suggests that any results using thiocyanate space as an estimate of the extracellular fluid space must be interpreted cautiously.

CONCLUSIONS:

The data suggests that either the extracellular space is quite variable or the precision of the method of measurement is poor.

The Effects of Nutrition and Environmental Stress Upon Work
Capacity and Nutritional Stress (Cont)

Interpretation of extracellular space data from the thiocyanate procedure must be treated cautiously.

RECOMMENDATIONS:

This is a poor technique and alternate procedures should be investigated.

PUBLICATIONS:

1. Consolazio, C.F. Physical Activity and Performance of the Adolescent. To be published as a chapter of the Proceedings of "Factors Influencing Nutrient Requirements in Rapidly Changing Adolescent Body," by Dept. of Health, Education and Welfare, Public Health Services, National Institutes of Health, Institute of Child Health and Human Development, Bethesda, MD.
2. Consolazio, C. F., H. L. Johnson, T. A. Daws, and R. A. Nelson. Energy requirements and metabolism during exposure to extreme environments. Chapter in: World Review of Nutrition and Dietetics. Edited by Dr. J. Bourne, Vol 18:177-194, Karger Basel, 1973.
3. Consolazio, C. F., H. L. Johnson, R. A. Nelson, J. G. Dramise, and J. H. Skala. Protein metabolism during intensive physical training in the young adult. Accepted for publication in Am. J. of Clin. Nutr.
4. Ward, G. M., H. J. Krzywicki, D. P. Rahman, R. L. Quaas, R. A. Nelson, and C. F. Consolazio. The relationship of anthropometric measurements to body fat as determined by densitometry, potassium⁴⁰ and body water. Returned to Am. J. of Clin. Nutr. after revision based on reviewer's comments.
5. Krzywicki, H. J., G. M. Ward, D. P. Rahman, R. A. Nelson, and C. F. Consolazio. A comparison of methods for estimating human body composition. Accepted for publication in Am. J. of Clin. Nutr.
6. Johnson, H. L., D. Wooldridge, H. J. Krzywicki, R. F. Burk, and C. F. Consolazio. Evaluation of sampling times for the determination of extracellular fluid space with NaSCN. This manuscript has been submitted to the Publications Review Committee.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL	
				DA OA 6377	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8. ORIGIN INST ⁶	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUMMARY
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ⁷	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY	61102A	3A161102B71R		02		166	
b. XXXXXXXX	61102A	3A061102B71R		02			
c. XXXXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ⁸							
(U) Design of Military Biomedical Research Information Systems (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁹							
004200 Computers; 009700 Mathematics and Statistics							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 07		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	20. FUNDS (in thousands)
a. DATES/EFFECTIVE:				PREVIOUS			
b. NUMBER: Not Applicable				FISCAL YEAR		8	51.5
c. TYPE:				CURRENT			
d. AMOUNT:				75		10	120.0
e. KIND OF AWARD:				75		10	120.0
21. RESPONSIBLE DOD ORGANIZATION				22. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: Teplick, R. S., MAJ, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-4740			
23. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: McCaa, T. C., E-4			
				NAME: Langley, W. H., DAC			
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To design, implement, and document computer programs to facilitate management of LAIR research data. These programs will provide the ability to load, update, and retrieve data. Included will be provisions to transcribe files, to alter file format, and to interface with different data analysis programs.							
24. (U) Two file management systems are available to LAIR through its computer service contract with Lawrence Berkeley Laboratory. They are the Remote File Management System (RFMS) and the Statistical Package for the Social Sciences (SPSS). RFMS is designed to load data and permit complex data retrievals, but its use is not cost effective when large file subsets are retrieved or updated. The SPSS systems file management routines are useful only when data can be loaded into fixed-length records and the subsequent data analysis is to be performed by the statistical routines which are an integral part of SPSS. Both of these systems are being studied and evaluated in terms of immediate and long range requirements. For immediate requirements, RFMS is being modified to enhance its efficiency. The long range objective is to establish a compromised data base format, with its associated file handling routines, that will be efficient to LAIR's particular file management requirements.							
25. (U) 73 07 - 74 06 All files which were managed on the RCA 301 computer are now operational on the CDC computers using RFMS. Enhancements are now being made to RFMS which substantially increases RFMS's efficiency to retrieve selected data and produce subfiles. The ability to update RFMS files efficiently still poses a problem. Prototypes of a new system have been developed and are being used to isolate the problems inherent in further development. Hopefully, the new system will permit us to simplify tasks which involve the simultaneous management of multiple data bases in a cost effective manner.							

ABSTRACT

PROJECT NO. 3A161102B71R Research in Biomedical Sciences
TASK NO. 02 Internal Medicine
WORK UNIT NO. 166 Design of Military Biomedical Research
Information Systems

The following investigations have been conducted under this work unit:

STUDY NO. 1 Data Processing Support to Biomedical Research
(General Support)

STUDY NO. 2 Direct Computer Support to LAIR Departments

File management systems are being adapted and/or developed for the standardization of loading, editing and reformatting biomedical research data files. The file management systems are being designed to utilize mass storage devices which were not formally available on the Institute's previous computer. Included in this report as a second study is a resume of the substantive ADP support provided the various departments.

BODY OF REPORT

WORK UNIT NO. 166

Design of Military Biomedical
Research Information Systems

STUDY NO. 1

Data Processing Support to
Biomedical Research (General
Support)

PROBLEM:

To maintain an ongoing ADP support facility for LAIR by making available the necessary computer hardware systems and supply general usage programs (software) to permit data file management and analysis.

RESULTS AND DISCUSSION OF THE RESULTS:

a. Computer conversion. A major computer change has required a massive effort to convert existing programs and data files to run on the new computer configuration. During FY 74 this has been particularly true because of the release of the outmoded USAMRNL RCA 301 computer and the relocation to LAIR where the batch oriented computer support would be supplied via a remote job entry terminal interfaced to the Lawrence Berkeley Laboratory (LBL) CDC 6600/7600 computer complex. The conversion is now technically completed in that all previously existing data files can now be loaded and analyzed. The new facility offers so many more options that the problem of optimization of operations will continue for a number of years.

b. File management systems. The most profound impact on the ADP of research data resulting from operations on the late generation LBL equipment is the ability to store and retrieve data from large extended core memories and randomly accessible storage mediums (i.e., disc and data cells). Now it is no longer required to sequentially process an entire data file to locate and/or modify selected elements of information. Theoretically now it is possible to manipulate any information fragment of a data file in what would appear to a human almost "instantaneous." To the computer, though, there is considerable overhead to perform such a task. Simply stated, sequential files are desired when entire files are processed, and totally "inverted" random access files are desired when single imbedded data elements are to be processed. In general no file fits an extreme. As a result one must compromise by designing a file management system that is not so highly inverted on a random access medium so that during processing groups of data are manipulated. Prototypes of new file definitions have been developed here and are

Design of Military Biomedical Research Information Systems (Cont)

being used to isolate problems inherent in further development of optimal file management at LAIR. Through its computer service contract with LBL, LAIR has available to it two file management systems. One is Remote File Management System (RFMS) which supports a highly inverted-tree structured data base. RFMS is designed to load complex data files for subsequent selective data retrievals and to format the selected output for printing and/or input requirements to analysis routines. The other file management system, Statistical Package for the Social Sciences (SPSS), is specifically designed to support data to be analyzed by its own statistical analysis routines and is restricted to fixed length records.

CONCLUSIONS:

The programming staff is studying various algorithms of storing and retrieving data that would be suitable for the rather extensive library file of data at LAIR. It appears that two standards are in order. New and live files can be more sequential in character because large amounts of new data are commonly being added or updated. Whereas old, archived files are best in an inverted structure.

RECOMMENDATIONS:

Studies be continued to decide on an optimal file format mix so that all file management and analysis routines can be standardized. Extreme care should be rendered in standardization procedures which do not unduly restrict operations to particular machine hardware configurations. While immediate requirements must be met in timely responsiveness and are subject to close cost scrutiny, the long range costs of inevitable future in-house machine conversions are equally as significant. Indeed software which is capable of being exportable to other government agencies would be a manpower and fund savings. Therefore it is recommended that general support programs be generalized and nonmachine specific. The Department of Information Sciences should take every possible avenue to share, give and take, with other government facilities.

STUDY NO. 2

Direct Computer Support to LAIR
Departments

PROBLEM:

Included in the mission of the Department of Information Sciences is the requirement to provide all departments of LAIR with substantive support in ADP. As such, this is an ongoing study area and varies in

Design of Military Biomedical Research Information Systems (Cont)

the degree and depth of support from department to department according to their current needs and staff capabilities. Support is given in systems analysis, methods of experiment design, data acquisition, program coding and execution to analyze experimental results.

RESULTS AND DISCUSSION OF THE RESULTS:

Computer support to the departments during FY 74 was principally in converting existing code and data bases to be compatible on the LBL computer complex. Additional support to the various elements of LAIR is described below. Support given to nutritional studies is a collaborative effort in which the Dept. of Information Sciences is one of the participating departments. As such, the support provided is described under Work Unit 086, Nutrition Studies in Support of DOD Food Program.

a. Program and Budget Office. The Decentralized Accounting System (DAO), finance and accounting system for LAIR, was implemented on the LBL CDC 7600 machine beginning 1 July 1973. These programs, written in COBOL, initiate, load, update, and process the daily F&A transactions.

Work has begun on a collection of programs which will distribute the Carrier Account (overhead) costs to the various direct, or productive, work units (FICs). Reports will be generated and transaction output files to be used as input to the DAO processing programs.

b. Department of Nutrition.

(1) Food Hygiene Division. Computer programs have been expanded to enhance the presentation of the analysis of food microbiology data. A large backlog of accumulated data is being key punched and processed.

(2) Bioenergetics Division. Specifications are being prepared for the procurement of a field portable mini-computer to automate ergometric experiment control functions and data acquisition. Additionally, an experiment is being designed to study rat feeding data by Newman-Keuls multiple comparison analysis techniques.

c. Department of Medicine. Statistical analyses were performed for the following studies: (1) aplastic anemia following viral hepatitis, (2) a preliminary study of the effects of in vitro incubation of cholera toxin on rabbit intestinal glycolytic and FDPase activities,

Design of Military Biomedical Research Information Systems (Cont)

(3) effects of diet and triiodothyronine on jejunal enzyme adaption in four normal subjects, and (4) effect of intravenous ethanol on hepatic enzymatic activities.

d. Department of Dermatology. The Dermatology Outpatient Data System, which was previously processed under an ADP service contract, is being implemented on the LBL machines. This involves transcribing old data to be compatible with new programs. Once operational, clinic data will be processed on a month by month basis to produce the required report tables.

e. Department of Comparative Medicine. Programs were written to facilitate the analysis of data from acute mountain sickness data. The analyses produced statistics on (1) the effects of altitude on body composition in mice, (2) nutrient intake and excretion patterns in humans exposed to high altitudes and, (3) acid-base regulation in humans during chronic altitude exposure.

f. Department of Logistics. Programs are supported and regular data file maintenance runs are made for the automated Property Book. Periodically selective retrievals are required resulting in the creation of special nonstandard programs.

g. Extramural Support:

(1) Statistical analyses were performed for Dr. Nelson, Fitzsimons Army Medical Center, on data from a study of the effects of ephedrine and epinephrine on glucose, free fatty acids, and eosinophil levels.

(2) Statistical assistance is being provided to the USA Medical Laboratory, Ft. Baker, in the analysis of certain viral infections in pregnant women and of the effects of these infections on the offspring.

CONCLUSIONS:

With the availability of computer services from Lawrence Berkeley Laboratory many data analysis routines are now readily accessible to LAIR researchers. In addition to standard program packages, special purpose programs are being developed to process data gathered from on-going research projects. As the number of personnel and projects at LAIR increases, so does the requirement increase to process different kinds of data. A significant proportion of the resources of the Department of Information Sciences is dedicated to supporting the ADP requirements of the various departments of LAIR.

Design of Military Biomedical Research Information Systems (Cont)

RECOMMENDATIONS:

Direct ADP support to the Institute must be continued in order to consolidate and coordinate the needed talent and facilities required to meet the sophisticated data processing demands of the modern researcher.

PUBLICATIONS:

1. Messa, C. J., E. B. Blair, A. H. Tull and D. H. Andres. Computer file and analyses of laboratory data from tuberculous patients. I. Data management system. Am. Rev. Respir. Dis. 108: 813, 1973.
2. Fowler, J. L., R. E. Thomas, J. J. Jorgensen and D. Stutzman. Microflora of prepared salads and specialty items procured for use by DOD installations. USAMRNL Report 338, September 1973.
3. Fowler, J. L., P. R. Ruckh, T. G. Murnane and W. F. Ganz. Report of analyses of 1972 microbiological data collection program. USAMRNL Report 339, September 1973.
4. Teplick, R. S. Problems with a compartment model for accessing human vitamin A kinetics. Proc. 19th Conf. on the Design of Experiments in Army R&D, 1973. (In press)
5. Lufkin, E. G., F. B. Stifel, R. S. Teplick and R. H. Herman. Permissive effects of testosterone on dietary adaptation of jejunal pyruvate kinase in hypogonadal males. J. Clin. Endocr. Metab. 38: 1130, 1974.
6. Sterner, R. T., R. S. Teplick and J. T. Wheeler. Interpretation of analysis of variance in designs yielding a subjects X treatment interaction. Proc. 19th Conf. on the Design of Experiments in Army R&D, 1973. (In press)
7. Teplick, R. Failure of the Sigma Phenomenon to account for the anomalous viscosity of blood. (Submitted to Publications Review Committee)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ³	2. DATE OF SUMMARY ³	REPORT CONTROL SYMBOL	
				DA OA 6379	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY ¹	4. KIND OF SUMMARY	5. SUMMARY SCTY ²	6. WORK SECURITY ²	7. REGRADING ³	8A. OMS'N INSTR' ³	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ³	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	61102A	3A161102B71R	02	167			
B. CONTRACTING	61102A	3A061102B71R	02				
C. CONTRACTING	CARDS 114 (F)						
11. TITLE (Precede with Security Classification Code) ³							
(U) Biochemical Factors Influencing Physiological Functioning (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ³							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
72 07		76 06		DA		C In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN TIME	
A. DATES/EFFECTIVE:				FISCAL YEAR		FUND YEAR	
B. NUMBER: ³ Not Applicable				74		3.0	
C. TYPE:				75		1.0	
D. KIND OF AWARD:				20. PERFORMING ORGANIZATION			
E. AMOUNT:				NAME: ³ Letterman Army Institute of Research			
F. CUM. AMT.				Dept. of Medicine			
19. RESPONSIBLE DOD ORGANIZATION				ADDRESS: Presidio of San Francisco, CA 94129			
NAME: ³ Letterman Army Institute of Research				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
ADDRESS: ³ Presidio of San Francisco, CA 94129				NAME: ³ Hierman, R. H., COL, MC			
RESPONSIBLE INDIVIDUAL				TELEPHONE: 415 561 4147			
NAME: Canham, J. E., COL, MC				SOCIAL SECURITY ACCOUNT NUMBER: 349-20-9755			
TELEPHONE: 415 561 3600				ASSOCIATE INVESTIGATORS			
21. GENERAL USE				NAME: Hagler, L., LTC, MC			
Foreign Intelligence not Considered				NAME:			
				DA			
22. REVISIONS (Precede EACH with Security Classification Code) (U) Fever; (U) temperature regulation; (U) adrenergic mechanisms; (U) adrenergic blocking agents; (U) hormones; (U) circadian rhythm							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.) (U) The soldier is subjected to stress particularly in combat. Some individuals develop tachycardia, palpitations, dyspnea, chest pain, malaise, weakness, anxiety, irritability and inability to work. This condition (hyperdynamic beta-adrenergic circulatory state (HBACS) appears to be related to abnormal function of beta-adrenergic receptors. Heat stress causes ineffectiveness, disability or death during military training, combat operations or febrile illness. Prevention of the HBACS or heatstroke would minimize the incidence of prolonged disability and death. Changes in day-night work sequences disrupts physiologically important circadian rhythms. Inability to adapt to acute changes in day-night shift work may be related to the disrupted circadian rhythms. Environmental stress (e.g., heat, time-phase changes) may be mediated in part through increased sensitivity to catecholamine excretion.							
24. (U) Selected patients with thermoregulatory defects will be investigated with regard to the generation of heat during exercise and certain therapeutic agents will be tested for their ability to control heat production. The pyrogenic steroid, etiocholanolone, will be studied with regard to its action on muscle membranes. Patients with abnormal beta-adrenergic function will be studied with provocative agents and adrenergic blocking substances. Changes of circadian rhythm will be studied and correlations with degree of adaptability will be measured.							
25. (U) 73-07 - 74-06 Several patients with HBACS have been studied and propranolol has been found useful in controlling symptoms. A few patients whose symptoms mimic those of the HBACS appear to respond to propranolol but have symptoms which cannot be provoked by isuprel, epinephrine or the monoamine oxidase inhibitor, parnate. A preliminary study has been completed where abrupt changes in day-night work were instituted and correlations measured between various parameters of circadian rhythm and adaptability to the abrupt changes. Preliminary data has been obtained but analysis is not complete.							

ABSTRACT

PROJECT NO. 3A061102B71R Research in Biomedical Science
TASK NO. 02 Internal Medicine
WORK UNIT NO. 167 Biochemical Factors Influencing
Physiological Functioning

The following investigations have been conducted under this work unit:

STUDY No. 6. Study of normal subjects on day-night shift rotation to evaluate the adaptative rate of selected cyclic physiological variables.

The study was conducted to determine: (1) the effects of day-night shift rotation on normal subjects and; (2) the feasibility of intensive measurements of a number of physiological parameters on the metabolic ward. The design utilized three consecutive five to six day periods; Period I - control work schedule; Period II - experimental work schedule; and Period III - post control work schedule. The work schedule during periods I and III were identical with regard to clocktime, while period II reversed the control sleep - work - leisure cycle. Performance measurements, symptomatic inventories and a number of cyclic physiological variables were obtained during each period. Variables were selected because they were known to be cyclic and were readily available. Control measurements, which were stationary in time will be compared to post control measurements which were non-stationary in time resulting from the phase shift during Period II. In addition, the measurements were correlated with the rate at which the variables became stationary in time during period III.

Measurements for all variables were taken at four hour intervals. An indwelling heparinized venous flush system was utilized for collection of the serum specimens. Performance measurements were obtained 3 times daily until baseline values were established following which daily measurements at midpoint of work period were obtained. A symptomatic inventory check sheet was prepared at the beginning and end of each work period. During all study periods a Read Universal Exposure meter reading and room temperature were recorded every four hours.

Preliminary observations demonstrated variable changes in each subject. The available data shows disruption in the occurrence of the maximum and minimum values of body temperature, serum osmolality, urine osmolality and body weight. These changes are in the process of being analyzed statistically by appropriate

Biochemical Factors Influencing Physiological Functioning (Cont.)

methods. Since laboratory and statistical analysis of the remainder of the data is not yet completed, the significance of the changes remains uncertain.

BODY OF REPORT

WORK UNIT NO. 167

Biochemical Factors Influencing
Physiological Functioning

STUDY NO. 6.

Study of normal subjects on
day-night shift rotation to
evaluate the adaptive rate of
selected cyclic physiological
variables.

PROBLEM:

There is evidence that there is a significant correlation between the efficiency of working and disruptions of specific cyclic physiological variables including urinary sodium, potassium and body temperature. However, it is not clear why some individuals develop subjective symptoms and show decreased work performance while others do not; and, why some individuals adapt with ease and others adapt with much difficulty, to disruption of specific cyclic physiological variables by day-night shift-rotation.

In order to determine the physiological effects of day-night shift-rotation volunteer subjects were stabilized on a day shift 30 days before the onset of the present study. During the study three periods of time were utilized: Period I - Study days 1-5 consisting of work (0800-1600), leisure (1600-2400), sleep (2400-0800); Period II - Study days 6-10 consisting of leisure (0800-1600), sleep (1600-2400), work (2400-0800); Period III - Study days 11-16 consisting of work (0800-1600), leisure (1600-2400), sleep (2400-0800). During each period physical activities and diet were specified. All subjects were maintained on a constant xanthine-free isocaloric diet which contained 15% protein, 45% carbohydrate, 40% fat and a constant mineral and vitamin supplement. Meal hours were consistent with the work-shift during all study periods and allowed for 4 equal feedings for each study day. A 24 hour oral intake of 2000 ml of fluids was equally distributed throughout the day. The following variables were considered: body weight; salivary pH; body temperature; heart rate; serum cortisol, inorganic phosphorus, calcium, osmolality and growth hormone; and urinary excretion of sodium, potassium, inorganic phosphorus, calcium, creatinine, 17-hydroxycorticosteroids, 17-ketosteroids and osmolality. Performance measurements were obtained by a research psychologist and included the Crawford Small Parts Dexterity Test and the Digit-Symbol substitution test. During the baseline period prior to the onset of the study twelve sessions were conducted to establish a level of practice. Performance test and symptomatic inventories were administered in the same order and under constant and controlled conditions.

Biochemical Factors Influencing Physiological Functioning (Cont)

Measurements for all variables were taken at four hour intervals. An indwelling heparinized venous flush system was utilized for collection of the serum specimens. Performance measurements were obtained 3 times daily until baseline values were established following which daily measurements at midpoint of work period were obtained. A symptomatic inventory check sheet was prepared at the beginning and end of each work period. During all study periods a Read Universal Exposure meter reading and room temperature were recorded every four hours.

RESULTS AND DISCUSSION OF THE RESULTS:

Seven normal subjects participated in the study. Despite the numerous inherent problems, under controlled conditions and with highly motivated personnel it is possible to execute a study of this complexity.

The first problem encountered concerned the use of an indwelling heparinized flush system. During the study, systems were changed approximately every 4th day. No inflammatory reactions occurred and few or no ecchymoses were encountered at the venipuncture sites. It was concluded that the indwelling flush systems were practical and free of deleterious effects. Indeed, some of the systems were effective and functional for longer than 4 days.

Although there was sufficient data in the scientific literature to serve as a precedent for the selection of the variables studied, there were no reported studies with the complexity of this study with regard to the number of variables. It was found that large volumes of data could be collected from a number of subjects in a relatively short period of time with the proper organizational system and high degree of motivation of the subjects. Differences between individual subjects did occur but whether or not these changes were statistically significant awaits further data analysis.

It was found that because of the time required for the collection and processing of samples the subjects did not receive precisely four hour sleep and leisure periods as set forth in the protocol.

It was concluded that the administration of performance tests required more coordination and control than was originally anticipated.

It was suggested that an appropriate dress should be designed for female subjects in order to reduce time spent in dressing, eliminate clothing over the indwelling flush system and for the esthetic appearance of the subject.

Biochemical Factors Influencing Physiological Functioning (Cont)

This pilot study demonstrated that it is necessary to use semi-micro-techniques for the analysis of blood serum parameters since anemia developed in the subjects because of the volume of blood drawn despite the use of adequate iron supplements.

It was found that the total involvement of the nursing staff resulted in the accurate and complete collection of multiple measurements on a recurring basis over a long period of time.

Preliminary observations demonstrated changes only in some subjects. The available data shows disruption in the occurrence of the maximum and minimum values of body temperature, serum osmolality, urine osmolality and body weight. These changes are in the process of being analyzed statistically by appropriate methods. Laboratory and statistical analysis of the remainder of the data is not completed.

CONCLUSIONS:

This has been a necessary pilot study from the standpoint of the mechanics of performance as well as from the results that may accrue from analysis of the data.

RECOMMENDATIONS:

It is recommended that a smaller number of subjects be studied in the future. If the results support given hypotheses then additional subjects should be studied over a longer period of time with the extended study time being in the third period, so that adjustment rates can be better analyzed.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OB 6301	74 07 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8A. ORIGIN INSTN ⁶	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
73 07 01	D Change	U	U	NA	NL	9. LEVEL OF SUM A. WORK UNIT	
10. NO./CODES ⁷		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		61102A	3A161102B71R	02	168		
b. SECONDARY		61102A	3A061102B71R				
c. TERTIARY		CARDS 114 (4)					
11. TITLE (Precede with Security Classification Code) ⁸							
(U) The Effects of Diet Upon Respiration Metabolism							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁹							
012900 Physiology, 006500 Food							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 02		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. FUNDS (In thousands)	
a. DATES/EFFECTIVE:				PRECEDING		b. PROFESSIONAL MAN YRS	
b. NUMBER: ¹⁰ Not Applicable				FISCAL YEAR		c. FUNDS (In thousands)	
c. TYPE:				74		1.9	
d. KIND OF AWARD:				75		20.0	
e. AMOUNT:				CURRENT			
f. CUM. AMT.							
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: ¹¹ Letterman Army Inst of Resch				NAME: ¹² Letterman Army Inst of Resch			
ADDRESS: ¹³ Presidio of San Francisco California 94129				ADDRESS: ¹⁴ Bioenergetics Division Department of Nutrition Presidio of San Francisco, California 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: ¹⁵ Canham, J. E., COL, MC				NAME: ¹⁶ Consolazio, C. F.			
TELEPHONE: ¹⁷ 415 561 3600				TELEPHONE: ¹⁸ 415 561 5092			
				SOCIAL SECURITY ACCOUNT NUMBER: ¹⁹ 352-24-4085			
22. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME: ²⁰ Dranise, J. G.			
				NAME: ²¹ Krzywicki, H. J. DA			
22. KEYWORDS (Precede Each with Security Classification Code) ²²							
(U) Humans; (U) Respiratory Function; (U) Diffusing Capacity; (U) Lung Compartments; (U) Nutritional Stress; (U) Dietary Modifications							
23. TECHNICAL OBJECTIVE, ²³ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number Precede text of each with Security Classification Code.)							
<p>23. (U) It is important that respiratory efficiency be enhanced, if possible, to enable the soldier to respond more adequately to physical and environmental stress. The respiratory system may be altered with various acute and chronic changes in diet and environmental conditions encountered by military personnel. Studies will be initiated to measure the effect of nutritional and environmental manipulation upon: (a) the respiratory system; (b) acid/base relationships; (c) blood gases; (d) cardiovascular responses, and (e) hemodynamic factors. The derived data may permit the development of recommendations to improve the respiratory efficiency and performance of the soldier.</p> <p>24. (U) Studies will evaluate: (a) the gaseous nitrogen exchange in humans during steady state conditions and with various protein intake levels; (b) the effects of varied levels of nutrient intake (protein, fat and carbohydrate) as they relate to changes in pulmonary function, primarily pulmonary diffusion capacity during rest and exercise, and (c) the hemodynamic responses resulting from dietary influence and pulmonary-respiratory efficiency.</p> <p>25. (U) 73 07-74 06 Two studies were reported under work unit 070, High Altitude Bioenergetics, which is being terminated.</p>							

Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A061102B71R **Research in Biomedical Sciences**
TASK NO. 02 **Internal Medicine**
WORK UNIT NO. 168 **The Effects of Diet Upon Respiratory Metabolism**

The following investigations have been conducted under this work unit:

STUDY NO. 1 The Effects of a Glucose Meal

STUDY NO. 2 The Effects of a High Fat Meal

These two studies are being reported in detail under Work Unit 070.

Study No. 1 and No. 2. The data indicates that a glucose meal is beneficial during acute altitude exposure. D_{LCO} was improved following glucose ingestion, while a high fat meal appeared to decrease diffusing capacity.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OA 6375	74 07 01	DD-DR&E(AK)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY ACTY ^b	6. WORK SECURITY ^b	7. REGRADING ^c	8A. OMB'S INSTR ^d	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^e		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		61102A		3A161102B71R		02	
b. XXXXXXXX		61102A		3A061102B71R		02	
c. XXXXXXXX		CARDS 114(f)				169	
11. TITLE (Precede with Security Classification Code) ^g							
(U) Comparative Pathology of Animals Maintained and Utilized in Biomedical Research(06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^h							
002600 Biology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
72 07		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRESENTING		FUND (\$ - Thousands)	
b. NUMBER: ⁱ				FISCAL YEAR		74	
c. TYPE: Not Applicable				CURRENT		5.0	
d. KIND OF AWARD:				75		6.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMER ORGANIZATION			
NAME: ^j Letterman Army Institute of Research				NAME: ^k Letterman Army Institute of Research		Pathology & Comparative Studies Div	
ADDRESS: ^l Presidio of San Francisco, CA 94129				ADDRESS: ^m Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL. MC				NAME: ⁿ Empson, R.M., CPT, VC			
TELEPHONE: 415 561-4714				TELEPHONE: 415 561-4714			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Bucci, T.J., LTC, VC		DA	
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Comparative Pathology; (U) Experimental Animals; (U) Human Analogues; (U) Military Research Support							
23. TECHNICAL OBJECTIVE, ^o 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Much biomedical research depends upon the proper use of healthy laboratory animals. The broad objectives of this work unit are: (1) to maintain and provide experimental animals for biomedical research for all the divisions of the Laboratory; (2) to provide pathology services, including gross, clinical, microscopic or special studies of spontaneous or induced diseases in animals; (3) to provide veterinary care to insure the health and well-being of the animals in the colony, (4) to study diseases of laboratory animals</p> <p>24. (U) Colonies of different animal species were maintained for the use of investigators. Pathology services were furnished and included necropsies, light and electron microscopy, autoradiography, serum and tissue enzyme studies and blood urine analyses. All cases requiring histopathologic assessment were accessioned sequentially and appropriate reports were rendered. Material of special teaching value was utilized to supplement didactic seminars and conferences.</p> <p>25. (U) 72 07-73 06. Some 4900 anima's, purchased from commercial sources or bred within the colony, were maintained for research during the report interval. These included 2600 rats, 1800 mice, 290 guinea pigs, 55 dogs, 30 cats and 119 rabbits. Cases accessioned numbered 650; these produced 3200 paraffin blocks, 7100 H&E stained microslides, 1100 specially stained slides, and 800 histochemical preparations.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A061102B71R Research in Biomedical Science
TASK NO. Internal Medicine
WORK UNIT NO. 169 Comparative Pathology of Animals
Maintained and Utilized in
Biomedical Research
STUDY NO. 2 Avian histologic atlas
STUDY NO. 3 Substrate specificity and selective inhibition of
alkaline phosphatase and adensine triphosphatase.

The objective of this work unit is to maintain an animal colony stocked with healthy animals of diverse species. Under this work unit, this Division supports all projects involving the use of research animals.

Study No. 2: The object of this study was to produce a brief yet comprehensive atlas of avian histology which would be extremely valuable to this and other laboratories utilizing avian species in biomedical research.

Study No. 3: Previous work demonstrated that histochemical reaction products from the activity of alkaline phosphatase and ATP-ase can mask each other in tissue sections. Addition of specific inhibitors selectively eliminates formation of reaction products and should permit specific identification of enzyme present in the tissue. Inhibitors proved to be specific for animal species and tissue.

BODY OF REPORT

WORK UNIT 169

Comparative Pathology of Animals
Maintained and Utilized in Biomedical
Research

STUDY NO. 2

Avian Histologic Atlas

PROBLEM:

Avian pathology is currently basically oriented towards the gross examination of necropsy specimens. With the increasing use of avian species as biomedical models of disease, certain problems have arisen due to the difficulty of identifying normal avian tissue at the histologic level. Therefore this study proposed to develop a basic reference atlas.

RESULTS AND DISCUSSION:

During the previous FY all animals had been sacrificed, tissues harvested, and histologic sections prepared. Within the first three months of FY 74, two adequate textbooks of avian histology were published by others, precluding the necessity for completion of this study. Coupled with this, departure of the senior investigator and pressing involvement of the alternate investigator in more timely and critical research effectively curtailed photomicrography.

CONCLUSIONS: NONE

RECOMMENDATIONS:

All histologic preparations be examined, replaced as necessary for technical quality, compiled as reference or study sets, and the study be terminated.

PUBLICATIONS: NONE

STUDY NO. 3

Substrate Specificity and Selective
Inhibition of Alkaline Phosphatase
and Adenosine Triphosphatase

PROBLEM:

Because of the widespread dependence of metabolism on phosphorylated compounds, the accuracy and specificity of the enzyme techniques in histochemistry which relate to alkaline phosphatase and ATP-ase activities are potentially of great significance. Since alkaline phosphatase and ATP-ase can reduce the same substrates, the reaction product cannot be said to be activity of either enzyme specifically

Comparative Pathology of Animals (Cont)

unless one is selectively inhibited. The action of each inhibitor is both species and tissue dependent. This study was designed to test the action of several inhibitors on enzyme activity in several animal species and tissues.

RESULTS AND DISCUSSION OF THE RESULTS:

During the previous fiscal year, the tissues were harvested, frozen and sectioned on a cryostat microtome. When inhibitors were introduced into incubating solutions, it was found that those which inhibited alkaline phosphatase activity in rat kidney had no effect on this enzyme in dog kidney. The same was true when comparing an inhibitor of intestinal ATP-ase and kidney ATP-ase. Due to departure of the senior investigator no further progress was made during FY 74.

CONCLUSIONS: NONE

RECOMMENDATIONS: Termination of study.

PUBLICATIONS: NONE

UNNUMBERED STUDIES

Included among the responsibilities of this work unit are maintenance of an animal colony and histopathologic support, as requested, for studies conducted by all other divisions. This work unit therefore actively supports, directly or indirectly, all projects involving the use of experimental animals. During FY 74 nine such projects generated by other divisions or organizations were supported.

During FY 74 approximately 4900 animals, purchased from commercial sources or bred within the colony, were maintained for research. These included 2600 rats, 1800 mice, 290 guinea pigs, 55 dogs, 30 cats and 119 rabbits. Cases accessioned numbered 650; these produced 3200 paraffin blocks, 7100 H&E stained microslides, 1100 specially stained slides, and 800 histochemical preparations. These figures are substantially lower than those for FY 73, reflecting the progressive decline in laboratory activity prior to consolidation of laboratories at LAIR-PSF. Interesting or representative material encountered during routine laboratory function was extracted for use in continuing education of the division's professional staff.

PUBLICATIONS:

Ford, G.H., P.R. Brown, R.N. Empson Jr., and C.G. Plopper. Equine and Feline Malignant Giant Cell Tumor of Soft Parts. American Journal of Pathology, 74:27a-28a, 1974.

Comparative Pathology of Animals (Cont)

Bucci, T.J.: A Morphological Study of Normal and Abnormal Glomeruli in Mice. Ph.D. Thesis, University of Colorado Medical Center, Denver Colorado, 1974.

ABSTRACT

PROJECT NO. **3A161102B71R** **Research in Biomedical Sciences**
TASK NO. **04** **Dentistry**
WORK UNIT NO. **135** **Oral Disease in Military Populations**

No specific single investigation has been carried out under this work unit since its inception in July 1966 as one of two "umbrella" type protocols under which all departmental research studies were conducted.

Each subproject allocated under this protocol bears its own specific work unit number and title, as follows:

- a) Work Unit No. 144 - Prevention of Post-Extraction Alveolitis
- b) Work Unit No. 147 - Bone Repair
- c) Work Unit No. 148 - Complications Following Intravenous Sedation in Military Dental Procedures

Details pertaining to each of these investigations can be found in the project report associated with the specific work unit number.

BODY OF REPORT

WORK UNIT NO. 135

Oral Disease in Military Populations

PROBLEM:

In July 1966, this umbrella-type protocol was established with the purpose of reducing morbidity and non-effectiveness of affected Army personnel by establishing methods, practical under military conditions, for achieving more efficient treatment, diagnosis and prevention of oral diseases prevalent in the military.

RESULTS AND DISCUSSION OF THE RESULTS:

By means of specific subprojects allocated under the category, "Oral Disease in Military Populations," but nevertheless bearing their own individual work unit numbers, various human clinical investigations and laboratory studies have been carried out.

During the present fiscal year, research has been conducted in three subprojects conducted under this protocol:

- a) Work Unit No. 144 - Prevention of Post-Extraction Alveolitis
- b) Work Unit No. 147 - Bone Repair
- c) Work Unit No. 148 - Complications Following Intravenous Sedation in Military Dental Procedures

Details relating to each of these investigations can be found in the project report associated with the specific work unit number.

CONCLUSIONS: None

RECOMMENDATIONS:

This protocol is terminated because of the closure of this department.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY A/C NUMBER	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL	
				DA OC 6795	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORG'N INSTR'M	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	K Completion	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61102A	3A161102B71R		04	144		
b. SECONDARY	61102A	3A061102B71R		04			
c. THIRDARY	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code)							
(U) Prevention of Post-Extraction Alveolitis (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
70 01		74 01		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	20. FUNDS (In thousands)
a. DATES/EFFECTIVE: EXPIRATION:				PRECEDING			
b. NUMBER: Not Applicable				FISCAL YEAR 74		.5	5.0
c. TYPE:				CURRENT 75		0	0
d. KIND OF AWARD:				e. AMOUNT:			
f. CUM. AMT.							
19. RESPONSIBLE DOC ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. / academic institution)			
NAME: Canham, J. E., COL, MC				NAME: Lilly, G. E., COL, DC			
TELEPHONE: 415-561-3600				TELEPHONE: 512 221-6224			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Rael, E. M., DAC			
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Antiseptic Mouthwash; (U) Military Dentistry; (U) Alveolar Osteitis; (U) Mandibular Third Molar; (U) Extraction							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Although the incidence has not been established in the U.S. Army, available data suggest that at least two percent of all tooth extractions are followed by local infection of the tooth socket (alveolitis or "dry socket"). Ninety percent of the cases of alveolitis that do occur follow the removal of a mandibular third molar. The objective of this study is to develop a simple, effective method of preventing this complication which would also be practical in military situations.</p> <p>24. (U) Prevention of post-extraction alveolitis by use of a topical antiseptic to temporarily reduce the bacterial population of the oral cavity. A collaborative study between Letterman Army Medical Center and Letterman Army Institute of Research.</p> <p>25. (U) 73 07 - 74 06 Incidence of alveolar osteitis in 2,195 third molar extractions was 9.1 percent. For further details see FY 73 Annual Progress Report, Letterman Army Institute of Research. Principal investigator has departed this facility. This study is completed.</p>							

* Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A161102B71R **Research in Biomedical Sciences**
TASK NO. 04 **Dentistry**
WORK UNIT NO. 144 **Prevention of Post-Extraction Alveolitis**

The principal investigator has departed this facility and studies conducted under this work unit have been completed. All details were reported in full in the FY 1973 Progress Report of this department. However, final preparation of data prior to publication was carried out by the associate investigator subsequent to the departure of the principal investigator.

BODY OF REPORT

WORK UNIT NO. 144

Prevention of Post-Extraction Alveolitis

PROBLEM:

Available statistics indicate that at least 2 percent of all tooth extractions are followed by local inflammation of the involved tooth socket (alveolitis or "dry socket"), although the precise incidence has not been established in the U. S. Army. Ninety percent of alveolitis cases which do occur follow the removal of a mandibular third molar. The purpose of this study was to develop a simple, effective method for preventing this complication which would also be practical for use in military dentistry.

RESULTS AND DISCUSSION OF THE RESULTS:

Incidence of alveolar osteitis in 2195 third molar extractions was 9.1 percent. Fewer cases of alveolitis occurred in extractions preceded by use of an oral lavage than in those not preceded by lavage. This difference in incidence was statistically significant (P less than 0.025). All details of this study were reported in the FY 1973 Progress Report of this facility. Subsequent to the departure of the principal investigator, final preparation of the data was carried out by the associate investigator.

CONCLUSIONS:

Preoperative lavage with a topical oral antiseptic can result in a significant reduction in the incidence of alveolar osteitis following extraction of lower third molar teeth.

RECOMMENDATIONS:

This study has been completed and the findings have been promulgated to the dental profession by means of publication in a national journal.

PUBLICATIONS:

Lilley, G. E., D. B. Osbon, E. M. Rael, H. S. Samuels and J. C. Jones. Alveolar osteitis associated with mandibular third molar extractions. J.A.D.A. 88: 802-806, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^b	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMPRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^c	6. WORK SECURITY ^d	7. REGRADING ^e	8. DIR'SN INSTR ^f	9. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
73 07 01	H Termination	U	U	NA	NL	A. WORK UNIT	
10. NO./CODES ^g		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		61102A		3A161102B71R		04	
B. XXXXXXXX		61102A		3A061102B71R		04	
C. XXXXXXXX		CARDS 114(f)				147	
11. TITLE (Precede with Security Classification Code) ^h							
(U) Bone Repair (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ⁱ							
003500 Clinical Medicine							
13. START DATE		71 03		74 03		14. FUNDING AGENCY	
				DA		15. PERFORMANCE METHOD	
						C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				FISCAL YEAR		B. FUNDS (in thousands)	
B. NUMBER: ^j Not Applicable				74		1.7	
C. TYPE:				75		0	
D. KIND OF AWARD:						0	
E. AMOUNT:							
F. CUM. AMT.							
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^k Letterman Army Institute of Research				NAME: ^k Letterman Army Institute of Research			
ADDRESS: ^l Presidio of San Francisco, CA 94129				ADDRESS: ^l Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: ^m Robert, R., CPT, DC			
TELEPHONE: 415-561-3600				TELEPHONE: 415 561-4042			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Payne, T. F., MAJ, DC			
				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Fractures; (U) Avulsion Wound; (U) Bone Repair; (U) Radioisotopes; (U) Mandibular Bone Grafts							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) a. To determine reliable quantitative and qualitative methods for assaying bone repair; b. To further develop more objective laboratory and clinical endpoints for examining and evaluating bone healing in order to reduce hospitalization time associated with bone injuries in military patients; c. To more accurately determine effects of different forms of therapy.							
24. (U) Laboratory studies on dogs relating physical strength findings to histologic, clinical and radiologic data.							
25. (U) 73 07 - 74 06 During a 16 week postoperative evaluation period, the strengths of union of non-irradiated (i.e. chemically sterilized) bone grafts (allogeneic and surface decalcified allogeneic bone, respectively) were consistently higher than the strengths of union of their respective, irradiated counterparts. This study is being terminated because of the closure of this department.							

* Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A161102B71R Research in Biomedical Sciences
TASK NO. 04 Dentistry
WORK UNIT NO. 147 Bone Repair

Considerable controversy exists as to the effect of radiation sterilization on the acceptability of allogeneic bone for use in bone grafting procedures. This study was undertaken to determine the influence of such radiation on the healing of bone grafts sterilized in this manner.

Since traditional criteria for assaying bone repair are either indirect or subjective, biomechanical strength of union (Instron Test Apparatus) was used to evaluate the healing process. Five osseous defects, 6 mm in diameter, were prepared bilaterally in tibias of 12 dogs and filled with cylinders of autologous bone, allogeneic bone, irradiated allogeneic bone, surface-decalcified allogeneic bone (SDAB) and irradiated SDAB. Sacrifice was at 4, 8 and 16 weeks.

Irradiated samples were sterilized at the Armed Forces Radiobiology Research Institute under the same conditions as those used by the Tissue Bank, Naval Medical Research Institute. Non-irradiated samples were sterilized by a modified ethylene oxide method developed at this facility.

Biomechanical strength data were obtained by shearing the graft cylinders from their respective sites. Data plots were subjected to an analysis of variance and found to be statistically significant (P less than .01).

By the eight week, both irradiated and non-irradiated allogeneic grafts had strength significantly greater than the respective SDAB grafts. Eighty percent of the combined non-irradiated grafts at all sacrifice dates showed strengths in excess of their irradiated counterparts, averaging as high as 40 percent greater. The data suggest that radiation sterilization causes changes in allogeneic bone which may adversely affect bone graft union.

BODY OF REPORT

WORK UNIT NO. 147

Bone Repair

PROBLEM:

The most commonly used method of sterilizing allogeneic bone for bone grafting is by irradiation. However, there is considerable controversy as to the effect of this irradiation on the healing of the graft. While several investigators have maintained that irradiation disrupts osteogenic induction, others have maintained that effects are minimal or nonexistent. This study was undertaken to ascertain whether irradiation used for sterilization influences the strength of bone graft union to host bone.

RESULTS AND DISCUSSION OF THE RESULTS:

Five round osseous defects, 6 mm in diameter, were created bilaterally in the medial diaphysis of the tibia in dogs by means of a Smedberg bone drill. These recipient sites were then filled with uniform graft cylinders as follows:

1. Surface decalcified allogeneic bone (SDAB).
2. Irradiation sterilized SDAB.
3. Autologous bone obtained from the radius of the recipient animal.
4. Allogeneic undecalcified bone.
5. Irradiation sterilized allogeneic bone.

The center of each plug was marked for orientation by a 0.031 inch amalgam retention pin. The non-irradiated allogeneic and SDAB grafts were sterilized by alcohol and ethylene oxide.

The bone used for comparing the union strength of irradiated and non-irradiated grafts was obtained from a single donor animal. Cutting and shaping of the bone grafts was accomplished in the laboratory under aseptic conditions and the bone grafts were divided into two groups: non-irradiated and irradiated. The non-irradiated bone grafts were further subdivided into allogeneic and SDAB groups. The allogeneic grafts received four 30 min. saline washes, were bathed in 95% ethanol for 2 hours at 5°C and were dried for 2 hours with sterile air prior to gas sterilization. The alcohol dessication step was instituted to enhance gaseous penetrability of ethylene

Bone Repair (Cont)

oxide. The SDAB grafts were similarly sterilized after their normal processing of defatting, decalcification, and washing. Both the allogeneic and SDAB groups were lyophilized for storage.

The irradiated bone grafts were subjected to 2.5 million rads via linear acceleration at the Armed Forces Radiobiology Research Institute. These are the same facilities and conditions under which bone is sterilized for the Tissue Bank of the Naval Research Institute. The grafts were divided into allogeneic and SDAB groups and processed as usual before lyophilization.

The bone grafts were surgically placed, as described above, in the tibias of a total of 12 dogs. Four dogs were sacrificed at each of three sacrifice intervals at 4, 8, and 16 weeks. At sacrifice, the tibias were surgically removed, the soft tissues excised, and were mounted for physical strength testing on an Instron Universal Testing Apparatus. Strength of bone graft union was determined by measuring the force required to shear the healing interface between the graft and surrounding host bone.

The shearing strength data, recorded in kg/cm^2 , were plotted against time to obtain time-strength comparisons at each interval (see Figure). As had been found in previous studies using this model, autologous grafts generally had higher values throughout the period studied than did allogeneic and SDAB grafts. However, the union strength of allogeneic grafts was second in magnitude to that of autologous grafts at each interval and was significantly greater than that of SDAB grafts at both the fourth and eighth weeks.

Examination of the relationship of the non-irradiated allogeneic grafts and the irradiated allogeneic grafts indicated that the union strength of the non-irradiated grafts was consistently higher than that of their irradiated counterparts. The data plots were subjected to a two-way analysis of variance with replication and were found to be different at a significance level of 0.05. Likewise, a comparison of non-irradiated SDAB grafts to irradiated SDAB grafts also indicated a difference of union strengths at the same level of significance.

The biomechanical strength data were found to correlate well with clinical and histologic observations. For instance, the low strength of irradiated SDAB graft union at four weeks was mirrored in its clinical appearance. Voids were noted around the periphery of the grafts and several were found to exhibit mobility within the graft sites. Although there was also a ring of decalcification around the non-irradiated SDAB grafts at four weeks, they did not exhibit the voids observed around the irradiated grafts.

Bone Repair (Cont)

The graft junctions of irradiated grafts usually differed in histologic appearance from those of non-irradiated grafts. For instance, resorptive lacunae were noted along the graft interface in the 8-week irradiated allogeneic grafts. There was little evidence of bridging or union between the graft and the host bone. However, in the non-irradiated allogeneic grafts, we observed good fusion of the graft to host bone. Indeed, the allogeneic grafts appeared to be as well fused as did the autologous grafts at this time. Microscopically, there was excellent union between the graft and host bone. The histologic observation reflects the similarity in strength between allogeneic and autologous grafts at the eighth week.

By the sixteenth postoperative week, all of the allogeneic and SDAB grafts (with the possible exception of the irradiated allogeneic graft) had undergone sufficient remodeling and replacement so that they had essentially equal strength of union. Microscopic examination revealed that fusion of all the grafts to host bone was virtually complete even in the irradiated SDAB grafts. Heretofore, all of the SDAB grafts, whether irradiated or non-irradiated, exhibited poorer fusion than allogeneic grafts, by both biomechanical and microscopic evaluation. Histologically, we found that new bone had closed the void between the graft and host bone. The union strength of the irradiated grafts was found to be statistically identical to that of non-irradiated grafts at 16 weeks.

Not surprisingly, in the non-irradiated allogeneic grafts, we also found new bone uniting the graft with host bone. However, it is in the early discrepancies in biomechanical strength of union among the various grafts at 4 and 8 weeks wherein lies the primary significance of our study. Early development of union strength is essential in order that periods of stabilization be minimized. It was during this time frame that the non-irradiated, gas-sterilized bone grafts showed greater union strengths than did their irradiated counterparts.

CONCLUSIONS:

Radiation sterilization of allogeneic canine bone grafts material prior to placement may adversely affect the strength of graft union, up to 8 weeks, post-operatively.

RECOMMENDATIONS:

Further work in this investigation is merited. This study is being terminated because of the closure of this department.

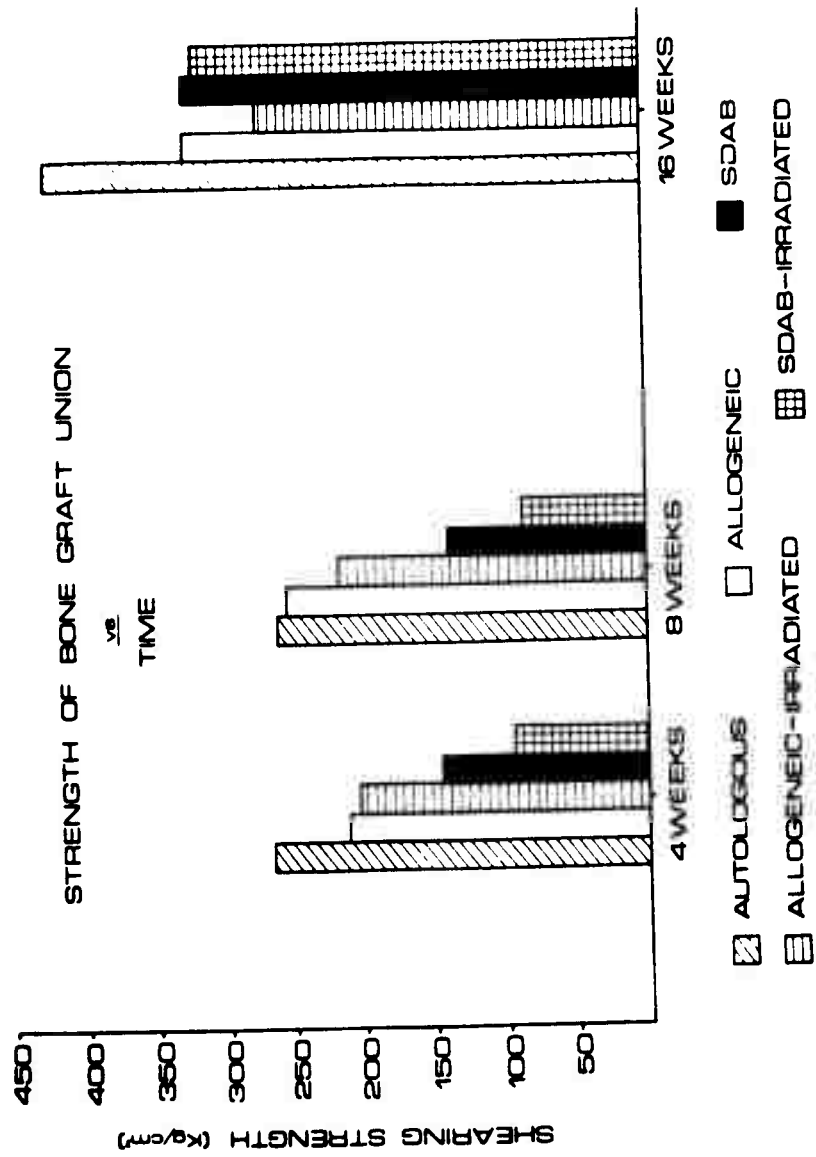
Bone Repair (Cont)

PUBLICATIONS:

1. Robert, R. C., T. F. Payne, J. T. Vincent, J. B. Richey and J. L. Cutcher. The effect of radiation sterilization on bone graft union. J. Dent. Res. 53: 185, 1974 (Abstract).
2. Robert, R. C., T. F. Payne, J. T. Vincent and G. E. Lilly. Comparison of healing strengths of autologous and allogeneic bone grafts. J. Dent. Res. 53: 249, 1974 (Abstract).

Bone Repair (Cont)

FIGURE 1



RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^b	REPORT CONTROL SYMBOL	
				DA OC 6932	74 07 01	DD-DR&E(AR)636	
3. DATE PREP SUMMARY	4. KIND OF SUMMARY	5. SUMMARY ACT ^c	6. WORK SECURITY ^d	7. REGRADING ^e	8. DES'N INST'N	9. SPECIFIC DATA CONTRACTOR ACCESS	10. LEVEL OF SUM A. WORK UNIT
73 09 25	H Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
11. NO./CODES ^f	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	61102A	3A161102B71R	04	148			
B. APPROXIMATE	61102A	3A061102B71R	04				
C. XREFERENCE	CARDS 114(F)						
11. TITLE (Precede with Security Classification Code)							
(U) Complications Following Local Anesthetic Sedation in Military Dental Procedures (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ^g							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 09		73 12		DA		C In-House	
17. CONTRACT GRANT				18. RESOURCES ESTIMATE		19. FUNDING PER YEAR	
A. DATES/EFFECTIVE:				FISCAL YEAR		PERCENT OF FUNDING	
B. NUMBER: Not Applicable				74		.3	
C. TYPE:				75		0	
D. KIND OF AWARD:						0	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Punch SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: Hourigan, M. J., LTC, DC			
TELEPHONE: 415-561-3600				TELEPHONE: 415 516-4042			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Dental Survey; (U) Dental Complications; (U) Intravenous Sedation							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Punch individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To establish guidelines for use of intravenous (IV) sedation in military dentistry by surveying types and incidence of complications occurring after use of such sedation techniques in conjunction with dental procedures.</p> <p>24. (U) A questionnaire will be utilized in record IV sedation data. The information will be transferred to IBM punch cards and tabulated by sorting machine. A statistical analysis of data will be made from a total of approximately 2,000 completed survey forms.</p> <p>25. (U) 73 09 - 74 06 A total of 1,872 dental patients who received IV sedation in the dental office were surveyed regarding complications during and after treatment procedures. Phlebitis was diagnosed in 2.8 percent of these patients overall. However, in women patients taking oral contraceptives, 35.7 percent developed phlebitis. The highest incidence of phlebitis was observed on the fourth post-operative day. Statistical analysis of data is in progress. This study is terminated due to discontinuation of the department.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

BODY OF REPORT

WORK UNIT NO. 148

Complications Following Intravenous Sedation in Military Dental Procedures

PROBLEM:

Complications during and following intravenous (IV) sedation in the dental office have been a source of controversy among general dentists, oral surgeons and dental educators, especially in the military dental services. Literature documentation concerning this matter is sparse. There currently appears to be a diversity of opinion in dentistry as to the type and occurrence of such sequelae. Figures range from 0-10 percent.

Use of IV sedation in dentistry is increasing. A recent study conducted at a public health hospital revealed that use of IV sedation combined with local anesthesia for oral surgery procedures had increased 35 percent in the past decade. In a comprehensive study dealing with postoperative alveolitis, the Letterman Army Institute of Research observed that 58 percent of patients undergoing third molar extractions received adjunctive IV sedation.

Among factors contributing to the upsurge in use of IV sedation are: 1) patient demand for sedation to neutralize the "fear and apprehension" of dental procedures; 2) research and marketing of drugs with a relatively high safety margin; 3) formal courses in IV sedation being taught in continuing education programs, internships and dental schools.

With the increased use of IV sedation in dentistry, especially among general dentists, it would appear that complications incident to such use would also increase. The undesirable sequelae may be related to the use of specific sedative drugs or combinations thereof, or with the manner in which these drugs are administered.

The purpose of this study was to establish guidelines for use of IV sedation in military dentistry by surveying types and incidence of complications occurring after use of such sedation techniques in conjunction with dental procedures.

RESULTS AND DISCUSSION OF THE RESULTS:

This study consisted of a survey of the following selected dental facilities:

- a. Military:

Complications Following Intravenous Sedation in Military Dental Procedures (Cont)

- (1) Letterman Army Medical Center
- (2) Oakland Naval Hospital
- (3) David Grant Hospital, Travis AFB
- (4) Hays Army Hospital, Ft. Ord
- (5) U.S.P.H.S. Hospital, San Francisco

b. Civilian:

- (1) University of the Pacific School of Dentistry
- (2) Loma Linda University School of Dentistry
- (3) University of California School of Dentistry
- (4) San Francisco General Hospital
- (5) Alameda County General Hospital
- (6) Mayo Clinic, Rochester, Minnesota

Using a prepared questionnaire, respondents reported the incidence and type of complications arising after administration of IV sedation prior to dental procedures in human patients. This information was transferred to IBM punch cards and tabulated by sorting machine.

A total of 1,872 dental patients who received IV sedation in the dental office were surveyed regarding complications during and after the treatment procedure. Of these subjects, 93 percent were outpatients; 50.7 percent were males; 14.7 percent were receiving antibiotics; 14.1 percent of female patients were taking oral contraceptives.

During the needle insertion, 3.9 percent of all patients complained of feeling "lightheaded or shaky" whereas 1.3 percent lost consciousness (syncope). The antecubital fossa was the most popular site for venipuncture (74.2 percent) followed by the forearm (19.1 percent) and the dorsum of the hand (12.5 percent). Most (85.1 percent) of the venipuncture attempts were successful on the first insertion; 10.6 percent of cases required a second insertion. Four or more venipunctures were necessary in 1.1 percent of cases. The most common means of initiating

Complications Following Intravenous Sedation in Military Dental Procedures (Cont)

the IV procedure was the direct drug to vein method (72.2 percent). An IV drip was used for sedation by 20.1 percent of the responding clinicians. In 20.3 percent of cases, dextrose in water (5 percent) was employed as the vehicle.

Respondents reported that phlebitis was diagnosed in 2.8 percent of the 1,872 patients; 94.7 percent of all patients stated that they were pleased with the use of IV sedation. Of the 54 patients who experienced phlebitis, 51.9 percent were males. In women patients taking oral contraceptives, 35.7 percent developed a phlebitis. The antecubital fossa was the site of phlebitis in 63.1 percent of patients who experienced this complication.

Of the single medications used, Valium[®] was associated with phlebitis most frequently (19.2 percent). A total of 50 drugs were employed either singly or in combination by reporting clinicians in this study. The most popular drug combination was Valium[®] and Demerol[®] (11.5 percent).

The highest incidence of phlebitis was observed on the 4th post-operative day (24.1 percent), followed by the 5th, 7th and 6th postoperative days (18.5, 16.7 and 14.8 percent, respectively).

Pain (either at venipuncture or postoperative) constituted the most common complaint associated with the IV sedation procedure. Of the patients experiencing phlebitis, 72 percent complained of pain and discomfort. Ecchymosis occurred in 40.7 percent and erythema in 14.3 percent of such cases, respectively. Of the 54 phlebitis patients, however, 88.9 percent stated that they were pleased with the technique. Of obvious interest is the high rate of phlebitis (35.7 percent) which developed in women receiving oral contraceptives. This is particularly notable when compared with a similar (i.e., threefold) increase in the incidence of alveolar osteitis observed by this department in earlier studies on women receiving oral contraceptives.

CONCLUSIONS:

None, the results represent a preliminary evaluation of the accrued data, especially with regard to analysis of internal relationships. No statistical analysis has as yet been performed.

**Complications Following Intravenous Sedation in Military Dental
Procedures (Cont)**

RECOMMENDATIONS:

This study has been completed. The principal investigator has been transferred and will submit a paper to a professional journal for publication.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL	
				DA OA 6312	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY ACT ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8A. ORG'N INSTR ⁶	8B. SPECIFIC DATA- CONTRACTOR ACCESS	
73 07 01	H Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ⁹		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
		61102A		3A161102B71R		05	
		61102A		3A061102B71R		080	
		CARDS 114 (E)					
11. TITLE (Precede with Security Classification Code) ¹⁰ (U) High Altitude Bioenergetics - Determination of Mechanisms Responsible for Acute Mountain Sickness (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ¹¹ 016200 Stress Physiology; 005900 Environmental Biology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
63 08		Terminate		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
4. DATES/EFFECTIVE:				PREVIOUS			
5. NUMBER: ¹² Not Applicable				FISCAL YEAR		21.3	
6. TYPE:				CURRENT		0	
7. KIND OF AWARD:				0		0	
8. AMOUNT:							
9. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ¹³ Letterman Army Inst of Rsch				NAME: ¹⁴ Letterman Army Inst of Rsch			
ADDRESS: ¹⁵ Presidio of San Francisco California 94129				ADDRESS: ¹⁶ Department of Nutrition Presidio of San Francisco, California 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: ¹⁷ Canham, J. E., COL, MC				NAME: ¹⁸ Johnson, H. L.			
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22. KEYWORDS (Precede EACH with Security Classification Code) ²⁴ (U) Hypoxia; (U) Altitude Stress; (U) Performance; (U) Balance Metabolic; (U) Blood Gases; (U) Glucose Metabolism; (U) Respiratory Function							
23. TECHNICAL OBJECTIVE, ²⁵ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To elucidate the biochemical and physiological changes in animals and man associated with abrupt altitude exposure and to measure acclimatization, dietary effects and the influence of training on these changes in order to improve the health, well-being and physical and mental capacities of soldiers having to function under conditions requiring abrupt exposure to high terrestrial altitude.							
24. (U) The effects of dietary alterations upon pulmonary diffusion capacity and other respiratory functions at 1600 m and 4300 m were investigated. Measurements included blood levels of hemoglobin, hematocrits, glucose, triglycerides, gases and blood pH and urinary levels of glucose and electrolytes.							
25. (U) 73 07-74 06 The catabolism of infused ¹⁴ C-glucose was compared in sea level natives during altitude exposure and in pair-fed men at sea level. The disappearance of plasma C ¹⁴ labelled glucose was increased at altitude. The expired C ¹⁴ CO ₂ excretion increased indicating enhanced carbohydrate metabolism. Fasting plasma glucose levels decreased with increased duration of altitude exposure. Altitude exposure enhanced the glucose-induced hyperglycemia. However, the duration of this hyperglycemia in men exposed to altitude for 40 hours was reduced and the plasma glucose levels were below initial values at 50 minutes after glucagon infusion suggesting a depletion of glycogen stores. Data are consistent with the hypothesis that glucose catabolism is beneficial during the first few days at altitude concomitant with an increased requirement for dietary carbohydrate, noted in previous studies. (Johnson, H.L. et al. Accepted for publication in Aerospace Medicine).							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A061102B71R Research in Biomedical Sciences
TASK NO. 05 Environmental Medicine
WORK UNIT NO. 080 High Altitude Bioenergetics -
Determination of Mechanisms
Responsible for Acute Mountain
Sickness

The following investigation has been conducted under this work unit:

STUDY NO. 12 Physiological and metabolic aspects of altering dietary carbohydrate-fat levels upon carbohydrate metabolism at altitude (enzymatic and electrolytes)

Three animal studies were conducted on the effects of dietary carbohydrate levels (30, 60 or 80%), exercise and altitude exposure on carbohydrate metabolism. Dietary consumption was reduced from 29 to 89% of control intake during the first 3 days at 4300 m altitude. Liver glucose-6-phosphate dehydrogenase activity increased with increasing dietary carbohydrate except when the rats were exposed to altitude and high carbohydrate simultaneously. Altitude exposure reduced this activity in all groups. In general, blood glucose values were decreasing throughout the first 3 days of altitude exposure. However, the exercised group had the total blood glucose decrease at day 1 of exposure, and these blood glucose values remained reduced for the 12 days of the study. Serum glutamic-pyruvic-transaminase appeared to peak at 6 days of altitude exposure while serum glutamic-oxalacetic activities were not significantly altered. Liver pentose phosphate metabolizing enzyme activity did not appear to be significantly affected by any of the variables. During a 12-day study, the water content of various rat tissues were increased during the first day of altitude (4300 m) exposure, while sodium, potassium, calcium and magnesium retention of the animals were significantly higher than rats at 1600 meters.

BODY OF REPORT

WORK UNIT NO. 060

High Altitude Bioenergetics -
Determination of Mechanisms
Responsible for Acute Mountain
Sickness

STUDY NO. 12

Physiological and metabolic aspects
of altering dietary carbohydrate-
fat levels upon carbohydrate metab-
olism at altitude (enzymatic and
electrolytes)

PROBLEM:

Anorexia, vomiting, insomnia and other symptoms (which could severely impair the combat effectiveness of men unless adequate allowances are made or means of alleviation are found) occur in both man and animals after abrupt altitude exposure. These detrimental effects of hypoxia can be alleviated by an adequate intake of carbohydrate and/or maintaining physical activity after abrupt exposure. These studies were designed to elucidate the mechanisms of the altitude effects in animals and of the carbohydrate and exercise ameliorating influences upon them in order to improve performance and well being of soldiers abruptly exposed to altitude.

Water and mineral balances during acute altitude exposure reported in human studies have been in disagreement. A study was conducted on rats sacrificed between 2 hours to 12 days after acute altitude exposure to gather additional information on tissue hydration and mineral balances.

RESULTS AND DISCUSSION OF RESULTS:

Three animal studies were conducted to observe the effects of 3 dietary carbohydrate levels, treadmill exercise, and altitude exposure of 12 days or less upon carbohydrate metabolizing enzyme activities. After 3 days of adaptation to 1600 m, while feeding a 60% carbohydrate diet, 150 to 175 gm rats were assigned to one of 3 dietary groups: A. 80% carbohydrate; B. 60% carbohydrate, and C. 30% carbohydrate. Seven days later, one-half of each dietary group was translocated to 4300 m and the remaining animals subjected to an equivalent transportation stress. Animals from each dietary group at each location were sacrificed on days 1, 2, 3, 6, 9 and 12. Diet consumption was reduced to 45% of control during the first day of altitude exposure but recovered thereafter. Hematocrit values increased from about 40% for controls to over 47% by the second day of altitude exposure. G-6-PD activity in the liver increased with increasing dietary carbohydrate levels, but was decreased by about

**High Altitude Bioenergetics - Determination of Mechanisms
Responsible for Acute Mountain Sickness (Cont)**

50% for the first 3 days at altitude. Serum G-PT activity increased during altitude exposure with a peak value on day 6 and then decreased, while serum G-OT values were unchanged. Blood glucose levels continued decreasing during the first 3 days at altitude and remained below control values for the remainder of the 12 days.

The second and third studies were similar to the first except that the animals were exercised daily on a motor driven treadmill after the initial 3-day adaptation. In the third studies, the experimental diets were not provided until altitude exposure. Results from these two studies were very similar to those from the first with the following exceptions. Food consumption was reduced to 29 and 31% of control during the first altitude day compared to 45% for Study 1 indicating that in the rat exercise does not ameliorate the anorexic effect of hypoxia. The Denver hematocrit values were higher (44 to 48%) than for the Denver rats of the first study, so that the increases observed at altitude were not consistently significant until day 3. G-6-PD values were greatly reduced for the 80% carbohydrate group at altitude in Study 3. The exercised animals (Study 2) had reduced blood glucose from day 1 at altitude.

Seventy-two male rats weighing 150 to 170 grams were exposed to 4300 m altitude. Food and water consumption was recorded and the average food intakes for each day was fed to "pair-fed" control rats maintained at 1600 m after an equivalent transportation stress. Six rats were sacrificed at each location at various time intervals after acute altitude exposure. A significant growth depression was observed only for the first day of altitude exposure. Significant increases in hemoglobin and hematocrit values were noted immediately (2 hours) after altitude exposure with red cell counts being significantly higher at days 9 and 12. Water contents of all tissues were increased during the first day of exposure and, with the exception of liver, remained increased throughout altitude exposure. Liver had an increased percent dry weight following the first day probably attributable to increased fat. Serum sodium levels were significantly increased and potassium significantly decreased during the 12-day exposure period. Although retention of sodium, potassium, calcium and magnesium was increased at altitude, retention of potassium and calcium were highest.

CONCLUSIONS:

Altitude exposure reduces carbohydrate metabolizing enzyme activities in rat liver. Therefore, exercise and high carbohydrate diets, which would induce increased enzymic activities, may exert their beneficial effects by this mechanism, i.e., by having increased activities prior to altitude exposure, the reduction due to hypoxia would only reduce these levels to normal rather than sub-normal.

**High Altitude Bioenergetics - Determination of Mechanisms
Responsible for Acute Mountain Sickness (Cont)**

Studies indicate a shift of body fluids from extracellular to intracellular compartments of rats, during the first 12 hours of acute altitude exposure.

RECOMMENDATIONS:

Prepare manuscripts of the animal studies for publication and terminate work unit due to discontinuation of this mission.

- 2. Schnakenberg, D. D. Hypoxic hyrophagia and hypodipsia in the rat. Ph.D. Dissertation, Univ. of California, Davis, CA, October 1973.**
- 3. Christensen, B. M., H. L. Johnson, and A. V. Ross. Organ changes and electrolyte excretion of rats exposed to high altitude. Submitted to Aerospace Med.**

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6339	74 07 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8A. DISEN INSTR ⁶	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
73 07 01	H. Termination	U	U	NA	NL		
9. NO./CODES ⁷		PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY		61102A	3A161102B71R	05	082		
b. SECONDARY		61102A	3A061102B71R				
c. TERTIARY		CARDS 114(F)					
11. TITLE (Provide with Security Classification Code) ⁸							
(U) Metabolic, Physiological and Psychological Effects of Altitude (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ⁹							
016200 Stress Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07		74 06		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	20. FUNDS (In thousands)
a. DATES/EFFECTIVE:				PRECEDING			
b. NUMBER: ¹⁰				FISCAL YEAR		1.0	16.8
c. TYPE: Not Applicable				CURRENT			
d. KIND OF AWARD:				75		0	0
e. CUM. AMT.							
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21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: ¹⁹ [REDACTED]			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
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				NAME: ²¹ Sullivan, F.L. DA			
22. KEYWORDS (Provide EACH with Security Classification Code) ²²							
(U) Altitude; (U) Adaptation; (U) Physiological; (U) Endocrine; (U) Biochemistry; (U) Physiology; (U) Military Stress							
23. TECHNICAL OBJECTIVE, ²³ 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Provide text of each with Security Classification Code.)							
<p>23. (U) Thirty to forty percent of soldiers exposed, under comfortable conditions, to an elevation of 4,300 m. experience a severe and incapacitating phenomenon called Acute Mountain Sickness (AMS). This results in a substantial group which cannot function effectively during the first several days at altitude. Studies designed to achieve a sound scientific basis for the treatment and/or amelioration of AMS can be conducted only partially in man. This work unit was developed, therefore, to study laboratory animals: a) to supplement the information acquired in human research, b) to test hypotheses that arose from human studies, c) to examine the various physiological, metabolic and psychologic defects and adaptations produced either directly or indirectly by hypoxic exposure, and c) ascertaining the basic physiological and metabolic mechanisms which underlie the observed defects and adaptation.</p> <p>24. (U) Laboratory animals were subjected to actual and simulated high altitude environments. Various physiologic, metabolic and psychologic techniques were used to determine the alterations caused by hypoxic exposure. Studies were conducted at cellular, organ and organism levels. Efforts to ameliorate or eliminate the defects were made with the use of drugs, special diets, training, etc. Two phase studies were conducted first, the acute response to hypoxia and second, the nature, extent and rate of adaptation of chronic exposure.</p> <p>25. (U) Defects in glycine and leucine metabolism of rats exposed to a simulated altitude of 5330 m. are largely, but not entirely, attributable to hypophagia. Changes in adrenocortical function at this elevation are minimal. Mice exposed to 6600 m. (simulated) exhibited a greater body weight and fat loss, but equivalent body water loss, when compared to mice exposed to 4300 m. This work unit is being terminated.</p>							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A061102B71R Research in Biomedical Sciences

TASK NO. 05 Environmental Medicine

WORK UNIT NO. 082 Metabolic, Physiological and Psychological Effects of Altitude

The following studies have been initiated or conducted under this work unit during the past year:

STUDY NO. 19 The effect of stress and hydrocortisone treatment on protein metabolism in rats

STUDY NO. 30 Body composition of mice during the early stages of altitude acclimatization

Effects of acute exposure to simulated high altitude, hydrocortisone injections and hypophagia induced by pair feeding were studied in a series of four interrelated experiments. Incorporation of ^{14}C -alanine and ^{14}C -leucine into CO_2 and tissue carbohydrate, lipid and protein were assessed in vivo and in vitro. Data, although still to be completely analyzed, show that most effects of altitude are attributable to hypophagia. Adrenocortical function appears to be slightly depressed. Exposure of mice to a simulated altitude of 6,600 meters led to greater losses in body weight, lean body mass, body fat and body water than did exposure to 4,300 meters. Most body weight loss at both elevations is attributable to a fat decrement. The protein and water fractions of lean body mass are altered, particularly at the higher elevation. This work unit is being terminated.

BODY OF REPORT

WORK UNIT NO. 082 Metabolic, Physiological and Psychological Effects of Altitude

This work unit is being terminated. As a consequence of transfer of the Medical Research and Nutrition Laboratory functions to San Francisco and dissolution of the Physiology Division the mission in Environmental Medicine has been withdrawn. During the past fiscal year, however, the following work was accomplished:

STUDY NO. 19 The effect of stress and hydrocortisone treatment on protein metabolism in rats.

PROBLEM:

Altitude exposure has been shown to suppress appetite, reduce efficiency of food utilization and alter normal gastrointestinal function. But even more important, evidence of defects in intermediary metabolism of various assimilated foodstuffs, particularly protein, has been accumulating. High protein diets, for example, are poorly tolerated; rats fed such diets not only fail to grow but actually lose weight. Negative nitrogen balances in both animals and man have been reported as have alterations in excretion patterns of nitrogenous metabolites. Serum concentrations of essential amino acids are reduced and turnover of serum albumin is increased. Finally, during the first few days of exposure incorporation of certain amino acids into tissue protein is suppressed, while oxidation of these same amino acids is enhanced. To a large extent the high altitude shift of amino acid metabolism toward catabolic pathways would appear necessary to support energy demands of the animal. Thus, hypophagia during the acute stage of exposure leads to an increased utilization of body and dietary amino acids as an energy source. The role of the adrenal cortex in this phenomenon has received little study. A similar lack of information pertains to effects of altitude-induced changes in appetite on other aspects of amino acid metabolism including gluconeogenesis and lipogenesis from amino acid precursors. Studies were therefore initiated to describe effects and interrelationships of caloric restriction, high altitude exposure and adrenocortical function on major pathways of amino acid metabolism.

RESULTS AND DISCUSSION OF THE RESULTS:

Four studies were conducted. In the first, rats were exposed to a simulated altitude of 4300m for a period of seven days. During this interval, body weight, food consumption, nitrogen excretion and adrenocorticosteroid excretion were monitored daily and compared to controls living at 1600m. In the second study, four groups of rats were used. The control group was fed ad libitum on a meal-feeding schedule. Another group was similarly fed but exposed to 4300m for two days. A third group was pair-fed to the high altitude animals for two days on a meal-eating schedule. The last group was fed for two days like the first group but was given daily injections of

Metabolic, Physiological and Psychological Effects of Altitude (Cont)

hydrocortisone sufficient to raise serum levels to twice normal. At the end of the two day experimental period the animals were sacrificed, liver and kidney tissue was excised, slices prepared, and in vitro incorporation of ^{14}C alanine into carbohydrate was measured. In the third study three groups of rats were studied under conditions which were identical to those used in the second study except that a hydrocortisone group was not included. At the end of the two day experimental period the animals were injected with ^{14}C alanine and subsequent distribution of label into glycogen, protein and lipid was measured in liver, kidney, heart, adipose and skeletal muscle tissue. In addition, oxidation of the label to $^{14}\text{CO}_2$ was also followed in the intact animal. The fourth study was identical to the third except for the amino acid label. In this instance oxidation and tissue incorporation of ^{14}C leucine label was monitored. The results of these studies are still being evaluated statistically, hence only certain qualitative effects are discernable. These include a marked reduction in food intake, growth and adrenocorticoid excretion in rats subjected to high altitude exposure. Gluconeogenesis appears to be enhanced in pair fed animals but not in high altitude animals whereas protein and lipid synthesis was suppressed in both groups to about the same extent. Tentatively, it would appear that most, but not all, effects of altitude on amino acid and protein metabolism are attributable to hypophagia.

RECOMMENDATIONS:

Data reduction from these studies should be completed, including appropriate statistical analyses. This will be accomplished even though the work unit is being terminated.

STUDY NO. 30

Body Composition of Mice
During the Early Stages of
Altitude Acclimatization

PROBLEM:

All mammalian species, including humans, exhibit a body weight loss during early stages of high altitude acclimatization. In large measure, this loss is attributable to hypophagia. In humans, indirect estimates of body composition by body densitometry and indicator-dilution techniques have yielded controversial results with respect to particular organic and inorganic components contributing to the weight decrement. Direct measurements, i.e., carcass analysis, in laboratory rats have shown fat and protein to be the major contributors, with little or no change in body water or mineral being apparent. Lack of change in body water is of particular interest since an increased loss might be expected, not only because of hypophagia and body protein loss but, perhaps more important, because of an increased rate of respiratory water loss. Thus, very small species would be operating nearer their maximum ventilatory capacity during altitude exposure and would be expected to exhibit high rates of evaporative loss.

Metabolic, Physiological and Psychological Effects of Altitude (Cont)

RESULTS AND DISCUSSION OF RESULTS:

Adult laboratory mice were exposed to simulated altitude of 6600m for periods of 3 and 7 days. At termination of exposure they were sacrificed and carcass analyses were performed. Results of these analyses were compared to results obtained in early studies conducted at 4550m (see FY 73 Annual Progress Report).

During the first 3 days at altitude mice exposed to 6600 meters lost considerably more weight than mice exposed to 4300 meters (16% vs 8%). At both elevations weight loss was attributable primarily to decrement in body fat content, fat loss being substantially greater at the higher elevation. Effects after 7 days' exposure were somewhat greater at both elevations than those observed at three days. Body water when expressed as a fraction of final body weight was not altered by exposure to either altitude. However, when expressed as a fraction of lean body mass, significant decrements were observed. These decrements were more pronounced at the higher elevation. In addition, exposure to 6600 but not to 4300 meters led to a reduction in fat-free dry mass. This would indicate a loss of body protein.

CONCLUSIONS:

Because of changes in lean body mass, particularly hydration and protein content of this mass, basic assumptions underlying estimates of human body composition at high altitude (by indirect procedures such as body densitometry) will probably need revision.

PUBLICATIONS:

Hannon, J.P., L.F. Krabill, T.A. Wooldridge and D.D. Schnakenberg:
Effects of high altitude and hypophagia on mineral metabolism. Jour. Nutr. (submitted for publication).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
73 07 01				DA OA 6356	74 07 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY ACTY ³	6. WORK SECURITY ⁴	7. SEPARATION ⁵	8. DRG'S INSTN ⁶	9. SPECIFIC DATA - CONTRACTOR ACCESS	
73 07 01	H. Termination	U	U	NA	HL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO. / CODES ⁷	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
	61102A	3A161102B71R	05	085			
11. TITLE (Precede with Security Classification Code)							
(U) Cardiopulmonary Effects of Altitude on Animals (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁸							
005900 Environmental Biology; 016200 Stress Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
67 04		74 06		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
17.1. DATE/EFFECTIVE:				PRECEDING			
17.2. NUMBER:				FISCAL YEAR		b. FUNDS (in thousands)	
17.3. TYPE: Not Applicable				74		3.0	
17.4. KIND OF AWARD:				CURRENT		11.9	
17.5. AMOUNT:				75		0	
17.6. CUM. AMT.				0		0	
18. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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21. GENERAL USE				ASSOCIATE INVESTIGATORS			
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				NAME: Persky, B.N., CPT, VC DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Altitude; (U) Subhuman Primates; (U) Military Stress; (U) Cardiovascular System; (U) Biological Sciences; (U) Respiratory System							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) The abrupt translocation of military personnel from low to high terrestrial altitudes evokes many physiological responses. Animal studies are conducted: 1) to elucidate cardiopulmonary changes and their causes in animals exposed to high altitude, 2) to describe the histological and chemical nature of these changes, 3) to determine whether these changes can be considered pathological or desirable adaptive processes, and 4) to explore more fully the extent that these changes may be extrapolated to man.</p> <p>24. (U) Animals were housed in heated facilities at 4300 m and at 1600 m to 1) compare their physiologic and pathologic response to altitude relative to man and 2) investigate changes in pulmonary vascular pressure and in lung structure during acute exposure to 4300 m from 1600 m. Right ventricular pressure was monitored by indwelling catheter, and lungs were examined by light microscopy.</p> <p>25. (U) 73 07-74 06. Study 9. Right ventricular pressure (Prv) was determined in unanesthetized cats at 1600 m and following rapid translocation (2 hrs) to 4300 m. Average Prv at 1600 m was 17.0 mm Hg 2 hrs after catheterization, and 25.2 mm Hg 6-8 hrs after catheterization. In cats relocated to 4300 m Prv was 22.1 mm Hg postoperatively (at 1600 m), 37.6 mm Hg 2-3 hrs later (immediately upon arrival at 4300 m), and 45.3 mm Hg after 8 hrs at 4300 m. Histologic study of fresh-frozen, freeze-substituted lung tissue revealed no difference between controls (1600 m) and those at 4300 m for 24 hrs. The baseline Prv (immediately post-catheterization at 1600m) differed between these groups, but the striking increase with acute exposure to altitude deserves further study. This work unit is being terminated.</p>							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A061102B71R **Research in Biomedical Sciences**

TASK NO. 05 **Environmental Medicine**

WORK UNIT NO. 085 **Cardiopulmonary Effects of Altitude on Animals**

STUDY NO. 9 **Effect of acute altitude exposure on the development of vascular hypertension in the awake domestic cat.**

The initial phases of right ventricular pressure elevation in response to high altitude hypoxia were studied in awake cats with surgically implanted right ventricular catheters. Right ventricular pressures were monitored over a 24-hour post-surgical period. Six cats were transported to 4300 m. Right ventricular pressure averaged 37 mm Hg at 1625 m, 39 mm Hg five hours after arrival at 4300 m, and 48 mm Hg after 17 hours of altitude exposure. Four cats monitored at 1625 m for 24 hours had average right ventricular pressures between 21 and 26 mm Hg. This work unit is being terminated.

Cardiopulmonary Effects of Altitude on Animals (Cont)

TABLE I

**Right Ventricular Pressures (mm Hg) in Awake
Cats with Right Ventricular Catheters**

	Time before alt.		Time following exposure					Hrs.
	5-6	3	1	5	9	17	21	
4300m	22	37	39	39	45	48	43	mmHg
Exposed (6)	Time following catheterization							
	0	4-6	10	14-18	20-24		Hrs.	
1625m	17	25.8	25	22	21		mmHg	
Control (4)								

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6920	74 07 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^b	6. WORK SECURITY ^b	7. REGRADING ^c	8. DISPN INSTR ^d	9. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
73 07 01	H Termination	U	U	NA	NL	A. WORK UNIT	
10. NO./CODES ^e		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62110A		3A162110A825		00	
b. SECONDARY		62110A		3A062110A825		00	
c. THIRDARY		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ^g							
(U) Early Management of Oral and Maxillofacial Wounds (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ^h							
003500 Clinical Medicine; 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
65 11		74 06		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	20. FUNDS (in thousands)
a. DATES/EFFECTIVE: EXPIRATION:				PRECEDING			
b. NUMBER: Not Applicable				FISCAL YEAR		74	2.5
c. TYPE: & AMOUNT:				CURRENT		75	0
d. KIND OF AWARD: f. CUM. AMT.						0	0
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
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22. KEYWORDS (Precede EACH with Security Classification Code) (U) Bone; (U) Maxillofacial Wounds; (U) Wound Incidence; (U) Wound Infection; (U) Bone Grafts							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Pursuit individual paragraphs identified by number. Precede text (1 each with Security Classification Code.)							
23. (U) a. To establish nature, incidence, cause and management problems of oral and maxillofacial injuries occurring in Army populations; b. To reduce individual morbidity and noneffectiveness and supportive care by developing methods and techniques for management of oral and maxillofacial wounds.							
24. (U) a. Documentation of nature, incidence and cause of oral and maxillofacial injuries by survey of selected treatment facilities in the Federal Dental Services; b. Laboratory studies directed toward development of methods which are practical under military field conditions for management of gunshot wounds of the maxillofacial area; c. Human clinical studies to validate laboratory findings.							
25. (U) 73 07 - 74 06 Investigations are being completed in one subproject: Oral and Maxillofacial Wound Infection. Because of the closure of this department on 30 June 1974, the following subprojects are being terminated: Mandibular Bone Grafts; Therapy for Oral Ulcers.							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A162110A825 **Oral and Maxillofacial Sciences**
WORK UNIT NO. 060 **Early Management of Oral and
Maxillofacial Wounds**

Although initiated in 1965 as one of two "umbrella" type protocols under which all departmental research has been conducted, no specific single investigation has been carried out under this work unit number.

At present, three subprojects are being conducted under this protocol, each of which bears its own work unit number. Their work unit numbers and titles are as follows:

- a) Work Unit No. 062 **Incidence of Oral and Maxillofacial Injuries**
- b) Work Unit No. 064 **Mandibular Bone Grafts**
- c) Work Unit No. 068 **Oral and Maxillofacial Wound Infection**

Details relating to each of these investigations can be found in the report associated with the specific work unit number.

BODY OF REPORT

WORK UNIT NO. 060

Early Management of Oral and
Maxillofacial Wounds

PROBLEM:

Before solution of research problems can be undertaken, the problems must first be identified, and, as far as possible, defined. This protocol was originally devised to: a) Establish the nature, incidence, cause and management problems of oral and maxillofacial injuries occurring in Army populations; b) Reduce individual morbidity, non-effectiveness and supportive care by developing methods and techniques for management of oral and maxillofacial wounds.

RESULTS AND DISCUSSION OF THE RESULTS:

Through the specific avenue of subprojects allocated to the category of "Early Management of Oral and Maxillofacial Wounds" but nevertheless bearing their own work unit numbers, various studies have been carried out to document the nature, incidence and cause of oral and maxillofacial injuries by means of surveys of selected dental treatment facilities in the Federal Services.

In addition, laboratory studies have been directed toward development of methods which are practical under military field conditions for management of gunshot wounds of the maxillofacial area. Human clinical studies have been performed in order to validate laboratory findings in some cases.

During the present fiscal year, research has been conducted in three subprojects conducted under this protocol:

- a) Work Unit No. 062 - Incidence of Oral and Maxillofacial Injuries
- b) Work Unit No. 064 - Mandibular Bone Grafts
- c) Work Unit No. 068 - Oral and Maxillofacial Wound Infection

Details relating to each of these investigations can be found in the project report associated with the specific work unit number.

CONCLUSIONS: None

Early Management of Oral and Maxillofacial Wounds (Cont)

RECOMMENDATIONS:

This protocol is terminated because of the closure of this department.

PUBLICATIONS: None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OC 6808	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. ORGN INSTR ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
73 07 01	H Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62110A		3A162110A825		00	
b. SECONDARY		62110A		3A062110A825		00	
c. THIRDARY		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ^a							
(U) Incidence of Oral and Maxillofacial Injuries (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ^a							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 09		74 06		DA		C In-House	
17. CONTRACT / GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	20. FUNDS (2nd thousands)
a. DATES/EFFECTIVE:				FISCAL YEAR		74	5.5
b. NUMBER: ^a Not Applicable				FISCAL YEAR		75	0
c. TYPE:				19. PROFESSIONAL MAN YRS		0	0
d. KIND OF AWARD:				20. FUNDS (2nd thousands)		0	0
e. AMOUNT:				21. PERFORMING ORGANIZATION			
f. CUM. AMT.				NAME: ^a Letterman Army Institute of Research			
21. RESPONSIBLE DOD ORGANIZATION				ADDRESS: ^a Presidio of San Francisco, CA 94129			
NAME: ^a Letterman Army Institute of Research				PRINCIPAL INVESTIGATOR (Provide SSAN if U.S. Academic Institution)			
ADDRESS: ^a Presidio of San Francisco, CA 94129				NAME: ^a Lilly, G. E., COL, DC			
RESPONSIBLE INDIVIDUAL				TELEPHONE: 512 221-6224			
NAME: Canham, J. E., COL, MC				SOCIAL SECURITY ACCOUNT NUMBER:			
TELEPHONE: 415-561-3600				ASSOCIATE INVESTIGATORS			
21. GENERAL USE				NAME: Rael, F. M., DAC			
Foreign Intelligence not Considered				NAME:			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Oral; (U) Maxillofacial; (U) Wounds; (U) Injuries; (U) Incidence; (U) Treatment							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Provide individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) This investigation was initiated to obtain information concerning the relative incidence and types of maxillofacial injuries treated by the Federal Dental Services. This information is considered to be essential for determination of the Federal Dental Services' commitment in this area which may be used as a guide for staffing, support required, orientation of training programs and direction of research activities.</p> <p>24. (U) Survey of oral and maxillofacial injuries at selected dental treatment facilities of the five Federal Dental Services.</p> <p>25. (U) 73 07 - 74 06 Over 8,000 cases of maxillofacial injuries have been reported. Tabulation is currently being accomplished under the direction of the principal investigator who has been transferred from this facility but who will publish this study in a professional journal upon completion of data analysis. This study is being terminated because of the closure of this department on 30 June 1974.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 66

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

BODY OF REPORT

WORK UNIT NO 062

Incidence of Oral and Maxillo-
facial Injuries

PROBLEM:

This study was undertaken to obtain data relating to the incidence and nature of oral and maxillofacial injuries treated by the Federal Dental Services. Such information is considered to be essential in the determination of the Federal Dental Services' commitment in this area with regard to staffing, support required, orientation of training programs and direction of research activities.

RESULTS AND DISCUSSION OF THE RESULTS:

The oral surgery services at selected hospitals of the five Federal Dental Services were furnished survey forms to be completed on all maxillofacial trauma cases treated at their respective facilities. The data were processed and tabulated by data processing equipment.

To date, over 8,000 cases have been reported on survey forms returned to this department. However, tabulation of this accrued data has remained under the responsibility of the principal investigator who had departed this facility as a result of a permanent change of station. Data from 7,200 forms have been tabulated thus far. Upon completion of data tabulation and analysis, final results will be published in a professional journal.

CONCLUSIONS: None.

RECOMMENDATIONS:

This study is being terminated because of the closure of this department.

PUBLICATIONS: None.

ABSTRACT

PROJECT NO. 3A162110A825 Oral and Maxillofacial Sciences
WORK UNIT NO. 064 Mandibular Bone Grafts

The following investigation has been conducted under this work unit:

**STUDY NO. 2 Gas Sterilization of Allogenic Bone for Grafting
 Procedures**

The probability of contaminating allogenic bone (AB) graft material during surgical procurement and subsequent processing has necessitated the search for a simple means of sterilizing this material without compromising its potential for grafting success. The purpose of this study was to evaluate, bacteriologically, the efficacy of ethylene oxide gas in sterilizing surface decalcified allogenic bone (SDAB) and AB samples.

Of 62 raw bone samples tested prior to chemical decalcification or desiccation, 61 were positive for bacterial growth. All bone samples tested at either of three stages during the chemical processing procedures were found to be uniformly free of bacterial growth during three weeks of aerobic and anaerobic incubation.

Thus, 122 samples assayed after washing in 95 percent ethanol, 122 samples assayed after exposure to ethylene oxide gas and 122 samples tested after lyophilization for 18 hours, respectively, were negative for bacterial growth.

BODY OF REPORT

WORK UNIT NO. 064

Mandibular Bone Grafts

STUDY NO. 2

Gas Sterilization of Allogenic
Bone for Grafting Procedures

PROBLEM:

Studies on dogs have been conducted in this laboratory in order to evaluate the effectiveness of allogenic bone (AB) as compared to surface decalcified allogenic bone (SDAB) in bone graft procedures. The probability of contaminating bone graft material during surgical procurement and subsequent processing has necessitated the search for a simple way to sterilize this material without compromising its potential for grafting success.

Bone graft material is usually sterilized by ionizing radiation prior to placement in the recipient site. Since the equipment required to produce the necessary high levels of radiation is expensive and complex, there are relatively few sites in the U.S. where such sterilization can be performed. Moreover, considerable controversy exists as to the effect of radiation sterilization on the acceptability of allogenic bone in bone grafting procedures.

The purpose of this study was to evaluate bacteriologically the efficacy of ethylene oxide gas in sterilizing SDAB and AB samples.

RESULTS AND DISCUSSION OF THE RESULTS:

A total of 366 samples (192 SDAB, 174 AB) of various canine bones (tibia, 162 samples; mandible, 156 samples; radius, 48 samples) were chemically treated and bacteriologically tested. In addition, 62 raw bone samples from the same sources were tested in order to obtain some assurance that test samples were indeed contaminated prior to initiation of chemical processing.

SDAB samples were tested bacteriologically at four different intervals and AB samples at three different intervals, respectively, before, during and after processing procedures required for each type of bone. All samples were approximately 0.5 cm³ in size and were washed in 95 percent alcohol for two hours at 5°C and dried with sterile air for two hours in order to enhance gaseous penetrability. All bone samples were then subjected to gas sterilization for 64 minutes at 60°C. Following a 12 hour passive aeration period, aseptic lyophilization was performed.

Gas Sterilization of Allogenic Bone for Grafting Procedures (Cont)

Of the 62 raw bone samples tested prior to chemical decalcification or desiccation procedures, 61 were positive for bacterial growth.

All bone samples tested at either of 3 intervals during the chemical processing procedures were found to be uniformly negative for bacterial growth during 3 weeks of aerobic or anaerobic incubation. Thus, 122 samples tested subsequent to washing in 95 percent ethanol, 122 samples tested after exposure to ethylene oxide gas and 122 samples tested following lyophilization for 18 hours, were negative for bacterial growth.

The results indicate that exposure of canine bone samples to a 95 percent ethanol wash is an effective means of destroying bacteria contaminating such samples as a result of handling during surgical procurement and the reduction of bone samples to achieve uniform physical dimensions. Such microorganisms as *Staphylococcus aureus*, *S. epidermidis*, diphtheroids and gram negative rods usually constituted the spectrum of contaminants encountered.

As a result of the unexpected uniformity of this effect of ethanol processing, the true role of ethylene oxide in this method remains undefined. Certainly, the tandem effect of exposing all bones to two separate sterilizing processes can be viewed as potentially additive in terms of the resulting destruction of bacterial contaminants.

CONCLUSIONS:

Exposure of canine allogenic bone samples to a 95 percent ethanol wash is an effective method for destroying bacteria contaminating these samples as a result of handling during procurement and processing. Because of the unexpected uniformity of this effect of exposure to ethanol, the true role of ethylene oxide gas in this procedure remains undefined.

RECOMMENDATIONS:

Future studies should focus upon deliberate contamination of bone samples with known microorganisms. Such specimens could then be used to test the efficacy of ethanol washes alone, as well as the effect of the tandem method employed in this study. An evaluation of the effect of this method on the grafting acceptability of the processed specimen has already been conducted and reported (see Work Unit No. 147, "Bone Repair"). This study is being terminated because of the closure of this department.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)836	
				DA OC 6923	74 07 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8. DISSEM INSTN ⁶	9. SPECIFIC DATA - CONTRACTOR ACCESS	
73 07 01	K Completion	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ⁷		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62110A		3A162110A825		00	
b. SECONDARY		62110A		3A062110A825		00	
c. TERTIARY		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ⁸							
(U) Oral and Maxillofacial Wound Infection (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ⁹							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 08		74 06		DA		C In-House	
17. CONTRACT ORIGIN				18. RESOURCES ESTIMATE		19. FUNDS (in thousands)	
a. DATE/EFFECTIVE:				EXPIRATION:		a. PROFESSIONAL MAN YRS	
b. NUMBER: ¹⁰ Not Applicable						b. FUNDS (in thousands)	
c. TYPE:				d. AMOUNT:		74	
d. KIND OF AWARD:				e. CUM. AMT.		1.0	
						75	
						0	
						0	
20. RESPONSIBLE DOD ORGANIZATION				21. PERFORMING ORGANIZATION			
NAME: ¹¹ Letterman Army Institute of Research				NAME: ¹² Letterman Army Institute of Research			
ADDRESS: ¹³ Presidio of San Francisco, CA 94129				ADDRESS: ¹⁴ Department of Maxillofacial Sciences Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Precede SSAN if U.S. Academic Institution)			
NAME: ¹⁵ Canham, J. E., COL, MC				NAME: ¹⁶ Cutcher, J. L., LTC, DC			
TELEPHONE: ¹⁷ 415-561-3600				TELEPHONE: ¹⁸ 415 561-5160			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: ¹⁹ Richey, J. B., DAC			
				NAME: ²⁰ Baker, J. K., SP4			
				DA			
23. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Wound Infection; (U) Antibiotic Susceptibility Testing; (U) Pathogenic Bacteria							
24. TECHNICAL OBJECTIVE, ²¹ 25. APPROACH, ²² 26. PROGRESS (Precede individual paragraphs identified by number. Precede rest of each with Security Classification Code.)							
23. (U) a. To reduce individual morbidity and mortality associated with oral and maxillofacial wound infections; b. To determine the most effective methods for treating infected oral and maxillofacial wounds.							
24. (U) Clinical and laboratory study of infected human oral and maxillofacial wounds, identifying all bacterial genera cultured from such infections and delineating the antibiotic susceptibility patterns of all microorganisms involved. Such information is collated with the clinical course of infection in order to evaluate the effect of drug therapy.							
25. (U) 73 07 - 74 06 A total of 262 infection samples were obtained from 142 patients. From this group, 882 pure culture strains were grown reflecting 48 different species (21 gram positive, 27 gram negative). The total number of penicillin-resistant strains (PRS) was 267/882 (30.2 percent). Gram positive PRS comprised 169/267 (63.2 percent) of these strains. In 121 infections samples obtained from patients not receiving antibiotics at culture nor for one month previously, 69 (57 percent) contained PRS. Of these 69 infection samples, 54 (78.2 percent) contained gram positive PRS. Knowledge of changing antibiotic susceptibility patterns of bacteria involved in oral and maxillofacial infections constitutes a primary clinical guideline for therapy in cases where the empirical use of antibiotics is required. This study is completed.							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

BODY OF REPORT

WORK UNIT NO. 068

Oral and Maxillofacial Wound
Infection

PROBLEM:

The rational use of antibiotics in therapy of oral and maxillofacial infections requires a knowledge of the microbiota of the anatomic area. Moreover, when empirical use of antibiotics is necessary, literature reports documenting the experiences of other clinicians can serve as important guidelines for initial administration of such therapy.

The literature relating to therapy for wounds of the maxillofacial area is not extensive. The purpose of this study was to survey the microbiota of oral and maxillofacial infections and thereby provide guidelines for therapy by identifying bacterial components, to include antibiotic susceptibility patterns of such bacteria.

RESULTS AND DISCUSSION OF THE RESULTS:

This study was performed on selected patients having oral and maxillofacial infections, who presented for treatment at the Department of Dentistry, Letterman Army Medical Center.

Prior to definitive therapeutic measures, a bacteriologic sample was obtained from the inflammatory site and inoculated directly into fluid thioglycollate medium. A dry culture swab sample was also obtained. Both specimen swabs, accompanied by pertinent data relating to the patient and the lesion, were immediately forwarded to the Oral Microbiology Laboratory, Department of Maxillofacial Sciences, Letterman Army Institute of Research.

Specimens were evaluated for identification of aerobic, facultative and anaerobic microorganisms cultured from these lesions. Antibiotic susceptibility patterns, where feasible, were determined according to the Kirby-Bauer disc diffusion method.

All laboratory finds relating to culture characterization and antibiotic susceptibility data were collated with the clinical course of each infection. Additional specimens were obtained and evaluated on individual patients as deemed necessary by the attending clinician.

Where possible, the presence of penicillin-resistant strains (PRS) was evaluated on the basis of the therapeutic status of the patient at the time the infection sample was obtained. Thus, the incidence

Oral and Maxillofacial Wound Infection (Cont)

of gram positive and gram negative PRS in these infections could be related to the type of antibiotic therapy, if any, that the patient was receiving at the time of culture.

A total of 262 infection samples were obtained from 142 patients. From this population, 882 pure culture strains of bacteria were grown, reflecting 48 different species (21 gram positive, 27 gram negative). Of these 882 isolations, 177 (20.0 percent) were gram negative.

The total number of PRS cultured was 267/882 (30.2 percent). Gram positive PRS comprised 169/267 (63.2 percent) of these strains.

Regardless of the antibiotic therapeutic status of these patients at time of culture, 157/262 infection samples contained one or more PRS, an incidence of 59.9 percent.

From the 262 infection samples, 206 were selected and divided into two groups, based upon antibiotic therapy. Group I (85 samples) were receiving penicillin at time of culture. Group II (121 samples) received no antibiotic at culture nor for one month previously. The excluded 56 cultures represented patients who were either receiving other antibiotics or whose therapeutic status was unknown.

In Group I: 54/85 samples (63.5 percent) contained PRS; 43/85 contained gram positive PRS (50.5 percent). In Group II: 69/121 samples (57 percent) contained PRS; 54/121 (44.6 percent) contained gram positive PRS.

Because of earlier indications that the species Staphylococcus epidermidis represented an increasing potential for pathogenic implications in these infections, the following observations on this species were noted: A total of 150 strains were grown, of which 97 were PRS (64.6 percent). Of 262 infection samples, 92 contained PRS of S. epidermidis (35.1 percent). Moreover, 44 of these 92 samples (47.8 percent) were obtained from patients not receiving antibiotic therapy.

For a variety of reasons, culture and sensitivity testing is frequently not utilized by the clinician in the initial administration of antibiotics. In the absence of the specific information obtainable from in vitro testing, the clinician must utilize an empirical approach based in large part upon a knowledge of epidemiological data relating to the infections which he encounters. Therefore, although currently controversial in many of its specific

Oral and Maxillofacial Wound Infection (Cont)

applications, the use of empirical or prophylactic approaches to antibiotic therapy in oral and maxillofacial infections is recognized as a valid, and at times, essential, factor in their management.

The general availability of various penicillins, however, has led to the widespread use, and abuse, of these drugs with resultant increases in resistance of bacteria formerly susceptible to this class of antibiotics.

In view of the widely promulgated operating principle that penicillin G is the unquestioned drug of choice in those oral and maxillofacial infections where an empirical approach is initially indicated, the data accrued in this study are of interest. Penicillin therapy in oral and maxillofacial infections is apparently related to a substantial incidence (63.5 percent) of PRS in test samples. Of potentially greater concern, however, is the incidence of gram positive PRS (44.6 percent) among infection samples derived from patients not receiving antibiotic therapy. In this respect, the relatively high incidence of penicillin-resistant *Staphylococcus epidermidis* (47.8 percent) in patients not receiving antibiotics, may be significant.

While no attempt has been made to analyze these PRS on the basis of classical concepts of pathogenicity, it must be recognized that any bacterial species cultured from a clinical infection should be regarded as at least potentially pathogenic. In view of increasing suspicions that *S. epidermidis* may possess ample pathogenic potential, the clinician employing an empirical approach in the initial administration of antibiotics therapy in these infections should be aware of the pitfalls in the arbitrary use of penicillin.

These findings emphasize the need for epidemiologic data as clinical guidelines for empirical use of antibiotics in the management of oral and maxillofacial infections, when circumstances dictate such an approach.

CONCLUSIONS:

Gram positive penicillin resistant strains of bacteria are frequently-observed components of the microbiota of oral and maxillofacial infections surveyed at Letterman Army Medical Center, regardless of the antibiotics therapy status of the patient.

Oral and Maxillofacial Wound Infection (Cont)

Periodic epidemiologic surveys of the infection floras of such wounds, together with antibiotic susceptibility screenings, are essential for clinical guidelines for empirical use of antibiotics in these injuries.

RECOMMENDATIONS:

This study is completed and will be submitted for publication in a scientific journal.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^b	REPORT CONTROL SYMBOL	
					74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^c	6. WORK SECURITY ^d	7. REGRADING ^e	8. ORG'N INSTR'N	9. SPECIFIC DATA - CONTRACTOR ACCESS	
73 07 01	D. Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ^g		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY		62110A		3A162110A830		00	061
b. SECONDARY		62110A		3A062110A830		00	
c. THIRDARY		CARDS 114(f)					
11. TITLE (Proceed with Security Classification Code) ^h							
(U) Nutritional Aspects of Military Dog Performance							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ⁱ							
002600 Biology; 012900 Physiology; 016700 Stress Physiology; 006500 Food							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
60 06		CONT		DA		C. In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	20. FUNDS (in thousands)
a. DATE/EFFECTIVE:		EXPIRATION:		PREVIOUS			
b. NUMBER: ^j				FISCAL YEAR		74	25.7
c. TYPE: Not Applicable		4. AMOUNT:		CURRENT		75	30
d. KIND OF AWARD:		5. CUM. AMT.				2.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^k Letterman Army Institute of Research				NAME: ^k Letterman Army Institute of Research			
ADDRESS: ^k Presidio of San Francisco, CA 94129				ADDRESS: ^k Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Publish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL MC				NAME: ^l Hannon, J.P.			
TELEPHONE: 415-561-3600				TELEPHONE: 415 561-4714			
				SOCIAL SECURITY ACCOUNT NUMBER: 558-32-6967			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence Not Considered				NAME: Bucci, T.J., LTC, VC			
				NAME: Parsky, B.N., CPT, VC DA			
22. KEYWORDS (Proceed EACH with Security Classification Code) ^m							
(U) Physical Performance; (U) Physiology; (U) Fatigue; (U) Physical Training; (U) Nutrition (U) Forced Exercise; (U) Stamina							
23. TECHNICAL OBJECTIVE, ⁿ 24. APPROACH, 25. PROGRESS (Publish - *Individual paragraphs identified by number. Proceed's text of each with Security Classification Code.)							
<p>23. (U) Dogs are used extensively by the U.S. Armed Forces for sentry, patrol and other duties. Although rigorously screened prior to selection and trained thereafter, a substantial number of dogs lack stamina. The cause is not understood but appears to be organic rather than psychic. There is a need for detailed information about the factors which influence performance, and how these might be improved by training, diet, selection or other procedures. The object of this study is to develop laboratory and field tests to assess nutritional and physiologic aspects of military dog performance.</p> <p>24. (U) Nutritional studies have been conducted comparing the efficacy of 3 different diets (Study No. 2) Studies to establish precise O₂ consumption and caloric requirements for known levels of energy expenditure, and to assess blood gas and acid-base status by capillary arterIALIZATION are planned or in progress.</p> <p>25. (U) 73 07 - 74 06 Data accumulated between July 1972 and June 1973 (Study No. 2, see Annual Report FY 73) was completed, tabulated and analyzed. A Numbered Laboratory Report was drafted and is being edited. Carotid artery loops were created in four dogs and the dogs were trained to exercise on a treadmill.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A062110A830 **Biosensor Systems**
WORK UNIT NO. 061 **Nutritional Aspects of
Military Dog Performance**

A laboratory report is in preparation of the results of Study No. 2, completed during FY 73, in which 3 different diets were compared in dogs exercised by swimming and treadmill running. All 3 diets were adequate at the levels of exercise employed. Studies are in progress to establish the precise relation between known amounts of energy expenditure and O₂ consumption and therefore caloric requirement, and to assess capillary arterIALIZATION as a simple means to determine acid-base and blood gas status.

BODY OF REPORT

WORK UNIT NO. 061

Nutritional Aspects of
Military Dog Performance

PROBLEM

Experience in Viet Nam and certain CONUS areas has demonstrated that military working dogs have insufficient stamina in hot humid environments. This laboratory has shown that decreased food consumption under those conditions contributes to weight loss and presumably to decreased endurance. Provision of a highly palatable calorie-dense diet enabled the dogs to ingest sufficient calories to sustain their weight.

These experiences emphasized the paucity of data concerning the nutritional requirements of working dogs and further studies were undertaken to clarify some of them. This study was initiated late in FY 70 and involved comparison of 3 diets in dogs exercised by swimming and by treadmill in a temperate environment, with evaluation of endurance, histochemistry of skeletal muscle, and of numerous biochemical characteristics as a function of exercise and diet.

Still needed in the field is some easily-measured determinant of caloric requirement. Heart rate and body temperature may be useful as a predictor of energy expenditure but the precise relation between O_2 consumption (and thus caloric requirement) and heart rate and body temperature must first be established in the laboratory for a spectrum of energy expenditure levels.

RESULTS AND DISCUSSION OF RESULTS:

Results of the diet comparisons have been described in preceding Annual Reports. During FY 74 the voluminous data were organized, analyzed and tabulated, and a numbered Laboratory Report was drafted and is in the final stages of being edited.

CONCLUSIONS:

Under temperate conditions and at the levels of exercise examined, all three diets were adequate.

RECOMMENDATIONS:

Because a number of diets may be adequate under moderate conditions of exercise, but special diets are indicated under particular circumstances, a field-expedient means to determine caloric requirement is necessary. A nomogram relating heart rate, body temperature and caloric requirement appears to be one means of fulfilling this need. The relation among these should be established in the laboratory over a broad range of levels of energy expenditure.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ³	2. DATE OF SUMMARY ³	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ⁴	6. WORK SECURITY ⁴	DA OC 6933	74 07 01		
73 07 01	D. Change	U	U	7. REGRADING ⁵	8A. DISEASE INSTR ⁵	8B. SPECIFIC DATA - CONTRACTOR ACCESS	8. LEVEL OF SUMMARY UNIT
				NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ⁶	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62110A	3A162110A830	00	066			
b. SECONDARY							
c. THIRDARY	CARDS 114(F)						
11. TITLE (Precede with Security Classification Code) ⁷ (U) Study of Chronic or Recurrent Diseases of the Military Working Dog (05) Study #1 Panosteitis							
12. SCIENTIFIC AND TECHNOLOGICAL AREA							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 09		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				FISCAL YEAR		b. FUNL. (In thousands)	
b. NUMBER: ⁸				74		2.0	
c. TYPE: Not Applicable				75		1.0	
d. KIND OF AWARD:				e. AMOUNT:		28.4	
f. CUM. AMT.						20.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ⁹ Letterman Army Institute of Research				NAME: ⁹ Letterman Army Institute of Research			
ADDRESS: ⁹ Presidio of San Francisco, CA 94129				ADDRESS: ⁹ Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ¹⁰ Kovatch, R.M., LTC, VC			
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-4714			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Ward, G., CPT, VC			
				NAME: Ford, G.H., CPT, VC			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ¹¹							
(U) Panosteitis; (U) Dogs; (U) German Shepherd Dogs; (U) Military Working Dog							
23. TECHNICAL OBJECTIVE, ¹² 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>3. (U) The pathogenesis and etiology of panosteitis has not been described. It is reported most frequently in the German Shepherd Dog, the breed used as a military working dog and currently being selectively bred to better fulfill divergent military requirements. Accurate definition of the etiology and pathogenesis may offer an opportunity to eliminate poor genetic stocks or improve methods of treatment and develop control measures.</p> <p>24. (U) Dogs with clinical and radiographic features consistent with panosteitis will be identified for additional study. Radiographic and hematologic findings and clinical chemistries will be monitored bimonthly. Biopsy specimens from involved bones will be examined for infectious agents and biopsy material transferred will be inoculated into other dogs. Siblings, with at least one of the partners' having had panosteitis, will be mated to evaluate genetic factors.</p> <p>25. (U) 73 07-74 06 Two cases of canine panosteitis developed during the report period. The lesions were characterized radiographically and by bone biopsy. In a third case progressive development of skeletal lesions was documented during routine radiographic monitoring; no clinical signs were evident, indicating the existence of a subclinical phase of this disease, previously unsuspected. Results of hematologic and biochemical analyses were noncontributory as were transmission and isolation studies. Electron-microscopy, attempts to isolate viral agents, and increased surveillance of susceptible dogs will be undertaken upon anticipated expansion of laboratory facilities.</p>							

* Available to contractors upon originator's approval

DD FORM 1498
1 MAR 66

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

ABSTRACT

PROJECT NO. 3A162110A830

WORK UNIT NO. 066

Study of the Etiology, Biological Parameters, Pathogenesis and Control of Panosteitis of Dogs

Panosteitis is a syndrome characterized clinically by debilitating, transient and recurrent lameness in young dogs. It is usually accompanied by fever, occasionally by eosinophilia. Radiographically, fully-developed lesions appear as densities in the marrow canal of long bones. The German Shepherd is the breed most affected and the disease occurs in dogs produced in the Army's Biological Sensor Research program. While the strong breed predilection has suggested a heritable cause for the condition, its cause is unknown. Elucidation of the cause may afford the opportunity for control of the condition.

Seven dogs were obtained from the Biosensor Research program, including two which had previously had attacks of the disease, and two of their siblings. Brother-sister matings were performed and all dogs were monitored clinically and radiographically. One had a recurrence; an attempt to transmit the condition with biopsy material from bone lesions was unsuccessful. Another dog had radiographic evidence of the disease in the absence of clinical signs. This is the first recognized case discovered with a subclinical phase.

BODY OF REPORT

WORK UNIT NO. 066

Study of the Etiology, Biological Parameters, Pathogenesis and Control of Panosteitis of Dogs

PROBLEM:

Panosteitis is a syndrome most frequently reported in the German Shepherd breed of dogs under 18 months of age, with a range age of two months to five years at time of initial diagnosis. It is characterized clinically as an acute-onset lameness followed by apparent recovery with recurrences in the same limb or involvement of the other limbs. The duration of the initial acute syndrome is 10-11 days but the entire clinical course may last 3 to 9 months. Lesions usually appear as radiographic densities in the marrow cavity near the nutrient foramen of long bones and spread rapidly in most cases to occupy the entire diaphyseal bone marrow. The cortex may be feathered on the periosteal surface. Histologically the lesion is described as an endosteal and periosteal reactive bone proliferation with or without local eosinophilia. Serum biochemical alterations and peripheral eosinophilia have been reported by some and discounted by other investigators. A variety of causes have been implicated including genetic and nutritional factors, bacterial and viral agents, and hormonal abnormalities. A definitive etiology has not been established.

The German Shepherd is used as a military working dog and is currently being selectively bred to fulfill divergent military requirements. The disease occurs among dogs bred by the Army. Elucidation of the etiology and pathogenesis of this syndrome may offer an opportunity to eliminate poor genetic stock, improve methods of treatment and develop control or preventive measures.

RESULTS AND DISCUSSION OF THE RESULTS:

Seven dogs were acquired from the Division of Biological Sensor Research (WRAIR), Aberdeen Proving Ground, Maryland. The following dogs were obtained during the period 17 August through 24 October: 2800, 2801, 2802, 2808, 2809, 2837, and 2848. Dogs 2800, 2837 and 2848 had had clinical and radiographic evidence of panosteitis prior to arrival, although all were symptom-free when received. Dogs 2801 and 2802 were female siblings of 2800 and were obtained to initiate an inbreeding program. Dogs 2808 and 2809 were included because they are offspring of 2800 by a sibling mating. The long bones of all dogs were radiographed twice monthly. On the same dates venous blood was collected for complete blood counts. Serum was obtained for the following serum chemistries: calcium, inorganic phosphorus, total protein, albumin, alkaline phosphatase, glucose, blood urea nitrogen, uric acid, cholesterol, total bilirubin, lactic dehydrogenase and glutamic-oxalacetic transaminase. Serum was frozen for possible future use.

Study of Etiology, Biological Parameters, Pathogenesis and Control of Panosteitis in Dogs (Cont)

During the period, two dogs experienced syndromes consistent with panosteitis. Canine male 2848 has recurrence of lesions that were first diagnosed at Aberdeen, Maryland, in early September 73, when he was nine months of age. During the subsequent months of November and December severe lameness developed in all limbs. Concomitant with severe pain, radiographic densities appeared in the marrow cavity of the diaphysis of long bones. Sequential biopsy specimens of the cortex and marrow were obtained to document the histologic lesions, to obtain material for transmission studies and to attempt to isolate an infectious agent.

On routine radiography of 21-month-old female canine 2801 a small endosteal density appeared, followed in two weeks by complete diaphyseal marrow involvement. These radiographic changes were consistent with those reported in the literature and with those observed in 2848. Acute clinical lameness was not observed in periods immediately prior, during or subsequent to radiographic diagnosis. Study of the history indicated lameness had been reported for a 5-day period two months prior to radiographic diagnosis. Study of

this case indicated severe radiographic changes can occur without identifiable clinical signs. It also points out the value of periodic radiography as an important adjunct to identify clinically silent cases. Macerated marrow was removed from 2848 and passed directly to the marrow cavity of a male juvenile German Shepherd. Transmission of the disease was unsuccessful. Additional bone and marrow has been frozen (-17°C) for future virus isolation attempts and other specimens were fixed in 2% glutaraldehyde for electron microscopic study when new facilities in Phase I LAIR permit.

CONCLUSIONS:

- (1) When the lesions of panosteitis are diagnostic by current methods (radiographic and histologic) the disease is of chronic duration and its cause not really identifiable. Attempts to isolate a causative agent may be successful only during earlier stages of the disease.
- (2) Serum concentration of enzymes, electrolytes and other metabolites does not aid in the diagnosis of panosteitis.
- (3) Peripheral eosinophil counts in affected and non-affected German Shepherd dogs in this study were variable and occasionally extremely high. They could not be correlated with the essential criteria for the diagnosis of the disease.
- (4) Routine radiographs can identify clinically silent cases.

RECOMMENDATIONS:

- (1) With the availability of increased space in Phase I, additional affected animals should be added to the study to better elucidate

**Study of Etiology, Biological Parameters, Pathogenesis and Control of
Panosteitis in Dogs (Cont)**

parameters currently under study.

(2) Continue periodic collections of serum for subsequent study but discontinue routine serum biochemical analysis.

(3) Expand the effort to identify pre-radiographically positive cases. The labeling of osteoblasts with radioactive technitium followed by bone scan may afford the opportunity to identify initial stages of the disease. In FY 75 the present group of dogs will be observed closely by a variety of techniques, in an increased effort to identify and study early stages of the disease. Attempts will be made to precipitate recrudescence of the disease by exercise and other stress. Matings between formerly-affected siblings will be performed in an attempt to obtain offspring with greater predilection for the disease.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(A)636		
3. DATE PREV SUMMARY		4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8. DIS'N INST'N ⁶	9. SPECIFIC DATA- CONTRACTOR ACCESS ⁷	10. LEVEL OF SUB A. WORK UNIT
73 07 01		H Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ⁸	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62758A	3A762758A827		00	070			
b. CONTRACTOR	62110A	3A062110A827		00				
c. CONTRACTOR	CARDS 114 (f)							
11. TITLE (Precede with Security Classification Code) ⁹ (U) High Altitude Bioenergetics - The Physiological Consequences of Altitude Exposure Upon the Soldier (06)								
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ¹⁰ 016200 Stress Physiology; 005900 Environmental Biology; 012900 Physiology								
13. START DATE			14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07			Terminated		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS		20. FUNDS (In thousands)
a. DATES/EFFECTIVE:				PRECEDING				
b. NUMBER ¹¹ Not Applicable				FISCAL		74		0.8
c. TYPE				YEAR		0		0
d. KIND OF AWARD:				AMOUNT:		0		0
e. CUM. AMT.								
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION				
NAME ¹² Letterman Army Inst of Rsch				NAME ¹³ Letterman Army Inst of Rsch				
ADDRESS ¹⁴ Presidio of San Francisco California 94129				ADDRESS ¹⁵ Bioenergetics Division Department of Nutrition Presidio of San Francisco, California 94129				
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)				
NAME: Canham, J. E., COL, MC				NAME ¹⁶ Consolazio, C. F.				
TELEPHONE: 415 561 3600				TELEPHONE: 415 561 5092				
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]				
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS				
				NAME: Johnson, H. L.				
				NAME: Krzywicki, H. J. DA				
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Hypoxia; (U) Stress; (U) Military Performance; (U) Balance Metabolic; (U) Respiratory Function								
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)								
23. (U) Soldiers, when abruptly translocated from sea level to altitudes of 10,000 to 18,000 ft., experience various degrees of acute mountain sickness symptoms, and of decreasing physical performance capabilities depending upon the altitude, ascent rate, the man's physical condition and activity. Studies were designed to (a) identify and quantitate these effects, and (b) to prevent or alleviate the effects through dietary alterations, drugs, physical training and activity, and changing other environmental factors.								
24. (U) Sea level soldiers were studied prior to altitude exposure, at various times after abrupt translocation to 4300 meters and again after return to sea level while manipulating their dietary intakes, physical activity and/or physical condition. Parameters monitored during all phases of the study included: a) pulmonary, cardiovascular and metabolic changes during rest, treadmill work of varying intensities and recovery; b) nutrient intakes and balances; c) clinical symptomology, and d) changes in body fluids and body composition.								
25. (U) 73 07-74 06 Two studies were completed to evaluate the effects of diet on pulmonary diffusing capacity (D _{LCO}). The effects of a glucose meal (410 kcal) on pulmonary function were observed in 9 healthy males at 1600 m and 4300 m. A significant increase of 7.7% over fasting diffusing capacity was noted at both altitudes following glucose ingestion, along with a significant decrease in serum triglyceride levels. The increased diffusing capacity values suggest an added advantage of a glucose meal for individuals moving to high altitude. A high fat meal was given to 8 young men with repeated measurements at 1600 m and 4300 m. Although the data is still being processed, it appears that the high fat meal decreases D _{LCO} . Further data evaluation is necessary.								

* Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A062110A827 Military Environmental Medicine
TASK NO. 05 Environmental Medicine
WORK UNIT NO. 070 High Altitude Bioenergetics - The
Physiological Consequences of
Altitude Exposure Upon the Soldier

The following investigations have been conducted under this work unit and Work Unit 168, "The Effects of Diet Upon Respiratory Metabolism."

STUDY NO. 1 The Effects of a Glucose Meal

STUDY NO. 2 The Effects of a High Fat Meal

Study No. 1. The effects of a glucose meal on pulmonary function were observed in 7 healthy males at medium (1,600 m) and high (4,300 m) altitude. Following the ingestion of 410 kcal of glucose, peak blood glucose values ($P < .05$) were noted 1/2 hour after glucose ingestion with a subsequent decrease to a level below fasting at both elevations. At the same time, triglyceride levels significantly declined ($P < .05$) from 104.2 to 83.3 mgm at 1,600 m and 103.7 to 80.5 mgm/100 ml at 4,300 m. Both expired minute volume (\dot{V}_E) and tidal volume (V_T) increased in response to translocation to altitude, while V_T increased by 10.9% and 13.3% at 1/2 hour for 1,600 m and 4,300 m, respectively. The oxygen uptake per minute (\dot{V}_{O_2}) increased ($P < .05$) during glucose elevation, while partial pressure of alveolar oxygen ($P_{A_{O_2}}$) remained essentially unchanged except for differences associated with translocation to altitude. A 13.9% increase was noted in pulmonary diffusion of carbon monoxide ($D_{L_{CO}}$) following glucose ingestion at 4,300 m along with a decreased ($P < .05$) triglyceride level. The increased $D_{L_{CO}}$ values suggest an advantage of a glucose meal for individuals transported to high altitude.

Study No. 2. A liquid high fat meal (70% corn oil) was given to 8 healthy male subjects followed by a series of respiratory function measurements including pulmonary diffusing capacity, membrane diffusion (D_m) and mean capillary volume (\bar{V}_C) at medium (1,600 m) and high (4,300 m) altitude. The processing of data is continuing, however it appears that even with a one-time high fat meal $D_{L_{CO}}$ values are decreased. Further data evaluation is necessary and follow up investigations are suggested.

BODY OF REPORT

WORK UNIT NO. 070

High Altitude Bioenergetics - The
Physiological Consequences of
Altitude Exposure Upon the Soldier

STUDY NO. 1

The Effects of a Glucose Meal

PROBLEM:

Tolerance to physiological stress is vital to the long term physical endurance of troops. Nutritional aspects may favorably influence the aerobic capacity of an individual and alter pulmonary function parameters to augment tolerance to hypoxic stress. With translocation to altitude, pulmonary diffusion capacity (D_{LCO}) and arterial oxygen pressure (P_{aO_2}) are known to decrease thereby limiting the individual's endurance capacity. During exposure to simulated high altitude, subjects given glucose were reported to have altered ventilation and increased alveolar oxygen pressures. The consequence of this change was a raised P_{aO_2} and a higher oxyhemoglobin saturation (S_{aO_2}).

Unfavorable pulmonary responses have been noted as evidenced by decreased D_{LCO} and P_{aO_2} , following the elevation of serum triglycerides associated with chylomicra. The mechanism for these decreases, although unresolved, is thought to be due to a barrier affecting O_2 diffusion and pulmonary blood shunting attributable to the high serum triglyceride levels. From these indications, glucose may be beneficial in facilitating pulmonary function particularly for gaseous exchange including D_{LCO} following acute translocation to altitude.

RESULTS AND DISCUSSION OF RESULTS:

Seven healthy males acclimated to 1,600 m were studied at 1,600 m and 4,300 m. Measurements were made in the fasting state and after a glucose meal (410 kcal).

No significant alterations in glucose values were attributable to elevation, however fasting and post meal values were usually lower but not significant at 4,300 m. In contrast, triglyceride levels generally declined throughout the 3-hour test period at both elevations. Although there was a significant altitude effect on P_{aO_2} , there was no significant difference across time at either location with a glucose meal.

There was no significant change in D_{LCO} across time with elevated glucose levels at 1,600 m. However, the D_{LCO} values were maintained above fasting measurements during the elevated blood glucose phase at 4,300 m and were not significantly different than the 1/2, 1 and 2 hour values at 1,600 m.

High Altitude Bioenergetics - The Physiological Consequences of Altitude Exposure Upon the Soldier (Cont)

Favorable ventilatory responses were seen at altitude along with increased D_{LCO} values ($P < .05$) following glucose ingestion. However, D_{LCO} appears to function independent of triglycerides under these circumstances since triglycerides continued to decrease linearly following glucose ingestion. Fasting D_{LCO} values decreased with acute altitude exposure. However, D_{LCO} increased across time during periods of elevated serum glucose measurements and returned to fasting values by the third hour. Similar responses in D_{LCO} were not observed at 1,600 m altitude since D_{LCO} remained constant across time. Although the oxygen uptakes were significantly raised (9.7%) indicating an increase in energy expenditure at 1,600 m and 4300 m, D_{LCO} did not significantly change at 1600 m, but the 17.4% increase of serum glucose at 4,300 m was accompanied by a 15.8% increase in D_{LCO} at 30 minutes post glucose ingestion.

CONCLUSIONS:

The results of the present study indicate a beneficial effect of a glucose meal at 4,300 m altitude. With acute translocation to altitude D_{LCO} was improved following glucose ingestion. These results suggest an increased carbohydrate requirement at altitude to increase pulmonary diffusion to pre-altitude levels.

RECOMMENDATIONS:

From the results obtained in this study further investigations of macro nutrient distribution in the diet is recommended, i.e., the effects of a protein meal and using glucose loading prior to acute translocation to altitude. This may provide extended endurance to hypoxic stress not accomplished from a single meal of 410 kcal (glucose).

PUBLICATIONS:

None.

STUDY NO. 2

The Effects of a High Fat Meal

PROBLEM:

Elevated serum triglyceride levels may decrease pulmonary diffusion capacity (D_{LCO}) similar to IV lipid infusion by modifying the rate of gas exchange from the alveolar to hemoglobin (θ). Transient decreases in D_{LCO} including P_{aO_2} and S_{aO_2} have been observed in recent studies following fat ingestion and is related to the increased chylomicra in the blood. Alveolar membrane diffusion (D_m) has not been observed under these conditions. Alterations in capillary membrane and alveolar tissue resulting from lipid

High Altitude Bioenergetics - The Physiological Consequences of Altitude Exposure Upon the Soldier (Cont)

aggregation may contribute to the reduction in D_{LCO} as well as changes in θ . D_{LCO} is a linear function of D_m and the capillary blood volume (V_c) θ , a relationship easily measured by basic D_{LCO} techniques. With reduced D_{LCO} values, aerobic capacity may be lowered and seriously limit the military man's physical endurance with acute translocation to altitude and low ambient O_2 .

RESULTS AND DISCUSSION OF RESULTS:

The subjects were 8 males from 19 to 25 years of age, who were acclimated for at least 6 months at 1,600 m altitude. After an overnight fast, blood samples and pulmonary measurements were acquired prior to and following the oral ingestion of a high fat meal.

The data at present is being processed for statistical analysis. However, it appears from the preliminary results that a high fat meal does in fact result in a decreased D_{LCO} at both medium and high altitudes.

CONCLUSIONS:

A single high fat meal appears to increase serum triglyceride levels sufficiently to reduce pulmonary diffusing capacity.

RECOMMENDATIONS:

Complete computer and statistical analyses of the data and publish the results.

PUBLICATIONS:

Dramise, J. G., C. M. Inouye, B. M. Christensen, R. D. Fults, J. E. Canham, and C. F. Consolazio. The effects of high carbohydrate diet on human pulmonary function at 1,600 and 4,300 meter altitudes. Submitted to Publications Review Committee.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OA 6350	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRATING ^a	8a. OMB INSTR ^a	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF R&D
73 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	WORK UNIT
10. NO./CODES: ^a		PROGRAM ELEMENT		PROJECT NUMBER		WORK UNIT NUMBER	
A. PRIMARY		62758A		3A762758A827		00 973	
B. BOOKHOLD		62110A		3A162110A827		00	
C. BOOKHOLD		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ^a (U) Physiological, Metabolic and Psychological Aspects of High Altitude Exposure (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ^a 013400 Psychology; 012600 Pharmacology; 012900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07		74 06		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				FISCAL YEAR		B. FUNDING (in thousands)	
B. NUMBER: ^a				74		3.0	
C. TYPE: Not Applicable				75		0	
D. KIND OF AWARD:				20. PERFORMING ORGANIZATION			
18. RESPONSIBLE DOD ORGANIZATION				NAME: ^a Letterman Army Institute of Research			
NAME: ^a Letterman Army Institute of Research				Dept of Comparative Medicine			
ADDRESS: ^a Presidio of San Francisco, CA 94129				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Hannon, J.P.			
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-4714			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Sullivan, F.J.			
				NAME: Sterner, R.T., CPT, MSC DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Environmental Stress; (U) Physiological Adaptation; (U) High Altitude; (U) Human Factors; (U) Biochemistry; (U) Military Stress							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Acute mountain sickness (AMS) can be a severe and incapacitating illness. At 4300 m. altitude, 30-40% of a group of soldiers will experience severe symptomatology and, hence, will not be able to function as combatants. This work unit was designed to study various aspects and correlates of symptomatology in humans at high altitude. Efforts were directed toward: a) obtaining quantitative estimates of symptom severity, b) establishing relationships between symptomatology and physiological and biochemical alterations caused by altitude exposure, c) investigating prophylactic and therapeutic measures to prevent or ameliorate AMS, d) elucidating criteria for prediction of individual susceptibility to AMS.</p> <p>24. (U) Human volunteers were subjected to actual and simulated high altitude environments for periods of short (days) and long (weeks) duration. Various physiological, biochemical and psychological measures were applied to describe the alterations - defects and adaptation - caused by exposure. Valid estimates of symptom severity were developed through subject self-rating, by paired comparison with previous illnesses and physician's interviews. Information gained should provide a better physiological and biochemical rationale to elucidate the mechanism(s) for AMS.</p> <p>25. (U) Two to four day staging at 1600 m. and 3400 m. alleviated certain AMS symptoms commonly associated with rapid exposure to 4300 m. The transitory changes and the temporal relationships of hemodynamic and acid-base variables were accessed during exercise at 1600 m. and over a 2-week period at 4300 m. High altitude acclimatization of pulmonary, cardiovascular and acid-base functions requires about 2, 4 and 10 weeks, respectively at 4300 m. This work unit is being terminated.</p>							

^a Available to Contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A062110A827 Military Environmental
Medicine

WORK UNIT NO. 073 Physiological, Metabolic and
Psychological Aspects of
High Altitude Exposure

The following studies have been conducted under this work unit during the past year:

STUDY NO. 5 Acute mountain sickness
symptomatology subscale

STUDY NO. 9 The interrelationships of
cardiopulmonary function and
performance during prolonged
altitude exposure of humans

Effects of two and four day staging at elevations of 1600 and 3400m on subsequent severity of Acute Mountain Sickness (AMS) at 4300m were studied in a collaborative effort by personnel from Letterman Army Institute of Research and the U.S. Army Institute of Environmental Medicine. Fifty-two subjects were studied. Four day staging at both 1600 and 3400m substantially alleviates symptoms of AMS commonly induced by rapid exposure of sea level subjects to 4300m. Studies of graded exercise in six Denver men exposed to 4300m for two weeks showed early stages of exposure were associated with transitory increases in cardiac output, heart rate and stroke volume. Later, these variables returned to, or below, values observed at low altitude. Temporal changes in blood gases and acid-base regulation in these subjects were studied at rest and during exercise. Analysis of data from 8 female subjects exposed for 78 days to 4300m showed a marked and sustained reduction in urinary acid and ammonia excretion. Flood acid-base chemistry was nearly, but not fully, returned to low-altitude characteristics at the end of 78 days. In contrast, pulmonary function and cardiovascular function appeared to acclimatize after two and four weeks, respectively. This work unit is being terminated.

BODY OF REPORT

WORK UNIT NO. 073 Physiological, Metabolic and Psychological Aspects of High Altitude Exposure

Due to the transfer of function from U.S. Army Medical Research and Nutrition Laboratory in Denver to Letterman Army Institute of Research, Presidio of San Francisco, and the consequent dissolution of the Physiology Division and loss of the Environmental Medicine mission this work unit is being terminated. During the past year the following work was accomplished.

STUDY NO. 5 Acute Mountain Sickness Symptomatology Scale

PROBLEM:

Development of a reliable and valid questionnaire for assessing subjective aspects of Acute Mountain Sickness (AMS) has been a primary concern of this work unit since 1965. To date, it has been shown that a 4-subscale breakdown (i.e., Arousal Level, Somatic Discomfort, Tired, and Mood) of Evan's General High Altitude Questionnaire (GHAQ) affords improved measurement characteristics of high-altitude induced symptomatology. Additionally, the potential for these subscales to reflect diurnal- and/or drug-induced alterations in AMS symptomatology has been shown. Continued study has centered upon: (a) collection of further data in a study which dealt with certain cardiovascular variables as they relate to maximum aerobic capacity (see Study No. 9), and (b) revision, expansion, and administration of the GHAQ in collaboration with USARIEM staff in a 1973 study entitled "The Effects of Staging on the Acute Adaptation of High Terrestrial Elevations."

RESULTS AND DISCUSSION OF THE RESULTS:

Fifty-two Army enlisted men were administered a 30-symptom, self-report questionnaire during a control period at San Antonio, Texas (200m) and subsequently during a four day experimental period on Pikes Peak Colorado (4300m). Four groups were studied: PP, direct ascent to Pikes Peak; CC2 and CC4, two and four days at Cripple Creek, Colorado, (3400m), respectively prior to ascent to PP; and D4, four days at Denver, Colorado (1600m) prior to PP. Factor analysis of the symptom data showed that select symptoms could be combined to form four subscales: headache, fatigue, cardiorespiratory and arousal. Subscale scores for headache and fatigue for groups PP and CC2, cardiorespiratory for CC2 and D4, and arousal for all groups increased significantly between 200m and 4300m. No other group by day differences were significant. Results indicate that delaying ascent at 1600m or 3400m elevation for four days substantially alleviates the symptoms of AMS commonly induced by rapid exposure to 4300m.

Physiological, Metabolic and Psychological Aspects of High Altitude Exposure (Cont)

CONCLUSIONS AND RECOMMENDATIONS:

The GHAQ continues to be an accurate tool for evaluating the symptoms of AMS. The present revised version is particularly useful in detecting subtle within and between subject differences. Staging for a period of four days or more at medium altitudes of 1600m or more is highly recommended as a means for alleviating AMS at higher elevations.

STUDY NO. 9

The Interrelationships of
Cardiopulmonary Function and
Performance During Prolonged
Exposure in Humans

PROBLEM:

EXERCISE:

Two types of steady-state, submaximal work have been extensively studied at high altitude. In one, workloads were arbitrarily selected with all subjects performing at the same absolute levels, e.g. 400 and 700 kpm., at both low and high altitudes. In the other, workloads were based upon preselected fractions, e.g. 25 and 50 percent of maximum oxygen consumption, the latter being determined in preliminary experiments conducted at low altitude. Neither type takes account of the fact that maximum working capacity is reduced at high altitude. Thus, in transition from low to high altitude, a given submaximal workload increases, relative to maximum working capacity. To evaluate this problem in experimental design, cardiopulmonary function was studied in a group of soldiers in which submaximal workloads were adjusted to preselected fractions of each subject's maximum work capacity, determined at both low and high altitude.

RESULTS AND DISCUSSION OF RESULTS:

Six young men, residents of Denver (1600m) were studied at that altitude and, over a 14-day period, on Pikes Peak (4300m). Maximum oxygen consumption was decreased by approximately 12 percent on day one of altitude exposure and remained at this level on days 6 and 13. On days 2, 7 and 14 hemodynamics and blood acid-base chemistry were measured at rest (sitting) and during bicycle ergometry for 10 minutes at 30 percent maximum oxygen consumption, followed by 10 minutes at 60 percent maximum and finally 100 percent maximum until exhaustion. These percentage values were based on maximum oxygen consumption values determined on the day immediately preceding submaximal measurements, i.e. in Denver and after 1, 6 and 13 days on Pikes Peak. On day two, resting and 30 percent values for cardiac output, stroke volume and heart rate were elevated,

Physiological, Metabolic and Psychological Aspects of High Altitude Exposure (Cont)

relative to Denver values; arterial pressure remained constant. At 60 percent of maximum oxygen consumption Pikes Peak values for all of these variables were equivalent to those observed in Denver. As the sojourn at high altitude was extended to two weeks, cardiac output and heart rate, particularly at maximum oxygen consumption, declined progressively. At 14 days, cardiac output and stroke volume were below control values at all work loads, where as heart rate remained slightly above Denver values during exercise at 30 and 60 percent of maximum. Arterial and mixed venous pH both declined similarly during exercise; no differences were observed from day to day. Resting arterial pH was elevated on the second day of altitude exposure and remained high during the 14 day period of exposure. Arterial and mixed venous P_{CO_2} and arterial P_{O_2} were less at high altitude; mixed venous P_{O_2} was similar to Denver values.

CONCLUSIONS:

Very consistent and equivalent decrements in mixed venous pH at both elevations and at all levels of exercise may be related to diminished performance noted at high altitude. This interpretation would be consistent with the decrease in blood buffering capacity noted in earlier high altitude studies.

PROBLEM:

ACID-BASE REGULATION

Acute effects of high altitude exposure on acid-base regulation have been extensively investigated and as a consequence are known in great detail. The same is true insofar as acid-base characteristics of the high altitude native are concerned. Briefly, the acute sojourner exhibits respiratory alkalosis, a decreased arterial P_{CO_2} , bicarbonate concentration, and hence buffering capacity. He partially compensates for these changes by reducing acid and ammonia excretion. The resident, on the other hand, does not exhibit respiratory alkalosis, even though his blood P_{CO_2} and bicarbonate values are low by sea level standards. Furthermore, he exhibits acid-base excretion characteristics similar to those observed in sea level residents. Up to the present time studies of high altitude sojourners have been too short in duration to delineate the transition of acid-base regulation from the partial compensation seen in the sojourner to full compensation seen in the high altitude resident. A phase of study No. 9 was directed to this question.

RESULTS AND DISCUSSION OF THE RESULTS:

Eight University of Oregon women were studied at low altitude (140m) in Oregon, and at the summit of Pikes Peak (4300m) over a 78 day period of exposure. Arterial P_{CO_2} oxygen saturation, P_{CO_2} , plasma hydrogen ion

Physiological, Metabolic and Psychological Aspects of High Altitude Exposure (Cont)

concentration all decreased upon exposure to 4300m. Arterial base deficit and lactate increased at altitude and then recovered slightly as exposure was extended to 78 days. The 24-hour urine volume, ammonia and potassium excretion all decreased early in the altitude sojourn while urinary hydrogen excretion and sodium to potassium ratio increased. The 24-hour excretion values for sodium, chloride, titratable acidity and phosphate declined transiently during the first week or two of exposure then gradually recovered to values similar to those observed earlier in Oregon. At the end of the altitude sojourn arterial P_{CO_2} , oxygen saturation, P_{CO_2} , plasma hydrogen ion and the bicarbonate concentration remained significantly below low altitude values, as did the 24 excretion values for urine volume, ammonia and potassium.

CONCLUSIONS.

An exposure interval of 78 days is not sufficient to acclimatize acid-base regulation to the same extent seen in the high altitude native. Acid-base acclimatization, therefore, is far slower than cardiovascular and pulmonary acclimatization in which major adjustments are seen within two to four weeks.

PUBLICATIONS:

1. S.M. Robinson, W.O. Evans, R.T. Sterner and D.A. Stamper. Effects of intermediate staging upon acute mountain sickness. Federation Proc. 33:307, 1974 (Abstract).
2. F.J. Sullivan, E.A. Morz, Jr., R.E. Miller and P.C. Weiser. Graded exercise at altitude Federation Proc. 33:307, 1974 (Abstract).
3. D. Sudman and J.P. Hannon. Acid-base regulation during altitude acclimatization of women. Federation Proc. 33:307, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OR 6790	74 07 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8A. DISSEM INSTR ⁶	8B. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
73 07 01	D. CHANGE	U	U	NA	NL		
9. NO./CODES ⁷		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62759A		3A762759A831		00	
b. SUBFUNCTIONAL		62110A		3A062110A831		00	
c. SUBFUNCTIONAL		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ⁸ (U) Delayed Type Skin Reaction and Lymphocyte Transformation in Cutaneous Diseases (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ⁹ 003500 - Clinical Medicine, 010100 - Immunology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
67 04		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE: Not applicable				PREVIOUS			
b. NUMBER: ¹⁰				FISCAL YEAR		b. FUNDS (in thousands)	
c. TYPE:				74		1	
d. KIND OF AWARD:				CURRENT		141.4	
e. AMOUNT:				75		2.5	
f. CUM. AMT.						80.5	
20. RESPONSIBLE JOG ORGANIZATION				21. PERFORMING ORGANIZATION			
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ADDRESS: ¹³				ADDRESS: ¹⁴			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ¹⁵ Greenberg, J.H., MAJ, MC			
TELEPHONE: 415:561-3600				TELEPHONE: 415:561-3006			
22. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Sharon Kerbs, Ph.D.			
				NAME: Joyce Inouye, B.S. DA			
23. KEYWORDS (Precede with Security Classification Code) ¹⁶ (U) Intra dermal skin test; (U) lymphocyte transformation (LT); (U) macrophage inhibition factor (MIF); (U) delayed hypersensitivity; (U) leukocytes							
23. TECHNICAL OBJECTIVE, ¹⁷ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To develop <u>in vitro</u> immunologic correlates to the cutaneous fungal infections in humans and guinea pigs. To develop a vaccine against cutaneous fungal infections in soldiers.							
24. (U) Human leukocytes from blood will be isolated and tested against LAIR trichophyton antigen to test for lymphocyte transformation. Guinea pig leukocytes from blood and lymph nodes will be reacted against LAIR trichophyton antigen to test for lymphocyte transformation and macrophage inhibition factor production. This will be done both prior to and during the course of infection. Macrophages from guinea pig peritoneum will be used as an index of how much macrophage inhibition factor is produced.							
25. (U) 73 07 - 74 06 A study has been completed which shows that the lymphocyte transformation test in humans who have been experimentally infected with <u>T. mentagrophytes</u> converts from nonreactive to reactive, while skin tests turn positive during the course of an infection. All guinea pigs infected with <u>T. mentagrophytes</u> develop a positive delayed intradermal skin test. The skin test reaction varies as to size but thus far no correlation can be made between skin test size and immunity to second infections. Macrophages have been isolated from guinea pig peritoneum after interperitoneal instillation of sterile mineral oil. Using these macrophages, we have worked on an agar drop method for measuring MIF development.							

*Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A762759A831

TASK NO. 00

WORK UNIT NO. 001

Delayed Type Skin Reaction and
Lymphocyte Transformation in
Cutaneous Diseases

Twenty-nine new Army recruits were studied for cell mediated immunity to Trichophyton mentagrophytes as measured by lymphocyte transformation. It was shown that only 21% had previous experience with the fungal antigen. This indicated that a vaccine would be useful to the Army.

Skin test results to trichophytin antigen were compared with lymphocyte transformation (LT) results in 22 individuals. The lymphocyte transformation test appears to be a better test for indicating an individual's previous experience to trichophytin antigen than the skin test.

Eight subjects were experimentally infected with Trichophyton mentagrophytes. These subjects had negative LT tests before the infection, and 6 had positive LT tests 44 days post infection, while two remained LT negative and skin test negative.

BODY OF REPORT

WORK UNIT NO. 001

Lymphocyte Transformation

PROBLEM:

Cutaneous fungal infections are important military problems, especially for troops stationed in the tropics. The immunological response of the host to cutaneous fungal infections is poorly understood, and this information would be helpful in evaluating and preparing vaccines.

RESULTS AND DISCUSSION OF RESULTS:

A pilot survey was made of newly enlisted men at LAIR to test their previous experience with T. mentagrophytes. If most individuals were experienced, as was shown to be the case with men at Vacaville Prison, then a vaccine would probably not be helpful to the majority of soldiers. One parameter of their immunological response to T. mentagrophytes infection was tested, namely, the ability of their lymphocytes to transform into large blast-like cells when cultured in vitro in the presence of trichophytin antigen, indicating previous experience with this antigen. Of the 29 men tested, 21% had a lymphocyte transformation ratio of three or greater which probably indicates previous experience with T. mentagrophytes (TABLE 1). This contrasts to men at Vacaville where 93% of the men were found to be experienced individuals. This pilot study indicated that a vaccine preparation would be useful to the Army since a large number of new recruits have not had previous experience as measured by this immunological test.

Since it is not always desirable to monitor the host delayed hypersensitivity response by skin testing, we attempted to show a correlation between skin test results and lymphocyte transformation results. Twenty-two individuals were skin tested on their forearms with 0.1, 1.0, or 10ug of trichophyton antigen, Lot #CM109. Their arms were examined after 20 minutes, 48 hours, 72 hours, and 2 weeks. Before skin testing, blood was withdrawn for lymphocyte transformation (LT) testing.

The 16 individuals with LT ratios of less than 3 were delayed skin test negative except for one individual who may represent either a false positive skin test or a false negative LT test. Three individuals in this group showed a positive skin test after two weeks, possibly due to a booster effect by the skin test antigen injection.

Of the six individuals with LT ratios greater than 3 (indicating past experience with this antigen) 4 individuals had positive delayed skin tests also indicating past experience. Two individuals that did not have a positive delayed skin test response had a positive immediate response and a history of trichophyton infections.

Therefore, while negative results for both assays correlated well except for one individual, the correlation for positive responses was not as high. For this group of individuals, the LT test appears to give a more accurate indication of the subjects past experience with trichophyton antigens since it gave a positive response in two individuals with negative skin tests and a history of infections.

Lymphocytes produce a number of soluble substances upon stimulation with non-specific antigens such as phytohemagglutinin, or with specific antigens to which the donor has had experience. What role these mediators, called lymphokines, have in the infectious process is unclear. We followed test subjects lymphocyte response to trichophyton antigen before, during and after an acute infection. We hypothesized that the beginning of remission of the infection would correlate with the subject changing from a negative to a positive LT response.

Infections were induced with either 6 or 300 spores of T. mentagrophytes applied to two sites of the same forearm of eight human volunteers. Blood was withdrawn and the lymphocytes separated for the LT test. The LT test became positive in one subject 10 days after applying the spores, and in 28-31 days in four other subjects. Three individuals did not have positive LT tests on any of the test days, and two of these were also skin test negative on day 44 and required therapy to clear their infections. The rest of the subjects were skin test positive on day 44, except for the negative controls and for one subject that was dropped from the experiment. Clinical readings of the infections could not clearly define the day of the beginning of remission, and it was not at the same time for all individuals. The LT test became positive at about the same time as the peak of infection in two individuals and about two weeks after the peak in four other individuals. Two subjects did not peak and remained LT negative.

Although most subjects changed from a negative LT test at the start of the experiment to a positive LT test at the end, a clear correlation between the onset of a positive test and the beginning of clinical remission could not be made.

WORK UNIT NO. 001

Lymphocyte Transformation

CONCLUSIONS:

A survey of 29 new enlisted men showed that only 21% had previous experience with T. mentagrophytes as measured by the ability of their lymphocytes to transform in the presence of specific antigen. This test (LT) appeared to reflect the individuals past experience with this fungus more accurately than skin testing. Subjects with negative LT tests before a fungal infection changed to positive LT tests after receiving a fungal infection.

RECOMMENDATIONS:

It should be established if any lymphokine, such as blastogenic factor, MIF, chemotactic factor, or lymphotoxin, correlates with the individual's ability to clear a trichophyten infection, or if any of these factors can be used for evaluating different vaccine preparations.

PUBLICATION: None

TABLE 1

SKIN TEST RESULTS TO 10ug T. MENTAGROPHYTES ANTIGEN LOT CM109

GROUP I: Subjects with lymphocyte transformation ratios less than 3.

<u>Subject</u>	<u>Immediate</u>	<u>48 Hours</u>	<u>72 Hours</u>	<u>2 Weeks</u>
1	-	0	0	0
2	40/10	0	0	0
3	-	0	0	0
4	-	0	0	0
5	-	7/0	7/0	8
6	40/15	0	0	7
7	-	0	0	-
8	-	0	0	7
9	-	0	0	0
10	-	0	0	0
11	40/14	0	0	0
12	0	0	0	0
13	32/10	0	0	0
14	-	0	0	0
15	-	0	0	10
16	30/20	0	0	0

GROUP II: Subjects with lymphocyte transformation ratios over 3.

17	6	24/13	22/12	0
18	45/12	0	0	0
19	-	10/0	4/0	0
20	6	10/0	8/8	10
21	7	15/15	14/12	13
22	20/4	0	0	0

Skin test results are reported as millimeters of erythema over millimeters of induration. Immediate reactions of less than 15 mm are not reported.

Subjects that expressed delayed hypersensitivity responses to 10ug CM109 did not consistently show positive responses to 1ug CM 109. These subjects gave negative responses to 0.1ug CM 109.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(A7)636		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8a. DISB'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		9. LEVEL OF SUM A. WORK UNIT
73 07 01	D. CHANGE	U	U	NA	NL			
10. NO / CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER				
a. PRIMARY	62759A	3A762759A831	00	004				
b. CONTRACTOR	62110A	3A062110A831	00					
c. CONTRACTOR	CARDS 114(F)							
11. TITLE (Precede with Security Classification Code) ^a								
(U) Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections								
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a								
002300 - Biochemistry, 010100 - Microbiology, 003500 - Clinical Medicine								
13. START DATE			14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
73 09			76 09		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS		b. FUNDS (In thousands)
a. DATES/EFFECTIVE: Not applicable				PREVIOUS				
b. NUMBER ^c				FISCAL YEAR		CURRENCY		
c. TYPE				74		3		54.9
d. KIND OF AWARD:				75		3		77.0
e. AMOUNT:								
f. CUM. AMT.								
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION				
NAME ^a Letterman Army Institute of Research ADDRESS: ^a Presidio of San Francisco, CA 94129				NAME ^a Letterman Army Institute of Research Department of Dermatology Research ADDRESS: ^a Microbiology Laboratory Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish SEAR if U.S. Academic Institution)				
RESPONSIBLE INDIVIDUAL				NAME ^a King, R.D., CPT, MSC				
NAME. Canham, J.E., COL. MC				TELEPHONE 415:561-3006				
TELEPHONE. 415:561-3600				SOCIAL SECURITY ACCOUNT NUMBER				
21. GENERAL USE				ASSOCIATE INVESTIGATORS				
Foreign Intelligence Not Considered				NAME: Jaeger, J.R., DA ^c				
				NAME: Khan, H.A., SP5				
22. KEYWORDS (Precede EACH with Security Classification Code)								
(U) skin; (U) pathogenicity; (U) enzymes; (U) vaccine; (U) inhibitors								
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)								
23. (U) 1) To elucidate biochemical mechanisms of pathogenesis in debilitating fungal skin infections in soldiers. 2) To determine the role of exo-enzymes in pathogenesis and host immunological response(s). 3) To develop novel therapeutic and immunological measures in preventing infections.								
24. (U) 1) Correlate exocellular enzyme production to pathogenicity by inoculation of non-enzyme and enzyme producing fungal strains on to susceptible guinea pigs. 2) Develop enzyme and sub-cellular component purification techniques utilizing biochemical procedures. 3) Determine efficacy of various exo-enzyme inhibitors in preventing fungal infections <u>in vivo</u> . 4) Determine effectiveness of exo-enzymes and other sub-cellular fractions as immunizing agents (vaccines).								
25. (U) 73 07 - 74 06 1) Initial results indicate that non-exo-enzyme producing fungal strains are less pathogenic than exo-enzyme producing strains. 2) A defined culture medium has been developed that will support the growth of most of the pathogenic skin fungi. 3) Growth conditions have been determined for optimal production of an extra-cellular proteinase and lipase. 4) These enzymes and a subcellular glycoprotein fraction have been partially purified for immunological (vaccine) studies. 5) Development of delayed hypersensitivity (immunity) reactions, to the exo-enzymes and to the isolated glycoprotein fraction, have been correlated to clearing of experimental animal infections.								

DA

* Available to contractors upon originator's approval

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

ABSTRACT

PROJECT NO. 3A762759A831

WORK UNIT NO. 004 Pathogenesis of Fungal Infections

The following investigations have been conducted under this work unit:

STUDY NO. 1 Biochemical Mechanisms of Pathogenesis in Fungal Skin Infections

Dermatophyte infections are prominent producers of medically-debilitating lesions in soldiers. The predominant etiologic agent for such infections in U.S. Army personnel in the Republic of Viet Nam has been identified as Trichophyton mentagrophytes var. granulares. Pathogenicity and the host's immunological response(s) have been correlated with the ability of this organism to produce exocellular enzymes. A defined medium for growth of the dermatophytes as well as cultural conditions for optimal elicitation of the exocellular enzymes have been developed. Development of enzyme isolation techniques and enzyme inhibition procedures are being pursued. Preliminary data indicate that development of delayed hypersensitivity reactions by the host, to the exocellular enzymes and an isolated subcellular glycoprotein fraction, can be correlated to clearing of experimental infections.

BODY OF REPORT

WORK UNIT 004

Pathogenesis of Fungal Infections

STUDY NO. 1

Biochemical Mechanisms of Pathogenesis in Fungal Skin Infection

PROBLEM:

Dermatophyte infections are prominent producers of medically-debilitating lesions in soldiers. The predominant etiologic agent for such infections in U.S. Army personnel in the Republic of Viet Nam has been identified as Trichophyton mentagrophytes var. granulares. Although there is a voluminous amount of literature written about these fungal infections, there remains a paucity of information regarding the specific mechanisms of pathogenesis.

Among the many possible mechanisms of pathogenesis, the most probably (logically and scientifically) is for the organism(s) to produce exocellular enzymes capable of hydrolyzing substances found in normal intact human skin (i.e. keratin, lipids, etc.). Production of these enzymes would allow the organism to gain necessary nutrients for growth and colonization on the skin.

During the past year, it has been the objective of this work unit to address the question as to what properties of T. mentagrophytes var. granulares enables it to colonize and cause disease on intact human skin. Specific emphasis was placed on determining the role of the exocellular enzymes in pathogenesis and the host's immunological response(s) to these enzymes. These studies could provide information necessary for the development and implementation of novel therapeutic and immunological measures in preventing dermatophyte infections.

RESULTS AND DISCUSSION OF THE RESULTS:

When necessary laboratory Trichophyton strains (e.g. T. rubrum, T. ajelloi, T. asteroides and T. mentagrophytes) were used to infect guinea pigs, marked differences in pathogenicity, as measured by the intensity and duration of the infection, were obtained. The best inflammatory lesions were produced by T. mentagrophytes, and the least by T. ajelloi. Initial enzyme studies revealed that there was quantitatively less proteolytic and lipolytic exocellular enzyme activity produced by the organisms with the least ability to produce disease. For example, T. mentagrophytes produced more exocellular proteolytic and lipolytic activity than T. ajelloi. These preliminary data indicate that the exocellular enzyme production by these organisms might be correlated to pathogenicity as well as the host's immunological response(s).

Pathogenesis of Fungal Infections (Cont)

These initial observations indicated to us the possible importance of the exocellular enzymes in dermatophytic infections and thus we developed a program to study these enzymes. In order to provide for an equal balance of basic as well as applied information with the least expenditure of time, the research work on the exocellular enzymes was divided into the following areas: (1) Development of in vitro cultural procedures for optimal production of these exocellular enzymes and to insure that these procedures are an accurate model of in vivo activities. (2) Identify and isolate the exocellular enzymatic activities in a purified form. (3) Study various agents or methods which might inhibit these enzyme activities and develop these methods for possible use as therapeutic procedures. (4) Determine the involvement of these exocellular enzymes in the host immunological response and determine whether or not these enzymes could be used as sensitizing agents (vaccines). Research work, to date, has been primarily centered in the first two areas; however, some experimentation has been accomplished in all areas.

To develop and isolate exocellular enzyme activities, it was imperative to develop a chemically defined medium since complex media contain various macromolecules which would hamper isolation techniques. In a chemically defined medium, macromolecules obtained after growth of the organism can be attributed to being produced by the organism and not a component of the medium. A defined medium has been developed that will support the growth of most dermatophytes. The cultural and environmental conditions for optimal exocellular enzyme production have been determined. The use of isolated human stratum corneum or delipidated sheep's wool keratin as a sole source of carbon and nitrogen in a defined medium induced T. mentagrophytes to elicit optimal proteolytic and lipolytic activities when grown in a shake culture at 30° C for 10 days. Currently, these media are being used to produce crude enzyme material for use in enzyme isolation techniques. Also, the proteolytic and lipolytic activities are being characterized as to the substrate they will attack.

Implementation of procedures for the isolation of the potent proteolytic exocellular enzyme has been accomplished. Methods for isolation of the lipolytic enzyme(s) are now being actively pursued. Isolation of the enzymes responsible for these two major activities (proteolytic and lipolytic) should be accomplished within the next fiscal year.

Although inhibition studies described previously for research area (3) are in their infancy due to lack of enough isolated enzymic material, some work has been accomplished in the last area. An initial pilot experiment has shown that development of delayed

Pathogenesis of Fungal Infections (Cont)

hypersensitivity (immunity) reactions by the host, to the exocellular enzymes and an isolated subcellular glycoprotein fraction, have been correlated to clearing of experimental infections.

CONCLUSIONS:

From preliminary data it appears that exocellular enzyme production by T. mentagrophytes can be correlated with the pathogenicity of the organism and the host's immunological response(s). Further investigations should confirm this relationship as well as provide possible novel therapeutic procedures and sensitizing agents (vaccines).

RECOMMENDATIONS:

It is recommended that this work be carried to completion in order to accomplish the work unit's objective -- namely, to describe the mechanisms of pathogenesis of cutaneous fungal infections.

PUBLICATIONS:

In preparation.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYM BOL DD-DR&E(AR)636	
				DA OC 6794	74 07 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DESIG INSTR ^a	9. SPECIFIC DATA CONTRACTOR ACCESS <input type="checkbox"/> YES <input type="checkbox"/> NO	10. LEVEL OF 'UM A. WORK UNIT
73 07 01	D CHANGE	U	U	NA	NI		
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62759A	3A762659A831	00	006			
b. SECONDARY	62110A	3A062110A831	00				
c. THIRDARY	CARDS 114(F)						
11. TITLE (Precede with Security Classification Code) ^a (U) Experimental Fungus Infections in the Skin of Man: A Therapeutic Model (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a 010100 - Microbiology, 003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
69 11		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE: EXPIRATION:				PRECEDING			
b. NUMBER ^a Not applicable				FISCAL YEAR		b. FUNDS (in thousands)	
c. TYPE: d. AMOUNT:				74		1.5	
e. KIND OF AWARD: f. CUM. AMT.				75		2.0	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129 ADDRESS ^a				NAME ^a Letterman Army Institute of Research Department of Dermatology Research Microbiology Research Laboratory Presidio of San Francisco, CA 94129 PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution) NAME ^a Greenberg, J.E., MAJ, MC TELEPHONE 415:561-3006 SOCIAL SECURITY ACCOUNT NUMBER			
RESPONSIBLE INDIVIDUAL NAME: Canham, J.E., COL, MC TELEPHONE: 415:561-3600				ASSOCIATE INVESTIGATORS NAME: Field, Russell, SP4 DA			
21. GENERAL USE Foreign Intelligence Not Considered							
22. KEYWORDS (Precede EACH with Security Classification Code) (U) dermatophytosis; (U) experimental infection; (U) skin; (U) human volunteers							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number; precede text of each with Security Classification Code)							
23. (U) 1) To perfect a reproducible and quantitative fungal infection in guinea pigs 2) To develop a vaccine that will prevent or minimize dermatophyte infections. 3) To evaluate prophylactic and therapeutic anti-fungal agents. 4) To completely quantitate the bacterial flora of the skin, and determine its role in fungal infections.							
24. (U) 1) A standard procedure for infecting humans with cutaneous fungal infection has been developed and the experience gained during these infections has been reported. 2) A quantitative infection in guinea pigs that is predictable and will allow evaluation of the therapeutic and immunizing capabilities will be developed. 3) An adaptation of the Lederberg replica-plating method will be developed to identify the resident flora. A linear scraping machine will be used for quantitative sampling.							
25. (U) 73 07 - 74 06 1) All parameters of a quantitative cutaneous guinea pig fungal infection have been evaluated as to predictability. These guinea pig infections seem to exactly mimic the course of infection in a non-experienced human. 2) Experiments have been started to delineate the part delayed hypersensitivity plays in dermatophyte infections. 3) <u>Staphylococcus aureus</u> is not involved in the pathogenesis of dermatophyte infections. <u>Staphylococcus</u> sub-groups II and IV predominate in infected sites, and staphylococci are more frequently isolated from the infected than non-infected sites.							

^a Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A762759A831
TASK NO. 00
WORK UNIT NO. 006 Experimental Fungus Infections in
the Skin of Man: A Therapeutic Trial

The following studies have been carried out under this work unit:

- STUDY NO. 1 Trichophyton mentagrophyte infections in human volunteers.
- STUDY NO. 2 Effect of the wodel infection on normal flora.
- STUDY NO. 3 Quantitative dermatophyte infections in guinea pigs.

A dermatophyte infection in nine human volunteers is described. This experiment was undertaken for two reasons: To test the influence of antecedent Trichophyton antigens skin testing on an experimental infection and to test what happens to the bacterial flora during such an infection. All infections were induced using a method previously described. Scrapings were made for quantitative bacterial cultures of the infection site. A control site on the opposite arm was also cultured. This infection did not follow a typical course. Possible reasons for this are outlined. Bacteriology of these lesions showed normal flora but penicillin resistance increased during the course of the infection.

A method is described for infecting guinea pigs quantitatively. This method has shown that immunity plays a protective role in re-infection. This immunity is not placentally transferred. A study on cytoxin caused some doubt on the traditional view that immunity to dermatophytes is solely of cell mediated type.

BODY OF REPORT

WORK UNIT NO. 006 Experimental Fungus Infections in
the Skin of Man: A Therapeutic
Trial

STUDY NO. 1 Trichophyton Mentagrophytes Infections
in Human Volunteer-s

PROBLEM:

Subjects experimentally infected with trichophyton mentagrophytes have always been screened first with trichophyton antigen. Possibly this skin testing has led to fallacious interpretation of the course of the induced infections.

RESULTS AND DISCUSSION OF THE RESULTS:

Nine volunteers were infected with either 6 or 300 spores. No volunteer followed the infection course predicted by previous studies. These subjects differed in the following ways, from all previously infected subjects; they had a younger average age, they were in a different environment, they had received no skin tests and they each had frequent scrapings taken from the lesion for bacterial culture. Since there were these other variables we could come to no conclusion about the influence of skin tests on fungal infections.

CONCLUSION:

None could be drawn from this experiment.

RECOMMENDATIONS.

Because of the uncertainties of this last human infection, the difficulty in obtaining human subjects for infection and the similarity in course between normal humans and guinea pigs, we have changed much of our thrust to experimentation in guinea pigs.

PUBLICATIONS:

None

STUDY NO. 2 Effect of the Model Infection
on Normal Flora

PROBLEM:

The role of the normal flora of the skin in the pathogenesis of dermatophytes has not been satisfactorily studied although clinical

Experimental Fungus Infections in the Skin of Man: A Therapeutic Trial (Cont)

evidence suggests a more than passive nature. Because the artificial infection procedure, employs wet, occlusive dressings, one could expect an artifactual alteration in the normal flora which might influence the pathology. For a better understanding of the experimental fungus infection, the normal flora must be investigated.

RESULTS AND DISCUSSION OF THE RESULTS:

A means for the total and definitive quantitation of skin flora was achieved by replica-plating of isolated colonies upon appropriate biochemical and fermentation test agars. This resulted in a clarification of the kinetics of microbial growth and survival on the skin. The procedure was reproducible and yielded data equivalent to the more time and labor-consuming standard methods.

To determine the effect of the wet dressing, a sham infection using no fungal inoculum was conducted on six volunteers. An increase in flora of 10^4 colony-forming units was detected in all subjects, but counts rapidly fell approximately 10^2 units once dressings were removed. Although similar types of bacteria were found on all subjects, the composition of each individual's flora during the recovery response appeared to be unique. Enterobacteriaceae were found on half the subjects with Enterobacter aerogenes being the most successful colonizer. Besides the expected presence of Baird-Parker Staphylococcus subgroup II, high numbers of subgroup IV and some colonies of subgroup III were also observed. Almost all cutaneous diphtheroids were lipophilic and lipolytic.

It must be emphasized that, although flora was partially reduced within two days after the removal of wet-occlusive dressings, the recovery had not stabilized and that ecological conditions were very fragile. Oscillations of decreasing amplitude regarding number and kinds of flora was the rule, and the skin probably had not returned to the normal degree of hydration by seven days.

Because the dressing was shown to produce a significant but artifactual alteration in the flora, sampling during the actual dermatophyte infection did not begin until the seven-day recovery period had passed. Nine volunteers participated in this study. There was no significant difference in total populations or in kinds of flora compared with untreated and positive skin-test control (opposite) forearms. The ratio of penicillin-resistant microorganisms, however, increased during the infection, the degree of which varied between individuals. All coccal groups were affected; resistant flora diminished when the fungus was no longer detected. Staphylococci were more frequently isolated than micrococci on infected areas, but Staphylococcus aureus was not found. Trends toward hierarchies in persistence and quantities

Experimental Fungus Infections in the Skin of Man: A Therapeutic Trial (Cont)

were observed with Staphylococcus subgroups II and IV dominating the infected sites. On both areas almost all diphtheroids were non-fluorescent, lipophilic, and lipolytic. Whereas other investigators had implicated Staphylococcus aureus as a significant contributor to the severe clinical lesions of ringworm, our data demonstrated that this Staphylococcus was not required. The results also showed the extensive effect of dermatophytes upon the antibiotic sensitivity of accompanying flora, indicating that ringworm lesions might be an important reservoir for penicillin-resistant strains of pathogenic or opportunist staphylococci and micrococci.

RECOMMENDATIONS:

We recommend that microbial interactions on the skin of healthy and infected soldiers be studied in detail. With respect to the therapeutic model, the microflora should be monitored during treatment of ringworm. As a long range goal, one could investigate the colonization on the skin of certain microorganisms which produce antifungal factors as a means of prophylaxis or therapy.

PUBLICATIONS:

1. Bibel, D. J., and Lebrun, J. R. Effect of Experimental Dermatophyte Infection on Cutaneous Flora. Abstracts of the Annual Meeting of the American Society for Microbiology, Chicago, Ill., 1974.

STUDY NO. 3

Quantitative Dermatophyte
Infections in Guinea Pigs

PROBLEM:

Dermatophyte infections were amongst the most troublesome problem faced in the Republic of Viet Nam, with up to 59% of men infected during some periods. These infections were a prominent cause of the number of days lost from combat duty. In the Mekong Delta up to 70% of the days lost were due to skin disease, with a large percentage of these being related to fungal infections.

The role immunity played in these infections is unclear. Whether immunity can be bolstered to prevent these infections has been disputed. In the past we have developed a human dermatophyte infection model to study many of these questions. A quantitative guinea pig infection model has been developed to permit investigation of many of these immunologic questions.

Experimental Fungus Infections in the Skin of Man: A Therapeutic Trial (Cont)

RESULTS AND DISCUSSION OF RESULTS:

A quantitative dermatophyte infection induction technique has been developed in guinea pigs. This technique was patterned after the experimental human infection technique developed at LAIR. This procedure allows 80% predictable infections when a 100 spore inoculum is used. A large number of previously uninfected (inexperienced) guinea pigs have been followed through the course of an infection to determine the following parameters: lesion first noted, maximal erythema, induration, scaling, crusting, size, duration of the clinical infection and the time that cultures stay positive. We have seen that guinea pigs re-exposed to 100 spore inoculums have a lower rate of re-infection, the infection covers less area and lasts a shorter period. Re-infection with 1000 spores gives a dermatitis that develops acutely and the duration is markedly decreased. Pregnant guinea pigs have been infected with 100 spores and their off-spring were infected in their first week of life. These new born guinea pigs had essentially the same course of infection as adults. Guinea pigs treated with cytoxin (20mg/kg/day) have had prolonged infections with marked spread of these infections.

CONCLUSION:

The following observations can be made: (1) A definite protective immunity develops during the first infection. (2) This immunity is relative and protects against infections with small inoculums and shortens, but does not prevent, infections with larger inoculums. (3) This immunity is not passed from mother to infant either transplacentally or through milk. New borns from infected and uninfected mothers have the same course as adult guinea pigs. (4) Cytoxin (a primarily B cell suppressor) inhibits the healing of these infections, and casts some doubt on the traditional view that immunity to dermatophytes is solely of cell mediated type.

RECOMMENDATIONS:

With this infective procedure compounds that may bolster immunity, and prophylactic and therapeutic agents should be evaluated.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ²	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV SUMMARY 72 07 01	4. KIND OF SUMMARY D Change	5. SUMMARY SCTY ² U	6. WORK SECURITY ² U	7. REGRADING ² NA	8. DISEM INSTR ² NL	9. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ²	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62760A	3A762760A822		00	074		
b. SECONDARY	62110A	3A062110A822					
c. TERTIARY	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ² (U) Nutritional and Metabolic Aspects of Nutrients (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ² 002300 Biochemistry; 003500 Clin. Medicine							
13. START DATE 66 07		14. ESTIMATED COMPLETION DATE CONT		15. FUNDING AGENCY DA		16. PERFORMANCE METHOD C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. FUNDS (in thousands)	
a. DATES/EFFECTIVE.		e. EXPIRATION:		PREVIOUS		a. PROFESSIONAL MAN YRS	
b. NUMBER* Not Applicable		d. AMOUNT:		FISCAL YEAR		b. FUNDS (in thousands)	
c. TYPE		f. CUM. AMT.		CURRENT		106.8	
d. KIND OF AWARD:				75		6.5	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME:* Letterman Army Institute of Research				NAME:* Letterman Army Institute of Research			
ADDRESS:* Presidio of San Francisco, CA 94129				ADDRESS:* Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL MC				NAME:* Raica, N., Jr			
TELEPHONE: 415 561-3600				TEL. PHONE:			
				SOCIAL SECURITY ACCOUNT NUMBER			
21. GENERAL USE ²				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME: Sauberlich, H. E.			
				NAME: Baker, E. M., COL			
22. KEYWORDS (Precede EACH with Security Classification Code) ² (U) Nutrients of Military rations; (U) Food Preservation (U) Nutritional requirements; (U) Metabolism; (U) Wholesomeness							
23. TECHNICAL OBJECTIVE. ² 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) (a) Provide essential information pertaining to the wholesomeness and nutritional adequacy of foods considered for military ration and feeding systems; (b) establish fundamental information concerning requirements for nutrients and factors that may alter these requirements including various military environments and infectious diseases; (c) study the metabolic interrelationships and functional aspects of nutrients that may influence the physical and mental performance of the individual soldier in any given military situation.</p> <p>24. (U) Studies will involve primarily animal and microbial experimentation for later application to human situations. Isotopically labeled nutrients will be employed to study their metabolism, requirements, interactions, turnover rates, etc., in animals under various controlled dietary and environmental conditions.</p> <p>25. (U) 73 07 - 74 06 Continued investigations have shown that ¹⁴C-2-riboflavin is metabolized by the rat and chromatographic fractions of urine and tissue extracts previously ignored contain significant amounts of ¹⁴C-compounds. An improved spectrophotometric method has been developed for erythrocyte transaminase assay. Preliminary results with rats indicate a substantial decrease in enzyme activity and a decided rise in stimulation coefficient associated with the consumption of a B-6 deficient diet. Recently developed microbiological, enzymatic and radioimmunoassay (RIA) procedures were used to measure vitamins B₆ and B₁₂ and folic acid in serum and erythrocyte samples from a rat study and three nutritional surveys. Preliminary evaluation of the data indicates a potential for routine use after completion of further testing.</p>							

*Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine
WORK UNIT NO. 074 Nutritional and Metabolic
Aspects of Nutrition

The following investigations have been conducted under this work unit:

- STUDY NO. 1 Ascorbic Acid: Chemistry and Biological Functions
- STUDY NO. 4 Tissue and Blood Enzymes
- STUDY NO. 14 Riboflavin Metabolism in the Rat
- STUDY NO. 15 Vitamin Assays

Study No. 1 Studies were initiated on ascorbic acid metabolism in the monkey. The oral administration of ascorbic acid-2-SO₄-1-¹⁴C resulted in about 95% of the radioactivity being recovered in the breath. A number of metabolites were also observed in the urine.

Study No. 4 Weanling rats were placed on semipurified diets ± pyridoxine. Erythrocyte GOT and GPT activity was determined with and without the addition of pyridoxal-PO₄ to the *in vitro* system using a revised spectrophotometric procedure. Very low GPT activity was found in erythrocytes of rats maintained on either diet, which precluded any accurate utility of this assay. However, GOT activity was substantially reduced and the stimulation coefficient was significantly higher in deficient rats by the second week of feeding. These differences became more marked during the remainder of the study.

Study No. 14 Isolation of ¹⁴C metabolites, some conjugated, from urine, feces and tissue homogenates of rats fed ¹⁴C-2-riboflavin demonstrate that riboflavin is metabolized. To further evaluate riboflavin metabolism new assay procedures must be established.

Study No. 15 Studies were conducted on new or improved microbiological and enzymatic procedures for the measurement of vitamin B₆ in serum and erythrocytes and on radioimmune assays (RIA) for the measurement of vitamin B₁₂ and folic acid in plasma.

BODY OF REPORT

WORK UNIT NO. 074

Nutritional and Metabolic
Aspects of Nutrients

STUDY NO. 1

Ascorbic Acid: Chemistry and
Biological Functions

PROBLEM:

Ascorbate-2-sulfate possess anti-scorbutic properties for the rainbow trout. The objective of this study was to determine whether the compound has biological activity in mammals. For this purpose, the monkey was selected as the experimental animal.

RESULTS AND DISCUSSION OF THE RESULTS:

In a preliminary study, ascorbate-2-SO₄-1-¹⁴C was administered by intubation to a young adult monkey. The animal was placed in a metabolism cage and urine and feces collected and expired air monitored for radioactivity. Approximately 95% of the ingested radioactivity was lost through the breath. DE-32 ion exchange chromatography of the urine collections showed the presence of several radioactive compounds. Several of the compounds behaved in manner to suggest that they were neither ascorbate-2-SO₄ nor 6-carboxy-ascorbate-2-SO₄. Efforts are continuing on the identification of these compounds.

CONCLUSIONS:

Ascorbate-2-sulfate is actively metabolized by the monkey. Additional studies will be required using ascorbic acid and other ascorbate derivatives to evaluate the significance of these preliminary findings.

STUDY NO. 4

Tissue and Blood Enzymes

PROBLEM:

The need exists for an accurate, reproducible, and practical procedure to aid in the evaluation of vitamin B₆ nutriture. A number of enzymes are known to require pyridoxal-PO₄ (P-PO₄) as the active vitamin B₆ cofactor, including the transaminase systems. The UV or spectrophotometric procedure appears to yield a more definitive determination of transaminase (GOT and GPT) activity than the colorimetric methods and warrants study. A priori, P-PO₄ is an obligatory cofactor of the GOT and GPT systems, hence a dietary deficiency in vitamin B₆ may well result in a reduced level of cofactor saturation of the transaminase enzymes. Therefore, GOT activity and GPT activity are assayed with and without the addition

Nutritional and Metabolic Aspects of Nutrients (Cont)

of 50 micrograms P-PO₄/ml of incubation volume and the stimulation coefficient (S.C. = $\frac{+P-PO_4}{-P-PO_4}$) is calculated.

Since the S. C. is an index of cofactor presence, it should be less affected by alterations in protein metabolism than the enzyme activity itself. The transaminase activity of erythrocytes has been studied since their activity is higher than plasma and appears to be a more reliable index of somatic activity.

RESULTS AND DISCUSSION OF THE RESULTS:

A preliminary study was conducted with male weanling rats fed 20% vitamin-free casein, semi-purified diets \pm 6 mg/kg of pyridoxine. Five animals on each diet were sacrificed at weekly intervals at 2 to 6 weeks on trial. The erythrocyte GPT activity (EGPT) activity was significantly lower on the deficient diet at all weekly intervals, however, the very low activity on either diet (0.04 - 0.24 I.U./ml red blood cells) prevented the analytical precision necessary for practical usage. Further, the S. C.s were not markedly changed due to diet, ranging from 1.07-1.28 across diets. The erythrocyte GOT activity (EGOT) was considerably higher than EGPT. The EGOT results are summarized in the table below.

Effect of Vitamin B₆ Deficiency on Erythrocyte GOT Activity

	<u>No. of Weeks on Diet</u>				
	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
<u>Activity (I.U./ml RBC)¹</u>					
-B ₆ Diet	0.720 ± 0.091	0.631 ± 0.043	0.384 ± 0.002	0.414 ± 0.018	0.328 ± 0.003
+B ₆ Diet	1.669 ± 0.122	1.678 ± 0.112	1.533 ± 0.077	1.921 ± 0.027	1.673 ± 0.077
<u>S.C. ($\frac{+P-PO_4}{-P-PO_4}$)</u>					
-B ₆ Diet	1.273 ± 0.013	1.545 ± 0.054	2.142 ± 0.208	1.736 ± 0.097	1.758 ± 0.112
+B ₆ Diet	1.091 ± 0.004	1.135 ± 0.012	1.266 ± 0.017	1.131 ± 0.010	1.104 ± 0.025

¹Values are means \pm S.E.M.

Nutritional and Metabolic Aspects of Nutrients (Cont)

The EGOT activity is substantially reduced at 2 weeks and falls further during the rest of the trial. A significant rise in the S. C. is shown by the second week on the deficient diet (27% vs 9%) and remains highly elevated through the 6th week when compared to the control animals.

CONCLUSIONS:

Stimulation coefficients derived from an improved spectrophotometric EGOT procedure of rats fed a vitamin B₆ deficient diet were found to increase substantially over controls. A diminished EGOT and EGPT activity was also associated with consumption of the vitamin B₆ deficient diet. Due to the extremely low EGPT activity observed in rats, efforts should be concentrated on the EGOT and other promising procedures. At present, the transaminase assays cannot be applied to a given situation without adequate controls. That is, until the effects of species, sex, diet and age are studied, and the influence of sample handling and storage are evaluated, no reported EGOT activity or S. C. can be regarded as a benchmark for the norm.

RECOMMENDATIONS:

Studies should be continued with concentration on erythrocyte glutamic-oxalacetic transaminase (EGOT) as a possible aid in evaluation of vitamin B₆ deficiency. Comparative analyses for EGOT, P-PO₄ and total vitamin B₆ should be achieved. Additional investigations on the effects of species, sex, age, and other dietary influences upon EGOT should be explored. A detailed examination of sample handling and storage influences should also receive priority attention.

STUDY NO. 14

Riboflavin Metabolism in
the Rat

PROBLEM:

This laboratory has shown that lack of dietary riboflavin has adverse effects on the performance of young men. Further it has been shown that ¹⁴C-2-riboflavin is metabolized by the rat. This investigation will provide techniques and procedures for the continued study of riboflavin metabolism.

RESULTS AND DISCUSSION OF THE RESULTS:

Studies were continued to establish the catabolic fate of riboflavin in rats fed ¹⁴C-2-riboflavin. A minimum of 10 ¹⁴C-metabolites, some conjugated, were isolated from tissue homogenates, urine and

Nutritional and Metabolic Aspects of Nutrients (Cont)

feces of rats. One compound containing 4 to 8% of the urinary ^{14}C was identified as urea. Many of the ^{14}C compounds did not fluoresce, thus routine assay procedures could not be used. At present the only assay to establish the pattern of the ^{14}C -2-riboflavin metabolites is thin layer chromatography (TLC) and scintillation counting of the TLC fractions. The rat tissue stores were repleted with ^{14}C -2-riboflavin after an initial riboflavin depletion period. After repletion, the rats received a riboflavin free diet for 10 days. Throughout the second depletion period, the rat feces contained significant levels of ^{14}C . The fecal ^{14}C would be from the metabolism of tissue bound riboflavin excreted in the bile into the intestines and/or from the constant replacement of the mucosa lining. Tissue ^{14}C compounds isolated by R-15 resin chromatography were different from the urinary ^{14}C compounds. Further, extensive protease digestion was required to release the ^{14}C activity from the tissues. Less than 37% of the total ^{14}C activity in liver was acid soluble.

CONCLUSIONS:

^{14}C -2-riboflavin is metabolized by the rat, thus a constant renewal of dietary riboflavin is required. Other metabolites will be isolated when uniformly labeled riboflavin is used. To further study riboflavin metabolism, new assays must be established as many ^{14}C -riboflavin metabolites do not fluoresce.

STUDY NO. 15

Vitamin Assays

PROBLEM:

This laboratory has the ability to manually assay prepared samples for vitamin B₆, folic acid, vitamin B₁₂, and other vitamins using modified microbiological methods developed earlier. Need continues for improved microbiological methods or alternate procedures to measure these nutrients in biological samples.

RESULTS AND DISCUSSION OF THE RESULTS:

Some preliminary studies were conducted on the automation of the microbiological assay using Saccharomyces uvarum ATCC 9080 and Lactobacillus casei ATCC 7469 and 7469-a, both the normal and chloramphenicol resistant strains. The main problem in the automation appears to be the long incubation coil needed to obtain adequate growth. The organisms tend to settle out or wash into the next sample and the bubble pattern is difficult to maintain. A proposed solution would be to automate parts of the sample preparation procedures and the assay set up including the dilutions and aliquoting of standard and sample and the addition of inoculated media. Each inoculated sample would be fed into a tube or

Nutritional and Metabolic Aspects of Nutrients (Cont)

sample cup or other stationary incubation system for the needed period of time. At the end of that incubation period the samples would be agitated and fed into the colorimeter. Using this approach, several portions of the procedure could be automated separately and then the system could be tied together in a continuous flow scheme. Although the automation of microbiological assays has various problems connected with each separate assay, a general scheme could be developed for one assay and modified to fit others. Automation of any part of the procedure from the sample preparation to the recording of results would save time and thus increase efficiency.

Both the vitamin B₁₂ and folic acid radioimmune assay (RIA) kits have been tested on plasma samples. The vitamin B₁₂ RIA kit was tested on fresh plasma samples and was found to be easy to use and reproducible. All values fell within the published normal range for both the microbiological and radioimmune assay. Preliminary work on stored plasma samples, both refrigerated and frozen, showed some decrease in activity, but it is not known whether this decrease was due to storage problems in the samples themselves or in the kit reagents. Since the amounts of vitamin B₁₂ in blood samples are so very small, and the potential for error in the microbiological assay is great due to the sample preparation procedure and the limitation of the instruments used in the assay, the RIA shows good potential for being used on a routine basis. The time required for the RIA is one-half to one-third the time required for the microbiological assay. With further testing on plasma samples as well as other types of samples, the limitations of the RIA can be better determined.

The folate RIA kit was tested on fresh plasma samples and on 140 frozen plasmas collected during the Ent AFB Nutrition Survey. The values of the fresh samples were within the normal range of the radioisotopic test, but in the frozen samples about 12% were deficient and 8% were borderline by the kit standards.

Since there is no published information on RIA folate kits tested on RBC samples, preliminary work was started using accepted RBC preparations for microbiological assays. There was binding under some conditions, but the values obtained were not within accepted microbiological ranges. Results varied with the RBC preparation, the buffer used, and the concentration of ascorbic acid added to the sample. RIA kits for folic acid tested on both plasma and RBC samples showed potential, but they are not ready to be used as a routine assay without further study and comparison to the microbiological methods.

Nutritional and Metabolic Aspects of Nutrients (Cont)

The main effort in vitamin assay work was on vitamin B₆ in various forms. Several small studies using published sample preparation procedures for plasma, RBC, and whole blood resulted in several modifications in the extraction procedure. Areas of change included acid strength, hydrolysis time, and use of a stronger deproteinizing agent such as heat for plasma and TCA for RBC and whole blood. The problem encountered with the whole blood was that the harsher treatment needed for maximum release of vitamin B₆ in the RBC fraction was too harsh for the plasma fraction. Thus, the plasma vitamin B₆ + RBC vitamin B₆ was always greater than analyzed whole blood vitamin B₆. This indicates that either plasma or RBC analysis would give a more accurate indication of vitamin B₆ status than whole blood. Animal studies are in progress to investigate these aspects.

CONCLUSIONS:

Vitamin B₁₂ and folic acid radioimmune assay (RIA) procedures have been tested on plasma samples and appear to have promise as a replacement or alternate procedure for the microbiological assay of these nutrients. Progress was made on new or improved methods for measuring vitamin B₆ in serum and erythrocytes.

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Nutritional and Metabolic Aspects of Nutrients (Cont)

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6337	74 07 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8. DDB'S INSTR ⁶	9. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
73 07 01	D Change	U	U	NA	NL		
10. NO./CODES ⁷		PROGRAM ELEMENT		TASK AREA NUMBER		WORK UNIT NUMBER	
a. PRIMARY		62760A		3A762760A822		00	
b. SECONDARY		62156011		3A025601A822		00	
c. TERTIARY		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ⁸							
(U) Analytical Biochemistry (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ⁹							
002300 Biochemistry; 003500 Clin. Medicine							
13. STARTY DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PREVIOUS		7.0	
b. NUMBER: Not Applicable				FISCAL YEAR		74	
c. TYPE:				CURRENT		75	
d. KIND OF AWARD:						3.5	
e. AMOUNT:						175.0	
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Punish DDB's Institution)			
NAME: Canham J. E., COL MC				NAME: Skala, J. H.			
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-4125			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Sauberlich, H. E.			
				NAME: [REDACTED]			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Analytical Biochemistry; (U) Instrumentation; (U) Automated Analyses; (U) Nutrition surveys; (U) Clinical Chemistry; (U) Mil Medicine							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Punish individual paragraphs identified by number. Precede last of each with Security Classification Code.)							
23. (U) Develop and adapt new concepts in analytical biochemistry to provide reliable and advanced procedures and services to military-oriented research programs of all divisions, 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. 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 in the evaluation of the nutritional requirements and dietary adequacy of military personnel. 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 and, whenever feasible and practical, automated and computer linked.							
25. (U) 73 07 - 74 06 Analytical support was provided to 22 research projects, requiring approximately 17,000 individual wet chemistries and physical method evaluations. A sample preparation system was fabricated and implemented for nitrogen ratios by isotope ratio mass spectrometry.							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine

WORK UNIT NO. 076 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

Analytical service amounting to 17,262 individual analyses was rendered to 22 protocol studies in a year in which operational status was abbreviated by transfer of function. A sample preparation system for stable nitrogen isotopes was designed and fabricated for use with the isotope ratio mass spectrometer extending the ratio capability of the instrument to carbon, hydrogen and nitrogen.

BODY OF REPORT

WORK UNIT NO. 076

Analytical Biochemistry

PROBLEM:

The Analytical Biochemistry Branch is called upon to support research projects requiring a broad spectrum of analytical services. This responsibility demands efficient laboratory function with assurance of accurate results.

RESULTS AND DISCUSSION OF THE RESULTS:

Commencing with the first day of the fiscal year, the Branch lost trained, experienced personnel through retirements, resignations and one long term training program grant. These losses were primarily due to refusals to accept the transfer of function offers augmented by the availability of alternatives such as other employment or early retirements resulting from the Department of Defense stress condition. In spite of the fact that only two of ten staff members remained at the time of relocation, a large number of analyses were performed during the first three quarters of the fiscal year.

Support was provided to 22 protocols and addenda resulting in a total of 13,305 laboratory automated and manual analyses plus 3,957 analyses on field studies as outlined in the following table.

<u>Analytical Service</u>	<u>Number of Analyses</u>
Blood Chemistry	
(1) Automated electrolytes, glucose, iron and uric acid	4920
(2) Semi-automated lipids, lactate and total iron binding capacity	3250
(3) Manual GLC fatty acids, porphyrins and electrophoresis	1648
(4) Field services for hemoglobin, hematocrit and serum protein	2074
Urine Chemistry	
(1) Automated electrolytes, uric acid and other nitrogenous constituents	1403
(2) Field services for screening specific gravity and osmolality	1883

Analytical Biochemistry (Cont)

<u>Analytical Service</u>	<u>Number of Analyses</u>
Diets and Stools	
Manual proximate analyses, bomb calorimetry, GLC fatty acids and cholesterol	2084

This record of performance was possible under the circumstances partly because of the efforts of two temporary personnel who, after training, performed admirably.

CONCLUSIONS:

Although minimum operational efficiency could not be maintained, service was rendered to the protocols on a priority basis.

RECOMMENDATIONS:

Preliminary effort must be placed on staff replacement and subsequent training in the areas required (food or biochemical techniques) and orientation to the instruments used.

PUBLICATIONS:

1. Askew, E.W., A.L. Hecker, W.R. Wise and G.L. Kuhl. Adipose tissue metabolism and turnover rate: response to exercise and dietary carnitine. Fed. Proc. 33:677, 1974.
2. Neldner, K.H., L. Hagler, W. Wise, F.B. Stifel, E.L. Lufkin and R.L. Herman. Aerodermatitis enteropathica: A clinical and biochemical survey. Accepted for publication in Arch. Dermatol.

STUDY NO. 2

Development of Analytical
Biochemistry Procedures

PROBLEM:

Innovation and/or improvement of analytical methods must be attempted in response to supported protocol requirements for new determinations or increased accuracy of existent techniques. Simplification of procedures and automation whenever possible increase the laboratory operational efficiency.

Analytical Biochemistry (Cont)

RESULTS AND DISCUSSION OF THE RESULTS:

The published TLC method for vitamin E quantitation proved unworkable with diet composites as presently collected in the studies being supported. Too great a quantity of non-saponifiable material which was not vitamin E resulted because a large amount of original sample had to be extracted to provide a quantity of vitamin E in the range of the method. This resulted in diffuse spreading on the plates and poor separation.

A sample preparation system for stable nitrogen isotopes was developed and fabricated for use with the isotope ratio mass spectrometer.

CONCLUSIONS:

The heavy per capita workload resulting from the attritional factors previously mentioned and the field studies conducted during the FY did not allow time for work on the vitamin E methodology problem, the use of the nitrogen isotope preparation system, or any of the carry-over projects from the previous year.

RECOMMENDATIONS:

The primary effort should be in restaffing as mentioned under Study 1. After analytical efficiency is achieved, senior staff chemists should resume work on the above mentioned problems and the carry-over projects, i.e., miniaturization of certain methodologies and automation of manual processing.

PUBLICATIONS:

1. Goad, W.C., J.H. Skala, R.S. Harding and H.E. Sauberlich. A semiautomated technique for the determination of vitamin C (ascorbic acid) in serum or plasma samples. USAMRNL Laboratory Report No. 337, September 1973.
2. Wise, W.R., J.H. Skala and H.E. Sauberlich. Automated determination of iron in biological materials. USAMRNL Laboratory No. 341, September 1973.
3. Sauberlich, H.E., R.P. Dowdy and J.H. Skala. Laboratory tests for the assessment of nutritional status. CRC Critical Reviews in Clinical Laboratory Science 4(3):215, 1973. Also published as a monograph by the CRC Press, Inc., Cleveland, Ohio; 1974.

Analytical Biochemistry (Cont)

4. Wise, W.R., R.S. Harding, J.H. Skala and H.E. Sauberlich. Semi-automated determination of serum lipids. USAMRNL Laboratory Report. Submitted to CO for final approval.
5. Knudsen, J.J., J.H. Skala and H.E. Sauberlich. A semi-automated method for the determination of total nitrogen in urine, feces and diets. USAMRNL Laboratory Report. Submitted to CO for final approval.
6. Sauberlich, H.E., W.C. Goad, J.H. Skala and R.S. Harding. Automated procedure for the measurement of serum ascorbic acid (vitamin C). Submitted for publication in Clin. Chem. and subsequently in Sel. Meth. of Clin. Chem.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OA 6324	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY ^a	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8a. DRG'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62760A	3A762760A822	00	077			
b. SECONDARY	62156011	3A025001A822	00				
c. SUBGROUP	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Nutritional Physiology (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
016200 Stress Physiology; 002300 Biochemistry; 005900 Environ. Biology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
64 10		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (in thousands)	
b. NUMBER: Not Applicable				FISCAL YEAR		74	
c. TYPE:				CURRENT		8.0	
d. KIND OF AWARD:				75		4.0	
e. AMOUNT:						177.9	
f. CUM. AMT.						100.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research			
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL MC				NAME: Klain, G. J.			
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-4305			
				SOCIAL SECURITY ACCOUNT NUMBER: ██████████			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME: Sterner, R. T., CPT, MSC			
				NAME: Sullivan, F. J. DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Military Nutrition; (U) Adaptation; (U) Metabolism; (U) Environmental Stress							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) The nutritional status, requirements and the nutritional-physiological characteristics of the military population may be quite different, particularly under combat conditions, than those of the civilian population. Troops in the field may be subjected to numerous stresses, and yet be required to complete successfully a variety of missions. These investigations will study the often-observed phenomenon of simultaneous metabolic adjustments, multiple stresses and their qualitative and quantitative effects upon nutritional requirements in animals, and the relationship of these responses to man.							
24. (U) The investigations will concentrate on studies of; responses common to one, two or more stresses; time sequence of the onset of the response to particular stresses; and the duration of these responses after removal of the stressing factor. Specific techniques will be: measurement of growth and/or food consumption; assay of enzyme activities; determination of levels of tissue and urinary metabolites; determination of metabolic pathways; and clinical observations.							
25. (U) 73 07 - 74 06 Administration of mannoheptulose to rats increases the plasma levels of alanine and glucagon which, in turn stimulates the biosynthesis of hepatic cyclic AMP. This nucleotide enhances the activities of the key hepatic gluconeogenic enzymes. Consequently, mannoheptulose induces temporary hyperglycemia. Incorporation of lysine into hepatic and adipose tissue proteins is markedly stimulated by refeeding after fasting. After two days of refeeding, lysine incorporation into hepatic proteins returns to the level observed in the fed controls. In contrast, lysine incorporation into adipose tissue proteins remains elevated even after five days of refeeding. Refeeding has essentially no effect on lysine incorporation into muscle, heart or kidney proteins.							
*Available to contractors upon originator's approval.							

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine
WORK UNIT NO. 077 Nutritional Physiology

The following investigations have been conducted under this work unit during the past year:

STUDY NO. 7 Metabolic Effects of Starvation - Refeeding

STUDY NO. 16 Gluconeogenic Response to Mannoheptulose

Lysine incorporation into hepatic, muscle and kidney proteins was markedly increased during the first and second day of refeeding after a prolonged period of fasting. Thereafter, the rate of lysine incorporation was similar to that observed in normally fed controls. In contrast, protein synthesis in the adipose tissue remained elevated throughout the entire five-day refeeding period. Fasting or refeeding did not affect protein synthesis in the heart.

Administration of mannoheptulose enhances hepatic gluconeogenesis as indicated by increased activities of the key gluconeogenic enzymes. These effects are apparently mediated by glucagon and cyclic AMP.

BODY OF REPORT

WORK UNIT NO. 077

Nutritional Physiology

STUDY NO. 7

Metabolic Effects of Starvation -
Refeeding

PROBLEM:

Results of the previous studies indicate a marked effect of fasting and subsequent refeeding on the metabolic rate of several amino acids. This apparently is the consequence of hormonal changes induced by the nutritional status of the animal. Since a prolonged period of fasting is associated with a rapid loss of cellular proteins from many of the tissues of the body, it appears that the relative hyper-alimentation associated with refeeding would affect amino acid incorporation into newly synthesized tissue proteins. The present study was directed at this problem.

RESULTS AND DISCUSSION OF THE RESULTS:

Male Holtzman rats ranging in weight from 200-220 gm were fed a complete casein-sucrose diet for ten days. After this period of dietary adjustment, the rats were randomly divided into three treatment groups. The first group continued on the ad libitum feeding schedule and the second and the third groups of animals were fasted for five days. Five animals each from the third group were subsequently refed for a period of one to five days. At the end of the fasting or refeeding periods, five animals from each dietary treatment groups were injected intraperitoneally with 2 μ Ci/100 gm body weight (BW) of L-U- 14 C-lysine. The animals were placed into the metabolism chambers and the expired 14 CO₂ was collected for two hours. Thereafter, the animals were sacrificed and lysine incorporation into tissue proteins was determined.

Compared to the fed controls, lysine oxidation was markedly increased during fasting, and remained elevated even after the first day of refeeding. Thereafter, lysine was oxidized at the rate similar to that observed in the controls. Fasting decreased protein synthesis in the liver, kidney, muscle and the adipose tissue. Lysine incorporation into the heart proteins was not affected by either fasting or refeeding. Lysine incorporation into liver, kidney and muscle proteins was markedly increased during the first two days of refeeding, and returned to the control levels thereafter. In contrast, lysine incorporation into adipose tissue proteins remained elevated throughout the entire five-day refeeding period.

CONCLUSIONS:

The metabolic rate of lysine depends on the nutritional state of the experimental animal and on the target tissue studied. During refeeding

Nutritional Physiology (Cont)

the marked increase in food intake stimulates insulin secretion, which results in an increased uptake of amino acids in tissues and increased protein synthesis. Differences in the amino acid pool size possibly affect the results of this study. The plasma levels of free lysine, and probably tissue levels as well, would be higher in the fasted and refed animals than in the ad libitum fed controls.

RECOMMENDATIONS:

1. Determine the size of the free lysine pool and the levels of RNA and DNA in the tissues studied and the molar ratios of plasma insulin to glucagon.
2. Further studies should be conducted to delineate the effects of refeeding on lysine metabolism in the adipose tissue.

STUDY NO. 16

Gluconeogenic Response to Mannoheptulose in the Rat

PROBLEM:

Ingestion or subcutaneous administration of mannoheptulose (MH), a seven-carbon sugar naturally occurring in avocado fruit, induces temporary hyperglycemia in man and in several animal species. The mechanism by which MH induces the transient diabetic state is believed to be partially an inhibition of insulin secretion from the pancreas and a direct stimulation of hepatic gluconeogenesis. Alternatively, these metabolic events could be the consequence of increased levels of plasma glucagon and hepatic cyclic AMP (cAMP) and of altered enzymatic activities in the gluconeogenic pathways. These possibilities, however, remain to be verified. Accordingly, we examined the effect of MH upon the activity of selected hepatic gluconeogenic enzymes, the level of hepatic cAMP, and the plasma levels of glucagon and alanine. In addition, incorporation of uniformly labeled ^{14}C -alanine into plasma glucose and hepatic glycogen was determined.

RESULTS AND DISCUSSION OF THE RESULTS:

Male Holtzman rats weighing from 250-280 gm were fed a complete casein-sucrose diet for ten days. After this period of dietary adjustment, a group of animals was injected intraperitoneally with a 20% solution of D-mannoheptulose in saline (200 mg/100 gm BW). The control animals were injected with a corresponding volume of saline. Three hours thereafter, the animals were sacrificed, blood was collected and livers were quickly excised. Activities of the following tissue enzymes were determined: glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), glucose-6-phosphatase (GPase), fructose-1,6-

Nutritional Physiology (Cont)

diphosphatase (FDPase), phosphoenol-pyruvate carboxykinase (PEPase), plus the concentration of cAMP.

Another group of control and MH-treated animals was injected with 3 μ Ci of uniformly labeled ^{14}C -alanine. Thirty minutes thereafter, the animals were sacrificed and ^{14}C incorporation into the plasma glucose and the hepatic glycogen was determined.

The results indicate that administration of MH stimulates gluconeogenesis by enhancing activities of hepatic CPase, FDPase and PEPase, and increases the concentration of hepatic cAMP, and the plasma levels of alanine and glucagon. In addition, MH stimulates glucose and glycogen synthesis from alanine.

The elevated levels of plasma glucagon and alanine, as well as an increase in the concentration of cAMP in MH treated animals, lend support to the possibility that glucagon and cAMP mediate gluconeogenic effect of MH. Alanine, as the principal endogenous precursor of glucose, participates in the "glucose-alanine" cycle which has been proposed as an important gluconeogenic system.

CONCLUSION:

Administration of MH produces a diabetic-like syndrome characterized by hyperglycemia and ketonemia. These effects are apparently brought about by insulin insufficiency, hyperglucagonemia and elevated levels of cAMP. This nucleotide, in turn, enhances the activity of the key gluconeogenic enzymes.

RECOMMENDATIONS:

The mechanisms by which MH inhibits insulin release and stimulates glucagon release should be investigated.

PUBLICATIONS:

- 1 Meikle, A. W., G. J. Klain and J. P. Hannon. Inhibition of glucose oxidation and fatty acid synthesis in liver slices from fed, fasted and fasted-refed rats by glucagon, epinephrine, and cyclic adenosine-3,5-monophosphate. Proc. Soc. Exptl. Biol. Med. 143: 379, 1973.
2. Klain, G. J. and P. C. Weiser. Changes in hepatic fatty acid synthesis following glucagon injection in vivo. Biochem. Biophys. Res. Commun. 55: 76, 1973.
3. Klain, G. J. and A. W. Meikle. Mannoheptulose and fatty acid synthesis in the rat. J. Nutrition 104: 473, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ³	2. DATE OF SUB. ARY ⁴	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ⁵	6. WORK SECURITY ⁶	7. REGRADING ⁷	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT	
73 07 01	H. Termination	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ⁸	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
A. PRIMARY	62760A	3A762760A822	00	078			
B. XXXXXXXXXX	62110A	3A062110A822	00				
C. XXXXXXXXXX	CARDS 114 (f)						
11. TITLE (Precede with Security Classification Code) ⁹							
(U) Metabolic Response of Man to Nutrition or Disease (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ¹⁰							
003500 Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
66 07		30 June 1974		DA		C In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. FUNDS (In thousands)	
A. DATES/EFFECTIVE				PRECEDING		A. PROFESSIONAL MAN YRS	
B. NUMBER [*]				74		6.0	
C. TYPE				CURRENT		0	
D. KIND OF AWARD				75		0	
E. AMOUNT							
F. CUM. AMT							
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME [*]				NAME [*]			
Letterman Army Institute of Research				Letterman Army Institute of Research			
ADDRESS [*]				ADDRESS [*]			
Presidio of San Francisco, CA 94129				Dept. of Medicine Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME				NAME [*]			
Canham, J. E., COL, MC				Herman, R. H., COL, MC			
TELEPHONE				TELEPHONE			
415 516 3600				415 561 4147			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER			
Foreign Intelligence not Considered				349-20-9755			
				ASSOCIATE INVESTIGATORS			
				NAME:			
				Hagler, L., LTC, MC			
				NAME			
				Stifel, F. B., DAC			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ¹¹							
(U) Gastrointestinal Disease in Combat Soldiers; (U) Ethanol; (U) Purines; (U) Jejunum; (U) Jejunal Enzymes; (U) Glycolytic Enzymes							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede last of each with Security Classification Code) ¹²							
<p>23. (U) Gastrointestinal (G.I.) and hepatic diseases are a significant cause of military ineffectiveness. Food and ethanol intolerance with the production of significant GI and hepatic disease is well known. It is necessary to study GI and hepatic metabolic processes in order to be able to identify the metabolic defects in susceptible people.</p> <p>24. (U) Normal human volunteer subjects and selected patients were studied with regard to the effect of carbohydrate diets, folic acid, ethanol and hormones (epinephrine, insulin and glucagon) on jejunal and hepatic enzyme activities. Similar studies were carried out acutely in rats and also in rabbit jejunum incubated <u>in vitro</u>.</p> <p>25. (U) 73-07 - 74-06 Several patients with reactive hypoglycemia have been found to have a deficiency of fructosediphosphatase. Treatment with folic acid has resulted in marked improvement. Administration of hormones and ethanol acutely to rats via the portal vein has shown marked effects on hepatic enzymes. Insulin caused an increase in phospho-fructokinase and pyruvate kinase activities and a decrease in fructosediphosphatase. Glucagon and epinephrine caused reciprocal changes. Fructosediphosphate aldolase was unaffected by these hormones. Ethanol increased cyclic-AMP levels but also decreased enzyme activities which suggest that ethanol has a biphasic action since the administration of cyclic-AMP alone increases fructosediphosphatase activity while ethanol decreases fructosediphosphatase activity. Similar effects of ethanol on rabbit jejunal enzymes were demonstrated using an <u>in vitro</u> incubation technique. The administration of purines to two patients with different types of GI disease gave encouraging results with regard to alleviation of symptoms and adaptive increases in jejunal enzyme activities. No such effects were seen in normal subjects or obese patients given purines orally. This work unit is being terminated. Further work in these areas will be conducted under other work units.</p>							

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine
WORK UNIT NO. 078 Metabolic Response of Man to
Nutrition or Disease

The following investigations have been conducted under this work unit:

- STUDY NO. 1. The effect of diet, drugs and sex steroids and other hormones on gastrointestinal enzymes of the jejunal mucosa.
- STUDY NO. 7. The effect of testosterone on jejunal glycolytic enzyme activities in male hypogonadism.
- STUDY NO. 15. Studies in acrodermatitis enteropathica.
- STUDY NO. 26. Studies of adaptive enzyme mechanisms in tissue incubated in vitro.

Study No. 1a. Insulin, glucagon and epinephrine cause rapid changes in rat hepatic formiminotransferase activity following intravenous administration. Intravenous glucagon and epinephrine produced rapid increases in activity, while insulin rapidly decreased formiminotransferase activity. Similar results occurred in the rat jejunum as well. The stimulatory effects of glucagon and epinephrine on hepatic formiminotransferase activity were mimicked by intravenous cyclic AMP. Preliminary data suggests a similar type of hormonal regulation in human jejunum and liver.

Study No. 1b. Oral administration of ethanol significantly decreased hepatic pyruvate carboxylase, fructosediphosphatase, fructosediphosphate aldolase and formiminotransferase activities. In each instance, the ethanol produced depression in enzyme activities was returned to normal levels by the addition of oral folic acid. Intravenous ethanol produced similar decreases in hepatic enzyme activities.

Study No. 1c. Preliminary data suggests that intravenous parathyroid hormone and calcitonin rapidly alter renal cortical pyruvate kinase and fructosediphosphatase activities, while fructosediphosphate aldolase activity is unchanged. Intravenous parathyroid hormone increased renal cortical fructosediphosphatase and decreased pyruvate kinase activities within 10 minutes, while intravenous calcitonin produced reciprocal changes in the same enzyme activities.

Study No. 7. A marked increase in dietary adaptation of jejunal pyruvate kinase occurred with the administration of oral

Metabolic Response of Man to Nutrition or Disease (Cont)

testosterone to male patients with hypogonadism. This permissive effect of testosterone was specific for pyruvate kinase since diet-drug interaction could not be demonstrated for jejunal hexokinase, fructose-1-phosphate aldolase or fructosediphosphate aldolase.

Study No. 15. Further studies have been performed in a patient with acrodermatitis enteropathica. Enzymatic mechanisms related to fatty acids and to fatty acid peroxides will be evaluated.

Study No. 24. Patients previously considered to have "idiopathic" reactive hypoglycemia were studied with regard to fructosediphosphatase deficiency. Abnormally low values of fructosediphosphatase were found in 8 out of 13 such patients. These patients had values ranging from 17.6 to 38.9 units. Normal values are 48.1 ± 4.2 . It may well be that a significant proportion of patients with "idiopathic" reactive hypoglycemia have mild types of fructosediphosphatase deficiency.

Study No. 26. Rabbit jejunal mucosa obtained by open biopsy was incubated in vitro and was found to be viable using a variety of index parameters for 24-hours. Such tissue responded to exposure to folic acid with a significant change in enzyme activities. Cholera toxin and ethanol increased cyclic-AMP levels in the jejunal tissue. Although enzyme activities changed as expected with exposure to cholera toxin such was not observed with ethanol. Instead enzyme activities were depressed by ethanol. This technique promises to be a powerful tool in studying the mechanism by which various agents may affect jejunal enzyme activities.

BODY OF REPORT

WORK UNIT NO. 078

Metabolic Response of Man to
Nutrition or Disease

STUDY NO. 1a.

The effect of diet, drugs and sex
steroids and other hormones on
gastrointestinal enzymes of the
jejunal mucosa.

PROBLEM:

Previous studies have clearly documented the rapid regulation of jejunal and hepatic glycolytic enzymes by glucagon, insulin and epinephrine. Folic acid also produces an increase in these same enzymes, but the folate effect occurs within hours, not minutes. To further understand the interrelationships between hormonal and folic acid regulation of enzymes we determined the effects of these hormones on the enzymes involved in folic acid metabolism. Because of the key role played by formiminotransferase in folate metabolism, we investigated the effect of the above hormones on this folate metabolizing enzyme.

RESULTS AND DISCUSSION OF THE RESULTS:

Glucagon, insulin and epinephrine produced rapid changes (within minutes) in hepatic and jejunal formiminotransferase activity in the rat. Limited evidence in human subjects and patients demonstrated that similar effects also occur in man.

Intravenous glucagon (0.0015- 0.5 mg) and insulin (0.015 - 1.5 units/kg) produced rapid increases and decreases, respectively, in hepatic formiminotransferase activity which were unaltered by pretreatment of the rats with either actinomycin D or puromycin. Intravenous insulin and glucagon significantly decreased and increased, respectively, rat jejunal formiminotransferase activity, as well. Intravenous epinephrine (1.0 - 2.0 μ g/min) also produced a rapid increase in hepatic formiminotransferase activity. The stimulatory effects of glucagon and epinephrine on hepatic formiminotransferase activity were mimicked by intravenous cyclic AMP suggesting that these hormonal responses may be mediated through increased cyclic AMP production.

In four children with fasting-induced hypoglycemia, intravenous glucagon (1 mg/min) significantly increased hepatic formiminotransferase activity within 2-3 min. In five normal human subjects, the administration of subcutaneous insulin (15 units) for three consecutive days significantly decreased jejunal formiminotransferase activity.

Metabolic Response of Man to Nutrition or Disease (Cont)

The results suggest that the rapid insulin, glucagon and epinephrine effects on formiminotransferase activity may be due to dephosphorylation-phosphorylation mechanisms analogous to that involved in the regulation of glycogen metabolism.

CONCLUSIONS:

It appears that the hormonal state of the rat plays a prominent role in the regulation of hepatic and extra-hepatic folate metabolism, just as it does in regulation of glycolysis and gluconeogenesis. In particular, glucagon and epinephrine, presumably mediated through increased cyclic AMP production, promoted rapid stimulation of formiminotransferase activity, while insulin exerted a reciprocal action on the same enzyme activity.

RECOMMENDATIONS:

Glucagon, insulin and epinephrine rapidly alter hepatic folate metabolism as well as hepatic gluconeogenesis and glycolysis through effects on certain key regulatory enzymes in these pathways. Continued investigation is needed to further define these complex regulatory interactions so that the alterations in carbohydrate metabolism and the role that folic acid plays as a therapeutic agent can be understood. Particular emphasis will be focused on the biochemical basis of the gastrointestinal maladaptation syndrome and "idiopathic" hypoglycemic states.

PUBLICATIONS:

1. Greene, H. L., O. D. Taunton, F. B. Stifel and R. H. Herman. The rapid changes of hepatic glycolytic enzymes and fructose-1,6-diphosphatase activities after intravenous glucagon in humans. *J. Clin. Invest.* 53: 44, 1974.
2. Stifel, F. B., O. D. Taunton, H. L. Greene, E. G. Lufkin, L. Hagler and R. H. Herman. Hormonal regulation of hepatic and jejunal formiminotransferase activity in man and rat. *Biochim. Biophys. Acta*, In press.
3. Greene, H. L., N. S. Rosensweig, E. G. Lufkin, L. Hagler, D. Gozansky, O. D. Taunton and R. H. Herman. Biopsy of the small intestine with the Crosby-Kugler capsule Experience in 3866 peroral biopsies in children and adults. *Am. J. Dig. Dis.* 19: 189, 1974.
4. Taunton, O. D., F. B. Stifel, H. L. Greene and R. H. Herman. Rapid changes in the activities of rat hepatic glycolytic enzymes

Metabolic Response of Man to Nutrition or Disease (Cont)

and fructosediphosphatase following insulin and glucagon injection. J. Biol. Chem., In press.

5. Stifel, F. B., O. D. Taunton, H. L. Greene, and R. H. Herman. Rapid reciprocal changes in rat tissue enzyme activities following epinephrine injection. J. Biol. Chem., In press.

STUDY NO. 1b.

The effect of diet, drugs and sex steroids and other hormones on gastrointestinal enzymes of the jejunal mucosa.

PROBLEM:

Alcoholism is a common disorder affecting military personnel as well as the general population. Although many of the biochemical effects of ethanol are known, the overall mode of action of this drug is poorly understood. We have shown that ethanol affects adenylyl cyclase. It is well-known that epinephrine and glucagon affect enzyme activities via the activation of adenylyl cyclase and the generation of cyclic-AMP, hence it seemed reasonable to assume that ethanol may alter physiological functions by affecting various enzyme activities. Since we have shown that ethanol actions are antagonized by folic acid it seemed reasonable to assume that effects of ethanol on enzyme activities might be counteracted by folic acid. The present study was designed to determine if ethanol affected certain of the enzymes involved in gluconeogenesis which might thus serve to explain ethanol-induced hypoglycemia, and if so, whether this might be prevented by folic acid.

RESULTS AND DISCUSSION OF THE RESULTS.

Our initial studies in rats demonstrated that the oral administration of ethanol by intubation (3 ml per day of 95% ethanol over a two day period) significantly decreased plasma glucose and insulin levels and the activities of two key regulatory enzymes of gluconeogenesis, pyruvate carboxylase and fructosediphosphatase, and one glycolytic enzyme, fructosediphosphate aldolase. In each instance, the administration of 800 µg daily of oral folate in conjunction with the ethanol prevented these alterations in carbohydrate metabolism. This is in contrast to oral folate alone which significantly increased plasma insulin levels and the activities of pyruvate kinase, fructosediphosphate aldolase, fructosediphosphatase and formiminotransferase. Folate failed to increase the activities of either pyruvate carboxylase or phosphoenolpyruvate carboxykinase.

Metabolic Response of Man to Nutrition or Disease (Cont)

The intravenous injection of ethanol into rats produced a rapid decrease (within 5 minutes) in the activities of hepatic phosphofructokinase, pyruvate kinase, fructosediphosphatase and fructosediphosphate aldolase. Intravenous ethanol significantly increased hepatic cyclic AMP concentrations approximately 60% within 10 minutes, while oral ethanol did not alter hepatic cyclic AMP concentrations. Our data substantiate the known antagonism between ethanol and folic acid.

The depression of two key regulatory enzymes of gluconeogenesis (pyruvate carboxylase and fructosediphosphatase) by oral ethanol helps explain why ethanol may produce hypoglycemia. Another possible contributing factor might be the increased sensitivity of the beta cell of the pancreas to glucagon in the ethanol-treated rats. At eight minutes post-glucagon injection, plasma insulin levels were increased approximately 170-fold in the ethanol-treated group, 27-fold in the ethanol plus folate treated group and only 5-fold in the control and folate-treated groups. This marked outpouring of insulin in response to glucagon could contribute to the production of a hypoglycemic state.

CONCLUSIONS AND RECOMMENDATIONS:

Our data suggest that oral folic acid might offer a protective effect against hypoglycemia in rats receiving alcohol. Additional studies are planned in humans (alcoholics) to determine whether chronic administration of oral folic acid has any beneficial effects.

PUBLICATIONS:

1. Greene, H. L., F. B. Stifel, R. H. Herman, Y. F. Herman and N. S. Rosensweig. Ethanol-induced inhibition of human intestinal enzyme activities: reversal by folic acid. *Gastroenterology*, In press.
2. Stifel, F. B., H. L. Greene, E. G. Lufkin and R. H. Herman. Acute effects of oral and intravenous ethanol on rat hepatic enzyme activities. *Fed. Proc.* 33: 709, 1974 (Abstract).

STUDY NO. 1c.

The effect of diet, drugs and sex steroids and other hormones on gastrointestinal enzymes of the jejunal mucosa.

PROBLEM:

The hormonal state of the experimental animal plays a fundamental

Metabolic Response of Man to Nutrition or Disease (Cont)

role in the regulation of both hepatic and intestinal metabolism. It is well known that parathyroid hormone and calcitonin are two hormones which are intimately involved in calcium metabolism and renal function. The purpose of these preliminary studies was to determine the effects, if any, of intravenous parathyroid hormone and calcitonin on renal cortical pyruvate kinase, fructosediphosphate aldolase and fructosediphosphatase activities in the rat.

RESULTS AND DISCUSSION OF THE RESULTS:

Intravenous parathyroid hormones (25 and 50 µg) significantly increased renal cortical fructosediphosphatase activity, decreased pyruvate kinase activity and had no effect on fructosediphosphate aldolase activity. The changes occurred within 5-10 minutes and persisted for at least 20 minutes. Intravenous calcitonin (25 and 50 µg) produced changes exactly opposite to those seen with parathyroid hormone: pyruvate kinase activity increased and fructosediphosphatase activities decreased. The greatest changes occurred within 10 minutes with calcitonin.

CONCLUSIONS:

Intravenous parathyroid hormone and calcitonin produce rapid changes in renal cortical enzymes which persist for at least 20 minutes.

RECOMMENDATIONS:

Additional studies are needed to determine the interrelationships between the parathyroid hormone and calcitonin effects on renal cortical enzyme activities, to determine the mediators of these hormonal effects and to determine the significance of these initial findings.

PUBLICATIONS: None.

STUDY NO. 7.

The effect of testosterone on jejunal glycolytic enzyme activities in male hypogonadism.

PROBLEM:

Previous studies showed that the activities of certain jejunal glycolytic enzymes (pyruvate kinase, PK; fructose-1-phosphate aldolase, FLPA; fructosediphosphate aldolase, FDPA; hexokinase, HK) were reduced in hypogonadal male adults. Adaptive changes in enzyme activities after dietary manipulation were less than in

Metabolic Response of Man to Nutrition or Disease (Cont)

normal males. To learn whether this lack of adaptive changes was related to testosterone deficiency, we performed further studies in 5 hypogonadal males.

Jejunal biopsies were performed serially during isocaloric formula diets, divided into 7-day treatment periods: carbohydrate-free, 40% glucose, 40% fructose, carbohydrate-free, carbohydrate-free plus testosterone, glucose plus testosterone, and fructose plus testosterone. The dose of testosterone was 10 mg daily in propylene glycol. An isocaloric amount of fat was substituted for sugar in the carbohydrate-free diets. Samples of jejunal mucosa were assayed for glycolytic enzyme activities.

RESULTS AND DISCUSSION OF THE RESULTS:

The results show that there were adaptive changes of all enzymes following dietary manipulation. A marked increase in dietary adaptation of PK occurred with administration of oral testosterone. This permissive effect of testosterone was specific for PK, since no diet-drug interaction could be demonstrated for jejunal HK, FLPA, or FDPA.

CONCLUSIONS AND RECOMMENDATIONS:

Further studies should be carried out in hypogonadal females. It is of interest that dietary maladaptation has been noted in two patients with cachexia and hypogonadism, presumably due to anorexia nervosa. It is likely that the jejunal enzyme abnormalities were somehow related to their hypogonadal state.

PUBLICATIONS:

1. Lufkin, E. G., F. B. Stifel, R. S. Teplick, and R. H. Herman. Permissive effect of testosterone on dietary adaptation of jejunal pyruvate kinase in hypogonadal males. *J. Clin. Endocr. Metab.* 38: 1130, 1974.

STUDY NO. 15.

Studies in acrodermatitis
enteropathica.

PROBLEM:

For background information see Annual Progress Report for FY 73, dated 30 June 1973. In the interim since the last annual report additional studies on this patient were performed. Tissue from the patient, and from her spontaneously aborted stillborn anencephalic fetus have been obtained and are being saved for future analysis.

Metabolic Response of Man to Nutrition or Disease (Cont)

It now seems clear that patients with acrodermatitis enteropathica (AE) have some defect in fatty acid metabolism. Whether this defect is related to an abnormality in prostaglandins, or to some other abnormality remains uncertain. It is also known that certain of the lipid classes are highly reactive and especially subject to peroxidation. These lipid peroxides are toxic, and could lead to cellular functional and structural defects, inflammatory changes, and potentially overt clinical manifestations. The metabolism of fatty acids is complex and incompletely understood. There are however, cellular mechanisms which operate to protect against peroxide formation. One such enzymatic mechanism is glutathione peroxidase, which serves to trap and destroy a variety of peroxides, including hydrogen peroxide and unsaturated fatty acid peroxides. Interestingly, this enzyme is present in skin and gut, while its exact function in these two organs remains unclear. It is postulated that a defect in glutathione peroxidase could lead to fatty acid abnormalities. Dysfunction in both skin and gut are the characteristics of acrodermatitis enteropathica.

RESULTS AND DISCUSSION OF THE RESULTS:

The assay of glutathione peroxidase in RBC's and tissue was being instituted when the studies were terminated in anticipation of the move to San Francisco.

CONCLUSIONS:

An accurate and fairly rapid assay system for glutathione peroxidase has been instituted.

RECOMMENDATIONS:

With resumption of work at LAIR, PSF, these studies should be completed. Tissue from the patient, her stillborn fetus, and one other unrelated child with acrodermatitis enteropathica are on hand, and can be analyzed with a minimum of effort. These studies are of potential significance not only in elucidating the pathogenetic mechanisms in acrodermatitis enteropathica, but in providing information as to the normal metabolism of fatty acids.

PUBLICATIONS:

1. Neldner, K. H., L. Hagler, W. Wise, F. B. Stifel, E. G. Lufkin and R. H. Herman. Acrodermatitis enteropathica: A clinical and biochemical survey. AMA Arch. Derm., In press.

Metabolic Response of Man to Nutrition or Disease (Cont)

STUDY NO. 24.

Hypoglycemia syndromes.

PROBLEM:

Previous work has shown that five patients had hypoglycemia related to a deficiency of hepatic and/or jejunal fructose diphosphatase, a disorder which responds, in some cases to the administration of folic acid. We hypothesized that if fructose diphosphatase deficiency causes an abnormality in gluconeogenesis, reactive hypoglycemia might occur in some patients in whom no other cause had been found.

Thirteen patients were selected who had been found on out-patient testing to have "idiopathic reactive hypoglycemia"; i.e., who had significant hypoglycemia (blood glucose below 45 mg/dl, accompanied by rise in plasma cortisol and growth hormone, and by symptoms of hypoglycemia) occurring usually 3-1/2 to 4 hours after glucose ingestion. In addition, studies were carried out in a small group of randomly selected normal subjects. The studies included insulin stimulation, glycerol ingestion, alanine infusion, a 5-hr glucose tolerance test, and jejunal biopsies while on carbohydrate-free feeding.

RESULTS AND DISCUSSION OF THE RESULTS:

Only partial results of these studies are available. The most remarkable finding is that jejunal fructose diphosphatase deficiency was found in 8 of 13 patients with "idiopathic reactive hypoglycemia". The deficiency was not severe in any patient, but in the 8 deficient patients ranged from 17.6 to 38.9 units (normal = 48.1 ± 4.2 SEM). No abnormalities in response to oral glycerol, alanine infusion or insulin injection were found. The results of glucagon assays are not yet available in these patients or their respective controls.

RECOMMENDATIONS:

A prospective study should be developed which would allow an evaluation of the use of folic acid in the treatment of these patients.

PUBLICATIONS:

1. Hagler, L., F. D. Hofeldt, E. G. Lufkin, and R. H. Herman. Reactive hypoglycemia. A clinical-physiologic approach to diagnosis and treatment. Rocky Mountain Med. J. 70: 41, 1973.
2. Hofeldt, F. D., E. G. Lufkin, L. Hagler, M. B. Block, S. Dippe,

Metabolic Response of Man to Nutrition or Disease (Cont)

J. W. Davis, P. H. Forsham and R. H. Herman. Are abnormalities in insulin secretion responsible for reactive hypoglycemia? Diabetes, In press.

STUDY NO. 26.

Studies of adaptive enzyme mechanisms in tissue incubated in vitro.

PROBLEM:

To date, the question of adaptive change of small bowel mucosal enzymes to a variety of stimuli has been approached in vivo using laboratory animals, patients and volunteer subjects. Now that a substantial body of data has been accumulated with respect to the in vivo situation, it is desired to see if in vitro experiments would be feasible. Advantages of this approach would include precise control of experimental conditions, convenience, reproducibility and ability to explore mechanisms not testable in the intact animal. A recently described in vitro organ culture technique for rabbit small bowel biopsies was utilized. The characterization of the system included light microscopic changes during the first 24 hours, stability of disaccharidases, protein determinations and the effect of folic acid on the soluble glucose metabolizing enzymes of the small bowel.

RESULTS AND DISCUSSION OF THE RESULTS:

It was determined that the technique was applicable and valid to determine enzyme changes. Exposure of rabbit jejunum to folic acid in vitro resulted in stimulation of enzyme activities and revealed striking correlation with in vivo data i.e. enzyme changes at 50 mcg/ml of culture media occurring at 4-6 hours and 50-100% increases in FDPase, FDPA and PK activities. Cholera toxin (cholera toxin, 10 mg/ml) caused a significant increase in the concentration of cyclic-AMP. There was a typical response to cyclic-AMP, i.e. no change in FDPA, a rise in FDPase and fall in PK. This was confirmed by using theophylline in 10^{-3} and 10^{-4} M concentration. Data dealing with the direct effect of cyclic nucleotides is pending.

Ethanol, which has been described in this laboratory to be a potent stimulator of adenylyl cyclase in lysed cells, was examined as a stimulator of cyclic-AMP formation in intact tissue. Ethanol at a concentration of 7 gm% but not 0.7 gm%, stimulated cyclic-AMP formation which increased in less than 15 minutes and was noted to return to normal at about 4 hours. In contrast to the experiments

Metabolic Response of Man to Nutrition or Disease (Cont)

where cholera toxin stimulated cyclic-AMP and then had a reciprocal effect on the gut enzymes studied, ethanol uniformly depressed all enzymes (PK, FDPA, FDPase) regardless of the cAMP levels. Combined stimulation of cultured jejunum with cholera toxin and ethanol revealed both the early increase of cyclic-AMP levels noted with ethanol as well as the late changes due to cholera toxin. Data for the representative enzymes is pending.

CONCLUSIONS:

It is possible to use in vitro incubation techniques to study the effect of various agents on jejunal enzymes. This technique could well be applied to study of human jejunal tissue obtained by per-oral biopsy to demonstrate biochemical abnormalities directly.

RECOMMENDATIONS:

These studies should be continued using animal jejunal biopsies and should be extended to jejunal tissue obtained from suitable patients.

PUBLICATIONS: None.

Note: This work unit is being terminated. The on-going studies will be continued under new work units designed to address specific defined research areas.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6357	74 07 01		
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^b	6. WORK SECURITY ^b	7. REGRADING ^c	8. DES'N INSTR ^d	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM A. WORK UNIT
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^e		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		62760A		3A762760A822		00	
B. XXXXXXXX		62110A		3A162110A822		00	
C. XXXXXXXX		CARDS 144(F)					
11. TITLE (Precede with Security Classification Code) ^f							
(U) Radioisotope Support for Military Medical Research (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^g							
008500 Isotopes; 013900 Radioactivity; 011000 Nuclear Instrumentation							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
64 05		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. FUNDS (in thousands)	
A. DATES/EFFECTIVE:				PREVIOUS			
B. NUMBER ^h Not Applicable				FISCAL YEAR		PROFESSIONAL MAN YRS	
C. TYPE:				74		.3	
D. KIND OF AWARD:				75		.3	
E. AMOUNT:						43.5	
F. CUM. AMT.						60.0	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME ⁱ Letterman Army Institute of Research				NAME ⁱ Letterman Army Institute of Research			
ADDRESS ^j Presidio of San Francisco, CA 94129				ADDRESS ^j Department of Nutrition Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME ^k Canham, J. E., COL, MC				NAME ^k Morrissey, R. L., CPT, VC			
TELEPHONE ^l (415) 561-3600				TELEPHONE ^l (415) 561-4770			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: 349-32-1087			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ^m							
(U) Military Research Projects; (U) Radioisotopes; (U) Instrumentation; (U) Data Acquisition							
23. TECHNICAL OBJECTIVE ⁿ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To provide radioisotope support to all projects requiring the use of radioisotopes and improve procedures and counting techniques where needed. Conduct radioactive counting procedures for cooperating 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.</p> <p>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 AEC licensure are conducted.</p> <p>25. (U) 73 07 - 74 06 Research support has been provided to 19 radioisotope investigators 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 AEC and Army regulations. Consultation concerning proper use and application of radioisotope technology has been provided as needed.</p>							

* Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO.	3A62110A822	Military Internal Medicine
WORK UNIT NO.	079	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.

BODY OF REPORT

WORK UNIT NO. 079

Radioisotope Support for Military
Medical Research

PROBLEM:

The use of radioisotopes in nutritional and medical research has proven to be very useful. The Radioisotope Branch is responsible for support of such use by procurement and storage of radioisotopes, radiation safety monitoring, decontamination of glassware, radioactive waste disposal and maintenance of logs and records as required by AEC and Army regulations. Advice and counsel is given to investigators regarding the use of radioisotopes. Beta and gamma counting instruments are maintained for the use of investigators throughout the laboratory.

RESULTS AND DISCUSSION OF RESULTS:

The above support functions were maintained in the current fiscal year. Current instrumentation includes 2 gamma counting instruments and 6 liquid scintillation counters. Disintegrations/minute and additional mathematical calculations are accomplished by computer with Radioisotope Division personnel providing the administrative support arrangements and Department of Information Sciences providing the computer programming and access support.

CONCLUSIONS AND RECOMMENDATIONS:

The use of radioisotopes is essential to the mission of the laboratory. 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.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OA 6364	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUPPLY	4. KIND OF SUMMARY	5. SUMMARY ACTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DIS'N INSTR ⁿ	9b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES: ^a	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62760A	3A762760A822	00	032			
b. CONTINGENT	62110A	3A062110A822	00				
c. XXXXXXXX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) Mathematical and Statistical Support of Military Biomedical Research							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
09700 Mathematics and Statistics; 002300 Biochemistry; 021900 Physiology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
70 02		CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE:				PRECEDING		b. FUNDS (In thousands)	
b. NUMBER: ^a Not Applicable				FISCAL		1	
c. TYPE:				74		106.1	
d. KIND OF AWARD:				YEAR		2	
e. AMOUNT:				75		140.0	
f. CUM. AMT.							
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research				NAME: ^a Letterman Army Institute of Research			
ADDRESS: ^a Presidio of San Francisco, CA 94129				ADDRESS: ^a Department of Information Sciences			
				Biostatistics & Applied Math Div.			
				Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: ^a Teplick, R. S., MAJ, MC			
TELEPHONE: 415-561-3600				TELEPHONE: 415-561-3773			
				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]			
21. GENERAL USE				ASSOCIATE INVESTIGATORS			
Foreign Intelligence not Considered				NAME: Hixson, J. T., E-4			
				NAME: Wensch, M. R.			
22. KEYWORDS (Precede EACH with Security Classification Code) ^a							
(U) Mathematics; (U) Statistics; (U) Research Data;							
(U) Processing and Analysis; (U) Support of Military Biomedical Research							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To provide mathematical, statistical and computer support for Military Biomedical Research at LAIR.							
25. (U) The RCA 301/355 computer has been phased out. It has been replaced with an RJE terminal and four teletypes which communicate with remote CDC 6000 and 7600 computer systems. These facilities are used to support all of the major mathematical and statistical data processing requirements for LAIR. Consultation services in these areas are also provided. Study No. 1 is concerned with developing and documenting program and system libraries relating to bio-mathematics, bio-statistics and data reduction. Special emphasis is placed upon orientating these systems towards the user without a mathematical or computer background.							
25. (U) 73 07 - 74 06 All systems previously on the RCA 301 have been converted to the CDC equipment. Many of these systems, such as GRASS and other programs used to calculate nutrient intakes from military nutrient surveys, have been greatly expanded and modified to take advantage of the immensely enhanced processing facilities. Work is underway in several new areas including a generalized experimental design package and a new system for simulating passive multi-compartment tracer kinetics. These programs and systems are designed to ease the investigator's burden in analyzing his experiment and to help the investigator to recognize problem areas before the experiments are conducted.							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A762760A822 Military Internal Medicine
WORK UNIT NO. 082 Mathematical and Computer Support
 of Military Biomedical Research

The following investigations have been conducted under this work unit:

STUDY NO. 1 Computerized Mathematical and Statistical Operations

STUDY NO. 2 Mathematical and Statistical Analysis Training Manual

Generalized statistical analysis routines are being assembled and documented for ready use by LAIR researchers. Additional programs to assist researchers in designing their experiments are being studied. A manual is being prepared to serve as a guide to the nature of statistics and ADP services that are available.

BODY OF REPORT

WORK UNIT NO. 082

Mathematical and Computer Support
of Military Biomedical Research

STUDY NO. 1

Computerized Mathematical and
Statistical Applications

PROBLEM:

This study is an ongoing one in that its purpose is to provide an ever expanding repertoire of computational routines to assist the LAIR researcher to evaluate and analyze his data.

RESULTS AND DISCUSSION OF THE RESULTS:

The Generalized Research Analysis Statistical System (GRASS) has been expanded to include many new routines which should facilitate data analysis. These include certain data manipulation routines, a number of transformation routines, and routines which automatically calculate probability levels associated with various statistics frequently used in analysis of data generated by investigators at LAIR. Also non-parametric statistical routines have been added. Additional routines are being developed.

Preliminary research has been conducted concerning the advisability and feasibility of developing a generalized experimental design package. Certain tasks necessary for such a package have been accomplished. A generalized experimental design package would be of great value to investigators in designing their experiments.

CONCLUSIONS:

There are now three statistical analysis program systems available to the LAIR investigator. They are GRASS, Statistical Package for the Social Sciences (SPSS), and the Biomedical Computer Program system (BMD). Between them, these three systems offer a rather comprehensive collection of programs to statistically analyze data supplied in a variety of formats.

RECOMMENDATIONS:

As the availability of computational support increases, so do the number of investigators seeking the support. It is imperative that the statistical routines be designed and documented adequately so as to create minimal dependence of the investigator on the Department of Information Sciences. Documentation of the routines and training classes should be initiated in the coming fiscal year.

Mathematical and Computer Support of Military Biomedical Research (Cont)

PUBLICATIONS:

None

STUDY NO. 2

Mathematical and Statistical
Analysis Training Manual

PROBLEM:

Efficiency in data collection and analysis is gained by the careful planning of experiments. A certain familiarity with statistical principles is required to comprehend many of the problems of experimental design. For this reason, it is desirable for investigators to have some exposure to basic statistical principles and techniques. The purpose of this study is to provide the exposure.

RESULTS AND DISCUSSION OF THE RESULTS:

A course covering basic statistical principles and techniques was developed with consideration of the particular problems faced by the investigators at LAIR. Topics covered include elementary probability, sampling theory, the normal distribution, formulation of hypotheses, significance levels and power associated with tests of hypotheses, tests of hypotheses about means and variances, and linear regression.

Part of the course was presented to investigators from the Bioenergetics Division. Presentation was terminated because of the transfer of facilities from Denver to San Francisco.

CONCLUSIONS:

Exposure to basic statistical principles should enhance investigators' ability to plan efficient experiments. The course developed includes the necessary topics and provides a medium for this exposure.

RECOMMENDATIONS:

It is difficult to arrange hours so that a number of investigators can attend an ongoing course. For this reason, and to avoid the necessity of giving the course repeatedly, a laboratory notebook is being prepared for Institute distribution. The laboratory notebook will include essentially the same material as the course. It may also include discussions of other statistical topics that LAIR's investigators should find helpful.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL	
				DA OA 6370	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECUM ⁴	7. REGRADING ⁵	8. ORGN INST'N	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO / CODES ⁶		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
						WORK UNIT NUMBER	
a. PRIMARY		62760A		3A762760A822		00	
b. XXXXXXXX		62110A		3A062110A822		083	
c. XXXXXXXX		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ⁷							
(U) Military Food Hygiene (06)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁸							
006500 Food, 007800 Hyg. & 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/JRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	20. FUNDS (in thousands)
a. DATES/EFFECTIVE:				EXPIRATION		PREVIOUS	
b. NUMBER ^a Not Applicable						FISCAL YEAR	CURRENT
c. TYPE				d. AMOUNT:		74	2
e. KIND OF AWARD:				f. CUM. AMT.		75	124.6
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ^a Letterman Army Institute of Research Department of Nutrition			
ADDRESS: ^a				ADDRESS: ^a Food Hygiene Division Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME: ^a Fowler, James L., COL, VC			
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-2878			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: ██████████			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Ladiges, Warren C., CPT, VC			
				NAME:			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) Wholesomeness; (U) Data Collection; (U) Microbiological Limits; (U) Safety; (U) Foodborne Diseases; (U) Food Contaminants; (U) Methodology							
23. TECHNICAL OBJECTIVE, ⁹ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) Perform applied and basic research in military food hygiene, including both microbiology and toxicology. Collects and assembles microbiological data, makes recommendations to OTSG for establishing microbiological guidelines for food products. Reviews new and revised military food specifications. Collaborates with governmental or regulatory agencies in developing microbiological standards and test methods for food products.</p> <p>24. (U) Research in the area of toxicants, both naturally occurring and as a result of food manufacturing processes, will be instituted. Collaboration with the American Public Health Association in the area of methodology will continue. In-house efforts on development or modification of microbiological methods, with particular emphasis of a microbiological data bank will be investigated and instituted if applicable. Liaison with other federal, industrial, and academic institutions will be continued or developed.</p> <p>25. (U) 73 07 - 74 06 During FY 74, preliminary investigations into the pathogenic <i>E. coli</i> problem were instituted and conducted. Research in this area will continue. Several microbiological methodology studies were completed. A reliability study of microbiological counting methods was conducted. A study on the survival of microflora in pre-cooked frozen meals, previously reported in FY 73, has been completed. Recommendations have been made to OTSG on the microbiological guidelines for products which will be prepared in Central Food Preparation Facilities which are to be constructed for DA use. Reviews of military food specifications have been accomplished and liaison with other Federal agencies maintained.</p>							

* Available to contractors upon originator's approval

DD FORM 1498
1 MAR 66

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

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine
WORK UNIT NO. 083 Military Food Hygiene

The following investigations have been conducted in FY74 under this work unit:

- STUDY NO. 3 Comparative Studies of Selective Media, Direct Plating Techniques, and Enrichment-Confirmatory Techniques for Detection and Enumeration of Experimentally Inoculated Staphylococcus aureus in Military Freeze-Dehydrated Foods
- STUDY NO. 6 Comparison of Media for Enumerating Fungi in Precooked Frozen Convenience Foods
- STUDY NO. 10 Bacterial Pathogens in Dry Dog Food
- STUDY NO. 11 Reliability Between Individual Persons in Performing the Standard Plate Count for Fluid Milk

Study No. 3. Three analytical procedures and six commercially available solid selective staphylococcal mediums were compared for recovery of coagulase positive staphylococci from experimentally inoculated foods. The highest mean counts were obtained with the pre-enrichment Most Probable Number Procedures and tellurite polymixin egg yolk agar. A modified second study was conducted, although not yet completely analyzed, the results obtained were almost identical to those originally found.

Study No. 6. Sabouraud dextrose agar (SDA) was compared to potato dextrose agar (PDA) acidified to pH 3.5 and to SDA containing 10 mg Kanamycin/100 ml and chloramphenicol (SDA+) in enumerating fungi from precooked frozen convenience foods. SDA yielded significantly higher fungal count in most foods even though it allowed a high degree of bacterial growth. No significant difference was found between the fungal counts on PDA and SDA+.

Study No. 10. The microbiological quality of commercially available dry dog foods was examined. Ten samples of 13 brands were obtained from local retail markets. Analyses of coliforms, Escherichia coli, fecal streptococci, Clostridium perfringens, Staphylococcus aureus, Standard Plate Count, yeasts and molds, and salmonella were performed on each sample. Organisms of the type listed were found, with the exception of salmonella.

Study No. 11. Standard methods for the Examination of Dairy Products

Military Food Hygiene (Cont)

list various criteria which technicians are required to meet in counting bacterial colonies when performing the Standard Plate Count. Unfortunately, these criteria do not appear to be based on valid reported research works and cannot be supported by proper references. In conjunction with the Chapter Chairman, Intersociety Council of The American Public Health Association, a study has been executed to statistically determine the limits of reliability of technicians performing Standard Plate Counts.

BODY OF REPORT

WORK UNIT NO. 083

Military Food Hygiene

STUDY NO. 3

Comparative Studies of Selective Media, Direct Plating Techniques, and Enrichment-Confirmatory Technique for Detection and Enumeration of Experimentally Inoculated Staphylococcus aureus in Military Freeze-Dehydrated Foods

PROBLEM:

Experiment No. 1. As reported in the Annual Progress Report for FY73. Illnesses due to the toxins elaborated by S. aureus have long been recognized. Statistics published by the National Center for Disease Control for the calendar year 1971 show that 28.8% of all outbreaks of food poisoning in the U.S. were caused by this organism. It is felt that such evidence indicates the continued importance of inspection-control measures and the need for standardized methods in the laboratory detection and enumeration of the organism. At the present time the laboratory method and media used to accomplish the latter varies from one worker to another, and is generally based on preference rather than specific merits of procedure and media. The purpose of the present study was to statistically compare three procedures and six commercially available media for their relative merits in the enumeration of S. aureus.

RESULTS AND DISCUSSION OF THE RESULTS:

Experiment No. 2. Upon review of the data from Experiment No. 1, questions pertaining to the methodology were raised. These were (a) use of streaking technique in lieu of pour plates for the direct plating method and (b) use of a 48-hour incubation in lieu of 24 hours in the selective enrichment MPN method. These objections were considered, and experiment 1 repeated. Results from experiment 2 were essentially the same as those of experiment 1, thus confirming the conclusions reached in the first experiment.

CONCLUSIONS:

In a test of three analytical procedures and six commercially available solid selective medium, the method of choice for enumeration of S. aureus in food products was the pre-enrichment Most Probable Number procedure using tellurite polymixin egg yolk agar for confirmation.

RECOMMENDATIONS:

None.

Military Food Hygiene (Cont)

PUBLICATIONS:

None.

STUDY NO. 6

Comparison of Media for Enumerating
Fungi in Precooked Frozen Convenience
Foods

PROBLEM:

The discovery of mycotoxins and the changing technology of food preparation has resulted in a review and testing of procedures used to isolate and count fungi from foods. Acidification of the media has long been used as a means of selectively inhibiting bacteria while allowing fungal growth but the low pH can be inhibitory to a portion of the fungal population. The addition of specific antibiotics to the media, in an attempt to more selectively inhibit bacteria and allow better fungal growth, has been used with good success.

Investigations were made comparing the ability of an acidified medium (Potato dextrose agar acidified to pH 3.5)(PDA), an antibiotic-containing medium (Sabouraud dextrose agar with chloramphenicol and Kanamycin) and Sabouraud dextrose agar to quantitatively isolate fungi from precooked frozen convenience foods.

RESULTS AND DISCUSSION OF THE RESULTS:

Nineteen (19) individual food items prepared specifically for military use and 17 commercial items were tested. Statistical analysis of the data (ANOVA) demonstrated that there was a significant difference between the media. Post-hoc Newman-Keuls comparisons showed that Sabouraud dextrose agar yielded significantly higher fungal and yeast counts than did potato dextrose agar or Sabouraud dextrose agar containing antibiotics (chloramphenicol and Kanamycin). Sabouraud dextrose agar allowed a higher number of bacterial colonies to develop on the media than did the other two media. However, this bacterial growth did not affect the efficiency of Sabouraud dextrose agar over the other two in enumerating fungi from the pre-cooked frozen meals tested.

CONCLUSIONS:

Fungal growth was not suppressed when bacterial growth was present, however the time and labor necessary to perform Gram staining for differentiation of bacteria and fungi points up the need for an inhibitory medium more selective than SDA. Acidified PDA appeared much too acidic for fungal growth, while Kanamycin and/or chloramphenicol

Military Food Hygiene (Cont)

PUBLICATIONS:

Ladiges, W. C., and J. F. Foster. Bacterial Pathogens in Dry Dog Food - A Clinical Item. In Press, J. AVMA.

STUDY NO. 11

Reliability Between Individual
Persons in Performing the Standard
Plate Count for Fluid Milk

PROBLEM:

Standard Methods for the Examination of Dairy Products is the official publication of the American Public Health Association which is used in performing laboratory examinations in regulatory dairy microbiology. It is updated regularly; the next edition is scheduled to be issued in July 1975. Many of the reliability indices used for counting techniques in the Agar Plate Method appear to be based on empirical rather than scientific data. The Chapter Chairman, Agar Plate Method, requested that this laboratory participate in a series of studies designed to provide scientific data, based on carefully controlled laboratory procedures and statistical analyses to establish the validity of the tests for inclusion in the next edition of Standard Methods for the Examination of Dairy Products.

RESULTS AND DISCUSSION OF RESULTS:

Five technicians were utilized in the study. Their experience with counting bacterial colonies ranged from several years to only a few months. Preliminary counting sessions were performed in order to establish similar reference points. The experiment was designed to statistically determine (1) reliability of technicians in preparing plates from identical samples; (2) reliability of technicians in counting their own and other technicians' plates from identical samples, and (3) long-term and short-term reliability in a given technician's counting of the same plates. In order to accomplish this, all plates were assigned blind numbers through a system of random numbers controlled by persons not performing the counting procedures.

The laboratory procedures in this study have been completed, however the statistical analyses have not been accomplished. It is anticipated that the results of this study will provide reliability data for the next edition of Standard Methods for the Examination of Dairy Products.

Military Food Hygiene (Cont)

CONCLUSIONS:

None as yet since the statistical analyses have not been performed.

RECOMMENDATIONS:

None.

PUBLICATIONS:

None.

ADDITIONAL PUBLICATIONS UNDER WORK UNIT 083

- STUDY NO. 2 Ladiges, W. C., J. L. Fowler, and J. F. Foster. Survival Time of Experimentally Inoculated Staphylococcus Aureus in a Military Freeze-Dehydrated Food Product. USAMRNL Laboratory Report, No. 344, 1973.
- STUDY NO. 4 Ladiges, W. C., and J. F. Foster. Incidence of Salmonella in Beef and Chicken. J. Milk and Food Technol. 37(4):213-214, 1974.
- Ladiges, W. C., J. F. Foster, and W. F. Ganz. Comparison of Salmonella Polyvalent H Antisera, Direct Fluorescent Antibody, and Cultural Procedures in Detecting Salmonellae from Experimentally Contaminated Ground Beef Under Frozen Storage. In Press, J. Milk and Food Technol, 1974.
- STUDY NO. 5 Ladiges, W. C., J. F. Foster, and J. L. Fowler. Survival of Microflora in Precooked, Frozen Meals During Frozen Storage. Cleared for publication as a LAIR Institute Report.
- STUDY NO. 7 Ladiges, W. C., J. F. Foster, W. F. Ganz, and M. L. Henderson. Microflora of Ground Beef. Submitted to Publications Review Committee as a LAIR Institute Report.
- Ladiges, W. C., J. F. Foster, and W. F. Ganz. Incidence of Viability of Clostridium perfringens in Ground Beef - A Research Note. In Press, J. Milk and Food Technol.
- STUDY NO. 8 Ladiges, W. C., W. F. Ganz, M. L. Henderson, and J. F. Foster. A Preliminary Study on the Antibiotic

Military Food Hygiene (Cont)

Resistance of Bacteria Isolated from Food Animal Products. USAMRNL Laboratory Report No. 343, 1973.

STUDY NO. 9 Ladiges, W. C., J. F. Foster, and W. F. Ganz. Evaluation and Use of the Mouse Intestinal Loop in Determining Enterotoxigenicity of Escherichia coli. Submitted to the Canadian Journal of Comparative Medicine, May 1974.

RESEARCH WORK PERFORMED ELSEWHERE BUT PUBLISHED DURING FY 74

1. Fowler, J. L., J. L. Young, R. T. Sterner, and R. C. Fernau. Dirofilaria immitis: Lack of correlation between numbers of microfilariae in peripheral blood and mature heart worms. JAAHA 9:391-394, 1973.
2. Ladiges, W. C., E. O. Dickenson, and J. R. Gorham. A clinical and pathological comparison of the pulmonary response during experimentally induced anaphylaxis in sheep and cattle. Am. J. Vet. Res. 35:389, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8a. DES'N INSTR ¹¹	8b. SPECIFIC DATA - CONTRACTOR ACCESS <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO		9. LEVEL OF SUM A. WORK UNIT
72 07 01	D Change	U	U	NA	NL			
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62760A	3A762760A822		00	085			
b. SECONDARY	62110A	3A062110A822						
c. THIRDARY	CARDS 114 (f)							
11. TITLE (Precede with Security Classification Code) ^a								
(U) Nutritional Requirements of Military Personnel								
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a								
00800 Agr. Economics; 002300 Biochemistry; 003500 Clin. Medicine								
13. START DATE			14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 07			CONT		DA		C In-House	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS		20. FUNDS (In thousands)
a. DATES/EFFECTIVE:		EXPIRATION:		PREVIOUS				
b. NUMBER: Not Applicable				FISCAL YEAR		7.0		164.4
c. TYPE:		d. AMOUNT:		CURRENT		6.0		135.0
e. KIND OF AWARD:		f. CUM. AMT.						
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION				
NAME: Letterman Army Institute of Research				NAME: Letterman Army Institute of Research				
ADDRESS: Presidio of San Francisco, CA 94129				ADDRESS: Biochemistry Div, Dept of Nutrition Presidio of San Francisco, CA 94129				
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Punish SSAN if U.S. Academic Institution)				
NAME: Canham, J. E., COL MC				NAME: Sauherlich, H. E.				
TELEPHONE: 415 561-3600				TELEPHONE: 415 561-4323				
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: [REDACTED]				
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS				
				NAME: Baker, E. M. COL				
				NAME: Raica, N., Jr DA				
22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Rations and Feeding; (U) Human Micro and Macro Nutrient Requirements; (U) Military Nutrition Surveys								
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Punish individual paragraphs identified by number. Precede text of each with Security Classification Code.)								
23. (U) To evaluate military rations, existent and experimental, in terms of chemical composition and macro- and micro nutrient content; to study the effect of such rations on the nutrient status of military personnel; to define the requirements for micro-nutrients in military situations and various environs and provide guidance for ration formulation on this basis; to investigate the parameters of nutrient status for various micro-nutrients to enable the early recognition of nutritional insufficiencies or excesses; to develop biochemical techniques to support these investigations and facilitate measurement of nutritional status.								
24. (U) A search for new urinary and blood parameters useful in defining nutritional status and the development of analytical methods to use these parameters will be accomplished. Following preliminary animal studies, volunteer human subjects will be studied under strict metabolic ward observation or during military field studies to permit more accurate definition of nutrient requirements under various conditions or environments common to the military.								
25. (U) 73 07 - 74 06 Metabolism studies with ¹⁴ C and ³⁵ S-ascorbic acid sulfate (AAS) in rats have shown AAS has little if any metabolic value when orally fed or injected intraperitoneally. These results must be evaluated with consideration that the rat does not require extrinsic ascorbic acid. Scurvy which had been produced in Rhesus monkeys required 250 mg/day for correction. T _{1/2} of AAS was 24 hrs in limited studies in Rhesus monkeys. The adult human male appears to require at least 600 µg/day of retinol to prevent or cure eye changes associated with a deficiency of vitamin A.								

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine
WORK UNIT NO. 085 Nutritional Requirements of
Military Personnel

The following investigations have been conducted under this work unit:

STUDY NO. 1 Vitamin C Metabolism and Requirement in Man

STUDY NO. 2 Vitamin A Requirement of the Adult Human

Study No. 1 Investigation of ascorbic acid-2-sulfate (AAS) metabolism has shown that AAS orally fed or injected intraperitoneally has little if any metabolic value in the rat. Chromatographic techniques have been established to isolate ascorbic acid, ascorbic acid sulfate and their metabolites; however, specific assays have yet to be established to assay the metabolites.

Study No. 2 A deficiency in vitamin A was induced experimentally in eight human volunteer subjects as evidenced by biochemical, clinical, ophthalmological or isotopic labeling findings. The adult human appears to require a minimal intake of 600 $\mu\text{g}/\text{day}$ of retinol although an intake of 1200 $\mu\text{g}/\text{day}$ is desirable to insure modest body stores of the vitamin. A daily intake of approximately 1200 μg of β -carotene will provide the minimal needs of the adult human for vitamin A. However, approximately 2400 $\mu\text{g}/\text{day}$ of β -carotene appear necessary to ensure plasma vitamin A levels above 30 $\mu\text{g}/100\text{ ml}$ which are judged desirable.

BODY OF REPORT

WORK UNIT NO. 085

Nutritional Requirements of
Military Personnel

STUDY NO. 1

Vitamin C Metabolism and
Requirement in Man

PROBLEM:

Investigation of the metabolism of ascorbic acid in man was continued to establish its physiological role. ^{14}C -ascorbic acid-2-sulfate (AAS) has been isolated from urine of man and the rat fed ^{14}C -ascorbic acid (AA). Further, equal molar amounts of AAS were shown beneficial to the trout as a replacement of dietary AA. Investigation of AAS metabolism in the rat would further the understanding of AA metabolism and might establish procedures for AAS metabolism studies in man. However in the interpretations of the derived data consideration must be given to the fact that the rat synthesizes ascorbic acid while man is dependent upon external sources of the vitamin.

RESULTS AND DISCUSSION OF THE RESULTS:

AAS has been isolated from human and rat urine. It was of interest to investigate the metabolic rate of AAS orally ingested or injected intraperitoneally (IP). Chromatographic procedures were established for the separation of AAS, AA and their metabolites in urine and tissue extracts. Ninety percent of the ^{14}C or ^{35}S was excreted in the urine within 24 hours following the IP injection of ^{14}C or ^{35}S -AAS into the rat. The compound excreted was not AAS, however, studies indicated the ^{14}C and ^{35}S were in the same molecule. Eight to 13% of the total ^{14}C and/or ^{35}S was recovered as a yet unidentified compound collected from rats fed ^{14}C and/or ^{35}S -AAS orally. The amount of ^{14}C or ^{35}S in the feces varied with time. Fifty to 70% of the ^{14}C from ^{14}C -AAS was recovered as $^{14}\text{CO}_2$ in 24 hours. Microbial degradation may have been partially responsible for the $^{14}\text{CO}_2$ release. Less than 0.1% of the ^{14}C or ^{35}S was found in any tissue examined.

CONCLUSIONS:

Ascorbate-2-sulfate injected intraperitoneally or fed orally is probably of little if any beneficial value to the rat but the rat is not an AA dependent mammal. The ascorbate sulfate isolated from urine of man and rat can be considered an ascorbic acid metabolite.

Nutritional Requirements of Military Personnel (Cont)

STUDY NO. 2

Vitamin A Requirement of the
Adult Human

PROBLEM:

Although vitamin A is one of the most important nutrients for the maintenance of life, health, vision, and reproduction, limited information is available for the adult human as to its metabolites, mode of action or minimal daily requirement. In view of this dearth of information, an extensive study was undertaken to induce a deficiency of vitamin A in adult male human volunteers and study their requirement and metabolism of the vitamin.

RESULTS AND DISCUSSION OF THE RESULTS:

As was previously reported, eight human volunteers were maintained in a metabolic ward and placed on a vitamin A depletion and repletion regimen. Because of the magnitude of data obtained from the study, extensive evaluations were necessary before interpretations and conclusions would be presented. Although the analysis of the data is not entirely completed, certain conclusions are now possible.

A deficiency in vitamin A was induced in the volunteer subjects as evidenced by biochemical, clinical, ophthalmological, or isotopic labeling findings. The clinical and ophthalmological changes were associated with decreased body pools of vitamin A, reduced utilization rates, and lowered plasma levels of the vitamin. An intake of 150 $\mu\text{g}/\text{day}$ of retinol corrected the dark adaptation impairment but was inadequate to reverse the observed abnormal electroretinograms. An intake of 600 $\mu\text{g}/\text{day}$ of retinol appeared to be marginal in correcting the electroretinogram changes present in two subjects. This level of retinol intake would probably result in a plasma retinol level of 20 $\mu\text{g}/100\text{ ml}$ or above, but a plasma level of 30 $\mu\text{g}/100\text{ ml}$ or above could not be ensured without an intake of 1200 $\mu\text{g}/\text{day}$ of retinol. The amount of β -carotene necessary to meet the vitamin A requirement of adult men appeared to be approximately twice that of retinol although in some instances the amount required appeared to be less than double. Based on the radiometric findings of body pools of vitamin A and on vitamin A utilization rates, the maintenance of a plasma vitamin A level above 30 $\mu\text{g}/100\text{ ml}$ would be necessary to ensure modest body stores of the vitamin. At this plasma vitamin A level, the utilization rate of vitamin A ranged from 570 to 1250 $\mu\text{g}/\text{day}$.

CONCLUSIONS:

A deficiency in vitamin A was induced experimentally in eight human volunteer subjects as evidenced by clinical, ophthalmological, bio-

Nutritional Requirements of Military Personnel (Cont)

chemical, or isotopic labeling findings. The ophthalmological and clinical changes were associated with decreased body pools of vitamin A, reduced utilization rates, and lowered plasma levels of the vitamin. The adult human male appears to require at least 600 µg/day of retinol to prevent or cure eye changes and perhaps more to reverse cutaneous changes. The requirement for β-carotene is approximately 1200 µg/day. These levels of retinol and β-carotene would not necessarily support optimal levels of plasma vitamin A. Intakes of 1200 µg/day of retinol or 2400 µg/day of β-carotene appear necessary to ensure plasma vitamin A levels above 30 µg/100 ml which are judged desirable.

PUBLICATIONS:

1. Baker, E. M., M. K. Knight, J. A. Tillotson, D. O. Johnsen and B. M. Tolbert. Comparative metabolism and nutrition of ascorbate-2-sulfate. Fed. Proc. 33: 257, 1974 (Abstract).
2. Neal, R. A., and H. E. Sauberlich. Thiamin. In: Modern Nutrition in Health and Disease, 5th edition, p. 186, 1973. Lea & Febiger, publishers.
3. Sauberlich, H. E. Pantothenic acid. In: Modern Nutrition in Health and Disease, 5th edition, p. 203, 1973. Lea & Febiger, publishers.
4. Sauberlich, H. E., and J. E. Canham. Vitamin B₆. In: Modern Nutrition in Health and Disease, 5th edition, p. 210, 1973. Lea & Febiger, publishers.
5. Sauberlich, H. E., R. P. Dowdy and J. H. Skala. Laboratory Test for the Assessment of Nutritional Status. 1974. CRC Press, publishers.
6. Sauberlich, H. E. Some aspects of vitamin metabolism and requirements. S. Afr. J. Med. In press.
7. Sauberlich, H. E., R. E. Hodges, D. L. Wallace. H. Kolder, J. E. Canham, J. Hood, N. Raica, Jr. and L. K. Lowry. Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. Vitamins and Hormones, vol. 32, 1974. In press.
8. Tolbert, B. M., and E. M. Baker. Ascorbic acid: Nutritional and biochemical aspects. Presented at joint meeting of American Institute of Nutrition and American Society for Clinical Nutrition held in Ithica, N.Y., Aug. 1973. In press in Fed. Proceedings.

Nutritional Requirements of Military Personnel (Cont)

9. March, S. C., B. M. Tolbert and E. M. Baker. Column chromatography of ascorbate sulfates II: Determination in natural products. Submitted for publication in Anal. Biochem.
10. Baker, E. M., D. C. Hammer, J. E. Kennedy and B. M. Tolbert. Interference by ascorbate-2-sulfate in the dinitrophenylhydrazine assay of ascorbic acid. Anal. Biochem. 55: 641, 1973.
11. Hodges, R. E., and E. M. Baker. Ascorbic acid. In: Modern Nutrition in Health and Disease, 5th edition, p. 245, 1973. Lea & Febiger, publishers.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1 AGENCY ACCESSION*	2 DATE OF SUMMARY*	REPORT CONTROL SYMBOL	
				DA OB 6302	74 07 01	DD DR&F(AK)636	
3 DATE PREV SUMMARY	4 KIND OF SUMMARY	5 SUMMARY SCY*	6 WORK SECURITY*	7 REGRADING*	8A DIBB'N INSTR'N	8B SPECIFIC DATA - CONTRACTOR ACCESS	9 LEVEL OF SUM
73 07 01	D Change	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A WORK UNIT
10 NO / CODES *	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	672760A	3A762760A822		02	086		
b. CONTRIBUTING							
XXXXXXXXXX	CARDS 114 (E)						
11 TITLE / Precede with Security Classification Code*							
(U) Nutrition Studies in Support of DOD Food Program (06)							
12 SCIENTIFIC AND TECHNOLOGICAL AREA* 002300 Biochemistry; 003500 Clin. Medicine; 009700 Mathematics and Statistics; 012900 Physiology; 006500 Food							
13 START DATE		14 ESTIMATED COMPLETION DATE		15 FUNDING AGENCY		16 PERFORMANCE METHOD	
73 07		CONT		DA		C In-House	
17 CONTRACT GRANT				18 RESOURCES ESTIMATE		19 PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE:				PRECEDING		B. FUNDS (In thousands)	
D. NUMBER* Not Applicable				FISCAL YEAR		74	
C. TYPE				CURRENT		10.1	
E. KIND OF AWARD						14.0	
F. CUM. AMT.						771.0	
18 RESPONSIBLE DOD ORGANIZATION				20 PERFORMING ORGANIZATION			
NAME* Letterman Army Institute of Research				NAME* Letterman Army Institute of Research			
ADDRESS* Presidio of San Francisco, CA 94129				ADDRESS* Departments of Nutrition, Information Sciences & Administration Presidio of San Francisco, CA 94129			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J. E., COL, MC				NAME* Sauberlich, H. E., & Consolazio, C. F.			
TELEPHONE 415-561-3600				TELEPHONE: 415-561-4323 & 415-561-5066			
21 GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER 393241897/352244085			
Foreign Intelligence not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Nelson, R. A.; Clastain, R., CPT			
				NAME: Skala, J. H.; Johnson, H. L. DA			
22 KEYWORDS (Precede EACH with Security Classification Code) (U) Military Rations; (U) Nutritional Requirements; (U) Military Medicine; (U) Clinical Chemistry; (U) Analytical Biochemistry; (U) Diet							
23 TECHNICAL OBJECTIVE, 24 APPROACH, 25 PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code)							
23. (U) (a) Provide essential information and guidance pertaining to the nutritional adequacy of the feeding systems, rations and dietary standards employed by the military services in various environs; (b) to evaluate the nutritional status, nutrient intake, work performance, body composition and work capacity of military personnel to ensure that performance is not impaired by improper nutrition; (c) to evaluate military rations, existent and experimental, in terms of chemical composition and nutrient content; and (d) to provide statistical and computer support for the indicated objectives.							
24. (U) Conduct nutrition surveys and other nutrition investigations of military bases, personnel groups and rations of the Armed Forces upon request to evaluate factors such as nutrient intake, nutritional status and work performance. Information would be obtained through procedures such as clinical examinations and histories, food intake measurements, nutrient analyses of food items and composites, biochemical measurements on blood and urine samples, body composition measurements and physical performance tests. Data will be subjected to mathematical and statistical evaluations through the use of developed computer programs, data files and data reduction systems. Specific and unique military nutrition and dietary problems would receive special attention through the conduct of workshops, symposia or research.							
25. (U) 73 07 - 74 06 A nutrition study was conducted throughout all three phases of a Ranger training cycle to evaluate the adequacy of the current ration allowance. Weight loss of 43% of the trainees exceeded 10% of the initial body weight. A nutrition survey was conducted at ENT AFB and Peterson Field with particular attention to the consumption of food outside the dining facility and the relationship of total food consumption to nutritional status of the personnel. Reports of nutrition surveys at Ft. Lewis, Lowry AFB, and Ft. Myer, VA which were delayed by loss of personnel and transfer of function from Denver to San Francisco are now in the final stages of preparation.							

ABSTRACT

PROJECT NO. 3A762760A822 Military Internal Medicine
TASK NO. 02 Nutrition and Wholesomeness Support
for DOD Food Program
WORK UNIT NO. 086 Nutrition Studies in Support of
DOD Food Program

The following investigations have been conducted under this work unit:

STUDY NO. 1 Nutrition Surveys of Military Populations and Installations:

- a. Lowry AFB, CO, 13-21 Jul 71
- b. Ft. Lewis, WA, 11 Oct-5 Nov 71
- c. Ft. Myer, VA, 15-26 May 72
- d. ENT-Peterson AFB, CO, Oct-Nov 73

STUDY NO. 2 Calculation of Nutrition Intake of American POWs from Recall Interviews

STUDY NO. 3 Study of the Rangers During Training, Ft. Benning, GA

STUDY NO. 4 Military Nutrition Studies: Biochemical Support

Study No. 1. The nutrition survey studies have all been listed under Study No. 1 of this work unit. The reports of the nutrition survey conducted at Lowry AFB, Colorado; Ft. Myer, Virginia, and Ft. Lewis, Washington are being completed. A survey was completed at ENT-Peterson AFB, Colorado. The data have been coded and keypunched; Statistical analyses are in progress.

Study No. 2. Based on dietary recall, histories obtained by the dieticians at participating US Army and US Navy hospitals, the dietary intakes of returning US prisoners of war have been calculated. Data on 241 POWs have been evaluated for various intervals of incarceration. Nutrient intakes for all men have been calculated for 1190 intake periods.

Study No. 3. A study was conducted to determine whether or not US Rangers in training at Ft. Benning, Georgia, require increased rations. Large body weight losses and significant decrements of work performance

Nutrition Studies in Support of DOD Food Program (Cont)

were observed in the men during phases of the training. These changes appear to justify a 10 to 15% increase in daily calorie allowances.

Study No. 4. An optimized erythrocyte transaminase procedure was utilized on samples obtained from three military nutrition studies. Selected mineral analyses were performed with the use of atomic absorption techniques on serum samples obtained from ENT AFB, CO.

BODY OF REPORT

WORK UNIT NO. 086 Nutrition Studies in Support of
DOD Food Program

STUDY NO. 1-a Lowry Air Force Base, Colorado

PROBLEM:

The effort by all three services to make the service operated dining halls and the military subsistence more appealing to the young military member coincides with the incorporation of many new food items which have not been thoroughly studied for nutritional quality or nutrient content. In addition, the incorporation of new foods and feeding systems has stimulated various concerned elements of the established food production industry, nutritionists and food scientists to question the adequacy of the new feeding systems. To adequately respond to these criticisms more information is required which can be supplied through surveys of DOD units and DOD dining facilities.

The military nutritional standards are designed to provide adequate nutrition to military personnel under normal conditions and to provide energy in sufficient quantities to maintain body weight most conducive to their well-being and health. However, the standards also allow a margin of safety for variations in body size.

RESULTS AND DISCUSSION OF THE RESULTS:

Study No. 1-a. The Lowry AFB survey was of limited scope and was designed to evaluate the acceptability and utilization of short order lines as compared to the standard serving line. The facility surveyed provided support to two training squadrons housed in the same building. The data (Table 1) indicate the "subsistence in kind" personnel (the trainees) utilized the facility as follows: 32% consumed only one meal a day; 42.9% eating two meals; and 25% consuming three meals. The overall utilization by the trainees was 63% for all days and up to 67.3% during the week days. This was a good dining facility and was used once a day by many individuals receiving a ration allowance. The inclusion of this group distorts the picture for the "entire population." An average of 888 total men were observed per day with an average of 778 of them receiving "subsistence in kind." The popularity and percentage of men utilizing various meal combinations throughout the day are depicted in Table 2. More than 45% of the personnel surveyed utilized the short order line for at least one meal during an average day and at least 16% received all their meals consumed in the dining facility from the short order lines. For the "subsistence in kind" group, the same two values are respectively 49% and 17%, which might indicate

Nutrition Studies in Support of DOD Food Program (Cont)

that those persons expected to utilize the dining facilities more throughout the day are more apt to favor the short order line for some part of the day but it may more reflect the eating habits of the youthful students as compared to the cadre who were utilizing the facility as a convenience.

In general, foods such as fruits, fruit juices, soups, gravies, dairy products, jams, jellies, soft drinks, most entrees, cereals, etc. were highly acceptable as demonstrated by fairly low plate wastes. However, items such as assorted cakes, pies, assorted potatoes, breads and most salads and vegetables had fairly high wastes.

CONCLUSIONS:

The daily caloric intake for the students subsisted within this one dining facility was 2265 kcal/day but no information was available to indicate the amount consumed outside of the facility. Caloric intake of meals from the short order line was higher than observed at the regular meals.

STUDY NO. 1-b Ft. Lewis, Washington (See USAMRNL Annual Progress Report, FY 72, for more details.)

PROBLEM:

The introduction of the short order and speciality houses in military dining halls was designed to provide military personnel the opportunity to obtain food items more in keeping with their previous eating practices and habits. At Ft. Lewis, the Centralized Army Feeding System (CAFE) was instituted to evaluate the economics of such a system and to determine the impact of the system upon food acceptability and meal attendance. The CAFE preparation area, a short order house; a specialty house, a conventional dining facility and a dining facility serving both short order and regular food items were surveyed along with a representative sample of the consumers. In addition basic trainees and the supporting dining facility were surveyed but the findings were reported previously.

RESULTS AND DISCUSSION OF THE RESULTS:

The experimental feeding system involved a central food preparation, with distribution of frozen, precooked foods to satellite dining halls for reconstitution (heating) and serving. A centralized scullery for dishwashing was an integral part of the study.

Nutrition Studies in Support of DOD Food Program (Cont)

The average man eating at Ft. Lewis CAFE consumed only 1.57 meals/day during the entire survey period, which represents a utilization rate of 52% based on a 3 meal/day authorization. The daily caloric intakes consumed in the dining facilities averaged 2475 kcal. Intakes of protein, calcium, vitamin A and vitamin C was adequate. See Annual Progress Report for FY 72.

The plate wastes calculated by food groups illustrated that the combination dining hall had, in most instances the highest wastes of any of the 4 dining halls. This could have been due to the great variety of foods served in that dining hall and limited effort at portion control of many items.

The combined waste for all dining halls showed 32 of the food items served had plate wastes above 20%. In all probability this was due to the heavily weighted waste data from the combination house.

CONCLUSIONS:

Despite the many innovations designed to increase dining hall utilization (availability of a large variety of foods for extended hours, remodeling the speciality and short order houses to resemble a restaurant and snack bar, including booths, tablecloths, juke boxes, etc., and serving short order items to order), the attendance did not appear to be greater than that observed on other posts. The daily head counts of all the dining areas indicated that the men ate less than 2 meals/day within the military dining facilities. This may also reflect the fact that even the majority of the enlisted personnel not authorized to draw quarters allowance were living away from the post under the, then new, liberalized pass policy.

STUDY NO. 1-c Ft. Myer, Virginia

PROBLEM:

The primary objective was to evaluate an all civilian catering service being utilized in the Tri-Service Dining Hall at Ft. Myer, VA, where the contractor was responsible for procurement, preparation, serving and clean-up (including waste disposal).

RESULTS AND DISCUSSION OF THE RESULTS:

This dining hall had an elaborate feeding system that was designed to feed up to 2200 individuals/meal period. Ten separate meals were possible. The overall dining hall attendance for the entire study

Nutrition Studies in Support of DOD Food Program (Cont)

indicated that 64.3% ate only one meal/day; 25.4% ate 2 meals/day, and only 8.4% ate 3 meals/day (Table 3). A further breakdown of the data shows that only 1.29% of all individuals ate the three regular meals (breakfast, lunch and supper); 5.29% ate the main breakfast and main supper meals, and 3.07% ate the main breakfast and main supper meals, and 3.07% ate the main dinner and supper meal (Table 4). The small attendance at these meals is due in part to the great variety of other meals being served during the day. However the major reason for low attendance was that the majority of personnel subsisted in this facility were assigned to duties throughout the District of Columbia and could not readily return for mid work-shift meals.

Although the regular breakfast, dinner and supper meals were most popular (Table 4), the attendance averaged only 32% of the total population for the main breakfast meal, 26% for the main lunch and 31% for the main supper. With the exception of the continental breakfast meal, the nutrient intakes based on the consumption of any three meals fulfilled the military allowances. (See Annual Progress Report FY 73.)

Milk intakes were low when compared to previous studies. The highest intakes for any three meals averaged 799 g/day. Although reported on a daily average, milk intake at Ft. Huachuca was 1119 g/day; Ft. Carson, 1150 g/day, and 1378 g/day - Ft. Benning Ranger study. In past studies, milk was readily available but not at Ft. Myer. The discrepancies could be related to the high consumption of carbonated and non-carbonated soft drinks which were available at every meal.

The data suggest fairly poor acceptability of many food items which appeared to be related to the use of leftovers. Although the use of leftovers may have been the contractor's means of economizing, the resulting high plate waste suggesting low acceptability were common. Items with apparent low acceptability included salads, soups, cakes, pies, puddings and Jello.

Edible plate wastes observed in this study were considerably higher than observed in past nutrition surveys at military installations. Frequently, food was prepared too far in advance for the dinner and supper meals and was usually stored in heated ovens and steamers for hours.

The kitchen work appeared programmed to create shortages. In general, "backing up the line" (food replenishing) was slow and portions of the more acceptable food items were small which necessitated going through the serving line again for seconds. The necessity of the

Nutrition Studies in Support of DOD Food Program (Cont)

individual consumer to sign the head count sheet a second time to receive seconds provided an additional source of revenue to the contractor, who was paid on the basis of the head counts.

CONCLUSIONS:

1. There is a major asset in utilizing a catering service to feed military personnel. Military personnel staffing to prepare meals and to support the dining facilities is drastically reduced.
2. In all recent surveys conducted the caloric intake from meals obtained in short order lines, a specialty house or a short order house has exceeded that normally consumed at a comparable conventional meal.
3. Recent surveys indicate that few military members will consume three meals a day within their military dining facility even when it is close by or when maximum effort is made to provide suitable meal hours.

STUDY NO.

1-d

ENT-Peterson AFB, Colorado

PROBLEM:

The objective was to evaluate the nutritional status of the troops and to compare the nutrient intake from the dining halls and to determine the amount and type of food consumed from sources outside the military dining facilities. The utilization of dining hall facilities was also evaluated.

RESULTS AND DISCUSSION OF THE RESULTS:

A nutrition survey was conducted of dining halls located at ENT AFB and Peterson Field, with particular attention given to the nutritional adequacy, quality, and selection of the foods served and the nutritional status of the individuals being served. Additional information was provided by 24-hour recall data and food diaries that indicated the food eaten away from the dining halls. Head counts and plate waste information were also gathered for evaluation. The head count data would provide information as to the number of meals consumed by each individual per day, and the type of meals consumed by these individuals (regular, short order, midnight supper, etc.).

Daily food intake and food waste data from the dining halls have been summarized and are now being evaluated. A sample of personnel receiving

Nutrition Studies in Support of DOD Food Program (Cont)

subsistence-in-kind was selected at random from eight units. The men were interviewed on day 1 of the survey concerning food intake for the previous 24 hours and given diary cards for 7 days. On day 7, another interview verified and completed the food intake information for the seven days and a second set of diary cards was given to the subject which was verified on day 15. In this manner, 15 days of detailed food intake information were obtained for each man.

CONCLUSIONS:

The data from the ENT AFB and Peterson Field nutrition survey have been coded and verified for keypunching. The keypunching has been completed and computer files have been established and are being verified for accuracy. Descriptive statistics and statistical analyses are currently in progress but have been delayed due to problems in conversion of previously available programs to conform to the capabilities of the current computer hardware.

RECOMMENDATIONS:

None.

PUBLICATIONS:

1. Consolazio, C. F., co-author with many others. Evaluacion Nutricional de la Poblacion de Centro America y Panama - Costa Rica. Institute of Nutrition for Central America and Panama, Guatemala and National Institutes of Health, Bethesda, MD. V-28, 1969.
2. Evaluacion Nutricional de la Poblacion de Centro America y Panama - Panama. INCAP, Guatemala and NIH, Bethesda, MD. V-30, 1969.
3. Evaluacion Nutricional de la Poblacion de Centro America y Panama - Guatemala. INCAP, Guatemala and NIH, Bethesda, MD. Guatemala V-25, 1969.

Nutrition Studies in Support of DOD Food Program (Cont)

TABLE 1. Lowry AFB, Colorado Nutrition Survey: Dining Hall Utilization, Percent of Personnel Surveyed.

Meals Eater No.	Entire Population			Subsistence in Kind Group		
	Work days 6	Weekends 2	All days 8	Work days 6	Weekends 2	All days 8
1	32.0	49.3	35.4	27.3	50.5	32.0
2	40.4	46.6	41.4	42.0	45.8	42.9
3	27.4	3.9	23.0	30.5	3.7	25.0
4	0.2	-	0.2	0.2	-	0.1
Mean meals/ man/day	1.96	1.54	1.86	2.02	1.53	1.90
Average daily % utilization	65.3	51.3	62.0	67.3	51.0	63.3

Nutrition Studies in Support of DOD Food Program (Cont)

TABLE 2. Lowry AFB, Colorado Nutrition Survey: Meal Combination Utilization - Percent of Personnel Surveyed.

Meal	Entire Population		Subsistence in Kind Group	
	Work days 6	Weekend 2	Work days 6	Weekend 2
Dinner	10.8	14.6	7.2	15.0
Dinner, supper	9.5	18.8	9.5	16.9
Supper	5.2	14.9	5.5	14.3
breakfast, dinner, supper	8.8	1.3	9.6	1.1
Dinner, *SO supper	5.2	10.4	5.7	11.4
SO lunch, SO supper	5.2	6.3	5.7	6.8
SO lunch	5.0	8.2	5.2	8.6
Breakfast	5.5	3.0	3.7	3.8
SO supper	4.0	8.5	4.7	8.8
SO lunch, supper	4.2	4.9	4.6	5.1
Breakfast, dinner, SO supper	4.7	1.0	5.2	0.9
Breakfast, SO dinner, SO supper	4.6	1.2	5.2	1.2
Breakfast, SO dinner, supper	4.2	0.4	4.7	0.4
Breakfast, SO dinner	2.7	1.2	2.9	0.9
Breakfast, supper	2.6	2.0	2.8	1.8
SO supper, midnight	2.6	-	2.7	-
Breakfast, SO supper	1.7	1.6	2.0	0.9
Dinner, supper midnight	2.3	-	2.7	-
Supper, midnight	1.9	-	2.1	-
Midnight	1.6	-	1.0	-
Dinner, SO supper midnight	1.3	-	1.5	-

NOTE: All other meal combinations were each utilized by less than 1% of the population.

* SO - Short Order

Nutrition Studies in Support of DOD Food Program (Cont)

TABLE 3. Ft. Myer, Virginia Survey: Dining Hall Utilization*.

Percentage eating	Weekdays 8	Weekend days 2	Total for all days 10
1 meal per day	64.1	65.3	64.3
2 meals per day	25.5	24.8	25.4
3 meals per day	8.6	7.9	8.4
4 meals per day	1.5	1.8	1.5
5 meals per day	0.2	0.2	0.2
More than 5 meals per day	0.1	0.0	0.1
Mean meals per man per day	1.42	1.39	1.42
Average daily % utilization	47.3	46.3	47.3

* Includes individuals who had second helpings.

Nutrition Studies in Support of DOD Food Program (Cont)

TABLE 4. Ft. Myer, Virginia Survey: Percent of Personnel Utilizing Various Meal Combinations*.

Meals	Weekdays 8	Weekend days 2	Total for all days 10
Main breakfast	16.8	10.8	15.9
Main dinner	12.7	15.2	13.1
Main supper	13.3	14.4	13.5
Late supper	9.2	5.8	9.7
Midnight supper	2.2	1.9	2.1
Continental breakfast	3.2	1.6	3.0
Short order lunch	2.2	7.3	3.0
Diet lunch**	1.6	-	-
Short order supper	3.7	4.4	3.8
Diet supper**	1.8	-	-
Main breakfast-dinner	2.1	0.7	1.9
Main breakfast-supper	5.9	1.8	5.3
Main breakfast-late supper	2.3	1.1	2.1
Main dinner-supper	2.6	5.8	3.1
Main supper-late supper	1.4	3.0	1.6
Main breakfast-dinner-supper	1.4	0.9	1.3

* All other 2-3 meal combinations showed percentage utilization to be below 1%.

** These meals only available weekdays.

Nutrition Studies in Support of DOD Food Program (Cont)

STUDY NO.	2	Calculation of Nutrient Intake of American POWs from Recall Interviews
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PROBLEM:

This unit was involved in the task of calculating estimates of nutrient intakes of repatriated American POWs (Project Homecoming). Information from recall dietary histories collected by dieticians at the various military hospitals was compiled and analyzed by computer techniques.

RESULTS AND DISCUSSION OF THE RESULTS:

Dietary histories of 241 Army, Navy and Marine Corps personnel were studied, calculated and coded for computer analysis. A total of 1190 dietary periods were evaluated and nutrient intake estimated. It became apparent that the time of capture was a major factor as the prisoners were in captivity for various periods. The longest period of captivity was more than 100 months and the least was only three months. A computer was utilized to break down each man's diet by one month periods with January 1965 as month one. The data in Table 1 show the number of individuals used in calculating the means and percentages of the various nutrient intakes.

In general, the daily diet consisted of two meals of a staple, either rice or bread, or both, a watery soup of vegetables and pork fat, and an occasional side dish. The side dishes were a mixture of vegetables, meat or fruit and many miscellaneous and exotic items. After 1969, a third meal was introduced and milk consumption increased.

Table 5 shows the average daily intake of several nutrients as they changed over time. Most of the increases reflect an increase in the quantity of food although the quality of food also improved during the later years. As can be seen in Table 5, the percent of POWs with diet deficiencies was quite low from 1969 through repatriation.

CONCLUSIONS:

There are many problems with interpretation of these data because many related factors are unknown. Such factors are diseases or gastrointestinal problems, parasite load, exercise or work load and nutrient losses in cooking. These factors plus the long recall period prohibit extensive interpretation of these data. In addition the data derived represent that obtained from the survivors of the captivity and may or may not represent that which would be pertinent

Nutrition Studies in Support of DOD Food Program (Cont)

TABLE 5. Estimated average daily nutrient intake for January of the following years.

NUTRIENT	1965	1967	1969	1971	1973
Number of PWs included	3	57	173	196	247
Energy (kcal)	581	1473	1600	2054	2105
% of PWs consuming <1800 Cal/day	100	75	66	43	47
PROTEIN, gm	14	40	50	70	76
% protein from animal source	23	14	15	24	29
% of PWs consuming <30 gm/day	100	28	18	7	6
FAT, gm	16	33	32	42	51
% fat from animal source	91	78	61	62	60
IRON, mg	4.7	14.5	13.5	15.9	15.1
% of PWs consuming <8 mg/day	66	19	23	13	16
VITAMIN A, IU	3458	9393	9327	11179	11163
% of PWs consuming <1500 IU/day	33	5	11	6	6
THIAMIN, mg	0.29	0.54	0.67	0.85	0.74
% of PWs consuming <0.5 mg/day	100	53	32	14	11
RIBOFLAVIN, mg	0.25	0.63	0.71	1.08	1.24
% of PWs consuming <0.5 mg/day	100	47	27	10	9
NIACIN, mg	6.17	7.04	8.70	11.38	13.37
% of PWs consuming <4.4 mg/day	66	7	10	3	2
VITAMIN C, mg	43	105	92	109	106
% of PWs consuming <15 mg/day	33	4	8	2	3

Nutrition Studies in Support of DOD Food Program (Cont)

Maximal oxygen uptakes were reduced immediately after the restriction or jungle phase, and although these values returned toward normal, they were still significantly reduced after 3 to 4 days, as were heart rates, pulmonary ventilation and oxygen uptakes in ml/kg/min, reflecting reduced work times (or lack of motivation).

CONCLUSION:

Large body weight losses and significant decrements of work performance (as measured by treadmill testing) were observed in the men during Ranger Training. These changes would justify a 10 to 15% increase in daily caloric allowances.

RECOMMENDATION:

Publish a laboratory report substantiating the recommendation for increased ration allowances during Ranger Training.

PUBLICATIONS:

None.

STUDY NO. 4

Military Nutrition Surveys:
Biochemical Support

PROBLEM:

To provide biochemical support to the military nutrition surveys. Additional studies were conducted as an aid in evaluating dietary adequacy of selected trace minerals (Mg⁺⁺, Cu⁺⁺, Zn⁺⁺). Information will also be compiled with respect to erythrocyte GOT as analyzed by an improved spectrophotometric procedure.

RESULTS AND DISCUSSION OF THE RESULTS:

Personnel and analytical support were provided the nutrition study conducted at Ft. Benning, Georgia (pre- and post Ranger Training study) and the nutrition survey conducted at Ft Air Force Base, Colorado during FT 74.

Serum samples from 452 subjects at Ent AFB have been analyzed for magnesium and zinc. Reconstituted chemistry control serum was employed throughout analyses as a quality control. The mean serum magnesium level was 20.1 micrograms/ml with a range of 13.6 to 26.6. Zinc determinations are currently being compiled for computer evaluation, while copper analyses must await A.A.S. equipment reestablishment at LAIR, PSF.

Erythrocyte glutamic-oxalacetic transaminase (EGOT) measurements derived from a revised spectrophotometric procedure were performed on 257 subjects prior to, and 127 subjects upon completion of Ranger

Nutrition Studies in Support of DOD Food Program (Cont)

Training at Ft. Benning, Georgia, and 451 subjects at Ent Air Force Base, Colorado. The EGOT procedure was performed with and without the addition of pyridoxal- PO_4 (P-PO_4) to the assay medium, so that the stimulation coefficient (S.C. = $\frac{4}{1-\text{PO}_4}$) could be calculated as a probable index of vitamin B_6 cofactor enzyme saturation. The results are summarized in the table below.

SUMMARY OF EGOT MEASUREMENTS
Ft. Benning, GA Rangers

<u>Measurement</u>	<u>Before Training</u>	<u>After Training</u>	<u>ENT AFB, CO Personnel</u>
Stimulation Coefficient:			
	1.992 (1.491-2.367)*	1.746 (1.521-2.352)	1.674 (1.368-2.188)
EGOT Activity (I.U./ml Erythrocytes):			
	1.214 (1.298-3.705)*	1.333 (0.857-2.169)	1.050 (0.503-2.099)

*Mean values with range indicated in parenthesis.

The data are currently being correlated with the information obtained from other vitamin B_6 measurements performed on blood and urine samples collected on the same subjects, to evaluate the possible relationships among the various assays. Summarized below are the findings on the vitamin B_6 content of plasma and red blood cells collected during the military nutrition studies conducted at Ft. Benning, Georgia, and Ent AFB, Colorado.

VITAMIN B_6 CONTENT OF PLASMA AND RED BLOOD CELLS
Ft. Benning, GA Rangers

<u>Vitamin B_6 Content (ng/ml)*</u>	<u>Before Training</u>	<u>After Training</u>	<u>ENT AFB, CO Personnel</u>
Plasma	15.1 (132)*	19.4 (134)	18.5 (459)
RBC	13.3 (132)*	22.9 (124)	24.5 (462)

*Total vitamin B_6 content as determined by Sacch. uvarum assay. Numbers in parenthesis indicate number of subjects studied.

Nutrition Studies in Support of DOD Food Program (Cont)

Due to the large number of samples processed, storage time became an unavoidable and disconcerting variable in the vitamin B₆ assays. The need exists for a thorough examination of storage conditions, sample handling, and dietary interrelationship influences upon the various vitamin B₆ assay determinations before any direct conclusions can be drawn from a given set of results. A detailed investigation involving a controlled human vitamin B₆ deficiency-repletion study is essential for a proper evaluation of current vitamin B₆ assays, including EGOT.

CONCLUSIONS:

Analytical support and personnel were provided the military nutrition studies conducted at Ft. Benning, Georgia, and Ent AFB, Colorado. Biochemical data derived from these surveys are tabulated by computer for storage and detailed evaluation.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OB 6800	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISSEM INSTR ^a	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	A. WORK UNIT
10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62760A	3A762760A822		00	155		
b. RESEARCH INDEX	62110A	3A062110A822		00			
c. RESEARCH INDEX	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^a							
(U) More effective topical repellents against malaria bearing mosquitoes (822)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
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				18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
a. DATES/EFFECTIVE: Not applicable EXPIRATION:				PRECEDING			
b. NUMBER: ^a				FISCAL YEAR		74	2.5
c. TYPE:				75		1.5	116.9
d. KIND OF AWARD:				f. CUM. AMT.		75	85.0
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ^a Letterman Army Institute of Research Department of Dermatology Research Presidio of San Francisco, CA 94129			
ADDRESS: ^a				ADDRESS: ^a			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Spencer, T.S., CPT, MSC			
TELEPHONE: 415:561-3600				TELEPHONE: 415:561-5485			
1. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Akers, W.A., COL, MC			
				NAME: DA			
21. REFERENCES (Precede with Security Classification Code)							
(U) wash-off; (U) sweat-off; (U) human volunteers; (U) repellent; (U) mosquito; (U) skin; (U) stratum corneum; (U) polymer formulations							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To discover a long-lasting, water and abrasion resistant, topical repellent formulation which will protect soldiers against malaria-bearing mosquitoes and other vectors of militarily important diseases. To develop <u>in vitro</u> test methods to determine physical properties of repellents and repellent formulations which are tested in the <u>in vivo</u> screening program. By correlation of <u>in vitro</u> and <u>in vivo</u> test results, to predict evaporation, penetration, and repellent-skin interactions, to aid in designing new repellents.							
24. (U) Repellents obtained from Army contractors and industry will be evaluated for physical properties and efficacy <u>in vivo</u> . Field trials of candidate repellents will be conducted to evaluate efficacy of repellents against Anopheles mosquitoes. Insufficient subjects to participate in testing might impede progress.							
25. (U) 73 07 - 74 06 Field trials of candidate repellents, conducted at Camp Lejeune, North Carolina, and Gainesville, Florida, indicate that test methods can effectively screen repellents which are more effective in the field than the standard military repellent. The four-site testing technique for screening repellents has been improved to reduce abrasion factors. Human variability in duration and minimum effective dose of repellents has been exhibited for a normal population. A new instrument for measuring the repellent volatility has been placed in operation and volatility has been determined for 9 known repellents at ambient conditions. Seven new repellents or repellent formulations have been evaluated.							

DA

^a Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A762760A822

TASK NO. 00

WORK UNIT NO. 155

More Effective Topical Repellents
Against Malaria Bearing Mosquitoes

The following investigations have been conducted under this work unit:

- STUDY NO. 1 Field trials of candidate mosquito repellents.
- STUDY NO. 2 Normal distribution of repellent protection time against mosquitoes.
- STUDY NO. 3 Moen-Chase guinea pigs as model hosts for mosquito repellent screening.
- STUDY NO. 4 Confirmation of validity of four-site repellent screening method.

Field trials of three candidate repellents showed that laboratory screening methods could predict the relative efficacy of repellents in the field. Laboratory screening of N,N-diethyl-m-toluamide (DEET) has indicated that DEET dry protection time (DPT) could be described by a reproducible normal distribution. Moen-Chase guinea pigs, which are also normally distributed in terms of DPT, have been shown to be a valid model host with DPT's longer than human DPT's. Guinea pigs were found to be difficult to handle in repellent screening tests.

BODY OF REPORT

WORK UNIT 155

More Effective Topical Repellents
Against Malaria Bearing Mosquitoes

STUDY NO. 1

Field Trial of Candidate Mosquito
Repellents

PROBLEM:

To determine if our laboratory results would predict a repellent's relative protection time under actual field conditions, a field study was undertaken to compare three repellents (cyclohexamethylene-carbamide, n-butane-hexamethyleneimine-sulfonamide and tryethylene glycol ether (SRI-6) to the standard military repellent, DEET.

RESULTS AND DISCUSSION OF THE RESULTS:

Preliminary testing was done to determine the feasibility of a field trial method which was found to be satisfactory. Actual field testing at Camp Lejeune, NC, using a four-site technique produced the same relative comparison to DEET as was determined in laboratory tests (Table 1).

CONCLUSIONS:

Carbamide and sulfonamide gave significantly longer protection time than DEET in the laboratory and significantly less bites than DEET under field conditions, while SRI-6 offered protection which was not significantly different from DEET. For the mosquito species and field conditions encountered, the laboratory screening procedure appears to have predicted the field results.

RECOMMENDATIONS:

The four-site and repellent testing technique should be instituted as a screening method which makes more efficient use of manpower.

PUBLICATIONS:

1. Shimmin RK, Bayles SF, Spencer TS, Akers WA, Grothaus RH: Four-site Method for Mosquito Repellent Field Trials. Presented at the National Meeting of the American Mosquito Control Association, Anaheim, CA, February, 1974. (To be published in the proceedings.)

Table 1

Ranking of Repellents: Lab and Field

	<u>LAIR</u>	<u>Camp Lejeune</u>	
	<u>\bar{X} Hrs Protection</u>	<u>Total Bites/Hr 6,7 Aug (0700)¹</u>	<u>Total Bites/Hr 8 Aug²</u>
Carbamide	17.4 ± 5.1*	0*	3*
Sulfonamide	14.3 ± 5.6*	2*	8*
SRI-6	7.8 ± 4.9	15	50
DEET	6.6 ± 1.7	21	66
Application rates	0.31 mg/cm ²	0.48 mg/cm ²	0.31 mg/cm ²
Number of volunteers	8-28	12	12

*Significantly different from DEET at 95% level.

1. 12 hours between time of application and one-hour test period.
2. 9 hours between time of application and one-hour test exposure.

STUDY NO. 2

Normal Distribution of Repellent
Protection Time Against Mosquitoes

PROBLEM:

To determine how repellent efficacy is affected by individual variability, dry protection times against mosquitoes were determined for a closed group of subjects. Information on relative ranking of individuals within the group was necessary for comparison with other physical attributes.

RESULTS AND DISCUSSION OF THE RESULTS:

Two groups of 32 volunteers were tested in February and October of 1973 using DEET at 0.31 mg/cm^2 . Test results in each case delineated a normal distribution of repellent dry protection times (DPT), the typical biological distribution expected from a normal population. Mean DPT's were 6.8 ± 1.9 hours for DEET applied at 0.31 mg/cm^2 in February and 7.1 ± 1.8 hours in October. Furthermore, relative ranks within the profile were the same for volunteers who participated in both tests, and the linear correlation was significant at the 5% level ($r = 0.559$, $N = 14$).

In a year of testing using the four-site method, the one-year mean average DPT afforded by DEET applied at 0.31 mg/cm^2 has been 6.7 hours, which is consistent with the profile tests.

CONCLUSIONS:

As a result of the consistency between the annual DPT average and both profile tests, DEET should be an adequate control test standard in the search for a repellent better than DEET.

RECOMMENDATIONS:

Test subjects should be randomly selected from a volunteer population which exhibits a normal distribution. Test results can then be evaluated more precisely since individual DPT's in a given test can be compared to the position in a known population profile.

PUBLICATIONS:

Spencer TS, Bayles SF, Shimmin RK, Gabel ML, Akeis WA: Interactions between mosquito repellents and human skin. Proceedings of the Ninth Army Science Conference, West Point, NY, 1974.

STUDY NO. 3

Moen-Chase Guinea Pigs as Model Hosts
for Mosquito Repellent Screening

PROBLEM:

To develop an animal host for mosquito repellent screening, the relationship between repellent efficacy on guinea pigs and man had to be determined.

RESULTS AND DISCUSSION OF THE RESULTS:

A testing procedure has been developed which permitted a direct comparison of repellent dry protection times (DPT) for guinea pigs and men. Using this method, we established a direct relationship between DPT's on guinea pigs and humans for the standard Army repellent, DEET.

The repellent used in each of the 5 replicates was DEET (N,N-diethyl-m-toluamide) applied at 0.08 mg/cm² dose rate. This concentration provided enough sensitivity in the test to allow differences to be easily observed. Test subjects, both human and animal, were randomly selected from normal populations. Repellent sites were tested each hour until two bites were received.

Dry protection time (DPT) afforded by DEET to guinea pigs was compared to men in 8 replications over one year. Each replication was on a different day and against a different population of mosquitoes (Table 2).

Standard deviations of mean DPT for 8 populations of guinea pigs appeared to be about twice as great as those for corresponding populations of humans. By pairing results from one replication on men which were tested against the same population of mosquitoes, an F ratio was determined (Table 2). F ratios for any of the 8 comparisons were significant at the 5% level indicating that populations of guinea pigs had the same degree of variation as human populations. Thus, the techniques used on the guinea pigs and men are similar statistically and may be compared directly to one another. A least squares plot of the differences between the mean DPT's for guinea pigs and the mean DPT's for humans versus the mean DPT's for the guinea pigs had an r-correlation of 0.94 which is significant at the 0.1% level.

CONCLUSIONS:

A standardized testing technique has been developed which permits comparing the DPT of a repellent on a guinea pig to the same repellent used on men. It is possible to estimate the mean DPT for DEET at 0.08 mg/cm² for a randomly selected sample of Moen-Chase guinea pigs.

Table 2
 Guinea Pig and Human
 Dry Protection Times in Hours

<u>Replicate</u>	<u>Guinea Pig</u>	<u>Human</u>	<u>Differences</u>
1	5.1 ± 6.3	2.8 ± 3.4	2.4
2	2.5 ± 3.0	1.3 ± 1.6	1.2
3	4.5 ± 4.5	1.5 ± 1.8	3.0
4	4.1 ± 5.0	1.7 ± 2.1	2.4
5	2.9 ± 2.6	1.9 ± 2.3	1.0
6	5.2 ± 1.0	1.9 ± 0.6	3.3
7	5.7 ± 2.3	2.2 ± 1.2	3.5
8	6.0 ± 1.4	2.2 ± 0.8	3.8

RECOMMENDATIONS:

Correlations between human dry protection times against mosquitoes and protection afforded other potential animal models should be carried out to extend the range of available model hosts.

PUBLICATIONS:

Bayles SF, Shimmin RK, Spencer TS, Akers WA: Moen-Chase guinea pigs as model hosts for mosquito repellent screening. Report No. 20, Task Force on Insect Transmitted Disease of the National Program for Dermatology, Department of Dermatology Research, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129, 1974.

STUDY NO. 4

Confirmation of Validity of Four-Site
Repellent Screening Technique

PROBLEM:

The significance of possible sources of variation in the four-site screening test method needed to be evaluated.

RESULTS AND DISCUSSION OF THE RESULTS:

A series of tests were carried out to identify sources of variation in the four-site repellent screening test method. (1) Cyclical rhythm of testing was investigated over a 60-hour interval. (2) The size of the test site on an individual's forearm was studied as a function of the area exposed to mosquitoes. (3) The effect of differing photoperiods on the test mosquitoes was studied.

Results show no cyclical effect was observed over a 60-hour period on the dry protection time afforded by DEET. Moreover, variation of the test site area exposed to mosquitoes did not affect repellent protection times determined in the four-site test technique.

Finally, changing the photoperiod of test mosquitoes from 24 hours of light to 8 hours of light and 16 of dark had little effect on test results. The 8/16-light/dark period, however, terminated the experiment at eight hours and prevented differentiation among repellents.

CONCLUSIONS:

The four-site test method for screening repellents has been shown to be effective and economical in terms of the number of man-hours necessary to carry out a screening test. Individual variability in repellent screening has been eliminated as a major factor.

RECOMMENDATIONS:

The four-site screening method for mosquito repellent formulations should be used as a standard method in the search for a better mosquito repellent.

PUBLICATIONS:

Brodell CF, Spencer TS, Akers WA: Evaluation of three mosquito repellent screening methods. Report No. 18, Department of Dermatology Research, Letterman Army Institute of Research, Presidio of San Francisco, CA, 1974.

Spencer TS, Shimmin RK, Bayles SF, Akers WA: Consideration of repellent screening standards. Report No. 20, Task Force on Insect Transmitted Disease of the National Program for Dermatology, Department of Dermatology Research, Letterman Army Institute of Research, Presidio of San Francisco, CA, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6907	74 07 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8. DES'N INSTR ⁶	9. SPECIFIC DATA- CONTRACTOR ACCESS	
73 07 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NC./CODES ⁷		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
						WORK UNIT NUMBER	
a. PRIMARY		62760A		3A762760A822		00	
b. CONTRACTOR		62110A		3A062110A822		00	
c. CONTRACTOR		CARDS 114(8)					
11. TITLE (Precede with Security Classification Code) ⁸							
(U) Studies on blistering produced by mechanical, thermal and chemical agents (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁹							
003500 - Clinical medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
65 03		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE: Not applicable				b. FISCAL YEAR		c. FUNDS (in thousands)	
b. NUMBER: ¹⁰				74		0.0	
c. TYPE:				75		0.2	
d. KIND OF AWARD:						15.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ¹¹ Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ¹² Letterman Army Institute of Research Department of Dermatology Research Presidio of San Francisco, CA 94129			
ADDRESS: ¹³				ADDRESS: ¹⁴			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: ¹⁵ Canham, J.E., COL, MC				NAME: ¹⁶ Akers, W.A., COL, MC			
TELEPHONE: ¹⁷ 415-561-3600				TELEPHONE: ¹⁸ 415:561-5485			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER: ¹⁹			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: ²⁰			
				NAME: ²¹ DA			
22. KEYWORDS (Precede each with Security Classification Code) ²²							
(U) friction; (U) blisters; (U) prevention; (U) feet; (U) skin; (U) blistering machine; (U) human volunteers							
23. TECHNICAL OBJECTIVE, ²³ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To study the epidermis, its chemical nature and physical properties when subjected to frictional injury in order to find ways to raise the threshold of susceptibility to skin frictional injury and reduce the disability and sequelae produced from blisters incurred by soldiers on long marches.							
24. (U) Experimental friction blisters by linear and twist rubbing will be used as a model of studying the prevention and treatment of friction blisters.							
25. (U) 73 07 - 74 06 Consultant and contractor attempts to construct a strain gauge to permit calibration of the friction blister rubbing machine have failed to date. We aided U.S. Air Force dermatologists in the conduct of a retrospective and prospective study on the incidence and morbidity from blisters during Air Force recruit training. Preliminary data from the retrospective study revealed of each 100 recruits, 9 had to be recycled in their training and one hospitalized from inability to perform training or because of infection of friction blisters on the feet. Laboratory studies will commence when the strain-gauge problem to measure 2000-3500 gm-cm torque is solved.							

*Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A762760A822

TASK NO. 00

WORK UNIT NO. 157

Studies on Blistering Produced by
Mechanical, Thermal and Chemical
Agents

In seeking ways to prevent friction blisters on the soldier's hands and feet and finding better treatment methods when they occur, we conducted studies on friction blisters in volunteers using twist and linear rubbing machines. With the basic studies completed, we are ready to search for friction reducing materials for boots, socks, and man's skin, but we need accurate measurements of the forces involved. Progress has been halted because our consultants have not been successful in incorporating a 4 mm strain gauge in the rubbing head of our machine that was capable of measuring up to 3500 g/cm of torque.

BODY OF REPORT

WORK UNIT NO. 157

Studies on Blistering Produced by
Mechanical, Thermal and Chemical
Agents

PROBLEM:

Friction blisters, often considered trivial, produce significant man-days lost from pain and secondary infection especially among recruits in training, and even in experienced soldiers who must march several miles during hot weather. We have developed machines to produce friction blisters on the heels and palms of volunteers and have published results on ascertaining frictional blistering thresholds, effect of moisture, blister fluid chemistry, histopathology, healing, treatment, and some physiological studies of blisters in man.

Our next effort concerns a search for footwear materials to reduce friction against the skin of men's feet. This requires accurate mensuration of the shear and torque forces involved. Our Mark VII friction blister machine can not be calibrated dynamically to provide data accurate enough for statistical analysis. Moving only 0.5 cm on a man's heel presents a different skin with its own unique frictional characteristics. We have demonstrated differences between similar sites on the right and left heels. Over the past 2 years, our consultants and their contractors have failed to produce a strain gauge to permit calibration of the head of our friction blister machine where it rubs against the skin. Several other calibration devices including a torque arm and a pony brake failed to give a reproducible calibration. The rubbing machine has a constant speed rotary motor with a crank to produce a to-and-fro motion to simulate the forces against the skin on walking.

RESULTS AND DISCUSSION OF THE RESULTS:

We aided U.S. Air Force dermatologists in the conduct of a retrospective and prospective study on the incidence and morbidity from blisters during Air Force recruit training. Preliminary data from the retrospective study revealed of each 100 recruits, 9 had to be recycled in their training and one hospitalized from inability to perform training or because of infection of friction blisters on the feet.

RECOMMENDATIONS:

Our consultant believes he can fabricate the necessary small strain gauge 4 mm in length to measure up to 3500 g/cm of torque.

Studies on Blistering Produced by Mechanical, Thermal and Chemical Agents (Cont)

PUBLICATIONS:

Brown RN, Blase GL, Akers WA, Griffin TB: Studies on experimental friction blister, II. A device to produce and measure friction blistering, Report No. 22, Letterman Army Institute of Research, Presidio of San Francisco, 21 June 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OR 6815	74 07 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. DISB'N INSTR' ^a	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM A. WORK UNIT
73 07 01	D. CHANGE	U	U	NA	NI	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES: ^a		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
a. PRIMARY		62760A		3A762760A822		00	
b. PROJECTOR		62110A		3A062110A822		00	
c. PROJECTOR		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ^a							
(U) Clinical evaluation of alpha-cyanoacrylates and treatment of friction blisters (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^a							
003500 - Clinical medicine, 012600 - Pharmacology							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
68 05		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE: Not applicable				b. FISCAL YEAR		c. FUNDS (in thousands)	
b. NUMBER: ^a				74		0.08	
c. TYPE:				CURRENT			
d. KIND OF AWARD:				75		0.1	
e. AMOUNT:						1.0	
f. CUM. AMT.						10.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ^a Letterman Army Institute of Research Department of Dermatology Research Presidio of San Francisco, CA 94129			
ADDRESS: ^a				ADDRESS: ^a			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Akers, W.A., COL, MC			
TELEPHONE: 415:561-3600				TELEPHONE: 415:561-5485			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Oestereich, O.C., COL, MS			
				NAME:			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) isoamyl-cyanoacrylate; (U) blister therapy; (U) topical							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To develop a method of therapy for friction blisters of the feet which can be used by the soldier in the field to reduce ineffectiveness, pain, infection, and to promote healing.							
24. (U) Clinical evaluation of the cutaneous reactivity and sensory response to the topical application of cyanoacrylate homologues on experimental friction blister bases.							
25. (U) 73 07 - 74 06 A collaborative clinical trial with U.S. Air Force dermatologists at Lackland Air Force Base was developed to compare treatment of raw friction blister bases on the feet with isoamyl cyanoacrylate to standard therapy. To estimate the population sample size needed, a survey of the incidence of foot blisters and the complications in recruits was necessary (work unit 157). Hopefully, the large clinical trial to test cyanoacrylate efficacy will be done in the coming year.							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A762760A822

TASK NO. 00

WORK UNIT NO. 160

**Clinical Evaluation of alpha Cyano-
acrylates and Treatment of Friction
Blisters**

A bandage to be used by the soldier in the field for raw blister bases, abrasions, and scratches to lessen pain and the chances of infection is being sought. Isoamyl cyanoacrylate, a liquid plastic material, has performed well in the laboratory and 2 small field studies. A definitive clinical trial is planned.

BODY OF REPORT

WORK UNIT NO. 160

Clinical Evaluation of alpha Cyanoacrylates and Treatment of Friction Blisters

PROBLEM:

We are seeking a field treatment for blisters and minor skin injuries on the feet of soldiers. The present antibiotic ointment-bandage treatment method is not carried to the field by soldiers and must be applied several times a day. The liquid tissue adhesive, isoamyl cyanoacrylate, upon one application to the raw blister base leaves a thin plastic film that reduces pain, permits walking, adheres for 4 to 6 days, and apparently reduces the chance of infection in laboratory and 2 small field trials. We are planning a collaborative study with the U.S. Air Force at the Lackland Air Force Base recruit training center to compare the present treatment of blister to isoamyl cyanoacrylate.

RESULTS AND DISCUSSION OF THE RESULTS:

To determine the population sample size necessary to compare the two treatments a survey of foot blisters and their complications was accomplished by 2 Air Force dermatologists from Wilford Hall Air Force Hospital under our guidance and advice. The results will be reported by them.

RECOMMENDATIONS:

When a date for the treatment study is determined the formal request to conduct the study will be made to the Army Investigational Drug Review Board, and appropriate Army and Air Force offices.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OA 6912	74 07 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ^b	6. WORK SECURITY ^b	7. REGRADING ^c	8. DES'N INS N ^d	9. SPECIFIC DATA- CONTRACTOR ACCESS	10. LEVEL OF SUP A. WORK UNIT
73 07 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NO./CODES ^e	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62760A	3A762760A822	00	162			
b. CONTINUING	62110A	3A062110A822	00				
c. CONTINUING	CARDS 114(f)						
11. TITLE (Precede with Security Classification Code) ^g (U) Studies on the effects of heat & humidity upon the human skin with particular emphasis on prickly heat & consequent disfiguring dermatoses (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ^h 003500 - Clinical medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
65 08		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. FUNDS (In thousands)	
a. DATES/EFFECTIVE: Not applicable				PREVIOUS			
b. NUMBER: ⁱ				FISCAL YEAR		74	
c. TYPE:				CURRENT		1.0	
d. KIND OF AWARD:						96.9	
e. AMOUNT:				75		1.0	
f. CUM. AMT.						45.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^j Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ^j Letterman Army Institute of Research Department of Dermatology Research Presidio of San Francisco, CA 94129			
ADDRESS: ^k				ADDRESS: ^k			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^l Akers, W.A., COL, MC			
TELEPHONE: 415:561-3600				TELEPHONE: 415:561-5485			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Schmid, P., Ph.D, DAC			
				NAME:			
				DA			
22. KEYWORDS (Precede EACH with Security Classification Code) (U) miliaria; (U) prickly heat; (U) rash; (U) sweating; (U) heat retention; (U) stratum corneum; (U) skin; (U) heat fatigue							
23. TECHNICAL OBJECTIVE, ^m 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To find the casual mechanism, means of prevention, treatment, and the inter-relationship of prickly heat rash (miliaria) to disturbances in sweating (hypohidrosis) due to heat retention and heat fatigue in soldiers.							
24. (U) Volunteers prone or resistant to experimental prickly heat and subsequent decrements in sweating are characterized regarding skin bacteria, skin surface fats, skin pH, and hydration dynamics of the horny cell layer.							
25. (U) 73 07 - 74 06 No progress has been made due to shortages of assigned dermatologists to conduct the studies.							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A762760A822

TASK NO. 00

WORK UNIT NO. 162

Studies on the Effects of Heat and Humidity upon the Human Skin with Particular Emphasis on Prickly Heat and Consequent Disabling Dermatoses

No investigator has been available to assign to this project. Prickly heat rash (miliaria) produces severe pruritis hypohidrosis lasting 3 weeks and which interferes markedly with body cooling especially in tropical climates. Miliaria is produced by placing an occlusive, pliable plastic film intimately against the skin for 48 hours. No effective therapy has been found to restore sweating rapidly, and prophylactic agents that work would be impractical or unacceptable to troops.

BODY OF REPORT

WORK UNIT NO. 162

Studies on the E-fect of Heat and Humidity upon the Human Skin with Particular Emphasis on Prickly Heat and Consequent Disabling Dermatoses

PROBLEM:

Besides robbing a soldier of sleep from incessant itching, prickly heat rash (miliaria) produces a profound hypohidrosis that persists for 2 to 3 weeks after the rash has disappeared. Experimentally induced miliaria involving as little as 20% of the body surface causes severe heat retention problems in soldiers performing in a tropical environment. We have found no effective treatment to restore normal sweating in less than 7 days. The only preventive measures are dehumidification and topical anhydrous lanolin, 2 of its constituents, chloramphenicol, and neomycin. Maintaining the acid pH of the skin by various substances lessens the severity and the consequent hypohidrosis of the individual miliaria.

RESULTS AND DISCUSSION OF THE RESULTS:

We have reached an impasse in finding prophylactic and therapeutic agents. Hopefully, work on the physical chemistry of stratum corneum (Work Unit No. 164) will provide new leads. We have not had an investigator to assign to the miliaria project.

RECOMMENDATIONS:

We expect to have an investigator assigned in August 1975 to work on the project.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ³	2. DATE OF SUMMARY ³	REPORT CONTROL SYMBOL	
				DA OB 6912	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY ¹	4. KIND OF SUMMARY	5. SUMMARY SCTY ⁴	6. WORK SECURITY ⁴	7. REGRADING ⁵	8A. DISEM INSTR ⁶	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM A. WORK UNIT
73 07 01	D. CHANGE	U	U	NA	NL	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	
10. NC	DES. ⁷	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER		
A. PRIMARY		62760A	3A762760A822	00	163		
B. CONTINUING		62110A	3A062110A822	00			
C. CONTINUING		CARDS 114(f)					
11. TITLE (Precede with Security Classification Code) ⁸ (U) Role of skin lipids in prevention and control of infectious disease in military personnel							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ⁸ 002300 - Biochemistry, 003500 - Clinical medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
68 07		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
A. DATES/EFFECTIVE: Not applicable				PRECEDING			
B. NUMBER: ⁹				FISCAL YEAR		b. FUNDS (in thousands)	
C. TYPE:				74		1.0	
D. KIND OF AWARD:				75		59.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ¹⁰ Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ¹⁰ Letterman Army Institute of Research Department of Dermatology Research Presidio of San Francisco, CA 94129			
ADDRESS: ¹⁰				ADDRESS: ¹⁰			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ¹¹ Schmid, P., Ph.D.			
TELEPHONE: 415:561-3600				TELEPHONE: 415:561-5485			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code) ¹² (U) fungal infections; (U) water immersion; (U) soldiers; (U) biochemistry; (U) lipids; (U) fatty acids; (U) glycerides							
23. TECHNICAL OBJECTIVE, ¹³ 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To determine and compare the composition of skin lipids in soldiers under normal and adverse conditions, such as before, during and after fungal infection, before and after prolonged water exposure, etc. To test or develop more effective therapeutic agents for the treatment or prevention of skin diseases.							
24. (U) The project will be developed in two areas: 1) develop improved techniques to determine the composition and structure of minute quantities of skin lipids; 2) apply the evolved techniques to study bacterial and mycotic diseases in military populations.							
25. (U) 73 07 - 74 06 The basic high performance liquid chromatography system is operational and has been improved by installation of a new sequential control system. Improvements were proceeding in several directions with the goal of better resolution, faster analysis and separations now considered 'impossible.' Quantitative infrared spectroscopy has been improved and computer aided data manipulation added to reduce time consuming calculations.							

DA

*Available to contractors upon originator's approval

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A762760A822

TASK NO. 00

WORK UNIT NO. 163

Role of Skin Lipids in Prevention and Control of Infectious Disease in Military Personnel.

The following investigations have been conducted under this work unit:

STUDY NO. 1 Computer Calculations of Lipid Concentrations From Integrated Band Intensities in the Infrared.

STUDY NO. 2 High Performance Liquid Chromatography of Lipids.

STUDY NO. 3 Precision Flow Rate Measurement in Liquid Chromatography.

STUDY NO. 4 Improvement and Automation of Solvent Changing in High Performance Liquid Chromatography.

Gains in productivity through semi-automation or automation of laboratory procedures is important by itself but especially so in this laboratory because of the high turnover rate of personnel. Improvements in the analysis of complex skin lipids are proceeding in several directions but all with the goal of better separations and smaller sample sizes. This is essential for the study of the role of skin lipids in prevention and control of infectious disease in military personnel. Analysis of skin lipids collected during previous fungal studies has begun.

BODY OF REPORT

WORK UNIT 163

Role of Skin Lipids in Prevention and Control of Infectious Disease in Military Personnel.

STUDY NO. 1

Novel Data Acquisition and Computer Calculations of Lipid Concentrations from Integrated Band Intensities in the Infrared.

PROBLEM:

Quantitation of lipids by infrared analysis, especially quantitation of impure lipids, can be performed more accurately with more selectivity and with smaller quantities than is now possible with microgravimetric techniques. Calculations are very time consuming if the primary data obtained from the spectrometer have to be used.

Band intensities, so far, have been obtained by well known methods such as those of Wilson and Wells or Ramsay. A modified Ramsay method is useful but depends on certain assumptions. For instance, the absorption curve is assumed to follow a Lorentz curve. Because of manpower limitations, much of the tedium and much work can be saved if modern data acquisition methods and computer calculations are used. This is especially true if infrared spectra of crude lipids are analyzed because corrections due to interfering compounds can then easily be made.

RESULTS AND DISCUSSION OF THE RESULTS:

The following new equipment and procedures were developed:

a. A scale expansion for modifying the secondary analog output from the existing infrared spectrometer was installed. This addition enables us to modify the sensitivity of the intensity of the difference spectrum at will over a wide range.

b. The analog signal was then modified by an integrating analog to digital conversion technique and the digital output from the intercoupler transmitted to an ASR 33 teletypewriter. The intercoupler allows recording of three-digit line numbers for series identification. The time interval between successive multiple readings can be chosen and optimized to the scanning speed of the spectrometer. Additional external signal controls including the formatting for number of observations per line number were installed. Digital data were recorded on paper tape during the scanning of an infrared spectrum. With proper calibration tapes for known lipids, spectra of unknown lipids were recorded and processed off-line with our

Hewlett-Packard 9820 system. The data collection system is fully operational. The data acquisition system was built around commercially available components and the design is such that it can be useful for data acquisition of almost any analog signal such as that from a pH meter.

c. Transmittance values of each individual data point recorded were formulated and corrected since deviation from Lambert-Beers law were observed. Corrections were made using conventional polynomial curve fitting techniques. Subroutines for corrections of transmittance values due to interference from non-lipid components were written and the necessary subroutines incorporated into the overall computer program.

d. Several troublesome but minor problems in sample handling were overcome and new and more resistant cells to chemical attack are now routinely used.

CONCLUSIONS:

Our current infrared techniques for quantitation of lipid samples have been greatly simplified, made more efficient and analysis can be done with less highly trained personnel. The results are calculated automatically in off-line mode using our minicomputer.

RECOMMENDATIONS:

Spectra of a number of skin lipid samples, collected during previous fungal studies, were recorded on paper tape. A number of these paper tapes await computer evaluation. Many samples previously collected await analysis. Use of the system for lipid studies of the Dermatology program should continue and a number of publications written.

PUBLICATIONS:

Schmid P, Hunter E, Calvert J. Extraction and purification of lipids: II. Why is chloroform-methanol such a good lipid solvent. *Physiol Chem Phys* 5:141, 1973.

Schmid P, Hunter E, Calvert J. Extraction and purification of lipids: III. Serious limitations of chloroform and chloroform-methanol in lipid investigations. *Physiol Chem & Physics* 5:151, 1973.

Schmid P, Calvert J, Steiner R. Extraction and purification of lipids: IV. Alternative binary solvent systems to replace chloroform-methanol in studies on biological membranes. *Physiol Chem & Physics* 5: 157, 1973.

STUDY NO. 2

High Performance Liquid Chromatography
of Lipids.

PROBLEM:

Spectacular advances in fundamental understanding of the chromatographic processes have been made during the past five years in many laboratories including our own. Improvements are proceeding in several directions but all with the goals of better resolution, faster analysis, smaller sample sizes and separation of mixtures now considered "impossible." In this study, improved separations of very small amounts of exceedingly complex skin lipids are investigated.

RESULTS AND DISCUSSION OF THE RESULTS:

We have recognized the importance and usefulness of microparticle liquid chromatography columns and have prepared adsorbents and ion-exchange resins for a number of years. It becomes increasingly clear that not only the adsorbents and ion-exchange resins but the packing of the column influences reproducibility of retention time and resolution of the separations. Within the last 18 months, several resins and adsorbents as well as packed columns have become commercially available. No reports on use in lipid analysis were available. Several of these packings were tested and found not to be superior to our currently used agents and columns.

In order to reduce analysis and quantitate the goodness of resolution, several improvements in our computer programs were made.

CONCLUSIONS:

A limited number of experiments did not conclusively prove that expensive commercial column packings for high performance liquid chromatography were superior to our presently used packings.

RECOMMENDATIONS:

Improvement of high speed, high performance chromatography is very important, not only for the studies of the dermatology program, but for medical research. This work should continue and chromatography of lipids already collected in a number of fungal studies initiated.

PUBLICATIONS:

None.

STUDY NO. 3

Precision Flow Rate Measurement in
Liquid Chromatography.

PROBLEM:

Knowledge of the flow rate of a liquid during high performance liquid

chromatography is very important. Changes in flow rate very markedly affect retention time and resolution in the chromatography of complex biological samples. In the past, flow rates were measured by simple, unsophisticated techniques on an intermittent basis. Nevertheless, this required full time use of a technician and valuable material was lost during the procedure.

RESULTS AND DISCUSSION OF THE RESULTS:

A new, low-cost detector was purchased. It is easy to use, has digital read-out and can be used on-line during chromatography. Although its accuracy and reliability was well proven in other laboratories, the instrument did not work well in our laboratory, but worked well at the manufacturer's plant. In consultation with our electronics consultant, it was finally established that high frequency noise due to line transients, electric ground, and people tending the equipment were the probable source.

Since similar difficulties had been experienced with other laboratory equipment, the problem needed to be investigated. At the suggestion of our electronic consultant, various voltage regulators, frequency filters and constant voltage transformers were tried to eliminate the problem. These devices proved satisfactory at times but not consistently. We have purchased a novel type of ultra-isolator which has been proven successful in computer installations plagued by similar problems. Installation is pending.

CONCLUSIONS:

A well proven, reliable instrument did not work in our institute because of transients injected over the AC-lines, personnel, etc. We are confident that the problem can be corrected shortly.

RECOMMENDATIONS:

N.A.

PUBLICATIONS:

None.

STUDY NO. 4

Improvement and Automation of Solvent Changing in High Performance Liquid Chromatography.

PROBLEM:

Competent operation of our high speed liquid chromatography system required two technicians in the past. One technician was needed to control manually sequential solvent changes and other functions. A competent electronic

control system should be able to perform these functions automatically, more reproducibly, and more reliably and eliminate the need of one technician.

RESULTS AND DISCUSSION OF THE RESULTS:

Our continuous market analysis of new trends in instrumentation and techniques for biomedical research suggested that the sequential timer made by HLS Industries, Sunnyvale, CA, was, on a cost-effectiveness basis, the most desirable system. The control system consists of a master timer and a series of timer modules which can be extended as needed. Each timer module can control four independent functions.

The exceptional flexibility suggested that the system will not become obsolete if requirements change. HLS, the electronics consultant at LAIR, and the principle investigator, together designed interfacing of the new system with the current manual system. After time consuming minor changes, the system became operational in late fall of 1973.

CONCLUSIONS:

A novel, automated sequential timing system has been successfully interfaced with the older timer system. Because of time limitations and other priorities, work was temporarily suspended.

RECOMMENDATIONS:

N.A.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ¹	2. DATE OF SUMMARY ²	7. REPORT CONTROL SYMBOL DD-DR&E(AR)636	
				DA OR 6913	74 07 01		
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY ³	6. WORK SECURITY ⁴	7. REGRADING ⁵	8. DISSEM INSTR ⁶	9. SPECIFIC DATA - CONTRACTOR ACCESS <input type="checkbox"/> YES <input type="checkbox"/> NO	
73 07 01	D CHANGE	U	U	NA	NI		
10. NO./CODES ⁸	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER			
a. PRIMARY	62760A	3A762760A822	00	164			
b. SUBPROGRAMS	62110A	3A062110A822	00				
c. SUBPROGRAMS	CARDS 114(8)						
11. TITLE (Precede with Security Classification Code) ⁹							
(U) Physical, chemical characteristics of human stratum corneum (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS ¹⁰							
003500 - Clinical medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
67 07		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE: Not applicable				PREVIOUS			
b. NUMBER: ¹¹				FISCAL YEAR		c. FUNDS (in thousands)	
c. TYPE:				74		1.0	
d. KIND OF AWARD:				CURRENT		44.3	
e. AMOUNT:				75		1.0	
f. CUM. AMT.						39.0	
19. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ¹² Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ¹³ Letterman Army Institute of Research Department of Dermatology Research Presidio of San Francisco, CA 94129			
ADDRESS: ¹⁴				ADDRESS: ¹⁵			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ¹⁶ Spencer, T.S., CPT, MSC			
TELEPHONE: 415:561-3600				TELEPHONE: 415:561-5485			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Rietschel, R.L., CPT, MC			
				NAME:			
22. REVWORDS (Precede EACH with Security Classification Code) ¹⁷							
(U) stratum corneum; (U) absorption; (U) permeability; (U) water; (U) water vapor; (U) chemicals; (U) persistence; (U) skin; (U) human volunteers							
23. TECHNICAL OBJECTIVE, ¹⁸ 24. APPROACH, ¹⁹ 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
23. (U) To define the physical-chemical characteristics of the stratum corneum and its interaction with water, chemicals, UV radiation and environment. These characteristics are fundamental to the etiology of several dermatological disorders caused by exposure of the soldier's skin to the environment and the behavior of topical preparations.							
24. (U) Stratum corneum will be harvested using different techniques. Techniques will be developed to assay the denaturing effects of harvest method, water, ultra violet radiation and chemical exposure in addition to environmental conditions on stratum corneum. Stratum corneum from individuals prone vs. individuals resistant to disabling dermatoses will be evaluated before and after experimental induction of such disabling conditions. The efficacy of protective formulations in preventing hydration and denaturation of stratum corneum will be studied.							
25. (U) 73 07 - 74 06 Effects of heat separation, aqueous trypsin solutions, and cantharidin on hydration of stratum corneum were determined. Results indicate that low concentrations of trypsin should be used in harvesting stratum corneum. The water content of stratum corneum at low relative humidities was shown to be temperature dependent, a contributing causative factor in chapping. Transepidermal water loss was shown to have an inverse correlation with mosquito repellent protection time, which indicates that penetration is a major factor in repellent loss from skin.							

* Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A762760A022

TASK NO. 00

WORK UNIT NO. 164

Physical Chemical Characteristics of
Human Stratum Corneum

The following investigations have been conducted under this work unit:

STUDY NO. 1 Temperature dependence of water content of human stratum corneum in vitro.

STUDY NO. 2 Transepidermal water loss and repellent protection time.

STUDY NO. 3 Spectroscopic study of stratum corneum.

Water content of human stratum corneum has been measured gravimetrically in vitro in relation to relative humidity (R.H.) and temperature. Stratum corneum water content decreased 50% when temperature was lowered from 35° C to 20° C at R.H. below 60%. Temperature dependence decreased with increasing R.H. until there was no detectable temperature dependence at 90% R.H. It was proposed that temperature changes could significantly affect water content and, hence, pliability of skin at R.H. below 60%.

When transepidermal water loss from the forearm was measured using stale air hygrometry, an inverse correlation was found between repellent protection time and transepidermal water loss. The correlation was interpreted as an increase in permeability of the skin to the repellent or an increase in repellent evaporation with increasing transepidermal water loss.

Total ultraviolet emission spectra of hydrated and dry stratum corneum have been studied. The spectra have been shown to result from tryptophan residues in stratum corneum. Phosphorescence lifetime studies of tryptophan in various solvents at 77° K have been measured.

BODY OF REPORT

WORK UNIT NO. 164

Physical Chemical Characteristics
of Human Stratum Corneum.

STUDY NO. 1

Temperature Dependence of Water Content
of Human Stratum Corneum In Vitro.

PROBLEM:

Previous work has indicated the importance of the water content of the skin in protecting the body from environmental assaults such as water, ultraviolet exposure, and chemical irritants. We have studied the relationship between water content of stratum corneum and changes in temperature and observed a previously undescribed dependence of water content on temperature.

A Cahn RG Electrobalance was used to monitor weight gain within a chamber kept at constant relative humidity and controlled temperature. Weight gain of the samples was measured in percent weight gain or mg water per 100 mg of dry stratum corneum. The experiment was so designed that four samples from the same source were monitored at each relative humidity at specified temperatures over the range 5-35° C. Hydration was monitored for ascending and descending temperature changes to eliminate one-way temperature and sensor hysteresis as variables.

RESULTS AND DISCUSSION OF THE RESULTS:

The mean water content of stratum corneum increases in an exponential type curve as relative humidity (R.H.) increases (Figure 1). For lower R.H., weight gain may also increase as absolute humidity increases; however, at higher relative humidities there is practically no increase in weight gain although absolute humidity increases exponentially. Since absolute humidity is a function of temperature, it is more appropriate to investigate the effect of temperature on weight increases. Below 90% relative humidity, increases in water content were observed with increases in temperature. As R.H. approaches 90%, the observed tendency becomes less prevalent.

At relative humidities below 60%, stratum corneum rapidly loses its ability to retain water with decreases in temperature. Heats of reaction show that greater energy is necessary for hydrating stratum corneum below 60% R.H. than at higher R.H. (Figure 2). This indicates that breaking of stronger bonds by water molecules is involved in hydration at low R.H. At high R.H., i.e., above 90%, relatively little energy is required for hydration which would indicate weaker bonds are involved in hydration. The first type of bond corresponds to 10% water molecules hydrating strong bonds between protein molecules of dry stratum corneum to give skin its pliability. The level of hydration observed experimentally at 60% relative humidity and normal skin temperature is approximately 10%. As R.H. is increased, hydration above 10% involves

water molecules which are bound less tightly. This additional hydration at R.H. above 60%, however, is not necessary for normal pliability of skin.

In considering chapping and dry skin conditions, we are concerned with the ability of skin to retain water necessary for pliability and extensibility. At 60% R.H. and below, temperature decreases have a significant effect on this 10% water content. At 60% R.H. and 30° C water content of stratum corneum is only slightly above 10% (Figure 1). For the same R.H. a decrease in skin temperature to 20° C reduces water content to about one-half this value. Thus, lower water content might be significant in the increase of chapping and dry skin conditions during colder months. Lower temperatures and relative humidity during those months decrease the ability of the stratum corneum to retain water and cause significant loss in water content, thereby reducing pliability and extensibility of the skin.

CONCLUSIONS:

The significance of temperature change on dry skin and chapping has been neglected previously, even though it may be an important consideration in understanding these skin conditions.

RECOMMENDATIONS:

Further work on hydration characteristics should be carried out to define how the membrane breaks down under environmental stress in disabling dermatological conditions.

PUBLICATIONS:

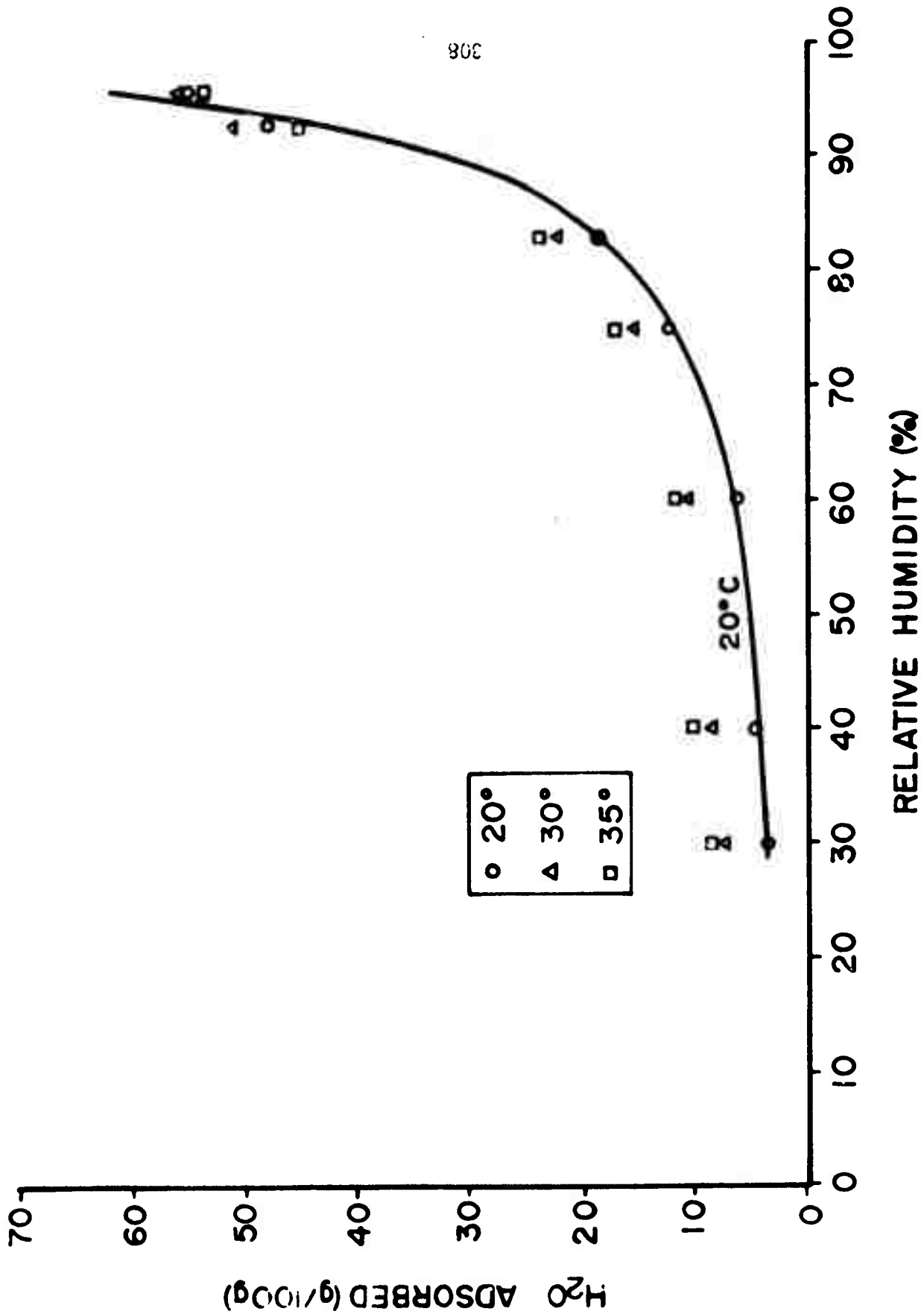
Spencer TS, Linamen CE, Akers WA, Jones HE: Temperature dependence of water content of stratum corneum. Clinical Research XXII (2), 160A, 1974.

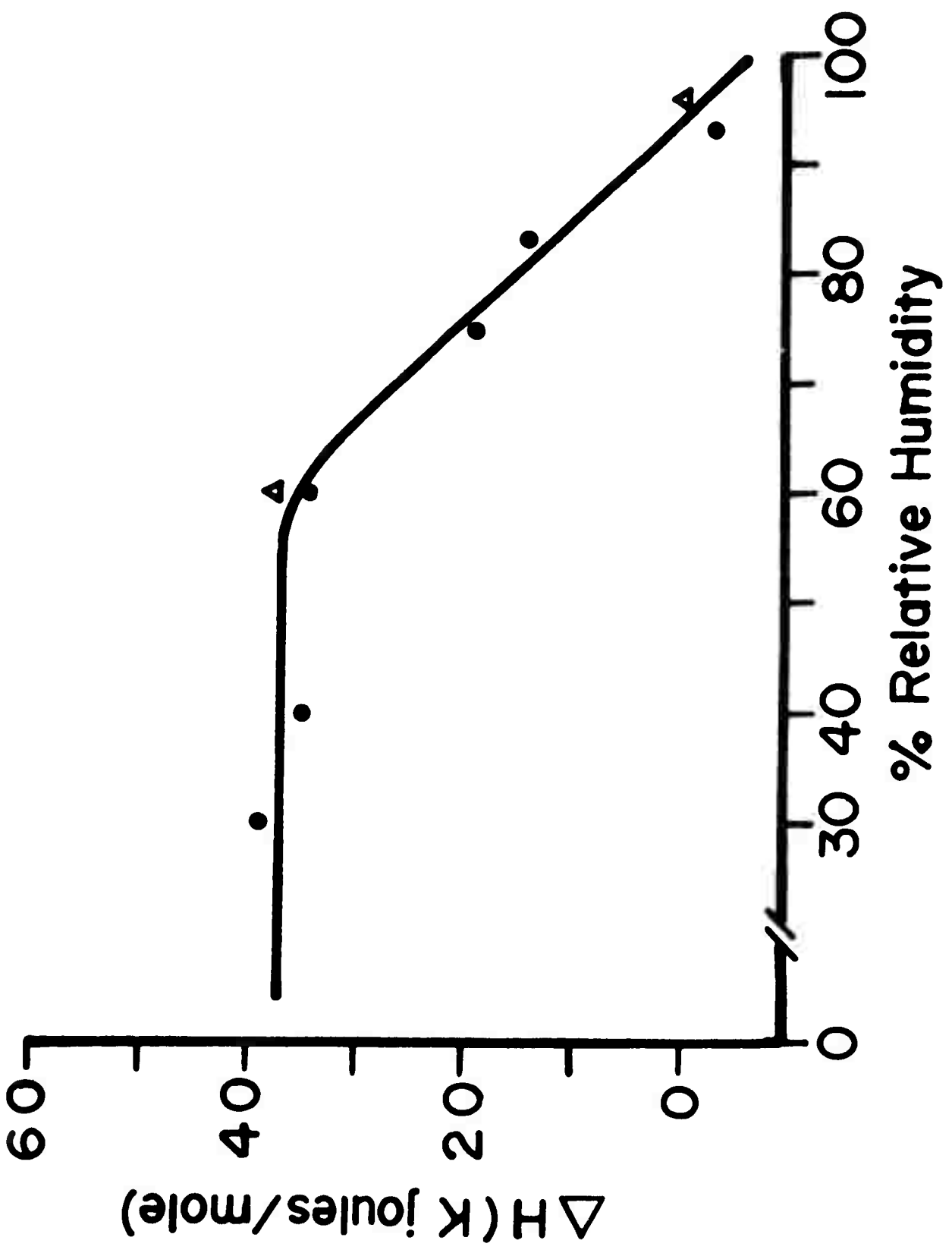
STUDY NO. 2

Transepidermal water loss and mosquito repellent dry protection time.

PROBLEM:

Hydrated skin is known to be more permeable to many compounds. Thus, individuals with higher transepidermal water loss (TEWL) could conceivably have greater stratum corneum permeability, thereby affecting repellent dry protection time against mosquitoes. TEWL was measured by stale air hygrometry, monitoring humidity in a plexiglass box of known volume placed on a subject's skin for approximately 10 minutes. The box contained a wide range humidity sensing element and thermistor both connected to a strip chart recorder. Knowing the area of skin under the hygrometer, the initial and final temperature and relative humidity, TEWL was calculated. This flux rate is expressed as $\text{mg cm}^{-2} \text{hr}^{-1}$. Skin temperature was monitored simultaneously by attaching a thermistor to the adjacent skin with micropore tape. Following this, TEWL was measured using an Aminco water analyzer.





RESULTS AND DISCUSSION OF THE RESULTS:

TWL's of 16 individuals showed an inverse correlation with repellent protection times determined for those same individuals tested on the same day (5% level of significance, $r = 0.588$).

Since individuals were under ambient conditions of 25-26° C during most of the DPT test, sweating was not considered to be a major factor in TWL measurement. Two possible explanations were proposed to explain the inverse correlation between TWL and DPT. First, increased TWL might have increased evaporation of repellent because of a steam distillation effect. Alternately, increased TWL might have indicated a higher water content of stratum corneum and higher permeability to repellent. Increased repellent loss by higher evaporation or penetration would have shortened repellent protection.

CONCLUSIONS:

Previous attempts to correlate individual mosquito attractancy to dry protection time afforded by a repellent have been unsuccessful. This TWL study showed one of the several factors which affect the protection afforded by a repellent, aiding in the search for a more effective repellent. In addition, variability of permeability of stratum corneum among individuals could in part be attributed to the TWL of water content of that individual's skin.

RECOMMENDATIONS:

Repellent formulations which are non-occlusive should be developed since occlusive formulations would tend to increase repellent loss due to penetration.

PUBLICATIONS:

None.

STUDY NO. 3

A spectroscopic study of stratum corneum.

PROBLEM:

The spectroscopic study of stratum corneum emission has centered around the identification and characterization of its luminescent centers. Experimental techniques have been established to study ultra violet emission and excitation spectra and excited state lifetimes of stratum corneum.

RESULTS AND DISCUSSION OF THE RESULTS:

Spectra were recorded at room temperature and at 77° K. Room temperature fluorescence showed a maximum at 335 nm and some small shoulders at 405 nm and 438 nm, all characteristic of tryptophan emission in a protein molecule. Low temperature emission showed a similar fluorescence and a more intense, well-resolved phosphorescence at 410 nm and 435 nm. These are characteristic

of tryptophan in the protein and in the matrix of stratum corneum solution. Triplet lifetimes of stratum corneum and tryptophan in various solvents are in good agreement with literature values (Table 1).

CONCLUSIONS:

The chromophore responsible for stratum corneum emission has been identified as tryptophan. Little or no contribution was attributed to tyrosine or phenylalanine. The fact that tryptophan in stratum corneum may be centered within the protein matrix and not in the cell membranes or lipid matrix makes it a unique and potentially powerful intrinsic environmental probe.

RECOMMENDATIONS:

We should develop the technique of using tryptophan as an intrinsic emission probe for stratum corneum.

PUBLICATIONS:

Spencer TS, Cunico RL, O'Donnell CI: Effect of hydrogen bonding on the emission of N-Heterocyclic compounds. Accepted by Rocky Mountain Spectroscopy Conference, Society of Applied Spectroscopy.

Cunico RL, Spencer TS: Tryptophan emission as an intrinsic environmental probe of human skin. Accepted by Rocky Mountain Spectroscopy Conference, Society of Applied Spectroscopy.

Table 1
 Lifetimes of Stratum Corneum and Tryptophan*

<u>Matrix</u>	<u>Mean lifetime (sec)</u>	<u>sd</u>
Stratum corneum	4.2	0.3
DMSO	6.0	0.3
D ₂ O	6.6	0.7
H ₂ O-MeOH	6.9	0.3
EPA	6.0	0.4

*Spectra at 77⁰ K, excitation 290 nm, emission 440 nm.

Tryptophan in various solvents at 1×10^{-3} M.

RESEARCH AND TECHNOLOGY WORK (R&T) SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL DD-DR&E(AR)636	
3. DATE PREV. SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCY ^b	6. WORK SECURITY ^b	7. REGRADING ^c	8. DISSEM INSTR ^d	9. SPECIFIC DATA - CONTRACTOR ACCESS <input type="checkbox"/> YES <input type="checkbox"/> NO	
73 07 01	D. CHANGE	U	U	NA	NL	A. WORK UNIT	
10. NO./CODES ^e		PROGRAM ELEMENT		PROJECT NUMBER		TASK AREA NUMBER	
A. PRIMARY		62760A		3A762760A822		00	
B. CONTRIBUTING		62110A		3A062110A822		00	
C. CONTRIBUTING		CARDS 114(f)				167	
11. TITLE (Precede with Security Classification Code) ^g							
(U) Skin diseases Among Soldiers							
12. SCIENTIFIC AND TECHNOLOGICAL AREAS							
003500 - Clinical Medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
68 01		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. FUNDS (in thousands)	
A. DATES/EFFECTIVE: Not Applicable				B. PROFESSIONAL MAN YRS		C. FUNDS (in thousands)	
B. NUMBER: Not Applicable				PREVIOUS		66.9	
C. TYPE: Not Applicable				FISCAL YEAR		74	
D. KIND OF AWARD: Not Applicable				75		1.0	
E. AMOUNT: Not Applicable				1.0		57.0	
F. CUM. AMT: Not Applicable				20. PERFORMING ORGANIZATION			
18. RESPONSIBLE DOD ORGANIZATION				NAME: Letterman Army Institute of Research			
NAME: Letterman Army Institute of Research				Department of Dermatology Research			
ADDRESS: Presidio of San Francisco, CA 94129				Microbiology Laboratory			
RESPONSIBLE INDIVIDUAL				Presidio of San Francisco, CA 94129			
NAME: Canham, J.E., COL, MC				PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)			
TELEPHONE: 415:561-3600				NAME: Greenberg, J.H., MAJ, MC			
21. GENERAL USE				TELEPHONE: 415:561-3006			
Foreign Intelligence Not Considered				SOCIAL SECURITY ACCOUNT NUMBER:			
				ASSOCIATE INVESTIGATORS			
				NAME:			
				NAME: DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) occupation; (U) patients; (U) diagnosis; (U) skin; (U) survey							
23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification C.-No.)							
23. (U) To ascertain the frequency and type of skin diseases among active duty soldiers, dependents and retirees seen in dermatology clinics at selected army posts. The relationship between these diseases and possible influencing factors will be sought.							
24. (U) A computer supported data collection in which each dermatology clinic visit at Walter Reed, Fitzsimons, Brooke and Letterman Army Medical Centers is indexed. Starting September 1974, this collection will be expanded to selected dermatology clinics at more isolated posts.							
25. (U) The information on frequency of diseases seen at the four hospitals involved has been summarized for the first year. This covered 63,342 patient visits. The second year's data is almost all collected but is not yet processed. A new data collection card has been planned to be more concise and to include morbidity data.							

^a Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A762759A831

TASK NO. 00

WORK UNIT NO. 167 Skin Diseases Among Soldiers

STUDY NO. 1 Skin Diseases Among Soldiers

This study was an attempt to get at prevalence data for dermatologic diseases amongst military personnel. Data cards were filled out for each dermatological patient seen at Walter Reed, Brooke, Fitzsimons and Letterman Army Medical Center. These cards were keypunched and the information was stored in a computer. The data gave a great amount of information about patients who come to dermatology clinics. It also helped gather patients for clinical studies but it did not give us true epidemiologic information on dermatologic diseases.

STUDY NO. 2 Biochemical Measurements on the Skin Surface with Particular Emphasis on Disabling Dermatoses in Soldiers

The following investigations have been conducted under this study:

- Experiment No. 1 Skin Surface Thermometry
- Experiment No. 2 Specific Ion Concentration at the Skin Surface
- Experiment No. 3 Safeguarding Biological Specimens During Field Studies
- Experiment No. 4 Anthropometric Studies on the Feet
- Experiment No. 5 Hydration at the Skin Surface

In a pragmatic sense, the five experiments demonstrate and test modern systems and techniques useful for field studies of the Dermatology Research Program relevant to current and potential needs of the Armed Forces.

BODY OF REPORT

WORK UNIT NO. 167

Skin Diseases Among Soldiers

STUDY NO. 1

Skin Diseases Among Soldiers

PROBLEM:

Little information has been gathered on the frequency of skin diseases in military and civilian populations seen in dermatology clinics.

Dermatologists recognize the varying skin diseases they see but there is little data pertaining to morbidity and time lost due to these diseases. Also a disease in one soldier with a certain MOS may not cause him to miss duty, whereas the same disease in a soldier with a different MOS may cause extensive hardship.

RESULTS AND DISCUSSION OF THE RESULTS:

Over the last two years information on diseases seen in the dermatology clinics at Walter Reed, Brooke, Fitzsimmons and Letterman Army Medical Center has been tabulated and stored in a computer. This represents 140,000 patient visits. Figures derived from these numbers tell us a great deal about the frequency with which diseases are seen in army dermatology clinics at class II hospitals. They do little to tell us the incidence, prevalence and morbidity of disease in a general military population.

From our first two years of data we have learned the following. The data collection system would best be bolstered by a survey of a military population for all dermatologic conditions, so a true prevalence can be figured. Possibly a more true picture of dermatologic disease experienced by military personnel may be obtained by using our data collection system at isolated class I hospitals. Most diseases seen in Dermatology Clinics cause minimal loss of duty and the main loss of duty is in the time taken for diagnosis and treatment of the condition.

Over these two years we have helped the participating hospitals to a great degree. By keeping a record of patients, by diagnosis we have supplied information for 16 different studies, many of which will generate publications of significant nature. This data bank has become invaluable to army residency programs and we have also supplied information to the National Program for Dermatology.

CONCLUSIONS:

As the Army data collection system was originally adapted it has been of great benefit for clinical research. Thus far it has not

Skin Diseases Among Soldiers (Cont)

led us to the prevalence of dermatologic diseases in the military population or to the loss of duty from these diseases.

RECOMMENDATIONS:

These are threefold. First we feel it may be of more value to take our dermatologic data collection to dermatology clinics at isolated class I hospitals. Secondly, a dermatologic survey on a valid statistical population sample at some of these same posts will then give us prevalence figures. Comparing actual prevalence to the number of cases seen in the dermatology clinic will give us an idea of discomfort or worry caused by the disease. Thirdly, a new data card to include a morbidity rating will be used. This will give us data on MOS related disease and amount of work lost because of specific disease.

PUBLICATIONS:

None.

STUDY NO. 2

Biochemical Measurements on the Skin Surface with Particular Emphasis on Disabling Dermatoses in Soldiers (P. Schmid, Ph.D., Investigator)

EXPERIMENT NO. 1

Skin Surface Thermometry

PROBLEM:

Previous work in this laboratory suggested that skin temperature, moisture content of the skin surface and microbial organisms are related and may result in diverse skin diseases in soldiers. In order to further clarify some of these parameters, a new temperature measurement system was designed and tested.

RESULTS AND DISCUSSION OF THE RESULTS:

Until recently, surface thermometry depended on slowly responding temperature probes connected to low precision needle-type meters. As a result of a commercial market survey, new temperature probes were found that are highly accurate, highly reproducible, and with very fast response times. Interfacing problems to a standard digital volt-Ohm meter were solved using advanced state of the art techniques. Cost of the new instrumentation is several times lower than that of commercially available thermometers. In order to prevent bias of the results in field studies, the digital read-out was chosen in Ohms rather than degrees centigrade.

Computer programs for the H.P. 9820 system were written to process primary data and derived information. The system was successfully tested in the laboratory and used in field trials in Alaska, during which time several hundred measurements were made.

Preliminary analysis of the field data indicated differences in temperature in various areas of the foot between the garrison population and the troops returning from a field exercise in the arctic winter.

CONCLUSIONS:

A highly sensitive temperature measuring system has been successfully field tested in Alaska.

RECOMMENDATIONS:

Analysis of data from Alaska should be completed and the system used in future field studies.

EXPERIMENT NO. 2

Specific Ion Concentration at the Skin Surface

PROBLEM:

Almost no data on pH and chloride of the skin surface of the foot are available for normal populations or for those with skin diseases. It was decided to initiate a program to measure pH at various anatomical sites such as the fibular plantar surface, toe webs, etc.

RESULTS AND DISCUSSION OF THE RESULTS:

A commercially available system was assembled which permits consecutive measurements of the pH and chloride concentration of the skin surface with ion-specific electrodes. Because of projected use of the system in the arctic winter, problems related to winterization were recognized. The influence of temperature and other parameters on the system and electrodes were investigated to safeguard interpretation of collected results.

The system was altered in such a way that untrained personnel could make measurements and check instruments and electrodes under double-blind conditions.

Data collection and retrieval systems were designed so that calculations and correction of data could be performed by computer (after the return of equipment and performance testing) at LAIR.

The system was used in Alaska to determine pH and chloride concentrations on the skin surface of feet of soldiers. At this time, statistical analysis has not been completed.

CONCLUSIONS:

A mobile instrument package to measure skin pH and skin chloride concentration has been successfully field tested in Alaska.

RECOMMENDATIONS:

Analysis of data from Alaska should be completed. Preliminary analysis of data suggest a number of in vitro and controlled in vivo tests to further the understanding of the biochemistry and ecology of the skin surface of soldiers. Baseline studies in moderate climates should be initiated. Other ion-specific electrodes may prove very advantageous and their potential use should be investigated.

EXPERIMENT NO. 3

Safeguarding Biological Specimens
During Field Studies.

PROBLEM:

In field studies, the Department of Dermatology Research and a contractor have found that transportation of biological specimens is a major problem. Specially insulated containers may alleviate some of the problems of extreme temperatures. In the past, maximum-minimum thermometers were used to record extreme temperatures; however, these measurements are inadequate since they do not indicate the duration or profile of the temperature stress. Significant alteration of the viability of biological specimens during transport may not be detected. As a consequence, data obtained from transported specimens may be misleading. In view of the cost and effort of these studies, an accurate record is essential.

RESULTS AND DISCUSSION OF THE RESULTS:

An extensive market survey for a miniature temperature recorder was initiated. An inexpensive recorder was found and tested. It is of the clock-type and does not require batteries or ink pens. The temperature range is from -40° F to +150° F and records over a period of at least 7 days without any maintenance. Time resolution and temperature response were tested under strenuous laboratory conditions and performance was found to be excellent.

CONCLUSIONS:

A very small and inexpensive temperature recorder was purchased and tested for use in containers that are transported to and from field studies. The recorder works well.

RECOMMENDATIONS:

To improve cost effectiveness, temperature recorders should be used whenever biological specimens are transported in future field studies.

EXPERIMENT NO. 4

Anthropometric Studies on the Feet
with the Aid of the Berkemann Imprinter

PROBLEM:

Foot disease in troops was a major problem during the Vietnam war. At that time, it was felt that anthropometric measurements of feet might correlate with certain clinical findings. A Berkemann Imprinter was used in previous field studies and a large number of foot imprints collected. However, no attempt was made to derive qualitative or quantitative information from these records.

RESULTS AND DISCUSSION OF THE RESULTS:

The Berkemann Imprinter was used to prepare imprints of model surfaces and feet under carefully controlled conditions. Numerous parameters such as applied pressure, amount of ink, etc, were investigated. Reproducibility, sensitivity and definition of area were evaluated.

CONCLUSIONS:

Experiments with the Berkemann Imprinter and foot imprints derived thereof suggest that previously obtained records can be analyzed, but accuracy of data derived from such analysis is questionable. In its present form, good anthropometric data cannot be obtained with the Berkemann Imprinter. Future analysis of data already collected may not be warranted.

RECOMMENDATIONS:

In order to salvage data already obtained in previous studies, a small amount of additional work should be done to decide if the study should be continued or terminated.

EXPERIMENT NO. 5

Hydration at the Skin Surface

PROBLEM:

Most of the knowledge on hydration of the skin surface is of two types. In vitro work is based mostly on gravimetric techniques and in in vivo work, outdated hygrometry techniques are used. Both types of measurement are inadequate to obtain new information on the biochemical properties and ecology of the living human skin.

RESULTS AND DISCUSSION OF THE RESULTS:

A new collection system using a new type of micro-container was developed and tested in the laboratory. The containers were used to collect micro samples of superficial skin scrapings from various regions of the foot. Gravimetric techniques were used to assess the state of hydration of the surface layers and lipid content was also measured.

Computer programs for efficient data calculation were written and tested.

The collection system was tested in field trials in Alaska and over 600 samples returned to LAIR. Hydration data have been obtained and calculations made; however, statistical evaluation has not been completed.

CONCLUSIONS:

Preliminary analysis suggests that meaningful data on surface hydration of skin can be obtained with very small quantities of skin samples that can be removed without trauma to human populations.

RECOMMENDATIONS:

Analysis of data should be completed. It appears that the basic system is working but some minor improvements may be needed. After possible modification, future field studies should be initiated to look at potential or real skin problems in military populations subjected to a variety of climates and stresses.

PUBLICATIONS FOR STUDY NO. 2:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION ^a	2. DATE OF SUMMARY ^a	REPORT CONTROL SYMBOL	
				DA OC 6913	74 07 01	DD-DR&E(AR)636	
3. DATE PREV SUMRY ^a	4. KIND OF SUMMARY	5. SUMMARY ACTY ^a	6. WORK SECURITY ^a	7. REGRADING ^a	8. ORG'S INSTN ^a	9. SPECIFIC DATA- CONTRACTOR ACCESS	10. LEVEL OF SUM A. WORK UNIT
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10. NO./CODES ^a	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	62760A	3A762760A822		00	168		
b. CONTRACTOR	62110A	3A062110A822		00			
c. CONTRACTOR	CARDS 114(8)						
11. TITLE (Precede with Security Classification Code)							
(U) The effects of prolonged water exposure on human skin (05)							
12. SCIENTIFIC AND TECHNOLOGICAL AREA ^a							
003500 - Clinical medicine							
13. START DATE		14. ESTIMATED COMPLETION DATE		15. FUNDING AGENCY		16. PERFORMANCE METHOD	
71 04		CONT		DA		C. In-house	
17. CONTRACT/GRANT				18. RESOURCES ESTIMATE		19. PROFESSIONAL MAN YRS	
a. DATES/EFFECTIVE: Not applicable				PRECEDENCE		b. FUNDS (in thousands)	
b. NUMBER: ^a				FISCAL YEAR		74	
c. TYPE:				CURRENT		1.0	
d. KIND OF AWARD:				75		1.0	
e. CUM. AMT.						60.0	
f. CUM. AMT.						55.0	
20. RESPONSIBLE DOD ORGANIZATION				20. PERFORMING ORGANIZATION			
NAME: ^a Letterman Army Institute of Research Presidio of San Francisco, CA 94129				NAME: ^a Letterman Army Institute of Research Department of Dermatology Research Presidio of San Francisco, CA 94129			
ADDRESS: ^a				ADDRESS: ^a			
RESPONSIBLE INDIVIDUAL				PRINCIPAL INVESTIGATOR (Publish SSAN if U.S. Academic Institution)			
NAME: Canham, J.E., COL, MC				NAME: ^a Allen, A.M., LTC, MC			
TELEPHONE: 415:561-3600				TELEPHONE: 415:561-5485			
21. GENERAL USE				SOCIAL SECURITY ACCOUNT NUMBER:			
Foreign Intelligence Not Considered				ASSOCIATE INVESTIGATORS			
				NAME: Rietschel, R.L., CPT, MC			
				NAME: Spencer, T.S., CPT, MSC DA			
22. KEYWORDS (Precede EACH with Security Classification Code)							
(U) immersion foot; (U) cold injury; (U) skin; (U) foot; (U) dermatophytes; (U) water; (U) human volunteer							
23. TECHNICAL OBJECTIVE, ^a 24. APPROACH, 25. PROGRESS (Publish individual paragraphs identified by number. Precede text of each with Security Classification Code.)							
<p>23. (U) To determine the clinical, physical, biochemical and microbiological changes that result from prolonged exposure of the skin to water; and to develop effective methods of preventing warm-climate water immersion injuries. Practical, effective preventive measures could prevent large numbers of "tropical immersion foot" casualties from occurring among ground combat troops operating in warm, wet areas, such as swamps and paddies as well as from accumulated sweat while wearing waterproofed footwear.</p> <p>24. (U) To develop model water immersion injuries in volunteers; to test the effects of changes in pH and other variables such as soaps and creams, in such model injuries; and to study naturally occurring water immersion injuries in troops.</p> <p>25. (U) 73 07 - 74 06. A previously described model immersion injury involving the skin of the back was retested and found to be an unsatisfactory model for warm climate immersion injuries of the feet. An improved method was devised for inducing one type of warm-climate immersion injury (i.e. warm water immersion foot). A field study was conducted among U.S. troops in the Arctic during the winter in order to assess the potential of the vapor barrier boot for producing warm, wet foot injury; no such injury was observed, and the risk of its occurrence was judged to be small.</p>							

^a Available to contractors upon originator's approval.

DD FORM 1498
1 MAR 68

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

ABSTRACT

PROJECT NO. 3A762760A822

TASK NO. 00

WORK UNIT NO. 168

The Effects of Prolonged Water
Exposure on Human Skin

The following investigations have been conducted under this work unit:

STUDY NO. 1 Continuous Exposure of Skin to Water

STUDY NO. 2 Model Immersion Foot Injury

STUDY NO. 3 Skin Conditions in the Arctic

Continuous exposure of human skin to water in small plastic cups produced a mild, transient dermatitis that was slightly greater at 144 than at 72 hours. The experimental injury did not resemble that of tropical immersion foot or other naturally occurring immersion injuries.

Warm water immersion foot (WWIF) was induced in volunteers by affixing a water-filled plastic intravenous fluid bag to the distal half of the foot for 19-43 hours. This method of inducing WWIF offers significant advantages over previously existing methods, including convenience and greater control over the cutaneous environment.

A survey was conducted of soldiers in the arctic winter to determine whether vapor barrier clothing produced measurable adverse effects on the skin of the feet and other susceptible body areas. No cases of "soggy foot" or other potentially disabling skin conditions were seen in troops who had just completed a 7-day field exercise.

BODY OF REPORT

WORK UNIT NO. 168

The Effects of Prolonged Water
Exposure on Human Skin

STUDY NO. 1

Continuous Exposure of Skin to
Water

PROBLEM:

Previous work suggested that a successful model for tropical immersion foot injury could be produced in volunteers by application of water-filled plastic cups to the skin of the back. In order to clarify certain points, the present investigators conducted pilot studies using the same cups in a slightly different manner. When no immersion injury appeared, it was decided to repeat the previous experiments in order to see if the results were replicable.

RESULTS AND DISCUSSION OF THE RESULTS:

Small, water-filled plastic cups were applied to the skin of the backs of 14 healthy young white men and left on continuously for 144 hours (6 days). One half of each man's back had been washed daily with Ivory soap for 14 days prior to application of the cups. Six cups were applied to each subject: two were filled with sterile water, two with water buffered to pH 3.5, and two with water buffered to pH 7.5. A balanced, randomized experimental design was used so that water at each pH was applied to both the Ivory and non-Ivory-washed sides of the back, and each part of the back received water of each pH an equal number of times. The cups were removed for one hour at the end of 72 hours (3 days), during which time the previously immersed area was assigned a clinical score; then the cups were reapplied for 72 hours more (total 144 hours).

Three different clinical scoring systems were used: (1) a 0 to 3+ global assessment system used by the previous investigators, (2) a 0 to 3+ system devised by the present investigators, which graded the severity of each of five clinical signs, and (3) a rank-order system.

Only 4 (5%) of the total of 84 immersed sites had lesions of greater than minimal severity at the end of 144 hours, as defined under grading system number one, and 44 (52%) had no lesions. Typical lesions consisted of slight, nonconfluent erythema and minimal edema.

No consistent relationships between production of lesions or lesion severity and pH or Ivory washing was detected using any of the three grading systems.

On average, lesions were slightly more severe at 144 than at 72 hours, but this did not pertain to every subject.

Hairs were thickly coated with waxy yellowish material at one site in 2 of 14 subjects.

No signs of dermatitis remained 24 hours after removal of the cups.

These results differ substantially from those described by the previous investigator, who stated that "striking inflammation" occurred under virtually identical conditions of water immersion. In this experiment, no subject developed marked inflammation similar to that of tropical immersion foot (TIF), and therefore the use of the water-cup technique to induce model TIF was not validated.

In contrast to the previous description, coating of hairs with waxy yellowish material (clumps of bacteria) was rare rather than common. Also, an apparent association between pH and lesion severity was not confirmed.

CONCLUSIONS:

The use of water-filled cups to induce dermatitis on the skin of the back does not produce a valid model of tropical immersion foot injury. There appears to be no consistent relationship between variations in water pH and Ivory soap washing on the one hand, and rate of induction of lesions or lesion intensity on the other.

RECOMMENDATIONS:

Use of the water-cup system as a model for immersion injury should be withheld at this time.

PUBLICATIONS:

None.

STUDY NO. 2

Model Immersion Foot Injury

PROBLEM:

An exact replicate of Warm Water Immersion Foot (WWIF) is inducible by immersing the feet of volunteers in swamp or swimming pool water for one to two days during the warm months of the year. Although successful, this method is inconvenient, inordinately time consuming, and restricted as to season. Moreover, it is difficult if not impossible to vary the content of the water in accord with an experimental protocol. A more convenient and easily variable method of inducing WWIF would facilitate controlled studies of this condition.

RESULTS AND DISCUSSION OF THE RESULTS:

A flexible, water-tight environmental chamber for the distal half of the foot was devised by cutting the end off of a 1000 ml plastic intravenous fluid bag and putting the bag on the foot like a sock. A water-tight seal was achieved using medical adhesive spray and plastic tape. Tap water was inserted into the inlet tube using a needle and syringe, and air was removed in the same manner. All of the foot's surface enclosed by the bag was in continuous contact with water.

Using this new method, water content could be sampled or varied via the inlet tube, while temperature and other measurements of the skin surface could be obtained via a larger, resealable tube running parallel to the inlet tube. A bag filled with 50 ml of water could be worn inside a standard men's shoe, and paired-foot studies could be conducted with an ambulatory subject.

Five young men wore a water-filled bag continuously on one foot until the soles became painful on walking. Pain developed in from 19 to 43 hours following immersion. At that time the soles had the pale, deeply furrowed appearance typical of WWIF. There were changes in the diameter of the first and second toes and in the temperature of the planter surfaces before and after immersion, but the changes were small and inconsistent. Relief of pain was experienced in from two to six hours after the bags were removed, and the signs disappeared shortly thereafter.

This convenient new method for inducing WWIF removes the time and place restrictions that formerly existed when investigators wished to study this form of immersion injury. It therefore clears the way for further studies concerning pathogenesis and prevention. Among the variables which may be tested using this method are pH, salt content, and microbial flora. With refinements of technique, it may be possible to provide a definitive answer to the question: Is WWIF due simply to hyperhydration of the plantar stratum corneum, or is there change in the deeper tissues as well?

CONCLUSIONS:

A convenient new method for experimentally inducing WWIF has been devised. Use of this method in further studies could provide valuable information concerning the pathogenesis and prevention of this form of warm-climate immersion injury.

RECOMMENDATIONS:

This method should replace previously existing methods for studying experimentally induced WWIF under controlled conditions.

PUBLICATIONS:

None.

STUDY NO. 3

Skin Conditions in the Arctic

PROBLEM:

The standard uniform for field use in the arctic includes a vapor barrier boot. Although this boot provides good protection from the cold, it has the disadvantage of creating an excessively warm and humid environment for the feet. This tropical microenvironment rapidly makes the feet feel itchy and uncomfortable, and it is conceivable that discomfort could increase to the point of disability if the boot is worn continuously for more than a few days. Little information exists about this potential source of disability in soldiers, and experimental studies cannot adequately duplicate the stresses of arctic field conditions.

RESULTS AND DISCUSSION OF THE RESULTS:

Skin disease surveys of soldiers were conducted at Fort Winwright (Fairbanks) Alaska in late February and early March 1974. Included were 79 infantrymen who had just completed a 7-day field training exercise and 100 support troops in garrison (total 179). A total-body clinical examination of the skin was performed. Physical measurements (temperature, pH and chloride), specimens for fungal and bacterial cultures, and stratum corneum specimens for water and lipid analysis were obtained from the skin of the feet.

Little was found which would implicate the vapor barrier boot in the production of potentially disabling "soggy foot" problems in infantrymen operating in the arctic winter. However, it was found that none of the men in the field exercise had worn their boots continuously for more than 24 hours, and that each man changed his socks daily. Consequently, it is doubtful whether this constituted an adequate test of the potential for disability. Commanders and troops uniformly denied the existence of disabling skin conditions other than frostbite.

Dry skin, especially of the upper arms, torso, thighs and ankles, was a common complaint in support troops as well as infantrymen, and approximately 20 percent of the men examined had dry skin conditions of minimal severity. Five soldiers (3%) had mild ringworm infections of the groin. Dermatophytes were recovered from 23 men (13%), of whom nine (39%) had lesions in the groin or toeweb. Of the 26 isolates, 7 (27%) were Trichophyton mentagrophytes; the rest were T. rubrum. There was no evidence of significant colonization of the toewebs by gram-negative bacteria.

The physical measurement data and the stratum corneum specimens have not yet been completely analyzed.

Only one study similar to this has been conducted among U.S. troops in the arctic and the results were similar. The significance of this information lies not in its arresting qualities, but in the fact that it is possibly the first and only information of its kind. A valid test of the vapor barrier boot's ability to induce severe and highly prevalent "soggy foot" conditions will not occur until men are subjected to far more rigorous stresses than those prevalent in these surveys.

CONCLUSIONS:

Latent fungal infections of the feet may become exacerbated under the tropical environmental conditions created by wearing vapor barrier boots; however, these could probably be adequately controlled by use of existing antifungal medications. Disabling "soggy foot" conditions appear to be nonexistent among troops wearing the vapor barrier boot in the arctic.

RECOMMENDATIONS:

If there are verified reports of significant disability resulting from skin conditions attributable to vapor barrier clothing, further studies of cutaneous diseases in the arctic would be warranted. Based on our current observations, such a study does not appear to be warranted.

PUBLICATIONS:

None.

APPENDIX A

PUBLICATIONS - FISCAL YEAR 1974

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PRESIDIO OF SAN FRANCISCO, CA
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(USAMRNL NUMBERED LABORATORY REPORTS)

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345. Kummerlin, A. J., B. L. Wilson, Y. M. Rhodes and J. E. Canham. Three decades of endeavor - a bibliography: 1944-1974. April 1974.

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3. Schmid, P., J. Calvert and R. Steiner. Extraction and purification of lipids: IV. Alternative binary solvent systems to replace chloroform-methanol in studies on biological membranes. Physiol. Chem. Physics 157, 1973.
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APPENDIX B

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