AD

U. S. ARMY MEDICAL RESEARCH & NUTRITION LABORATORY

FITZSIMONS GENERAL HOSPITAL
DENVER, COLORADO 80240

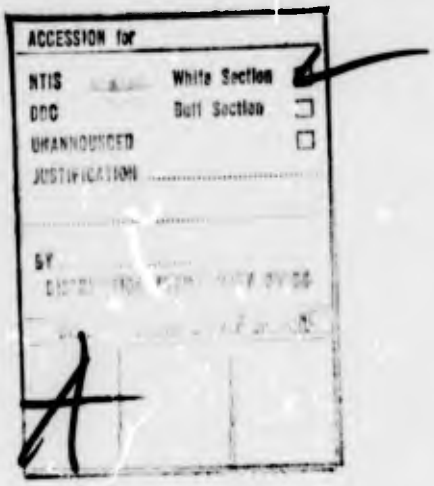
ANNUAL RESEARCH PROGRESS REPORT

(REPORTS CONTROL SYMBOL MEDDH 288 (R1))

30 JUNE 1972

APPROVED FOR PUBLIC RELEASE; DISTRIBUTION UNLIMITED

381



UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author)

U. S. Army Medical Research & Nutrition Laboratory
Fitzsimons General Hospital
Denver, Colorado 80240

2a REPORT SECURITY CLASSIFICATION

2b GROUP

3 REPORT TITLE

U. S. Army Medical Research and Development Technical Report

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

Annual Research Progress Report 1 July 1971 - 30 June 1972

5 AUTHOR(S) (Last name, first name, initial)

6 REPORT DATE	7a TOTAL NO OF PAGES	7b NO OF REFS
30 June 1972		

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10 AVAILABILITY/LIMITATION NOTICES

Approved for Public Release; Distribution Unlimited

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	U. S. Army Medical Research and Development Command Washington, D. C. 20314

13 ABSTRACT

During Fiscal Year 1972 progress was obtained at the U. S. Army Medical Research and Nutrition Laboratory in the following research areas; basic nutritional biochemistry; basic biochemical processes of metabolism; basic and applied nutrition; clinical nutrition; basic and applied aspects of the influence of environment on man; the metabolism of normal man and as altered by disease; work performance of man and military dogs; the microbiology of pulmonary disease; and research computer science. The progress made in this fiscal year is described in the reports of the thirty-five work units presented.

DD FORM 1 JAN 64 1473

UNCLASSIFIED

Security Classification

Reports Control Symbol MEDDH 288 (R1)

US Army Medical Research
and Development Technical Report

FISCAL YEAR 1972

30 June 1972

US ARMY MEDICAL RESEARCH
AND NUTRITION LABORATORY

Fitzsimons General Hospital

Denver, Colorado 80240

FOREWORD

The research conducted at the U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado, was accomplished in Fiscal Year 72 under the following projects and Task Areas:

3A061101A91C - In-House Laboratory Independent Research

3A061102B71P - Basic Research in Support of Military Medicine

01 - Biochemistry

3A061102B71R - Research in Biomedical Sciences

02 - Internal Medicine
05 - Environmental Medicine

3A062110A822 - Military Internal Medicine

3A062110A827 - Military Environmental Medicine

3A062110A830 - Bio Sensor Systems

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

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.

Appendix A, Publications, lists seven USAMRNL Laboratory Reports, sixty papers published in the open scientific literature and twenty-five published abstracts. This does not represent the entire productivity of the Laboratory for FY 72. Additional productivity has been manifested by the many staff members who are active teachers in the intern and residency training program at Fitzsimons General Hospital and the University of Colorado Medical Center and in graduate student education at the University of Colorado and Colorado State University. Many of the staff have been invited participants in national and international scientific seminars sponsored by governmental agencies or private organizations. Training of physicians, veterinary officers, graduate students, medical students on research clerkships, and reserve officers is conducted in-house.

The Annual Research Progress Report for FY 72 undoubtedly will represent the last full year in which maximum research at USAMRNL can be completed with the authorized and assigned staff. The research effort in FY 72 remained high despite the many turnovers in personnel necessitated by National policy. Effective 1 July 1972, the Microbiology Division, including 19 personnel, will be officially transferred to the Clinical Research Service, Fitzsimons General Hospital. Moreover, the impending move of USAMRNL to the Western Medical Institute of Research in FY 73 will impact upon the total amount of research that can be accomplished. Personnel losses are occurring and more can be projected, particularly for personnel in those grade levels where the economic impact of the transfer will be most felt, i.e., wage grade and GS 3 through 9. However, FY 73 will permit completion of a number of studies under the described work units and hopefully, will provide time for compilation, collation, and reporting of data which has been delayed due to ongoing research studies.

The division chiefs, scientific investigators, the technicans and all USAMRNL personnel are to be congratulated for their continually high effort, morale and productivity through the past few years, particularly, in view of the knowledge that this Laboratory was to be transferred. However, such research effort would have been impossible without the outstanding support and guidance provided by the U. S. Army Medical Research and Development Command and the logistical support provided by Fitzsimons General Hospital.

During FY 72 a total of $241,783 of USAMRNL funds were expended for purchase of equipment for installation at the Western Institute of Medical Research - Phase I. This equipment is essential to permit orderly transfer of ongoing research that will be continued in San Francisco. The breakdown of these purchases by project and task area is given below and is reflected in the 1400's for the work units under these projects and task areas.

3A061102B71P Basic Research in Support of Military Medicine	01 Biochemistry	$114,274
3A061102B71R Research in Biomedical Sciences	02 Internal Medicine	54,217
3A062110A822 Military Internal Medicine		72,313
3A062110A830 BIO Sensor Systems		979
		$241,783

Praise and credit for the painstaking attention in summarizing and reporting the research described in this report belongs to the individual investigators and division chiefs, however, additional credit must be given for the assemblage and publication of this report to Mr. Walter O. Keinsley and Miss Elaine Watson, Management Services Branch, USAMRNL.

TABLE OF CONTENTS

Table of Contents (Cont'd)

Table of Contents (Cont'd)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6378	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 04 01	D Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	041
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Wholesomeness Aspects of Military Subsistence (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

006500 Food, 007800 Hyg. & Sanitation, 010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 04	CONT	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	.3	2
FISCAL YEAR CURRENT 73	2.7	8

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J.E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Food Hygiene Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Fowler, James L., COL, VC

TELEPHONE: 303 366 5311 X22223

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

21. GENERAL USE

Foreign Intelligence Not Considered

ASSOCIATE INVESTIGATORS

NAME: Ladiges, Warren C., CPT, VC

NAME: Romer, James C. DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Public Health; (U) Food Poisoning; (U) Microbiology; (U) Bacteriological Techniques; (U) Food

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

23. (U) In the past few years, much interest has been evidenced in the public health aspects of precooked frozen "convenience" foods. The DoD is one of the largest single users of these items. The need for established microbiological specifications or standards is evident due to the highly perishable hazardous nature of frozen convenience foods.

DoD has the ability to develop microbiological specifications under which large quantities of convenience foods are purchased and has the capability of agencies to perform necessary inspections and laboratory testing. In addition, a laboratory testing system is available by which the microbiological quality of these items, in terms of specifications, methodology, and techniques may be investigated. The purpose of this protocol is to perform the latter investigation.

24. (U) Menus of precooked frozen convenience meals, both specification and brand-name, will be analyzed for: a) standard plate count, b) coliform count, c) E. coli count, d) Staphylococcus aureus enumeration, e) fecal streptococcus count, f) Cl. perfringens counts, g) other clostridial counts, h) yeast and mold counts, and i) salmonellae count. In addition, specific identification of isolates will be made in order to obtain insight into the microflora in the food. Data will be expressed as range, arithmetic average, and geometric mean, as applicable. Particular emphasis will be placed on detection of pathogens.

25. (U) Pilot studies to establish initial methodology and media to be used have been accomplished. Initial results indicate widely varying microflora and microbiological population in different menus of brand-name convenience foods purchased by DoD. Specification meals from the latest production are being procured through DPSC, and non-specification products are being selected from local commissary stock. A statistically sound sampling program is being accomplished, but data is to preliminary to summarize.

* Available to contractors upon submittal of approval

DD FORM 1 MAR 66 1498

ABSTRACT

PROJECT NO.	3A061101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	041	Wholesomeness Aspects of Military Subsistence

The following investigations have been conducted under this work unit:

STUDY NO. 1 Microflora of Meals, Precooked, Frozen, and Non-Specification "Convenience" Foods Procured by DoD

STUDY NO. 2 Computerized Microbiological Data Collection Program on Subsistence Items Analyzed by DoD Food Testing Laboratories

The Department of Defense has long been a leader in promoting the mass-feeding concept, and has taken advantage of the numerous technological advances in the field of "convenience" or quick-serve meals. The microbiological specifications developed and used by DoD for monitoring the quality of precooked, frozen meals purchased for its use have done much to insure the quality and safety of this type of food served to armed forces personnel.

Food safety has traditionally been the responsibility of the persons preparing the food. With the advent of widespread use of convenience foods, however, this responsibility has been transferred to the manufacturer or processor. Food hygiene in the preparation of large quantities of meals is far different than that practical in the preparation of conventional meals. Unfortunately microbiological standards, accepted by consumers and producers alike, are still not established.

Investigations of the microbiological quality of specification and non-specification precooked, frozen meals is considered necessary in order to have a firm research data base on which to advise on microbiological specifications and enumeration techniques. Particular emphasis is being placed on detection and enumeration of pathogens.

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 microbiological information available today is not sufficient basis on which regulatory agencies can satisfactorily base microbiological standards. DoD has had microbiological specifications in effect for quite a few years, although many of these have been based on "state of the art" judgment rather than on research data. The military services require and use many products which have no counterparts in the civilian economy. A research effort has been designed to accomplish the following objectives:

A. To investigate the microbiological quality of precooked, frozen convenience foods, both specification and non-specification, being purchased by the DoD.

B. To investigate the complete microbiological flora of Meals, Precooked, Frozen, both specification and non-specification.

C. To investigate the methodology, including technique and media, best suited for enumeration and identification of microbiological flora of frozen convenience foods.

RESULTS AND DISCUSSION OF THE RESULTS:

The results of analyses have varied quite widely on a number of products produced by commercial companies. In general, fish and other marine products have had a higher microbiological burden than other types of meats. Many fruits and vegetables have approached sterility, although others have had a significant number of enterococci and molds. Data on specific organisms are being generated through definitive identification of isolates. Both specification and non-specification products are being analyzed. Information generated is expected to give a basis to formulate recommendations for microbiological standards of precooked, frozen convenience foods.

CONCLUSIONS:

Research in this area has not been in progress long enough to formulate conclusions or recommendations. As data is generated, a firm basis for making recommendations should evolve.

STUDY NO. 2 — Computerized Microbiological Data Collection Program on Subsistence Items Analyzed by DoD Food Testing Laboratories

PROBLEM:

The Department of Defense has within its structure several laboratories which perform testing of food items for procurement purposes and for detection of pathogens potentially dangerous to human health. These laboratories are mainly facilities of the U.S. Army Medical Department, although one large laboratory is operated by the Defense Supply Agency. A large amount of valuable microbiological data is generated by these laboratories, but unfortunately has been lost due to the lack of a centralized tabulating agency. A Computerized Data Collection Program has been developed which provides for the collection of data from the performing laboratories, the tabulation of data into a computerized system, and for the retrieval of data in several forms as required. The data collected is microbiological information of food products other than fresh dairy products, and is divided into procurement testing and testing for special purposes. Information stored is expected to provide a basis for defining problem areas as well as providing a listing of food items tested within the DoD system. The program is a joint effort of the Computer Division and the Food Hygiene Division.

CONCLUSIONS:

A computer program for storage, manipulation and retrieval of data generated from many laboratories performing food microbiological evaluation has been developed.

RECOMMENDATIONS:

The data bank should continually be expanded and statistical programs written to provide the user with information necessary for procurement evaluation and specification development.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6346	72 06 30	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H. Termination	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	049
b. CONTRIBUTING	61130011	3A013001A91C	00	
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)
(U) The Mechanism of Body Temperature Control (06)

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

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07		DA	C In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	1.1	40
FISCAL YEAR CURRENT 72	1.5	33

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240
RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
Metabolic Division
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Herman, R. H., COL, MC
TELEPHONE: 303 366 5311 X25193
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]
ASSOCIATE INVESTIGATORS
NAME: Hagler, L., LTC, MC
NAME: DA

21. GENERAL USE
Foreign Intelligence not Considered

22. KEYWORDS (Procede EACH with Security Classification Code)
(U) Heat Stress; (U) Body Temperature Regulation; (U) Heat Production in Exercise; (U) Temperature; (U) Fever; (U) Etiocholanolone

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

23. (U) Heat stress causes ineffectiveness, disability or death in military personnel during basic training or combat operations. A significant proportion of military ineffectiveness is caused also by febrile illnesses which often require prolonged hospitalization and extensive diagnostic study. This is especially true of the medical entity, fever of unknown origin. The regulation of body temperature by appropriate therapeutic agents to prevent or minimize the adverse effect of heat production would materially enhance the effectiveness of basic training and combat operations by postponing fatigue, reducing the time required for heat adaptation and preventing heat injury. These therapeutic agents may also reduce the morbidity and ineffectiveness seen in a variety of febrile illnesses. Knowledge of the mechanism of body temperature control would permit the design of such therapeutic agents and enable the physician to effectively diagnose and treat fevers of unknown origin.

24. (U) The effect of various therapeutic agents on fever induced by pyrogenic substances in normal human volunteers have been studied. Selected patients with thermoregulation defects have been investigated with regard to returning temperature regulation to normal and in determining the site of the defect in the regulating mechanism.

25. (U) 71 07 - 72 06 Six patients with different types of fever have been studied on a continuing basis. The data suggest that periodic fever represents a heterogeneous group of diseases which may be due to defects in different parts of a common metabolic pathway. Computer programs have been developed for storing, plotting and analyzing human body temperature data. This work unit is terminated. A portion of the work will be completed under 3A061102B71B Research in Biomedical Sciences 02 Internal Medicine, Work Unit 167 Biochemical Factors Influencing Physiological Functioning.

Available to contractors upon originator's approval

ABSTRACT

PROJECT NO.	3A061101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	049	The Mechanism of Body Temperature Control

The following investigations have been conducted under this work unit:

STUDY NO. 2. Studies in patients with fever.

STUDY NO. 4. Application of computer techniques for the plotting and analysis of human body temperature data.

Study No. 2. Six patients with disordered temperature regulation have been studied. Four of them appear to have periodic fever while the other two have residual thermoregulatory abnormalities following severe heat stroke. Provocation of fever by a variety of measures has been attempted in normal volunteer subjects. The fever in one of these patients has responded to a variety of therapeutic agents. The two patients who were studied following heat stroke manifested residual thermoregulatory abnormalities, with occasional intermittent fever and abnormal diurnal temperature cycles. In addition both of them showed excessive elevations in muscle temperature during exercise. These studies further support the concept that the periodic fevers and other thermoregulatory abnormalities are a heterogeneous group of disorders which may represent defects along a final common pathway mediating normal temperature regulation.

Study No. 4. We have continued to record and store (in the computer) all human body temperature data which has been obtained on the metabolic ward during various studies over the past year. Preliminary computer analysis of some of this data has been accomplished.

BODY OF REPORT

WORK UNIT NO. 049 — The Mechanism of Body Temperature Control

STUDY NO. 2. — Studies in patients with fever

PROBLEM:

The present studies in patients with periodic fever represent a continuing effort to classify the perodic fevers which appear to be a heterogeneous group of disorders. The studies in patients following heatstroke also allows us to gain an understanding of various types of thermoregulatory abnormalities. All of these studies are directed at the elucidation of the various mechanisms of temperature regulation in the normal individual.

RESULTS AND DISCUSSION OF THE RESULTS:

Studies were performed on 4 patients with periodic fever and 2 patients who had recovered from heatstroke. Two of the patients with periodic fever had remarkably similar histories. Both had evidence of periodic fever prior to their entry into service. Despite this history, and despite the history of periodic fever in the mother of one of the patients, both were inducted into active military duty. In the past both have undergone extensive studies and respond paradoxically with fever and abdominal pain following the administration of adrenal corticosteroids. Neither patient responds to uronic acids. Both patients were found to have continuing but unpredictable febrile episodes during their hospitalization. Neither patient responded to licorice extract, which is known to inhibit beta-glucuronidase. In neither patient has there been evidence of progression of the disease, and there is, thus far, no evidence of amyloidosis or renal involvement in either patient.

The third patient with periodic fever was a 29 year old female of Italian and Mediterranean ethnic origin. Her illness began in her early 20's with fairly typical attacks of fever and abdominal pain. Characteristically, she was free of symptoms during her first pregnancy and for several months thereafter. She has also been free of symptoms while on a certain oral contraceptive but not on others. In the past year she had had frequent and severe episodes of fever, abdominal pain, nausea, anorexia and incapacitation with several prolonged hospitalizations, one of which resulted in a negative exploratory laporatomy. Extensive studies revealed exquisite sensitivity to etiocholanolone injection which resulted in 5 to 6 days of fever, severe abdominal pain, anorexia, nausea and vomiting, all of which similated quite closely the spontaneous episodes. Her entire study was complicated by the presence of abdominal pain and nausea without clear cut evidence of fever. She did not respond to various

antipyretic measures. While it appears fairly certain that she does in fact have periodic fever the precise nature of her thermoregulatory defect remains uncertain. To this time no satisfactory form of therapy is available, however therapeutic trials with several of the oral contraceptives are planned in the hope that one can be found which will control her symptoms.

The fourth patient with periodic fever was a 15 year old Arapahoe Indian boy who has a 10-plus year history of recurring episodes of fever. Within the past year he apparently has had episodes of polyserositis including pleurisy and pericarditis associated with the fever. In the interim between the febrile episodes he is perfectly well. He is undergoing evaluation at this time, but no conclusions can be drawn.

Two patients underwent evaluation following heatstroke. Both of them have very similar histories. Both developed severe heatstroke during basic training and were subsequently transferred to Fitzsimons General Hospital where they received intensive treatment for several months before recovering. Following this they were evaluated on the Metabolic Ward and were found to have residual thermoregulatory abnormalities consisting of intermittent, unpredictable temperature elevations and predictable and reproducible febrile responses to the infusion of epinephrine and following exercise. During a recent hospitalization their residual thermoregulatory abnormalities were again found, having persisted for two years since the original heatstroke. Leg muscle temperature was measured in both of these patients during exercise and both were found to have an abnormally high rise in muscle temperature which corresponded to the severity of the exercise. The frequent abnormal temperature elevations seen in these patients do not respond to ordinary antipyretic measures.

CONCLUSIONS:

Four patients with apparently three different forms of periodic fever have undergone evaluation. The patients spontaneously develop fever by mechanisms which are not well understood. Furthermore they have been found to hyper-respond to etiocholanolone, or paradoxically develop fever and abdominal pain following the administration of adrenal corticosteroids. No satisfactory means of treatment has yet been found although it appears that oral contraceptives may be of value in selected female patients. These studies provide additional evidence supporting the concept that the periodic fevers represent a heterogeneous group of disorders which may be due to differing defects along a final common pathway which regulates normal temperature. The studies in patients following heatstroke demonstrate continuing thermoregulatory abnormalities persisting long after the original insult. Excessive rises in muscle temperature resulted from mild exercise. This suggests, but does not prove, that there may have

been a defect of temperature regulation already present which predisposed these individuals to heatstroke during exercise. The studies in these patients provide important ancillary evidence regarding selected abnormalities in thermoregulation. It is hoped that studies such as these provide insight into the mechanisms of temperature regulation in the normal and to the various thermoregulatory abnormalities which may contribute to the production of fever in various disease states.

RECOMMENDATIONS:

These studies have been conducted on an In-House basis, the support for which terminates in FY72. While some information of value has been obtained the marked heterogeneity of this group of patients and the capricious nature of their symptoms makes their study difficult, makes the interpretation of results relatively uncertain and prevents drawing conclusions regarding them as a group. While studies in this group of patients are important, further investigation should be defered until a better theoretical approach can be developed.

PUBLICATIONS: None.

STUDY NO. 4.

Application of computer techniques for the plotting and analysis of human body temperature data.

PROBLEM:

Studies concerning the mechanisms of body temperature regulation in normal subjects and in selected patients with thermoregulatory abnormalities have continued to provide large amounts of temperature data. The amount and type of data lends itself particularly well to computer analysis. We have continued to enter all relevant human temperature data obtained on the Metabolic Ward over the past year.

RESULTS AND DISCUSSION OF THE RESULTS:

A large amount of additional human temperature data has been provided to the computer over the past year. Preliminary computer analysis of this data has been undertaken. Since the computer analysis is in the preliminary stages no results are available at this time.

CONCLUSIONS:

We have accumulated a vast amount of human temperature data both in normal volunteer subjects and in selected patients with thermoregulatory abnormalities (periodic fever, heatstroke) the analysis of which is just getting underway. No conclusions can be drawn from the data at this time.

The Mechanism of Body Temperature Control (Cont'd)

RECOMMENDATIONS:

This was an important adjunct to the studies conducted in work unit 049 the In-House support for which will terminate in FY72. The computer analysis of the data should provide insights into temperature controlling. mechanisms and the allow the development of mathematical models which can be tested against the temperature patterns obtained in normal subjects and patients. This portion of the work unit will be continued under a regularly funded work unit.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6362	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	054
b. CONTRIBUTING	61130011	3A013001A91C	00	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Ultrastructure of Normal and Diseased Animal Tissue (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 002600 Biol.; 006500 Food; 010100 Microbiology; 016800 Toxic

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 01	CONT	DA	C In-House

17. CONTRACT/GRANT

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	2.5	11
FISCAL YEAR CURRENT 73	2.5	11

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Pathology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Demaree, R. S., CPT
TELEPHONE: 303 366 5311 X23230
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME: Trevino, G. S., LTC
NAME: DA

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Precede 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

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text 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 are: (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 will be studied by EM and the ultrastructural morphology will be correlated with routine histopathology on the same tissue; this approach reveals changes not clearly visualized by light microscopy since the identity and significance of structures poorly visualized can be confirmed with the electron microscope. Sequential studies can reveal processes or mechanisms and help relate structure to functional changes which are observed. Considerable experimentation with fixation, embedding and staining for EM examination may be required.

25. (U) 71 07 - 72 06 Study 1: Previous work demonstrating a pigment identical to secondary lysosomes in hepatic and reticuloendothelial cells of rabbits receiving 20cc/kg of intravenous lipid emulsion was reported in MRNL Laboratory Report No. 332, April, 1972. Lipid emulsion accumulates in pulmonary capillaries of rabbits immediately after infusion and can temporarily coalesce and occlude capillaries but with subsequent clearing over six hours. This concludes Study 1. Study 3: "Electron Microscopy of Frozen Skeletal Muscle: Emphasis on Morphologic Preservation with Possible Enzyme Demonstration" has just been initiated.

*Available to contractors upon originator's approval

FORM MAR 68 1498

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. 1 A sequential study of the ultrastructural changes within the hepatic and reticuloendothelial cells following administration of parenteral lipid emulsions.

STUDY NO. 3 Electron microscopy of frozen skeletal muscle: emphasis on morphologic preservation with possible enzyme demonstration.

Previous work demonstrating a pigment identical to secondary lysosomes in hepatic and reticuloendothelial cells of rabbits receiving 20cc/kg of intravenous lipid emulsion was reported. Additional study revealed that fresh lipid emulsions accumulate in pulmonary capillaries of rabbits immediately after infusion and can temporarily coalesce and occlude capillaries. But six hours after infusion very little lipid was detectable in pulmonary capillaries. That concluded Study 1.

The protocol for Study 3 has just been approved and research is being initiated.

BODY OF REPORT

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

STUDY NO. 1 — A sequential study of the ultrastructural changes within the hepatic and reticuloendothelial cells following administration of parenteral lipid emulsions.

PROBLEM:

Fat emulsions could be a very useful component of parenteral alimentation regimens. However, emulsions evaluated to date produce a pigment which is deposited within reticuloendothelial cells and hepatic parenchymal cells. The functional significance of this pigment has not been established.

Previous electron microscopic studies have shown that alterations exist at both the cell surface and within the cell, in association with uptake and assimilation of parenterally administered fat emulsions. The specificity of these studies with respect to total dose and to time after administration has hitherto not been as complete as desired.

This study was initiated to examine by light and electron microscopy the uptake, accumulation, and assimilation of lipid particles by reticuloendothelial and hepatic cells following intravenous fat administration. Since potential candidates for lipid emulsions might have some degrees of pulmonary dysfunction, additional study was conducted to determine if any observable changes could be detected in lung after intravenous fat administration.

RESULTS AND DISCUSSION OF RESULTS:

Previous work demonstrating a pigment identical to secondary lysosomes in hepatic and reticuloendothelial cells of rabbits receiving 20cc/kg of intravenous lipid emulsion was reported in the Annual Research Progress Report, FY 71.

Study of lung tissue collected at the time of the above study and six additional rabbit lungs revealed that lipid droplets accumulate in pulmonary capillaries immediately after infusion. By 30 minutes after infusion lipid droplets "coated" capillary endothelium. One hour after infusion complete occlusion of some pulmonary capillaries had occurred by the coalescence of lipid droplets. But, six hours after infusion very few lipid droplets were detectable in pulmonary capillaries and no permanent occlusion was observed.

CONCLUSIONS:

This study has shown that the fate of intravenous lipid emulsions varied considerably from tissue to tissue. Lipid was incorporated into lysosomes in liver and spleen and remained there for the duration of the study (8 weeks), while it had practically disappeared from lung six hours after infusion.

RECOMMENDATIONS:

Since capillary occlusion caused by lipid droplet coalescence was demonstrated, certain patients with specific categories of pulmonary insufficiency might limit the use of intravenous fat emulsions.

STUDY NO. 3 — Electron microscopy of frozen skeletal muscle: emphasis on morphologic preservation with possible enzyme demonstrations

PROBLEM:

The engineering advances with the electron microscope have far exceeded the techniques for preservation of tissues. The drastic chemical and physical changes imposed on the tissues result in poor histochemical preservation. The ultrastructural patterns seen in conventionally prepared tissues may not be the same as in living tissue.

Frozen ultrathin sectioning is an alternative to the use of organic solvents and the plastic embedding procedure which causes protein denaturation and loss of histochemical reactivity. Recently equipment has been developed which makes ultrathin frozen sectioning possible. This equipment has been obtained and techniques are being developed to utilize this approach to histochemistry at the electron microscopy level.

For some time this and other divisions have been studying mammalian skeletal muscle using electron microscopy. It is possible that dehydration and embedding of skeletal muscle may have altered the subtle experimentally-induced morphologic changes. This study will be a pilot study to determine if subtle changes can be lost during routine tissue preparation.

Ultrastructure of Normal and Diseased Animal Tissue (Cont'd)

RESULTS AND DISCUSSION OF THE RESULTS:

The protocol for Study 3 has just been approved and research is being initiated.

PUBLICATIONS:

1. Demaree, R. S., Jr. and W. C. Marquardt. Avian Trypanosome division: a light and electron microscope study. J. Protozoology 18:388, 1971.

2. Demaree, R. S., Jr., P. L. Senger, M. B. Bischoff and L. D. Jones. A sequential ultrastructural study of the effects of an intravenous lipid emulsion. Report No. 332. Denver, Colorado, 1972. U. S. Army Medical Research and Nutrition Laboratory.

3. Greene, H. L., D. R. Hazlett, J. B. Dramise, R. S. Demaree, and R. H. Herman. Effects of Intralipid on pulmonary function. Clin. Res. 20:274 (Abstract), 1972

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6369	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8a. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	058
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Peripheral Blood Flow Studies (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	71 06	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	2.5	27
FISCAL YEAR CURRENT 73	2.0	20

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Physiology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Sullivan, Francis J.

TELEPHONE: 303 366 5311 X22119

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Corneil, N. J., CPT, VC

NAME: DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Peripheral blood flow; (U) Blood viscosity; (U) Ischemia; (U) Frostbite; (U) Combat Trauma; (U) Hemorrhage; (U) Shock

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

23. (U) Soldiers subjected to battlefield injury frequently incur compromised blood flow to various tissues and organs of the body caused by the injury, the resultant hemorrhage and/or the surgical repair of the injury. Other potential hazards to the soldier, e. g., frostbite, chilblains, trenchfoot, hypovolemia caused by hemorrhage and vascular occlusion, also result in partial or total ischemia of the body. Reversal of this ischemia is dependent upon reestablishment of occluded flow or augmentation of reduced flow concurrent with treatment. This work unit will be directed toward two aspects of peripheral blood flow: methods and adjuncts of augmenting reduced flow in patients with battlefield injuries complicated by compromised flow and hemorrhage; and the determinants of peripheral flow and the relationship between peripheral flow and metabolism.

24. (U) Selected patients where reduced or occluded flow has compromised tissue or organ function will be monitored before, during and after vascular surgery to study various vasoactive agents, blood volume adjuncts and agents that increase peripheral blood flow. Laboratory animals will be used to study the determinants of peripheral blood flow, and to test agents and methods that may have a clinical application in the treatment of patients.

25. (U) 71 07 - 72 06 Experiments infusing low molecular weight dextran into dogs have shown that cardiac output and peripheral blood flow rises faster than plasma dextran concentrations. Subsequent to infusions, plasma dextran concentration falls faster than peripheral flow. Studies in patients at Fitzsimons General Hospital, currently in progress, indicate some agents can delineate the reactivity of the peripheral vasculature. Studies on indocyanine green dye have shown that electronic calibration of cardiac output equipment is possible.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498

ABSTRACT

PROJECT NO.	3A061101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	058	Peripheral Blood Flow Studies

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

STUDY NO. 1 The effects of low molecular weight dextran on the cardiovascular system in dogs

STUDY NO. 2 Studies of low molecular weight dextran following surgical reconstruction in occlusive vascular disease

STUDY NO. 3 Studies on vasoactive drugs in man

STUDY NO. 4 Some aspects of indocyanine green in dye-dilution measurements

During and after an infusion of low molecular weight dextran (LMD) the relationship between circulating dextran levels and the magnitude of peripheral blood flow was completed. The data has shown that during infusion, peripheral flow increases faster than plasma LMD concentration whereas after the cessation of infusion, plasma LMD concentration decreases faster than peripheral blood flow. Studies on the effects of LMD in occlusive vascular disease are not complete. However, preliminary results have demonstrated LMD consistently doubles cardiac output intraoperatively while blood flow through reconstructed vessels is increased to a lesser extent. Reactive hyperemia and vasoactive drugs (e.g., papaverine) are demonstrating the extent of reactivity of the vasculature distal to the reconstructed vessel. These studies are also incomplete at this time. A study to electronically calibrate cardiac output equipment was completed. The stability of indocyanine green in the frozen state was examined.

BODY OF REPORT

WORK UNIT NO. 058 Peripheral Blood Flow Studies

STUDY NO. 1 The Effects of Low Molecular Weight Dextran on the Cardiovascular System in Dogs

PROBLEM:

Reduced volume blood flow is a paramount factor in the establishment and continuation of ischemia in metabolizing tissues. Injured soldiers frequently incur compromised blood flow to various tissues and organs of the body as a result of injury, associated hemorrhage and/or surgical repair of injury. Reversal of this ischemia is dependent upon re-establishment of occluded blood flow or augmentation of reduced flow to the involved areas(s) of the body. One method that has been proposed to augment blood flow has been the use of low molecular weight dextran (LMD). Previous work has demonstrated injection or infusion of LMD (1 g/kg) increased cardiac output and peripheral blood flow without affecting heart rate or arterial blood pressure with a concomitant decrease in limb vascular resistance. The present study was designed to investigate the relationship between the increased flow subsequent to LMD infusion and the plasma concentration of LMD.

RESULTS AND DISCUSSION OF THE RESULTS:

During the infusion of 1 g LMD/kg in 60 minutes the serum concentration of LMD rose to 16.2 $\pm$ 0.6 mg/ml while 8.3 g were excreted into the urine. At the end of infusion, limb blood flow had risen 62%. During the hour after infusion circulating levels of LMD had decreased to 11.6 $\pm$ 0.6 mg/ml whereas limb blood flow remained 56% higher than control. Thus the augmented cardiac output and limb blood flow are not directly attributable to circulating levels of LMD. Rather, it is related probably to the oncotic properties of the molecule. Although 72% of the infused dose had been excreted during the experimental time, the remainder was responsible for the maintenance of limb blood flow.

CONCLUSIONS :

The increase in cardiac output and limb blood flow is related to several things; increase in circulating volume, increase in central venous pressure and the oncotic properties of the circulating dextran molecule.

RECOMMENDATIONS:

Complete analysis of data.

STUDY NO. 2 Studies of Low Molecular Weight Dextran Following Surgical Reconstruction in Occlusive Vascular Disease

Peripheral Blood Flow Studies (Cont)

PROBLEM:

Direct revascularization of the heart is presently an attractive procedure in the management of obstructive coronary artery disease. A saphenous vein by-pass graft from the aorta to the coronary artery below the obstruction is a surgical procedure presently being used. Similarly, autologous and synthetic grafts have been emplanted between the aorta and femoral artery and the femoral and the popliteal arteries. The object of these procedures is to increase blood flow to the ischemia areas distal to the occlusion. Little information is available concerning volume flow through the reconstructed vasculature. Thus, this study was conducted to assess blood flow through the grafts intraoperatively following the infusion of 500 ml of 10% LMD in 5% glucose.

RESULTS AND DISCUSSION OF THE RESULTS:

Although the studies are incomplete, preliminary analysis of the data is possible. Three candidates for aorto-coronary saphenous vein by-pass grafts have been studied to date. Following construction of the graft and after stabilization of blood pressure and heart rate the patients were hypovolemic and vasoconstricted. Saphenous blood flow in the three patients were 60, 55 and 35 ml/min. In the first two patients LMD doubled cardiac output and increased saphenous blood flow four times within 30 minutes of infusion. The third patient did not respond to LMD.

In seven patients having aorto-femoral artery bypass grafts constructed, LMD doubled both cardiac output and femoral blood flow. Lumbar sympathectomy improved femoral flow in 3 of 4 patients.

Postoperatively, LMD therapy was seemingly beneficial, based upon intraoperative findings, because it augments basal flow and, thus, allows a greater chance to maintain patency during the immediate postoperative time when the opportunity for clotting is greatest.

CONCLUSIONS:

The required number of studies to complete an analysis are not yet performed.

RECOMMENDATIONS:

These studies should be completed.

STUDY NO. 3 Studies on Vasoactive Drugs in Man

PROBLEM:

The use of vasoactive drugs in the maintenance of blood pressure and flow in man is a widespread practice. Cursory evaluation of isoproteranol and

Peripheral Blood Flow Studies (Cont)

papaverine injections has shown conflicting results. In an attempt to augment peripheral flow following vascular reconstructive surgery, adjuncts to flow seem to be of value to maintain vessel patency. Thus, studies were conducted in patients mentioned in Study 2 as well as others undergoing vascular surgery.

RESULTS AND DISCUSSION OF RESULTS:

This study is incomplete at present. However, several important points have been noted. Intra-arterial papaverine injections seem to delineate vascular reactivity. Secondly, papaverine-induced vasodilatation is considerably augmented by pretreatment with LMD.

CONCLUSIONS:

The required number of studies to complete the analysis of the data are not yet performed.

RECOMMENDATIONS:

Complete the study.

STUDY NO. 4 — Some Aspects of Indocyanine Green Dye-Dilution Measurements

PROBLEM:

In recent years cardiac output has become a frequent and necessary determination in clinical diagnosis as well as in patient management. Dye-dilution studies with indocyanine green are readily performed and perhaps offer the most accurate estimation of cardiac output when proper attention is given to methodological details. Using special purpose analog computers, the calculation of cardiac output can be done while the dye curve is being recorded. However, standard methods of calibration of cardiac output equipment are slow, tedious and seldom free of pipetting errors. Secondly, and more significantly, they also require exposure of the blood to the non-sterile atmosphere. Therefore, a method has been devised to calibrate the equipment electronically. In this way, the problems and the time required in the standard way could be eliminated. Thus, cardiac output could be measured in a patient in minimal time.

RESULTS AND DISCUSSION OF RESULTS:

In a series of experiments indocyanine green was diluted in blood concentrations of 1, 2, 4, 8 and 16 mg/liter. The voltage output from the cuvette-densitometer was monitored at the time of mixing dye with diluent, 2 and 4 hours later. From this data a voltage-concentration curve was constructed. This was repeated six times

over a 30-day period. With instruments used the values showed linearity through 8 mg/l with the intercept going to zero. At 16 mg/l, the linear regression line (calculated with values from 1 to 8 mg/l) exceeded the value by less than 8%. An increase in background dye in blood seemed to move the origin of a voltage-concentration curve along the curve determined with dye-free blood.

CONCLUSIONS:

1. Cardiac output monitoring equipment can be calibrated using standard procedures relying upon the maintenance of the stability of gain setting. In this way, measurement of cardiac output, especially in a clinical setting, can be made with minimal delay.

2. Electronic calibration of cardiac output equipment is valid for at least 30 days.

3. Recalibration of equipment with dye and blood should be performed monthly until a different time interval is attained.

RECOMMENDATIONS:

1. Use of this procedure, especially in a clinical situation, is valid. As a result a cardiac output determination can be made as soon as catheters are placed.

2. Mixing indocyanine green with diluent should be as exact as possible.

PUBLICATIONS:

1. Sullivan, F. J. and N. J. Corneil. The relationship between serum low molecular weight dextran (LMD) and peripheral blood flow. Federation Proc. 31:392, 1972 (abstract)

2. Sullivan, F. J., E. A. Mroz and R. E. Miller. Electronic calibration for indocyanine dye-dilution curves. American Heart Journal (submitted)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6372	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 08 21	D Change	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	059
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Performance, Fatigue, and Exhaustion (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 016200 Stress Physiology; 013400 Psychology; 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

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	2.0	25
	CURRENT 73	2.0	23

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Physiology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Weiser, P. C., MAJ

TELEPHONE: 303 366 5311 X24198

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Sterner, R. T.

NAME: Sullivan, F. J. DA

21. GENERAL USE

Foreign Intelligence not Considered

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.)

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. To develop means for improving and/or maintaining performance effectiveness and for ameliorating fatigue and exhaustion.

24. (U) The studies will fall into three general categories: 1) organismic study 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.

25. (U) 71 06 - 72 07 Experimental procedures for the assessment of regional blood flow and energy metablism in dogs running to exhaustion at two work loads were completed. Collection of experimental data was started. A pilot experiment on trained dogs showed that at 8 km/hr, 20% grade, maximum run times averaged 102 and 110 min, while at 10 km/hr, 20% grade, these dogs could run 51 and 42 min, respectively. Cluster analysis of reported symptomatology resulting from prolonged bicycle riding at 56% estimated $\dot{M}O_2$ was further refined. At least four clusters were identified and named: Task Aversion, Motivation, Leg Fatigue, and General Fatigue. Analysis of the data with respect to ride duration suggests that riders of shorter duration tend to have greater heart rate increments as well as larger scores for Leg Fatigue and General Fatigue than do riders of longer duration. Additional analyses of Vitamin A data are pending development of a 4-compartment storage model deriving original A level estimates for subjects. Finally, prolonged ribflavin restriction (i.e., 39-56 days of <0.07 mg riboflavin/day) produced behavior-specific effects in adult males.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498

ABSTRACT

PROJECT NO. 3A061101A91C In-House Laboratory Independent Research

WORK UNIT NO. 059 Performance, Fatigue and Exhaustion

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

STUDY NO. 1 Effects of prolonged, exhaustive exercise on the control of energy metabolism

STUDY NO. 2 Performance efficiency during prolonged exercise

STUDY NO. 3 Effect of gait on treadmill walking on the above-knee amputee

STUDY NO. 4 Vitamin A requirement of the adult human: relationship to performance

STUDY NO. 5 Performance and Psychological Effects of Ribflavin Deficiency

All experimental procedures for assessing changes in regional blood flow and energy metablism in dogs running to exhaustion at two work loads were completed, and collection of experimental data was started. A pilot experiment on two trained dogs showed that at 8 km/hr, 20% grade, maximum run times averaged 102 and 110 min, while at 10 km/hr, 20% grade, these dogs could run 51 and 42 min, respectively. Cluster analysis of reported symptomatology resulting from prolonged bicycle riding at 56% estimated $\dot{M}VO_2$ was further refined. At least four clusters were identified and named: Task Aversion, Motivation, Leg Fatigue, and General Fatigue. Analysis of the data with respect to ride duration suggests that riders of shorter duration tend to have greater heart rate increments as well as larger scores for Leg Fatigue and General Fatigue than do riders of longer duration. Additional analyses of Vitamin A data obtained from Study No. 4 are planned, pending development of a 4-compartment storage model deriving original A level estimates for subjects. Finally, counter to earlier reports (Keys et al., 1944), prolonged riboflavin restriction (i.e., 39-56 days of <0.07 mg riboflavin/day) produced behavior-specific effects in adult males.

BODY OF REPORT

WORK UNIT NO. 059 — Performance, Fatigue, and Exhaustion

STUDY NO. 1 — Effects of Prolonged, Exhaustive Exercise on the Control of Energy Metabolism

PROBLEM:

Physical fatigue has long been recognized as a problem for the combat soldier and patrol dog. Yet the responsible factors remain to be clearly described. The location of impairment(s) causing exhaustion is not known. It is thought to be either in the nervous system, the myoneural junction, or the exercising muscle (or a combination of these). With respect to the exercising muscle, the most specific mechanisms appear to be metabolic in nature. One metabolic factor which appears to limit prolonged performance is the resting level of muscle glycogen. Another is the ability of working muscles to oxidize fatty acids. This study will focus on describing alterations in the control of energy metabolism in dogs during prolonged, exhaustive treadmill running.

RESULTS AND DISCUSSION OF THE RESULTS:

Two dogs were trained to run for four hours on a treadmill at 6 km/hr, 15% grade. Subsequently, they were run to exhaustion at two different work loads. Each dog showed a practice effect with run duration ultimately rising, respectively, to average 102 and 110 min at 8 km/hr, 20% grade and 51 and 42 min at 10 km/hr, 20% grade. All pilot tests were completed, and the training of dogs and collection of experimental data were started.

RECOMMENDATION:

With the projected closing of USAMRNL at the end of FY 73, this study should be completed as soon as possible.

STUDY NO. 2 — Performance Efficiency During Prolonged Exercise

PROBLEM:

During continuous, prolonged physical work, progressive alterations in physiological functioning and associated subjective feeling of fatigue eventually lead to exhaustion. Typically, exhaustion has been operationally defined as the point at which the subject, animal or human, refuses to continue a specific work task. As such, it is likely that both psychological and physiological factors interact to determine the point of exhaustion. From a psychological standpoint, tolerance to discomfort may be a major variable affecting this point at least in the human subject. Physiologically, the ability

to maintain a steady work pulse may also be an indicator of endurance fitness.

The onset and progression of physical fatigue during strenuous exercise will be examined and a Physical Fatigue Scale (PFS) will be developed. It is expected that the degree of physical fatigue and severity of various symptoms will be exaggerated for those individuals with a low discomfort tolerance relative to their capacity to do physical work.

RESULTS AND DISCUSSION OF THE RESULTS:

Phase I. The Perceived Discomfort Questionnaire (i.e., a 62-item, 7-point subjective questionnaire of recreational interests) has been cluster analyzed. Several sets of items reflecting sport interests were found (i.e., Physical Contact, Level of Exertion, and Aerobic Requirements). Initial attempts to relate these subjective responses to a variety of performance tasks (e.g., bicycle ergometer and prolonged hand-grip performance) yielded low correlations. However, further analyses directed toward improving the measurement characteristics of the questionnaire items are planned. Ultimately, the item clusters should provide a reliable means for predicting performance on a variety of behavioral tasks.

Phase II. Following prolonged strenuous bicycle riding at 56% estimated $M\dot{V}O_2$, 57 male subjects reported increased scores for symptoms that grouped into clusters named Fatigue and Task Aversion. They exhibited decreased scores for a Motivation cluster. All clusters had high coefficients of stability and test-retest reliabilities. The Fatigue cluster was further shown to be comprised of relatively independent Leg Fatigue and General Fatigue item groups. Heart rate from the 5th to 13th min was correlated -.41 with ride duration, .38 Leg Fatigue, and .35 with General Fatigue.

CONCLUSION:

This suggests that riders of shorter durations tend to have greater heart rate increases and larger scores for the Leg and General Fatigue components than do longer riders.

RECOMMENDATIONS:

1. In studying the factors influencing physical work performance, the use of reported symptomatology should be encouraged since it can yield important information on perceptual limiting factors of performance.

2. Further analysis of personality factors, heart rates, and symptomatology collected from subjects riding at 56% and 75% estimated $M\dot{V}O_2$ should be completed.

Performance, Fatigue, and Exhaustion (Cont)

3. A time course study of heart rate, blood metabolites, reported symptomatology, and reaction time during a ride to exhaustion at 75% $M\dot{V}O_2$ should be investigated.

STUDY NO. 3 — Effect of Gait on Treadmill Walking of the Above-Knee Amputee

PROBLEM:

The ideal gait pattern for the lower-extremity amputee is considered to be that of a normal individual. Not only is a "normal" gait desirable from a cosmetic standpoint, but also it is tacitly assumed to be energetically the most efficient.

When not under the supervision of a physical therapist, amputees frequently are observed to discard their cosmetic gait. They assume instead a swaying, choppy pattern, leaning toward their prosthetic side with both legs slightly abducted. If, then, the therapist's assumption that the cosmetic gait is the most efficient, why does the amputee revert to a gait which is assumed to be more difficult for him energetically and is uncosmetic for him as well?

RESULTS AND DISCUSSION OF THE RESULTS:

Data on heart rate, oxygen consumption and Physical Activity Questionnaire changes of seven normal subjects walking at 1.2 km/hr, 5% grade have been collected. Only one uniliateral above-knee amputee has been studied, walking first in a cosmetic gait and then walking uncontrolled. Future study of amputees is restricted since fewer are being treated at Fitzsimons General Hospital and practically all are retired from the Army soon after fitting of their prosthesis.

RECOMMENDATION:

Due to the nonavailability of amputee subjects this study should be terminated.

STUDY NO. 4 — Vitamin A Requirement of the Adult Human: Relationship to Performance

PROBLEM:

This study was conducted in collaboration with Chemistry Division, USAMRNL (see Work Unit No. 072; Study No. 2). Briefly, subjects were administered a set of 8 Behavioral Measures (i. e., Minnesota Multiphasic Personality Inventory, Fleishman Physical Fitness Battery, Reaction Time, Minnesota Turning Test, Crawford Small Parts Dexterity Test, Brightness Discrimination, High Frequency Audiometry, and Ortho-Rater) during 8, 3-day sessions to assess potential performance decrements induced by hypovitaminosis A.

Performance, Fatigue, and Exhaustion (Cont)

RESULTS AND DISCUSSION OF THE RESULTS:

Data analyses to date confirmed earlier reports of high intercorrelations among measures categorized as Personality, Performance and Perception as well as the lack of linear relationship between blood-level A values and test-session behavioral scores. One-way, repeated-measures analyses of variance for each measure by test sessions yielded no significant changes. These results are indicative of high between-subject variance. Additional analyses involving stratification of subjects on the basis of stored vitamin-A estimates are pending. In view of the long depletion period required for vitamin A (i.e., 12-30 months), as well as the fact that only 5 of the 8 subjects met the criteria for severe depletion, such subject stratification was viewed as the only feasible means of data analysis.

CONCLUSIONS:

Additional analyses of the data are planned based upon a 4-compartment storage model of vitamin A. Final conclusions regarding the behavioral consequences of hypovitamosis A upon performance are reserved pending results of these analyses.

STUDY NO. 5 Performance and Psychological Effects of Riboflavin Deficiency

PROBLEM:

The current study was conducted as part of a larger study by Chemistry Division, USAMRNL (see Work Unit No. 072, Study No. 12) designed to assess erythrocyte glutathione reductase activity coefficient as an index of human riboflavin status.

Nine Behavioral Measures were administered to six conscientious-objector volunteers (i. e., Brightness Discrimination, Crawford Small Parts Dexterity Test, Ortho-Rater, Hand Dynamometer, Pursuit Rotor, Reaction Time, Wechsler Adult Intelligence Scale, Minnesota Multiphasic Personality Inventory, and Rorschach Test) during 6 selected test periods distributed over a 14-day Control period, a 56-day Depletion period and a 14-day Repletion Period.

RESULTS AND DISCUSSION OF THE RESULTS:

During the 56-day period of riboflavin restriction (i.e., less than 0.07 mg/day) significant test-day differences were found for five personality subscales of the MMPI (i.e., Hypochondriasis, Depression, Hysteria, Psychopathic-Deviate and Hypomania). In addition, handgrip (Dynamometer) strength was reduced. These effects were noted by the 39th and 52nd day of restriction, respectively, in the absence of any clinical symptoms. They were not readily reversible during a 14-day repletion period. The MMPI data are viewed as reflecting

subjective reports of somatic symptomatology and situational reactions associated with the stress of riboflavin restriction. Decreased hand-grip strength is viewed as a residual effect of reduced flavoprotein levels in body musculature. Incidental findings showed an erythrocyte glutathione reductase activity coefficient between 1.20-1.30 to be a potentially conservative range not related to hyporiboflavinosis; and, select behavioral measures afford sensitive means for assessing the onset and recovery of a specific vitamin deficiency.

CONCLUSIONS:

Counter to earlier reports (Keys et al., 1944), results of the present study indicate that severe, prolonged riboflavin restriction (i.e., 39-56 days of $\leq$0.07 mg riboflavin/day) has behavior-specific effects in adult males.

RECOMMENDATIONS:

Behavioral criteria of recovery from vitamin-restriction regimen should be used in determining adequacy of repletion.

PUBLICATIONS:

1. Kinsman, R. A., P. C. Weiser and D. A. Stamper. Multidimensional Analysis of Subjective Symptomatology During Prolonged Strenuous Exercise. Ergonomics (submitted)

2. Sterner, Ray T. and W. R. Price. Restricted Riboflavin Within-Subject Behavioral Effects in Humans. Am. J. of Clinical Nutrition (submitted)

3. Weiser, P. C., R. A. Kinsman and D. A. Stamper. Task-Specific Symptomatology Changes Resulting from Prolonged Submaximal Bicycle Riding. Medicine and Science in Sports (submitted)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6373	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A06110A91C	00	060
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Vitamin D, Calcium and Phosphorus Metabolism (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 002600 Biology, 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 04	74 06	DA	C In-House

17. CONTRACT/GRANT

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	.7	5
FISCAL YEAR CURRENT 73	.7	8

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Administrative Division
Fitzsimons General Hospital
Denver, Colorado 80240

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Morrissey, R. L., CPT, VC
TELEPHONE: 303 366 5311 X26111
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Combat Bone Injuries; (U) Vitamin D; (U) Calcium; (U) Phosphorus, (U) Mineral Metabolism

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

23. (U) It is hypothesized that Vitamin D_3 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 effect on intestine, including induction of calcium binding protein (CaBP) and increasing 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 peridontal and dental health.

24. (U) Data preliminary to the clinical use of Vitamin D metabolites are being generated; an assay for human CaBP is being developed; and the factors which control the rate of Vitamin D metabolite formation are being studied.

25. (U) 71 07-72 06 25-HCC is at least 10 fold and possibly 100 fold greater in toxicity than Vitamin D_3 as manifested by renal tubule calcification. Dosages above 0.8 µg 25-HCC/Kg body wt. should not be used without extreme caution. Samples (1,200) from the study of the sites of tissue localization and the dynamics of metabolism of Vitamin D_3 and 25-HCC have been oxidized, counted, and statistically evaluated. Final analysis will result when column chromatography of selected pooled tissues specimens is completed. Data from tissue uptake studies indicate that liver and spleen exhibit marked uptake specifity for the non-hydroxylated vitamin. Analysis of samples from ST-3, "Control of 25-HCC conversion to 1,25-DHCC," is also complete, except for statistical evaluation and preparation of graphs.

Failure to contractors upon originator's approval.

FORM AR 66 1498

ABSTRACT

PROJECT NO. 3A06110A91C In-House Laboratory Independent Research

WORK UNIT NO. 060 Vitamin D, Calcium and Phosphorus Metabolism

The following investigations have been conducted under this work unit:

STUDY NO. 1 Toxicity and Tissue Localization of Vitamin D_3 and 25-Hydroxycholecalciferol (25-HCC)

STUDY NO. 2 Assay for Human Calcium Binding Protein

STUDY NO. 3 Control of 25-Hydroxycholecalciferol Conversion to 1,25-Dihydroxycholecalciferol (1,25-DHCC)

Study No. 1 was designed to elucidate factors involved in the control of mineralization and determine the safe limitations of 25-HCC as a therapeutic agent. The sites of tissue localization and the dynamics of metabolism of Vitamin D_3 and 25-HCC were studied in chicks by injection of ^{14}C-Vitamin D_3 and ^{3}H-25-HCC and sampling tissues over a period from 1.0 minute to 72 hours. During the first 15 minutes, liver and spleen showed a marked preference to the non-hydroxylated form of the vitamin while all other tissues examined either preferentially took up the hydroxylated form or showed no preference. This result suggests that when a single dose of 25-HCC is given instead of Vitamin D_3, the amount of vitamin stored for future use would probably be markedly reduced. The relative toxicity and metabolic effectiveness of Vitamin D_3 and 25-HCC were evaluated by feeding six graded levels of each and observing gross and microscopic pathology as well as several metabolic parameters of calcium metabolism. Vitamin D_3 was fed for up to 14 days at the rate of 1.0 mg/kg of diet without renal tubule calcification or elevated kidney calcium concentration. When 25-HCC was fed at the same rate both renal tubule calcification and elevated kidney calcium concentration occurred at 3, 6, and 14 days and renal tubule calcification also occurred at all three times in chicks fed 0.1 mg of 25-HCC/kg of diet. Thus, a 10 to 100 fold increase in toxicity appears to result when the hydroxylated form of Vitamin D_3 is fed.

Study No. 2, Assay for Human Calcium Binding Protein (CaBP), remains a continuing effort. Efforts to purify human CaBP from postmortem tissue were unsuccessful and viable surgical specimens have not been obtainable.

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

Study No. 3 has been initiated and sample assays are nearly completed. The study was designed to confirm or deny the hypothesis that serum calcium concentration controls the rate of 25-HCC conversion to 1,25-DHCC in renal tissue. Although the results of the study are consistent with the hypothesis, they are questionable because adaptation of the rate of calcium absorption failed to occur. It did occur in an earlier study which was similar with the exception that 1000 IU of Vitamin D/kg diet was fed instead of the 400 IU of Vitamin D/kg diet used in this study. The study should be repeated at the higher dietary Vitamin D level.

BODY OF REPORT

WORK UNIT NO. 060 — Vitamin D, Calcium and Phosphorus Metabolism

STUDY NO. 1 — Toxicity and Tissue Localization of Vitamin D_3 and 25-Hydroxycholecalciferol

PROBLEM:

From January 1965 through September 1970, 2,347,464 man days have been lost due to hospitalization of 59,472 soldiers in Vietnam with bone and joint injuries. Vitamin D_3 is converted to 25-hydroxycholecalciferol (25-HCC), a more active metabolite, prior to its functional activity of increasing calcium absorption from the intestine. It is hoped that 25-HCC might prove effective in the prevention and/or treatment of the demineralization of bone which accompanies disuse during the prolonged convalescence involved in extensive wounds. Ultimate aim is to decrease the period of convalescence. Demineralization also occurs naturally with advancing age (senile osteoporosis) and 25-HCC may be effective in its therapy.

The relative toxicity and metabolic effectiveness of Vitamin D_3 and 25-HCC were studied by feeding six graded levels of Vitamin D_3 and six graded levels of 25-HCC to chicks and performing gross and microscopic pathologic evaluations after being fed the diets for 3, 6, and 14 days. Calcium binding activity, electrophoretic pattern, ^{45}Ca and ^{32}P uptake, 3',5'-cyclic AMP concentration, adenyl cyclase activity, and membrane ATPase activity were assayed in duodenal mucosa. Serum, calcium inorganic phosphate and alkaline phosphatase concentrations were also determined. Calcium concentration in kidney and percent bone ash were also determined.

The sites of tissue localization and the dynamics of metabolism of Vitamin D_3 and 25-HCC were studied in chicks by injection of ^{14}C Vitamin D_3 and ^{3}H-25-HCC and sampling a total of 20 tissues from each chick over a period from 1.0 minute to 72 hours. These samples were oxidized and assayed for ^{14}C and ^{3}H. Liver and duodenum and kidney were also collected, frozen in liquid nitrogen, and stored at -90°C for later chromatographic assay of ^{14}C and ^{3}H labeled Vitamin D_3 and its metabolites.

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

RESULTS AND DISCUSSION OF THE RESULTS:

The relative toxicity of Vitamin D_3 and 25-HCC can be ascertained by the occurrence of histopathologic lesions, mortality incidence or by monitoring the concentration of calcium in renal tissue to determine the dietary concentration of vitamin that will induce sufficient renal tissue calcification to elevate the tissue concentration of calcium. Renal tubule calcification occurred at 3, 6, and 14 days in chicks fed 10 mg of Vitamin D_3/kg diet while similar lesions occurred at the same times in chicks fed only 0.1 mg of 25-HCC/kg of diet, indicating a 100 fold difference in relative toxicity. After being fed the diets for 14 days, only one chick from the Vitamin D_3 fed group had died, and it was receiving 100 mg of Vitamin D_3/kg diet, while all five chicks fed 100 mg of 25-HCC/kg diet had died prior to the 14th day and one of the chicks fed the 10 mg/kg diet level of 25-HCC had also died. A 10 fold difference in toxicity is indicated by this criterion. The highest dietary concentration of Vitamin D_3 consistent with normal renal tissue calcium concentration was 1.0 mg/kg diet while only 0.1 mg of 25-HCC/kg diet was tolerated, again indicating a 10 fold difference in toxicity between the vitamin forms. Plasma calcium concentration was elevated in chicks fed both Vitamin D_3 and 25-HCC at the 10 mg/kg diet level but not at the 1.0 mg/kg diet level, suggesting no difference in toxicity of the two vitamin forms. Thus, monitoring of plasma calcium concentration should not be considered as a particularly sensitive means of monitoring the toxic effects of the vitamin.

The results of assays for concentration of cyclic AMP, membrane ATPase and adenyl cyclase in duodenal mucosa did not allow correlation of these criteria with dietary vitamin level. Duodenal calcium uptake and CaBP concentration was increased with increasing dietary vitamin concentration for both forms of the vitamin.

The tissue distribution of ^{14}C-Vitamin D_3 and ^{3}H-25-HCC was also studied. Liver and spleen were distinguished from all other tissues by the fact that the uptake of ^{14}C (%dose/gm dry tissue) was 5 to 10 fold greater than uptake of ^{3}H. Since this effect was apparent at 15 minutes, it is quite likely that the labeled vitamins were still in the form originally injected and thus that these tissues have a markedly greater affinity for Vitamin D_3 than 25-HCC. It was also noted that ^{14}C concentration in liver and spleen increased during the first 15 minutes after injection and then decreased exponentially between 15 minutes and 12 hours. Conversely, the ^{3}H concentration in these tissues was maximal at 1.0

minute and decreased in a relatively exponential manner during the first hour, but at 3 and 6 hours a distinct shoulder on the curve exists, suggesting that some limited conversion of 25-HCC to Vitamin D_3 may occur in vivo. This possibility will be confirmed or denied when the frozen samples are extracted and chromatographed to identify the form of the vitamin for each of the isotope labels.

The concentration of ^{3}H in kidney peaked at 15 minutes and decreased exponentially thereafter, while the concentration of ^{14}C peaked at 1.0 hour and decreased exponentially thereafter. The concentration of ^{3}H and ^{14}C in duodenum peaked at 30 minutes and 3.0 hours respectively. These observations are quite consistent with the current theory that Vitamin D_3 is converted by liver to 25-HCC and then 25-HCC is converted by kidney to 1,25-dihydroxycholecalciferol (1,25-DHCC), which initiates a metabolic effect in the duodenum.

Aorta has been previously reported as one of the sites of calcification as an effect of hypervitaminosis D.[3] It was observed in this experiment that the concentration of ^{3}H in aorta was approximately 2 fold greater than the concentration of ^{14}C at all time periods.

CONCLUSIONS:

25-Hydroxycholecalciferol is at least 10 fold and possibly 100 fold greater in toxicity than the non-hydroxylated form of Vitamin D_3.

Liver and spleen have a 5 to 10 fold greater capacity for storage of Vitamin D_3 than 25-HCC.

RECOMMENDATIONS:

Dosages above 0.8 mg (0.8 mg of Vitamin D=24 IU)/kg body weight/day should not be used without extreme caution. Monitoring of serum calcium concentration as an indication of Vitamin D toxicity is not an adequate precaution since histopathologic lesions occur in renal tubules prior to the elevation of serum calcium. Urinary calcium concentration may be a more sensitive means of monitoring for toxic effects, but this recommendation is based on expectations from knowledge of renal physiology rather than data from this study.

PUBLICATIONS:

None.

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

STUDY NO. 2

Assay for Human Calcium Binding Protein

PROBLEM:

A means of monitoring intestinal calcium absorptive capacity on a routine basis in man is an important prerequisite to the study of dietary means of preventing decalcification and promoting calcification in appropriate clinical situations. A Vitamin D dependent calcium binding protein (CaBP) has been demonstrated in the intestinal mucosa, and the concentration of CaBP is closely associated with changes in calcium absorptive capacity. The primary objective of this study is to develop an assay for CaBP which is applicable to human jejunal biopsy specimens.

RESULTS AND DISCUSSION OF RESULTS:

The first approach was to attempt to purify human CaBP from autopsy specimens and use it to develop a radioimmunoassay. Material obtained in this manner was usually 12 to 24 hours postmortem and proved to be unsuitable for this work. Attempts to obtain surgical specimens have not been successful thus far. Analytical disk gel electrophoresis has been performed on portions of human biopsy specimens obtained coincidental to another study. Since the migrational characteristics of pure human CaBP have recently been reported (Hoppe-Seyler's Z. Physiol. Chem. Bd. 352, S. 1480-1486, Nov. 1971) this approach can be used to assay for human CaBP. However, the assay would require most or all of a single biopsy specimen.

CONCLUSIONS:

None.

RECOMMENDATIONS:

None.

PUBLICATIONS:

None.

Vitamin D, Calcium and Phosphorus Metabolism (Cont)

STUDY NO. 3 — Control of 25-Hydroxycholecalciferol Conversion to 1,25-Dihydroxycholecalciferol

PROBLEM:

The body is able to adapt to a fairly wide range of dietary calcium and phosphorus intakes. There is currently considerable concern about the effects of excess dietary phosphorus on mineralization of bones and teeth. Without the above-mentioned adaptation ability, the effects of excess dietary phosphorus would be considerably more drastic than those noted. Vitamin D is required for the above adaptation to occur and parathyroid hormone is also involved. The actual mechanism of adaptation is unknown. One possibility might be via the control of 25-hydroxycholecalciferol (25-HCC) conversion to 1,25-dihydroxycholecalciferol (1,25-DHCC) in kidney, which in turn would control the rate of calcium absorption from the gut. It has been suggested by some investigators (Proc. Nat. Acad. Sci. USA 68(9):2131, 1971) that serum calcium is the regulator of this kidney hydroxylase. However, this theory is not consistent with the earlier observation that rickets induced by low dietary phosphorus and normal or high dietary calcium (diet contained 1000 IU of Vitamin D_3/kg) will result in relatively high serum calcium levels while in the same chicks, the rate of calcium absorption and the concentration of CaBP in duodenal mucosa was greatly elevated. Since an understanding of the basic mechanisms involved in the regulation of calcium absorption is considered as a prerequisite to the modification of this capacity in clinical situations, the present study was designed to confirm or deny the theory that serum calcium is the regulator of the kidney hydroxylase.

RESULTS AND DISCUSSION OF THE RESULTS:

The experiment has been completed with the exception of approximately one week's worth of column chromatography of Vitamin D metabolites in tissue specimens and statistical analysis of the results. Preliminary results will be discussed.

It had been reported earlier that excess dietary Vitamin D would inhibit the kidney hydroxylase. Thus, considerable thought was given to the dietary level of Vitamin D that should be used and it was decided to use a minimal amount (400 IU/kg diet) consistent with normal growth at normal levels of dietary calcium and phosphorus. It was also recognized that adequate Vitamin D was required for the desired adaptation in calcium absorption to occur and thus

the concern about the appropriate dietary Vitamin D concentration for the experiment. The results indicated that the amount of Vitamin D fed (400 IU/kg diet) was not sufficient to support adaptation as measured by the rate of calcium absorption and the concentration of CaBP in duodenal mucosa. However, the chicks fed the high dietary calcium (2.32%) and low dietary phosphorus (0.25%) did have an elevated serum calcium concentration (mean of 11.98 compared to 10.28 mg calcium/100 ml serum in controls) as expected. The rate of conversion of 25-HCC to 1,25-DHCC can be measured by column chromatography of chloroform-methanol extracts of duodenal mucosa three hours after administration of ^{3}H-25-HCC and determining the ratio of ^{3}H present as 25-HCC to ^{3}H present as 1,25,DHCC. The ratios obtained were .614, .710, and .961 for normal calcium-normal phosphorus (NC-NP) diet, high calcium-high phosphorus (HC-HP) diet and high calcium-low phosphorus (HC-LP) diet respectively. The markedly reduced rate of hydroxylation of 25-HCC in the chicks fed the HC-LP diet is consistent with the theory that serum calcium concentration does regulate the rate of 25-HCC hydroxylation. However, since adaptation of the rate of calcium absorption did not occur (probably because of inadequate dietary Vitamin D level) as expected, this experiment is probably not an adequate test of the hypothesis. The most pertinent observation from this study is that the level of Vitamin D that was sufficient for chicks fed a NC-NP diet was not sufficient to allow the adaptation in calcium absorption that would occur with higher dietary Vitamin D, suggesting that the Vitamin D requirement may be markedly altered by the calcium: phosphorus ratio in the diet.

CONCLUSIONS:

None, because only preliminary data are available.

RECOMMENDATIONS:

Repeat the study at a higher level of dietary Vitamin D, such as 1000 IU/kg diet.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6335	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	01	059
b. CONTRIBUTING	61145011	3A014501B71P	01	
c. CONTRIBUTING	CDOG 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

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING	72	1.5	114
FISCAL YEAR — CURRENT	73	3.4	50

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Metabolic Division
Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Herman, R. H., COL, MC

TELEPHONE: 303 366 5311 X25193

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Hagler, L., LTC, MC

NAME: Greene, H. L., MAJ, MC DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Lipid Utilization by Muscle; (U) Carbohydrate Utilization by Muscle; (U) Muscle Function, Combat Soldier; (U) Lipids; (U) Steroids

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

23. (U) Maximum muscular activity is essential to 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 necessary to serve as the best energy source for muscle function. The absorption of carbohydrate occurs as monosaccharide which is water soluble. Lipid can only be absorbed from the small intestine when emulsified by bile steroids. The mechanism of production of bile steroids by the liver is not well known. Failure of production of adequate amounts of bile steroids leads to malabsorption of dietary lipid, chronic diarrhea, weight loss, and general debility. Knowledge of the mechanism of production of bile steroids will provide the basis for enhancing lipid absorption from the gastrointestinal tract.

24. (U) Hyperlipemic patients (non-obese, obese, diabetic and non-diabetic) will be investigated with regard to the action of clofibrate which decreases the hypertriglyceridemia of many patients. Clofibrate inhibits adenyl cyclase and we have hypothesized that the mechanism of action is by virtue of its effect on adenyl cyclase. Knowledge of the mechanism of action of clofibrate may provide a better understanding of lipid metabolism and its regulatory controls.

25. (U) 71 07 - 72 06 Studies of hyperlipemic patients showed no consistent change in jejunal or adipose tissue, adenyl cyclase or cyclic AMP with clofibrate treatment. Heparin, however, caused a significant decrease in adipose tissue cAMP.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498-1

ABSTRACT

PROJECT NO.	3A061102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biochemistry
WORK UNIT NO.	059	Basic Studies in Lipids

The following investigations have been conducted under this work unit:

STUDY NO. 6. The effect of corticosterone and diet on rat duodenal and liver adenyl cyclase activity and cyclic AMP concentrations.

STUDY NO. 7. Study of patients with Type IV hyperlipemia.

Study No. 6. The effect of corticosterone and diet on rat duodenal and liver adenyl cyclase and cyclic AMP concentrations was studied. Cyclic AMP determinations have been completed and the concentrations in duodenum and liver were not altered by adrenalectomy.

Study No. 7. In type IV hyperlipemic patients jejunal and adipose tissue adenyl cyclase and cyclic AMP levels did not change consistently after clofibrate treatment. Heparin, however, caused a significant decrease in adipose tissue cyclic AMP.

BODY OF REPORT

WORK UNIT No. 059 — Basic Studies in Lipids

STUDY NO. 6. — The effect of corticosterone and diet on rat duodenal and liver adenyl cyclase activity and cyclic AMP concentrations.

PROBLEM:

The role of corticosterone in the adaptive response of rat gut and liver to diet is unknown. It has been postulated that cyclic AMP might play a role in this adaptation. The effect of corticosterone on the glycolytic response to diet and its effect on adenyl cyclase activity and cyclic AMP concentrations were measured simultaneously.

RESULTS AND DISCUSSION OF THE RESULTS:

No difference was found in rat duodenal and liver adenyl cyclase activity in normal, adrenalectomized and adrenalectomized rats treated with corticosterone on a high casein, high glucose or high fructose diet. However, adenyl cyclase activity only in the adrenalectomized animals was greater than in the normal or adrenalectomized animals given corticosterone after fasting for 24 hours. Cyclic AMP concentrations were the same in each group of animals.

CONCLUSIONS:

Corticosterone does not affect adenyl cyclase activity or cyclic AMP levels in rat duodenum and liver after feeding different diets. However, the data suggests that adenyl cyclase activity is increased during fasting in adrenalectomized animals. These data are consistent with the present concept that steroid hormones work directly on chromatin after being transported to the nucleus by specific cytoplasmic steroid binding proteins.

RECOMMENDATIONS:

This work should be continued in connection with our studies with other hormones as indicated in Work Unit 078, studies #1, 20 and 24.

PUBLICATIONS: None.

STUDY NO. 7. — Study of patients with Type IV hyperlipemia.

PROBLEM:

In previous studies in 2 patients with type IV hyperlipemia (Annual Research Progress Report, FY71) there was the suggestion that adenyl

cyclase activity and cyclic AMP concentration could be lowered in adipose and jejunal mucosal tissues by clofibrate administration. This was based on studies demonstrating that clofibrate inhibits rat jejunal, hepatic and adipose tissue adenyl cyclase. Seven patients were studied. These patients were given liquid synthetic diets containing 3,000 calories, 80% as sucrose, 10% as protein (sodium caseinate) and 10% as fat (corn oil). Jejunal and adipose adenyl cyclase and cAMP were measured serially before and after clofibrate administration. In 4 patients adipose cAMP was measured before and after heparin, 10 mg intravenously.

RESULTS AND DISCUSSION OF THE RESULTS:

A significant decrease in the adipose tissue concentration of cAMP was produced by heparin in the 4 patients studied. Heparin may thus have a direct effect upon adipose adenyl cyclase activity, resulting in decreased cAMP and hence indirectly decreasing the activity of triglyceride lipase.

There were no consistent alterations in plasma lipids following high sucrose feeding or clofibrate administration in the present studies. There were no consistent changes in the activity of jejunal or adipose tissue adenyl cyclase or in the concentation of jejunal or adipose tissue cyclic AMP. The hyperglycemic response of 4 patients to a glucagon infusion (2 mg given over 7 minutes) was not altered by clofibrate administration.

RECOMMENDATIONS:

The interesting results concerning the effects of heparin on adipose cAMP should be studied in additional patients and in normal subjects.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6344	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	01	060
b. CONTRIBUTING	61145011	3A014501B71P	01	[redacted]
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Precede with Security Classification Code)*

(U) Basic Studies of Nutrition and Metabolism (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

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

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:* Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	4.2	134
	CURRENT 73	4.0	120

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
Chemistry Division

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME:* Ziporin, Z. Z.

TELEPHONE: 303 366 5311 X24214

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Huston, R. L.

NAME: Dowdy, R. P. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Nutrition; (U) Military Rations; (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.)

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 effects 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.
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.
25. (U) 71 07 - 72 06 Serum ceruloplasmin oxidase activity was markedly increased in both trained and exhaustively-exercised rats. The influence of magnesium on calcium absorption appears to be dependent upon the gut lumen calcium concentration. Cyclic AMP reduces the amount of calcium found in intestinal tissue, under in vitro conditions, probably by reducing uptake from the medium.

*Available to contractors upon originator's approval

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

The following investigations have been conducted under this work unit:

STUDY NO. 2	The Biochemistry of Calcium and Phosphorus Metabolism
STUDY NO. 3	Studies on Mineral Metabolism and Interactions

Study No. 2. Experiment 2. Studies on the absorption of calcium and phosphorus using actinomycin D ARN 484. Studies on the influence of actinomycin D on vitamin D metabolism and calcium and phosphorus absorption have been completed and a manuscript submitted for publication.

Experiment 3. An investigation of a possible relation between vitamin D and 3',5' - adenosine monophosphate in controlling the uptake of calcium by the small intestine. Intestinal tissue was labeled with ^{45}Ca *in vivo* and incubated *in vitro* in a medium containing non-radioactive calcium. Cyclic AMP in the medium appeared to reduce the movement of calcium from the tissue but did significantly decrease the amount of calcium accumulated from the medium. The studies suggest that the mode of action is to inhibit the movement of calcium from the medium into the tissue while at the same time possibly reducing the passage of calcium from the tissue to the medium. These findings have implications which warrant investigations into membrane phenomena responsible for the above results.

Experiment 4. The relation of vitamin D status to the synthesis of proteins. The relation of vitamin D to protein synthesis in intestinal mucosa and kidney will be studied.

Experiment 5. A study of the relation between hypophosphatemia and calcium status on red blood cell metabolism. Hypophosphatemia may be induced in humans by (1) oral administration of aluminum hydroxide gels; (2) intravenous administration of nutrients such as amino acids or glucose; (3) dialysis of a patient. Low blood phosphorus levels have also been found in rickets, osteomalacia, primary hyperparathyroidism, diabetic coma and phosphate diabetes. One of the effects of hypophosphatemia is an alteration in the metabolism of the red blood cell leading to an

impairment in the delivery of oxygen to the tissues. From our work with phosphorus-depleted rats, we know that the simple administration of phosphorus to these animals does not overcome the deficiency. There is a need for calcium when the phosphorus is given. The relation of calcium to phosphorus in the phosphorus-depleted animal using the red blood cell as the tissue will be investigated.

Study No. 3. Experiment 3. Metabolic interactions between magnesium and calcium. The calcium-magnesium interaction was studied using an *in situ* ligated intestinal segment in the rat. When the calcium concentration in this segment is low, increased magnesium appeared to inhibit the absorption of calcium-45 (as measured by tissue levels of ^{45}Ca). Conversely, at a nearly physiological concentration of calcium in the segment, increased magnesium enhanced ^{45}Ca absorption.

Experiment 5. Development of copper deficiency in rats. A 12-week training schedule of treadmill running resulted in significantly elevated serum ceruloplasmin oxidase activity (CPA) in the rat. Similarly, exhaustive exercise significantly increased serum CPA in both the trained and untrained rat.

BODY OF REPORT

WORK UNIT NO. 060 — Basic Studies of Nutrition and Metabolism

STUDY NO. 2 — The Biochemistry of Calcium and Phosphorus Metabolism

PROBLEM:

Experiment 2. Studies on the absorption of calcium and phosphorus using actinomycin D ARN 484. Results obtained at this laboratory did not support the published findings of other investigators that actinomycin D inhibited the action of vitamin D on intestinal uptake and transport of calcium. Additional investigations were designed and conducted to elucidate these discrepancies.

Experiment 3. An investigation of a possible relation between vitamin D and 3',5' - adenosine monophosphate in controlling the uptake of calcium by the small intestine. According to one current theory, cyclic AMP may be synthesized in response to vitamin D and may be the agent responsible for the increased permeability of calcium across intestinal membranes. If this could be established, a mechanism may be offered whereby vitamin D increases the passage of calcium into an intestinal slice in vitro.

Experiment 4. The relation of vitamin D status to the synthesis of proteins. To determine the extent to which vitamin D affects protein synthesis in specific target tissues. If it can be shown that vitamin D initiates the synthesis of proteins, other than calcium-binding protein, it should be possible to isolate these newly-formed proteins so that their possible relation to calcium and phosphorus metabolism might be investigated.

Experiment 5. A study of the relation between hypophosphatemia and calcium status on red blood cell metabolism. Hypophosphatemia is a specific disease entity with a defined therapeutic course of treatment which reveals the involvement of calcium. This entity and its treatment provide an area in which calcium-phosphorus interrelations may be studied.

RESULTS AND DISCUSSION OF THE RESULTS:

Ex. 2. Results of this investigation have been summarized in a manuscript submitted for publication.

Ex. 3. When intestinal slices were removed from vitamin D-deficient as well as vitamin D-repleted rats for incubation in a medium

containing radiocalcium (^{45}Ca), our criterion for assessing the effects of the various treatments was the accumulation of ^{45}Ca from the medium into the intestinal slice. Our finding that cyclic AMP reduced the amount of ^{45}Ca in the tissue after incubation indicated that this nucleotide reduced the ability of the tissue to accumulate this cation. This result might lead to the conclusion that cyclic AMP decreased the permeability of intestinal epithelial cells to the passage of calcium. This was at variance with results reported in the literature which stated that cyclic AMP increased the permeability of intestinal cells to calcium. Perhaps our finding of decreased amounts of ^{45}Ca in the tissue after incubation in the presence of cyclic AMP might be due to an increased permeability, as reported, which would lead to calcium leaking out at a faster rate than it was taken up but with both directions being enhanced over those occurring in the tissue incubated without cyclic AMP. More recent studies were undertaken in which the intestinal tissue was labeled with ^{45}Ca *in vivo*. Slices of the tissues were incubated *in vitro* in media with and without DBCAMP. From the fact that ^{45}Ca left the tissue mixed with the non-radioactive calcium in the flask and returned to the tissue, it was possible to calculate the extent to which this was taking place. The results are summarized in the following table:

Treatment Group[1]	% counts remaining in tissue (actual)	Fractional increase in Ca in tissue	% of original counts leaving tissue, mixing with pool and returning to tissue (calculated)
-D-DB	40.1 ± 10.2[2]	4.17 ± 1.99	76.0
-D+DB	36.4 ± 5.5	2.57 ± 0.78	72.5
+D-DB	45.0 ± 10.3	4.89 ± 1.50	73.5
+D+DB	42.9 ± 11.1	2.82 ± 0.87	68.0

[1] D - vitamin D
DB - dibutyryl cyclic adenosine 3'5' monophosphoria acid
- absence
+ presence

[2] Mean ± SD

While no absolute conclusions are possible from the above data, it would appear that: (1) Both vitamin D and dibutyryl cyclic AMP show a trend toward decreasing the fraction of counts leaving the tissue, mixing with

the medium and returning to the tissue; (2) DBCAMP in the medium causes a significant reduction in the amount of calcium found in the tissue after a 1-hour incubation in vitro. A more precise explanation of the mechanisms involved will require short incubation times to measure rates of influx and efflux, as well as the application of statistical methods to evaluate the contribution of each variable to the total picture.

Ex. 4. The protocol has recently been activated. Experiments will begin shortly.

Ex. 5. Hypophosphatemia in rats has been produced by rearing animals on two different diets: (1) Hi Ca (1.2%): 0 P (0.02%) and (2) 0 Ca (0.02%): 0 P (0.02%) for 20 days. Repletion of phosphorus was then instituted by placing these animals on either of two diets: (1) Hi Ca (1.2%): adeq P (0.3%), or (2) 0 Ca (0.02%): adeq P (0.3%). At sacrifice, blood was drawn for blood pH and blood gas measurements as well as total Ca and inorganic P in the blood. Red blood cell content of 2,3 diphosphoglycerate was also measured. Too few experiments and animals have been run to arrive at any conclusions.

CONCLUSIONS:

Ex. 2. With the manuscript regarding this work submitted for journal publication, this study has been terminated.

Ex. 3. Dibutyryl cyclic AMP significantly reduces the amount of calcium found in an intestinal slice incubated in vitro. It appears to reduce the fraction leaving the tissue, mixing with the calcium in the medium and returning to the tissue. Additional studies are needed before a more definitive description of the mechanisms involved can be provided.

PUBLICATIONS:

1. Zinorin, Z. Z., S. T. Aminoto and P. P. Waring. Acute effects from shifting rats on a low phosphorus diet to one containing adequate phosphorus. Fed. Proc. 31: 707 Abs (1972).

STUDY NO. 3 Studies on Mineral Metabolism and Interactions

PROBLEM:

Experiment 3. Metabolic interactions between magnesium and calcium. Many conflicting reports are in the literature concerning the effect of magnesium on calcium absorption. Some reports show that magnesium inhibits calcium absorption, some show no effect, and some show that magnesium enhanced calcium absorption. Perhaps some of these anomalies

could be attributed to variations in intestinal calcium concentrations. This study was designed to test such a hypothesis.

Experiment 5. Development of copper deficiency in rats. Ceruloplasmin (CP) is a copper-containing protein found in serum which shows oxidase activity toward a number of substrates. It has been proposed that CP activity may serve as an index of copper nutriture. However, CP activity is reportedly altered under a variety of conditions including certain pathological states, stress conditions and, perhaps, in high-performance athletes. The study to be reported here was designed to determine the effect of training and exhaustive exercise in trained and untrained rats on CP activity.

RESULTS AND DISCUSSION OF THE RESULTS:

Ex. 3. Adult rats weighing approximately 250 g were used in a 3 x 4 (Ca x Mg) factorially-designed experiment to study the effects of calcium and magnesium on the absorption and tissue distribution of calcium. An *in situ* ligated intestinal segment technique was used wherein the absorption solution being tested was delivered into a finite section of the gastrointestinal tract. The absorption and tissue distribution of ^{45}Ca was used as an index of calcium absorption. The absorption solution contained 50 μCi of ^{45}Ca along with the various concentrations and combinations of Ca and Mg. The three concentrations of Ca used were 0.2, 0.5 and 0.8 percent of the test dose; the 4 Mg concentrations were 0.0, 0.2, 0.5 and 0.8 percent of the test dose. Ca and Mg were supplied as the chloride salts and all solutions were formed in 0.9% saline. Two hours following dosage, the rats were sacrificed and the blood (for serum), liver, kidney and isolated gut segment were removed for isotopic assay. At the low concentration of Ca (0.2%), increasing the Mg from 0.5% to 0.8% significantly reduced (some 25-30%) the amount of ^{45}Ca found in serum, kidney and liver. This suggested that, at low Ca concentrations, increased Mg had an inhibitory effect on Ca absorption. Conversely, at the more physiological Ca concentration (0.8%), increasing the Mg from 0.5% to 0.8% significantly increased (approximately 45%) the ^{45}Ca concentration in the three tissues assayed. Data concerning the amount of ^{45}Ca remaining in the intestinal segment supported the tissue observations in that, where tissue ^{45}Ca was increased, residual gut ^{45}Ca was reduced and vice versa.

Ex. 5. Young adult rats (weighing approximately 110 g at the beginning of the study) were used in this experiment. Half of the rats were subjected to a training regimen of treadmill running 5 days/week while the other half of the rats were maintained as sedentary controls. The training schedule lasted for 12 weeks with sacrifice intervals at 1, 4, 9 and 12 weeks. To test the effect of training on serum CP

activity, the trained rats (no treadmill running for 24 hours prior to sacrifice) were compared to the sedentary controls. To test the effect of exercise on serum CP activity, some sedentary rats were run to exhaustion on the treadmill immediately prior to sacrifice and these rats compared with trained rats pair-run for the same length of time as it took to exhaust the sedentary rats. At the 12-week interval, some of the trained rats were also exercised to exhaustion. There was no effect of either training or exhaustive exercise on serum CP activity at the 1- and 4-week sacrifice periods. By the 9-week period, training and exhaustive exercise resulted in significant increases in CP activity over the sedentary rats (31% and 52%, respectively). Similar significant differences were observed at the 12-week sacrifice period. In addition, exhaustive exercise in the trained rats (at 12 weeks) resulted in a significant increase (32%) in serum CP activity over the trained control rats.

CONCLUSIONS:

Ex. 3. The concentration of calcium in the gut lumen appears to play an important role on the effect of magnesium on calcium absorption. Data from this study indicate that, at low concentrations, magnesium is inhibitory toward calcium absorption. Conversely, at near physiological calcium concentrations, magnesium appears to enhance calcium absorption.

Ex. 5. Serum ceruloplasmin oxidase activity (CPA) appears to be significantly increased in rats as a result of an extensive training regimen. Similarly, the stress of exhaustive exercise seems to be severe enough to markedly elevate CPA in trained, as well as untrained, rats.

RECOMMENDATIONS:

Ex. 3. Continue to study the nutritional and metabolic interaction between calcium and magnesium to more completely delineate the conditions under which magnesium is antagonistic to calcium absorption and the conditions where magnesium enhances calcium absorption. This information is needed to better define precise nutritional requirements.

Ex. 5. Additional studies should be performed to ascertain the consequences of elevated serum ceruloplasmin oxidase activity which results from either training or the stress of exhaustive exercise. This could be important from the point of view that ceruloplasmin has oxidase activity toward iron and may, in turn, influence hemoglobin and oxygen transport functions in highly physically-trained individuals such as combat soldiers.

Basic Studies of Nutrition and Metabolism (Cont'd)

PUBLICATIONS:

1. Dowdy, R. P., and G. L. Dohm. Effect of training and exercise on serum ceruloplasmin in rats. Proc. Soc. Exptl. Biol. Med. 139: 489, 1972.

2. Dowdy, R. P., and F. H. Nielsen. Effect of histidine, histamine, and aspirin on sulfur-35 metabolism in zinc-deficient chick bone. J. Nutr. 102: 529, 1972.

3. Dowdy, R. P., and F. H. Nielsen. Effect of histidine, histamine, and aspirin on sulfur-35 metabolism in zinc-deficient chick bone. Fed. Proc. 31: 667 Abs (1972).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6341	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	01	061
b. CONTRIBUTING	61145011	3A014501B71P	01	[redacted]
c. CONTRIBUTING	CDOG 114 (f)			[redacted]

11. TITLE (Procede with Security Classification Code)* (U) Mineral Metabolism - The Requirements of Trace Minerals in Man Under Various Stresses (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

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

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:* Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	2.5	40
FISCAL YEAR CURRENT 73	2.5	18

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
Bioenergetics Division

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME:* Johnson, H. L.

TELEPHONE: 303 366 5311 X25222

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Consolazio, C. F.

NAME: Burk, R. F., CPT, MC DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Human Mineral Balances; (U) Trace Mineral Requirements; (U) Rations and Minerals; (U) Selenium and Vitamin E

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

23. (U) (1) To study the interaction between macro mineral metabolism and water balances as affected by nutritional and environmental stresses encountered during mili tary manuevers; (2) to determine the essentiality and requirements of trace minerals which may become limiting in rations; (3) to study the distribution and function of selenium (Se).

24. (U) Mineral balances including sweat losses, whenever feasible, are being conducted on military volunteer and conscientious objector subjects during studies on the effects of diet and environment upon physical performance, physiological function and body composition. Correlations are made between mineral balances, body fluid changes and other nutrient balances. Studies in the distribution and metabolism of selenium are progressing.

25. (U) 71 07 - 72 06 Two selenium-containing proteins have been identified in rat plasma and several binding studies have been done. Selenium is attached to the mid-piece of rat spermatozoa and appears to be essential for spermatogenesis. Analysis of samples and calculation of mineral balances during a study on the effects of ingesting electrolyte solutions upon physical performance are in progress. Evaluation of mineral status during studies on altering protein and carbohydrate contents of diets is progressing. Studies of urinary selenium metabolites are continuing. Purification and amino acid analyses of rat plasma proteins and identification and characterization of selenium-containing human plasma proteins will be completed.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498-1

ABSTRACT

PROJECT NO.	3A061102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biochemistry
WORK UNIT NO.	061	Mineral Metabolism - The requirement of Trace Minerals in Man Under Various Stresses

Blood, urine, fecal and sweat samples were collected during the study to evaluate the effect of ingesting electrolyte solutions upon work performance. Mineral balances, including sweat excretions, for sodium, potassium, calcium and magnesium (also, trace minerals, copper, zinc and nickel) will be calculated on the men consuming constant diets for 12 weeks. These diets were altered with various electrolyte solutions during 4 hours of exercise per day in the hot room. Mineral balances will be correlated with changes in the body water status of the men.

The normal physiology of selenium in the rat has been studied with the aid of ^{75}Se. Over 90% of the ^{75}Se given as $^{75}SeO_3^=$ was absorbed from the GI tract when the dietary level was 0 to 3 ppm Se. Two selenium-containing plasma proteins have been discovered. It has been shown that the tracer selenium concentrates heavily in the midpiece of the rat spermatozoan when the animal is fed a low-selenium diet. Finally, data have been obtained which suggested that the liver responded to increased dietary selenium by making a selenium metabolite which was excreted in the urine.

BODY OF REPORT

WORK UNIT NO. 061

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

PROBLEM:

The state of body hydration is correlated with fluid and electrolyte balances, and is affected by nutritional, physical and environmental stresses. Measurement of complete intakes and excretions provide the data for calculation of mineral balances which are then correlated with fluid balance and fluid compartment shifts. The results obtained will provide information on the mechanisms involved and possibly on methods of counteracting the detrimental effects of these changes. Hypohydration contribution to large weight losses have been consistently observed during calorie restriction, abrupt altitude exposure and often occurs under conditions of profuse sweating, e.g., during heavy work and/or in hot environments. Further studies on these phenomena are required.

With advancing technology for elemental analyses and diet purification the requirements for additional trace elements are being established. One of these minerals that has been found to be essential in several species of animals, but not as yet in man, is selenium. Selenium will prevent specific degenerative diseases in various animals.

Except for the observations that serum selenium levels were reduced in some kwashiorkor cases, and that it is difficult to attain positive nitrogen balances until the selenium levels were increased, essentially no information has been obtained in humans. There is a distinct possibility that selenium may have a role in human liver disease; however, more information is needed on normal metabolism of selenium before studying abnormal states. Therefore, a series of experiments has been initiated on the intestinal absorption, plasma transport, tissue distribution, and urinary excretion of this element.

RESULTS AND DISCUSSION OF THE RESULTS:

(Under Work Unit 073) Weight losses of one to two kilograms were observed for men exercising for 4 hours in a hot room to test the effects of various commercial "anti-fatigue" drinks and electrolyte solutions upon energy expenditure and physical performance. The volunteer subjects were on a constant diet throughout the 12-week study. The only variations in their mineral intakes were in the fluid supplements consumed during the exercise periods. Twenty-four hour urine and fecal collections

and daily sweat samples during work, were taken throughout the study. These samples will be analyzed for sodium, potassium, calcium and magnesium (also zinc, copper and nickel,) so that balances can be determined and related to the water losses. A pilot study of intestinal absorption using tracer doses of $^{75}SeO_3$ administered by stomach tube showed 90+% absorption of the label. No influence due to dietary selenium level was found. This is in contrast to work reported in the Annual Research Progress Report - FY 71 and will be studied further.

Two selenium-containing plasma proteins have been identified in the rat. Binding studies show that one of these did not bind selenium when the animal was depleted of selenium. Selenium can be removed from both proteins by alkaline dialysis. Studies of selenium distribution in the tissues of rats have shown that selenium was incorporated into the midpiece of the spermatozoan and was probably essential for spermatogenesis. The brain and thymus concentrate selenium well in selenium-depleted animals. All studies completed indicate that the kidney was the major route of selenium excretion in the rat under physiologic conditions. It has been shown that urinary selenium was directly related to dietary selenium level and that the liver was mainly responsible for the formation of urinary metabolites of the element. It has been determined that the production of the metabolites does not begin until dietary selenium reaches 0.05 ppm.

CONCLUSIONS:

During studies on the effects of "anti-fatigue" or energy drinks on energy expenditure and physical performance, complete balances of sodium, potassium, calcium and magnesium will provide information on body water changes. Analyses of sweat samples will reveal the contribution of these losses to balance studies.

Selenium has a unique metabolism. It is efficiently absorbed in the selenite form and is rapidly taken up by many tissues. The liver appears to have a central role in selenium metabolism. It likely binds the element to the selenium-containing plasma proteins and produces its urinary metabolites in proportion to intake.

RECOMMENDATIONS:

Continue mineral balances incorporating more of the trace elements in human studies involving any stress which may affect the water or electrolyte status of the man. It is recommended that these basic studies be continued particularly in the area of selenium-containing proteins and consideration be given to designing appropriate human studies.

PUBLICATIONS:

1. Burk, R. F. and C. F. Consolazio. Selenium-containing rat plasma proteins. Fed. Proc. 31: 692 Abs., 1972. (Abstract)

2. Brown, D. G. and R. F. Burk. Selenium retention in tissues and sperm of rats fed a Torula yeast diet. Fed. Proc. 31 692 Abs., 1972. (Abstract)

3. Burk, R. F., D. G. Brown, R. J. Seely, C. C. Scaief. Influence of dietary and injected selenium on whole body retention, route of excretion, and tissue distribution of $^{75}SeO_3^=$ in the rat. J. Nutr. (in press).

4. Brown, D. G., R. F. Burk. Selenium retention in tissues and sperm of rats fed a Torula yeast diet. (Cleared for publication)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6338	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	01	062
b. CONTRIBUTING	61145011	3A014501B71P	01	[redacted]
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Precede with Security Classification Code)

(U) Haemopoietic Metabolism as Related to Nutrition, Genetics and Metabolic Disease (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

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

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	0.1	32
FISCAL YEAR CURRENT 73	0.3	18

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Metabolic Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Herman, R. H., COL, MC

TELEPHONE: 303 366 5311 X25193

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Hagler, L., LTC, MC

NAME: DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Vitamin Requirements of the Combat Soldier; (U) Red Blood Cell Enzymes; (U) Red Blood Cell Membrane; (U) Blood Cell Metabolism

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

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 one must consider the fact that different tissues require different amounts of vitamins under varying environmental condition. Vitamins interact with one another which affects the requirement of each vitamin. The human red blood cell is the most technically feasible system for determining a specific tissue requirement of vitamins and vitamin interaction. Folic acid increases the activity of glycolytic enzymes of jejunum. There is a suggestion that this may be true in the RBC. Thus, the red cell will be used as a model to investigate the mechanism of action of folic acid on red cell enzymes.

24. (U) Administration of folate orally to a patient with formiminotransferase (FIT) deficiency resulted in an increase to a minimal in red cell F.I.T. Administration of folate orally to normal subjects and to patients with various metabolic problems resulted in an increase in F.I.T. activity in a dose related fashion suggesting that the folate effect was directly on the red cell since the time span during which changes took place were too short to allow for red cell replacement by cells synthesized in the bone marrow. On this basis red blood cells will be incubated in vitro and the effect of folic acid on red cell and platelet enzyme activities will be investigated.

25. (U) 71 07 - 72 06 Folate appears to affect red cells directly and therefore further studies on red cells and platelets will be carried out.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3A061102B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biochemistry
WORK UNIT NO.	062	Haemopoietic Metabolism as Related to Nutrition, Genetics and Metabolic Disease.

The following investigations have been conducted under this work unit:

STUDY NO. 2. The effect of folic acid on glycolytic enzymes in human red blood cells.

Study No. 2. Since the glycolytic enzymes of human jejunum increase in activity after the administration of folic acid it has been postulated that this increase is due to activation of protein synthesis. On this basis plans have been formulated to test the system in red blood cells since there may be residual components of this system available in red cells to demonstrate red cell enzyme increases. There is some experimental data to support this since folic acid administration to individuals results in an increase in formimino-transferase activity in red blood cells at a rate quicker than would be expected if the increase were due to the effect of folic acid on developing red cells in bone marrow and release of these red cells containing increased activity into the peripheral blood. This study is still in a formative stage.

BODY OF REPORT

WORK UNIT NO. 062 — Haemopoietic Metabolism as Related to Nutrition, Genetics and Metabolic Disease.

STUDY NO. 2. — The effect of folic acid on glycolytic enzymes in human red blood cells.

PROBLEM:

In the study of a patient with formiminotransferase deficiency of jejunum, red blood cells and liver it was found that folic acid administration increased the level of formiminotransferase to a small degree in the gut and red cell and decreased the FIGLU aciduria that was occurring. In normal individuals and certain other patients who had a normal formiminotransferase activity in the jejunum it was noted that there was an increase in formiminotransferase activity in red cells when folic acid was administered in large amounts. This increase seemed to occur at a rate far more rapidly than could be accounted for by a release of red cells into the peripheral blood from the bone marrow that had increased formiminotransferase due to an effect of the folic acid on the developing red cells within the bone marrow.

It has been postulated that folic acid increases enzyme activities by generating tetrahydrofolate which then is metabolized by formiminotransferase to the formiminotetrahydrofolate which then forms the N^{10}-formyltetrahydrofolate. This latter compound serves as a cofactor for formylating methionyl-transfer RNA to form formylmethionyl-transfer RNA which serves as an initiator of protein synthesis. This mechanism has been proven to occur in microorganisms and in certain plant systems but it is not generally accepted to be a mechanism occurring in mammalian tissue although certain of the components of this mechanism exist in mammalian systems. It may well be that this is a system that occurs only in certain tissues such as jejunum. Since folic acid is neither a cofactor nor a substrate for the glycolytic enzymes of the jejunum, induction-repression mechanisms would not seem to be the appropriate mechanism whereby folic acid increases enzyme activities.

Folic acid is necessary for pyrimidine and purine biosynthesis. Two folate cofactors are necessary for purine biosynthesis but the formation of purines would have to be the limiting factor in supplying nucleotides for mRNA synthesis if one were to suppose that the folate-effect occurred via increased mRNA formation. Of course, a pre-existing pyrimidine pool could be present and serve as the source of the increased requirement for pyrimidine should purine synthesis be increased. The overall effect of stimulating purine synthesis then would be to increase the ability to make more messenger RNA. Since red cells seem to be stimulated by folic acid in vivo and yet lack any of the usual protein synthesizing machinery and certainly lack a nucleus

Haemopoietic Metabolism as Related to Nutrition, Genetics and Metabolic Disease (Cont'd)

whereby messenger RNA could be made it may well be that certain of the remnants of the protein synthesizing machinery (for example messenger RNA, ribosomes) would be present in sufficient quantity to cause an increase in red cell glycolytic enzyme activities in the presence of large amounts of folic acid. Thus, it would seem reasonable to utilize red cells as a test system to investigate the mechanism whereby folic acid increases glycolytic enzyme activities. Should folic acid be able to cause an increase in red cell glycolytic enzymes this would tend to eliminate the repression-induction mechanism and an increased synthesis of messenger RNA as mediators of the folate effect and would tend to support the hypothesis that initiation is the mechanism of action of folic acid. Thus, we have devoted considerable time to the development of a theoretical approach to the study of folic acid effect on enzymes and are presently formulating plans for studying the effect of folic acid on red cell enzymes.

RESULTS AND DISCUSSION OF THE RESULTS:

The study has not yet been carried out experimentally. We are still formulating the approach to this problem.

CONCLUSIONS:

This study is in a process of being carried out.

RECOMMENDATIONS:

None at this time.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6321	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	02	058
b. CONTRIBUTING	61145011	3A014501B71R	02	[redacted]
c. CONTRIBUTING	CDOG 114(f)			[redacted]

11. TITLE (Precede with Security Classification Code)*

(U) Nutritional and Metabolic Adaptations and Interrelationships (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

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

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	5.0	68
FISCAL YEAR CURRENT 73	4.5	80

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
Chemistry Division
ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Sauberlich, H. E.
TELEPHONE: 303 366 5311 X24214
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME: Huston, R. L.
NAME: Askew, E. W., CPT, MSC DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Exercise; (U) Metabolism; (U) Diet; (U) Adaptation; (U) Enzymes; (U) Lipogenesis; (U) Fatigue; (U) Military Physical Performance

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

23. (U) To study adaptive changes which occur in laboratory animals as a result of exercise and physical training and to determine the effects of environment and nutrition on these adaptive changes. Obtain information on these aspects that may provide an improved rational program of physical training and nutrition of man in order to improve his ability to cope with various environmental and military situations.

24. (U) Rats conditioned by a standardized 12-week program of treadmill running were utilized to study the effect of physical exertion and diet on performance and cellular metabolic adaptations. The effect of physical training and diet on key enzymes of glycogen mobilization, glycolysis, lipogenesis, ketogenesis, gluconeogenesis, glyceride synthesis, TCA cycle, fatty acid oxidation, oxidative phosphorylation, plasma glucose, FFA, ketones and glucocorticoids was measured in both trained and untrained rats in the rested and exhausted states.

25. (U) 71 07 - 72 06 Feeding normal, high-fat, high-carbohydrate or carnitine diets to rats undergoing a physical conditioning program did not significantly affect running time to exhaustion but was associated with marked changes in serum metabolites and tissue enzyme activities. Main energy-generating metabolic pathways were enhanced by physical training. Evidence was obtained indicating that gluconeogenesis and possibly plasma glycocorticoids were related to the enhanced performance of trained rats.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498-1

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. 7	Influence of Diet and Exercise on Energy Metabolism and Performance

Study No. 5. The biochemical adaptations of muscle mitochondria due to training and subsequent exhaustive exercise were compared to the changes induced in mitochondria isolated from trained and untrained rats exposed to hypoxia (25,000 ft. ASL) for 6 hours. No changes were noted in the mitochondria obtained from untrained rats due to hypoxia, but those from trained animals exhibited changes similar to those found in mitochondria from rats run to exhaustion. Feeding a control diet, high fat diet or a high carbohydrate diet to trained and untrained rats for a period of 4 weeks did not alter the effects of training or exhaustive exercise on mitochondria isolated from muscle.

Study No. 6. The effect of physical training and exhaustion was extended to include synthetic as well as degradative pathways of lipid metabolism. While exhaustion tended to decrease certain oxidative pathways, it had an opposite effect on processes providing substrate for these pathways agreeing with the concept that exhaustion is not related to a shortage of substrate for lipid oxidation. The synthesis of glycerides by muscle and adipose was enhanced by training indicating that local as well as depot stores of lipid are important energy sources during exercise. Ketone body oxidation was increased in muscle of trained animals implying that ketones may play an important role in providing muscular tissue an alternate form of the energy contained in fatty acids.

Study No. 7. Exercise and training resulted in enhanced enzyme activities of both aerobic and anaerobic pathways. The dietary changes employed did not alter these adaptations. Hepatic gluconeogenesis was increased by training especially in animals fed high fat diets. Gluconeogenesis may be important in prolonging work performance by maintaining blood glucose levels above the point where an animal may

become hypoglycemic hence delaying hypoglycemic shock and a subsequent cessation of performance. Exhaustive exercise caused different results in trained compared to untrained animals. The former were exhausted with a more intense, longer work load and exhibited a classic metabolic picture of an exhausted animal. The latter were exercised with a lighter, shorter work load and were mobilizing and metabolizing substrate faster than their rested controls. Hence, untrained subjects had the capacity, energetically speaking, to perform longer but for some reason could not.

BODY OF REPORT

WORK UNIT NO. 058 — Nutritional and Metabolic Adaptations and Interrelationships

STUDY NO. 5 — Biochemical Adaptation to Exercise

PROBLEM:

An effort was made to demonstrate a relationship between the effects of hypoxia and exhaustive exercise at the cellular level. During strenuous exercise, the demand for oxygen in the muscle may be greater than the supply causing lower oxygen tension in the muscle. Thus, intracellular hypoxia may occur during exhaustive exercise and could be similar to the conditions induced by a hypoxic environment. It was the purpose of this study to investigate effects of hypoxia on the oxidative capacity of muscle mitochondria from exhausted rats and to determine if low oxygen tension could be a factor causing the changes noted in the annual report for FY 71.

The effects of dietary alterations on muscle mitochondrial oxidation rates were investigated. Similarly, the influence of dietary changes on the effects of acute exercise on mitochondrial properties was studied.

RESULTS AND DISCUSSION OF THE RESULTS:

The annual report for FY 71 described results obtained from trained and untrained animals exposed to altitude for 24 hours. It was concluded that anorexia induced by short-term hypoxia influenced our biochemical observations. Subsequently, we chose a short-term hypoxic exposure at 25,000 feet in an altitude chamber to circumvent the effects of anorexia and more closely approximate the time required to run trained animals to exhaustion at 1.25 mph.

Following a 12-week period of treadmill running employed to condition the animals, both trained and untrained animals were placed in an altitude chamber for 6 hours without food or water. During this time, trained animals were also run to exhaustion at 1.25 mph while untrained rats were exhausted at 0.5 mph. Appropriate control animals were sacrificed in a rested state (no exercise for at least 24 hours). Mitochondria were isolated and incubated with substrate to measure oxygen uptake rate with a Clark oxygen electrode.

The results in Table I show that hypoxia did not affect oxidative capacity in skeletal muscle mitochondria from untrained rats regardless of substrate. However, mitochondria from hypoxic trained rats respired less actively indicating some impairment in Krebs cycle and fatty acid oxidative capacities.

These results are similar to those reported in the previous annual report and would tempt one to conclude that exercise and hypoxia affect the oxidative capacity in skeletal muscle mitochondria similarly. It could be argued that the lower oxygen tension in skeletal muscle produced during strenuous exercise causes changes in the oxidative capacity of muscle mitochondria. It is also conceivable, however, that the effects of strenuous exercise and hypoxia are mediated through a common process and that intracellular hypoxia itself may not be the causative factor. Conclusive data is not yet available to determine which of the above two possibilities is the answer.

Control, high fat (60%) and high carbohydrate (76.5%) diets were fed during the last 4 weeks of the 12-week training period. No changes in the effects of exhaustive exercise upon skeletal muscle mitochondria were evident when comparing the results from animals on each of the three diets. Dietary effects were absent in both trained and untrained animals.

TABLE I

Effect of Hypoxia (6 hrs at 25,000 ft) on Pyruvate and Palmitate Oxidation by Skeletal Muscle Mitochondria from Trained and Untrained Rats

	Oxygen uptake (µg atoms/hr/mg protein)	
	Normoxic	Hypoxic
Pyruvate		
Untrained	5.62 ± .27 (15)[1]	6.16 ± .94 (6)
Trained	6.16 ± .44 (11)	4.92 ± .66 (5)
Palmitate		
Untrained	3.68 ± .26 (15)	3.31 ± .57 (5)
Trained	3.60 ± .25 (11)	1.78 ± .26 (4)[2]

[1]Mean ± standard error, number of observations in parentheses.

[2]Significantly different from trained normoxic ($P < .01$).

CONCLUSIONS:

1. Hypoxia induced no changes in the mitochondria obtained from untrained rats, but mitochondria prepared from trained animals exhibited changes similar to those observed with animals run to exhaustion.

2. Feeding rats diets high in fat or carbohydrate did not influence the effects of training or exhaustive exercise on mitochondria isolated from muscle.

Nutritional and Metabolic Adaptations and Interrelationships (Cont'd)

PUBLICATIONS:

1. Dohm, G. L., R. L. Huston, E. W. Askew, P. C. Weiser. The effect of acute exercise on the activity of heart and skeletal muscle mitochondria. Accepted by Am. J. Phys. 1972.

STUDY NO. 6 Dietary Control of Lipid Metabolism

PROBLEM:

The oxidation of fat provides the majority of the calories expended during exercise of sub-maximal intensity of long duration. Lipid metabolism is responsive to dietary alterations. Conceivably, alteration of the flux of substrate through the synthetic and degradative pathways of lipid metabolism could influence physical performance. We have attempted to identify the biochemical adaptations of lipid metabolism to physical training and determine if dietary adaptive response is additive or, in some manner, complementary to the training response.

RESULTS AND DISCUSSION OF THE RESULTS:

1. Synthetic pathways.
Glyceride synthesis was increased by training in skeletal muscle, heart, and adipose tissue but not in liver. Exhaustion decreased glyceride synthesis in muscle, adipose and liver. These results indicate that local as well as depot stores of glycerolipids are important energy stores in the exercising animal. The ability to store fatty acids as glycerides in time of inactivity to be called upon during physical exertion appears to be an adaptation to physical training. The decrease in glyceride synthesis observed following exhaustive exercise appears to be a logical mechanism preventing the expenditure of ATP for synthetic purposes when it is needed for muscular contraction.

Lipogenesis by adipose tissues and liver as reflected by malic enzyme, citrate cleavage enzyme and glucose-6-phosphate dehydrogenase activities was not affected by physical training or exhaustion. Lipogenesis was increased by feeding a high carbohydrate diet to rats. In the case of the liver, the lipogenic response of the high carbohydrate-fed rats was greater in sedentary than in trained rats. This would be compatible with a smaller acetate pool available for fatty acid synthesis in trained rats due to increased utilization of acetate for production of energy.

Acetoacetyl-CoA thiolase and HMG-CoA synthetase activities in the liver were unaffected by training and exhaustion although plasma ß-hydroxybutyric acid was increased by both variables. The ketogenic capacity of the liver may be of such magnitude that ketogenesis is controlled by supply of FFA to the liver.

2. Oxidative pathways.
Both palmityl-carnitine oxidation by skeletal muscle mitochondria and ketone body oxidation (3-oxoacid-CoA transferase) by disrupted skeletal muscle mitochondria were increased by training and decreased by exhaustion. The effect of training on these two pathways of fatty acid oxidation indicates that the partioning of the energy of fatty acids into ketone bodies prior to oxidation by skeletal muscle may be advantageous to the intact animal. This effect may be related to a deleterious effect of high concentrations of fatty acids upon muscle cellular enzymic processes and represent an attempt to provide the energy contained in fatty acids in an alternate form. It is not known if the adverse effect of exhaustion on fatty acid and ketone body oxidation is caused by or contributes to exhaustion. Further definitive studies are required to elucidate this problem.

CONCLUSIONS:

The effect of physical training and exhaustion was extended to include synthetic as well as degradative pathways of lipid metabolism. Glyceride synthesis was increased by training in skeletal muscle, heart and adipose tissue but not in liver. Exhaustion decreased glyceride synthesis. Both palmityl-carnitine oxidation by skeletal muscle mitochondria and ketone body oxidation by disrupted skeletal muscle mitochondria were increased by training and decreased by exhaustion.

PUBLICATIONS:

1. Askew, E. W., G. L. Dohm, R. L. Huston and W. H. Doub, Jr. Effect of diet, exercise and physical training on lipid metabolism in the rat. Fed. Proc. 31: 719 Abs (1972).
2. Askew, E. W., R. L. Huston, G. L. Dohm and P. C. Weiser. Biochemical adaptation of energy metabolism to exercise training. J. Colo.-Wyo. Acad. Sci. VII: 41 Abs (1972).

STUDY NO. 7 Influence of Diet and Exercise on Energy Metabolism and Performance

PROBLEM:

Earlier assessments of biochemical adaptations to exercise were made on intact mitochondria. Additional studies have been initiated to investigate enzyme activities in skeletal muscle (glycolysis and TCA cycle) and liver (gluconeogenesis) as influenced by strenuous physical exercise and how these adaptations are altered by diet. The object was to gain insight into which key enzymes might be influenced by both diet and exercise.

Nutritional and Metabolic Adaptations and Interrelationships (Cont'd)

RESULTS AND DISCUSSION OF THE RESULTS:

Male Carworth rats were trained by treadmill running for 12 weeks. During the last 4 weeks of this period, trained and untrained rats were fed either a control, high fat (60%) or high carbohydrate (76.5%) diet, each diet formulated to contain 176 calories per gram of dietary nitrogen. Prior to sacrifice, one half of the trained rats were exhausted at 1.5 mph and one half of the untrained rats exhausted at 0.6 mph while the remainder in each group was sacrificed in a rested state. It should be emphasized that trained and untrained animals exhausted in this manner exhibit deep body temperatures of 41-42°C (indicating a true state of exhaustion) when they can no longer be forced to run after repeated electrical stimuli. The comparisons reported here between trained and untrained animals were considered valid in that both were sacrificed in an exhausted state even though the conditions used to reach exhaustion were different.

Glycolysis (muscle): No dietary effects were found on the glycolytic enzyme activities. Training increased phosphorylase, phosphofructokinase (PFK) and hexokinase activities significantly ($P < .01$) indicating an enhanced capacity to mobilize glycogen, an elevated overall glycolytic activity (PFK is rate limiting) and a greater ability to phosphorylate blood glucose for energy metabolism. These adaptations to training were detected in rested animals that had not been exercised for 48 hours.

Other glycolytic enzymes were measured with no response to training. These included aldolase, pyruvate kinase and lactic dehydrogenase all of which turn over much greater quantities of substrate per gram of tissue than those earlier in the glycolytic scheme. The adaptations to training, therefore, are manifested in the rate-limiting enzymes of glycolysis.

Exhaustion caused different biochemical effects in trained animals compared to untrained rats. In fact, it would appear that trained animals were adversely affected whereas untrained animals were not. The trained rats exhibited higher phosphorylase and PFK activities than untrained-rested animals, but trained-exhausted and trained-rested rats exhibited similar activities. Upon the termination of their exhaustive exercise period (approximately 1 hour), untrained rats were apparently mobilizing muscle glycogen stores faster and possessed higher overall glycolytic activity than untrained-rested animals. Also, blood glucose levels were much higher in untrained-exhausted than in untrained-rested rats. Biochemically speaking, untrained animals did not appear exhausted but, as indicated earlier, were unable to exercise beyond 1 hour at 0.6 mph. Conversely, trained animals were not mobilizing and metabolizing carbohydrate any faster in the exhausted as compared to the rested state and exhibited

plasma titers indicative of hypoglycemic shock. We interpret this to mean the trained-exhausted animals were depleted of carbohydrate stores and represent the classical metabolic picture of an exhausted animal. However, trained rats ran much longer (approximately 3-4 hours) at a higher work load. Even though both trained and untrained animals were physically exhausted, the trained animal reflects the expected metabolic picture. Perhaps the lower intensity and/or shorter duration exercise period employed to exhaust untrained individuals was not sufficent to induce the expected metabolic results, or there was some other factor causing the untrained animal to terminate his exercise period that was not reflected in the metabolic parameters we measured.

Hexokinase was lowered by exhaustion in both trained and untrained animals. This may be a mechanism to limit glucose utilization by exercising muscle because glucose may be better utilized by brain tissue where it is the major metabolic fuel. This mechanism would help delay hypoglycemic shock and avoid complications in the central nervous system since glucose would be burned more rapidly by exercising muscle.

Gluconeogenesis (liver): Pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEP CK) were elevated in trained-rested animals fed a high fat diet but no other dietary effects were evident. Subsequent exhaustion reduced the former activity but increased the latter. PEP CK activity was reduced by exhaustion in rats fed the other two diets also. It would seem that training increased the capacity for glucose synthesis from non-carbohydrate precursors. Since the caloric yield from carbohydrate is considerably less than from fat and the latter is the major metabolic fuel in exercising muscle anyway, perhaps the glucose thus formed is used to maintain blood levels and to prevent hypoglycemic shock.

After a sufficient period of exercise, however, when the animal starts drawing more upon anaerobic pathways for additional ATP, blood glucose stores are finally utilized for energy and thus depleted. Oddly enough, this was true for trained rats but not untrained rats run to exhaustion. In fact, untrained-exhausted animals exhibited no increased gluconeogenic capacity as did trained-exhausted rats. Plasma glucocorticoids were elevated in both trained and untrained exhausted animals which should act to increase gluconeogenic enzyme capacity. The reasons for gluconeogenic enzymes in untrained rats not responding to exhaustion or the glucocorticoids are not understood at this time.

TCA cycle (muscle): Once again no TCA cycle enzymes were influenced by the dietary treatments. Training increased citrate synthetase, isocitrate, succinic dehydrogenase, succinate oxidation by intact muscle mitochondria, malic dehydrogenase and cytochrome oxidase. The latter was measured using intact mitochondria. Consequently, the most efficient

energy-producing pathway in muscle, i.e., aerobic metabolism, has a greater capacity as a result of the training stimulus.

Although food consumption information was obtained, pair-feeding was not employed in these studies because of the personnel demands such practices would impose. Moreover, other investigators have found that pair-feeding of animals to equal food intakes or the equal body weights did not influence the results obtained in exercise and training studies (e.g., Molé, Oscai and Holloszy, J. Clin. Invest. 50: 2323, 1971; Oscai and Holloszy, J. Biol. Chem. 246: 6968, 1971).

CONCLUSIONS:

Enzyme activities of both the aerobic and anaerobic pathways were enhanced by exercise and training. These effects were not altered by feeding animals diets high in fat or carbohydrate. Gluconeogenesis may be important not only in the production of substrate for energy production in muscle but also in supplying blood glucose which may help delay hypoglycemic symptoms and cessation of performance.

PUBLICATIONS:

1. Huston, R. L., G. L. Dohm, J. B. Boyd and E. W. Askew. Effect of exercise training and exhaustion upon mitochondrial function in skeletal muscle. J. Colo.-Wyo. Acad. Sci. VII: 41 Abs (1972).

2. Huston, R. L., G. L. Dohm, P. C. Weiser, J. Boyd and E. W. Askew. The effect of diet, exercise and training on skeletal muscle glycolytic and gluconeogenic enzymes of the rat. Fed. Proc. 31: 719 Abs (1972).

3. Dohm, G. L., R. L. Huston, E. W. Askew and H. L. Fleshood. The effects of diet, exercise and training on Krebs cycle enzymes in skeletal muscle of the rat. Fed. Proc. 31: 719 Abs (1972).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6334	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	02	062
b. CONTRIBUTING	61145011	3A014501B71R	02	
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Precede with Security Classification Code): (U) Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet, and Steroids in Normal Man and Disease (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 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
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	1.4	67
FISCAL YEAR CURRENT 73	0.5	66

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240
RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
Metabolic Division
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Herman, R. H., COL, MC
TELEPHONE: 303 366 5311 X25193
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]
ASSOCIATE INVESTIGATORS
NAME: Hagler, L., LTC, MC
NAME: DA

21. GENERAL USE
Foreign Intelligence not Considered

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

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

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 patients with a variety of muscle diseases and muscle cell membranes will be isolated. The protein components will be examined by various techniques and compared to normal muscle obtained from patients undergoing thoracotomy.

25 (U) 71 07 - 72 06 Studies of a patient with myoglobinuria has demonstrated that not only myoglobin leaks from the muscle but also the muscle-type of pyruvate kinase. This suggests that the defect in myoglobinuria resides in the muscle cell membrane.

*Available to contractors upon originator's approval

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. 2. Studies in a patient with idiopathic myoglobinuria.

Study No. 2. Laboratory investigation of muscle specimens obtained from a patient with idiopathic myoglobinuria have continued since the discharge of the patient. In these studies we have determined the following: 1) The pigment in the urine is myoglobin and is identical to myoglobin which was isolated from the patient's muscle. The identity of the pigment has been convincingly demonstrated by scanning spectrophotometry, sephadex column chromatography, and polyacrylamide gel electrophoresis. 2) The elevated serum level of pyruvate kinase (PK) was shown to originate from muscle since its electrophoretic mobility corresponded to that of muscle PK, which is distinctly different from the mobility of liver or red blood cell PK. Techniques have been perfected and studies are now underway to further characterize the myoglobin by "fingerprinting", and to further evaluate the muscle membrane structure.

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. 2. — Studies in a patient with idopathic myoglobinuria.

PROBLEM:

To continue laboratory investigation of specimens obtained during the study of a patient with idiopathic myoglobinuria. Much of the background information concerning this patient and the rationale for the studies which were undertaken were documented in the Annual Progress report dated 30 June 1971.

RESULTS AND DISCUSSION OF THE RESULTS:

Continuing evaluation of laboratory specimens obtained during the patient's hospitalization have revealed the following: 1) The dark pigment passed in the urine by the patient during symptomatic episodes is myoglobin. This was differentiated from hemoglobin by appropriate methods of extraction and evaluation on the scanning spectrophotometer, on sephadex gel column chromatography, and by polyacrylamide gel electrophoresis in 5 and 10% gels. These techniques serve to distinguish hemoglobin from myoglobin and conclusively identify the pigment in the patient's urine as myoglobin. 2) We postulated that the patient's elevated serum pyruvate kinase level had its origin in muscle and that the "leaky" muscle membrane allowed pyruvate kinase which is normally contained in muscle access to the serum. To prove this we developed a method of pyruvate kinase electrophoresis in acrylamide gels using specific enzymatic histochemical detection methods. These methods established that the pyruvate kinase in the serum was primarily the muscle isoenzyme which has an electrophoretic mobility different from the pyruvate kinase found in liver and/or red cells. 3) Techniques for making peptide maps ("fingerprints") of tryptic digests of myoglobin are nearly perfected and will be ready to apply to a muscle biopsy sample from the patient in the near future. 4) We have been developing methods for the isolation and solublization of muscle membrane proteins which can then be subjected to electrophoresis. Under these circumstances the muscle membrane proteins from this patient can be compared with the muscle membrane proteins from normal muscle in an attempt to identify the underlying problem in idiopathic myoglobinuria.

CONCLUSIONS:

The following conclusions have now been drawn: 1) The abnormal urinary pigment in this patient with idiopathic myoglobinuria is definitely myoglobin. 2) The elevated serum pyruvate kinase is derived from

muscle, presumably via leakage through a defective membrane and 3) membrane protein studies and myoglobin fingerprinting will complete the planned studies of this patient. It is hoped that an abnormality in one or both of these parameters will be found which might provide further clues for evaluation in other patients with myoglobinuria.

RECOMMENDATIONS:

The information which the study of this patient has provided affords us some insight into normal muscle structure and function. These studies should be continued, studies of other patients with myogobinuria or other potential muscle membrane defects should be conducted along similar lines.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6328	72 06 30	DD-DR&E(AR)636

3 DATE PREV SUM'RY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8A DISP'N INSTR'N	8B SPECIFIC DATA-CONTRACTOR ACCESS	9 LEVEL OF SUM
71 07 01	R Termination	U	U	NA	NL	☒ YES ☐ NO	A WORK UNIT

10 NO CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	02	063
b. CONTRIBUTING	61145011	3A014501B71R	02	
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Precede with Security Classification Code)

(U) Studies in Microbial Metabolism - Pathogenic Mycobacteria (06)

12 SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13 START DATE	14 ESTIMATED COMPLETION DATE	15 FUNDING AGENCY	16 PERFORMANCE METHOD
64 10	30 June 1972	DA	C In-House

17 CONTRACT GRANT

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER — Not Applicable

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18 RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 71	2.0	64
FISCAL YEAR CURRENT 72	2.0	58

19 RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20 PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Microbiology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: O'Barr, T. P.

TELEPHONE: 303 366 5311 X25223

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Preston, K. A.

NAME: DA

21 GENERAL USE

Foreign Intelligence not Considered

22 KEYWORDS (Precede EACH with Security Classification Code)

(U) Mycobacteria; (U) Metabolism; (U) Drug-Resistance

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

23. (U) Military personnel returning from duty in areas of high tuberculosis risk, where there is a prevalence of drug-resistant strains, alter the scope of tuberculosis in military, military dependent and civilian populations. Therefore, studies are needed to provide a base for the development of chemotherapeutic agents, to improve methods for the isolation and identification of pathogenic mycobacteria, and to establish the biochemical factors involved in the pathogenesis of tuberculosis.

24. (U) Nutritional and metabolic differences between drug-resistant and drug-susceptible M. tuberculosis were sought by examining the growth response to selected compounds, the enzyme content, and intermediary metabolism. Biochemical changes in host tissue were studied to establish the metabolic consequences of tuberculosis infection.

25. (U) 71 07 - 72 06 Evaluation in mice of hepatic in vivo ribonucleic acid (RNA) and protein metabolism with progressively severe pulmonary tuberculosis has been completed. As measured by incorporation of L-leucine-^{14}C into microsomal protein, protein synthesis was depressed at all stages of infection. Labeling of RNA with orotic acid-^{14}C was also depressed after an initial period of stimulation. Rates of protein synthesis by polyribosomal preparations from normal and infected animals were comparable. However, polyribosomes from infected animals were not compatible with soluble enzymes from controls. This project terminated 30 June 1972 due to transfer of the Microbiology Division to Fitzsimons General Hospital and OMA funding.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498

ABSTRACT

PROJECT NO.	3A061102B71R	Research in Biomedical Sciences
WORK UNIT NO.	063	Studies in Microbial Metabolism - Pathogenic Mycobacteria

The following investigations have been conducted under this work unit:

STUDY NO. 1 Enzymes in Mycobacterium tuberculosis

STUDY NO. 3 Pathways of amino acid synthesis in Mycobacterium tuberculosis

STUDY NO. 4 Biochemical transformation of ascorbic acid

(1) Changes in hepatic protein and RNA metabolism have been evaluated in mice with respect to progressively severe pulmonary tuberculosis. Using the incorporation of leucine-^{14}C into microsomal protein as a measure of protein synthesis, less radioactivity was present in protein from infected animals at all stages of disease as compared to controls. Infected animals initially incorporated greater levels of orotic acid-^{14}C into RNA but in time this value dropped below the level observed with controls. Protein synthesis at the subcellular level as measured with polyribosomal preparations showed little difference between control and infected.

(3) An evaluation of the in vivo effectiveness of DL-α-amino-n-butyric acid (AABA) against M. tuberculosis has been completed. Although mice receiving an 8% supplement of AABA showed normal weight gain, some changes in levels of plasma amino acids were observed. AABA was found to be concentrated in a variety of tissue and organs as the DL-isomer. Serum from animals receiving supplements of AABA showed enhanced ability to inhibit the in vitro growth of M. tuberculosis. Lungs from mice challenged via aerosol with M. tuberculosis and placed on a laboratory diet supplemented with 8% AABA contained fewer viable M. tuberculosis organisms than controls after ten days.

(4) The decomposition of ascorbic acid by an intestinal strain of Escherichia coli has been examined. Ascorbic acid was transformed by actively growing cells and by washed cell suspensions. Ascorbic acid was not utilized in the presence of glucose. Intermediates of ascorbic acid metabolism were separated by column chromatography with DEAE cellulose and Dowex 1. By comparing radioactive compounds formed by growth of E. coli on glucose and ascorbic acid an acidic component specifically related to the metabolism of ascorbic acid

was recognized. Buffered extracts of cells incubated with sodium ascorbate-sodium hydrosulfite contained a 2,4-dinitrophenylhydrazine reacting component which was eluted from DEAE-cellulose with dilute sulfuric acid in an area assigned to ascorbate sulfate.

BODY OF REPORT

WORK UNIT NO. 063 — Studies in Microbial Metabolism - Pathogenic Mycobacteria

STUDY NO. 1 — Enzymes in Mycobacterium tuberculosis

PROBLEM:

To study ribonucleic acid and protein metabolism in animals infected with Mycobacterium tuberculosis.

RESULTS AND DISCUSSION OF THE RESULTS:

Completed studies in this area have yielded knowledge of some of the biochemical and metabolic events occurring in the animal host as the result of infection by M. tuberculosis. Because of its high metabolic activity, the liver was chosen as a likely site to look for disease-related changes in protein and ribonucleic acid metabolism. In the study of protein synthesis mice, at various stages of infection, were injected with 5 μc of L-leucine-^{14}C. After two hours, livers were removed and the amount of ^{14}C present in microsomal protein was determined. From the earliest stages of infection less radioactivity was found in protein from infected animals as compared to controls. This ratio of infected to control remained constant at 0.8 over a two-month observation period. The labeling of ribonucleic acid with orotic acid-^{14}C appeared to be considerably influenced by the degree of infection. Initially 30% more orotic acid-^{14}C was incorporated by infected animals but as the diseased state became more severe, this trend was reversed until the value for the infected was 53% less than the control.

Examination of in vitro protein synthesis with polyribosomal preparations from livers of infected and control animals corroborated to some extent the labeling data obtained with leucine-^{14}C. Rates of protein synthesis were similar with the two preparations. However, protein synthesis was significantly reduced when ribosomes from infected animals were mixed with cell-sap enzymes from controls.

CONCLUSIONS:

Infection with M. tuberculosis depresses protein synthesis in the liver while ribonucleic acid synthesis was initially stimulated and finally depressed.

RECOMMENDATIONS:

The noncompatability of polyribosomal preparations from livers of infected animals with soluble enzymes from controls implies a disease induced modification of the protein synthesizing apparatus. Because of its important theoretical implication this finding should be expanded and verified by additional work.

STUDY NO. 3 — Pathways of amino acid synthesis in drug-susceptible and drug-resistant *Mycobacterium tuberculosis*

PROBLEM:

To study the antagonistic effect of DL-α-amino-n-butyric acid (AABA) on the growth of *M. tuberculosis*

RESULTS AND DISCUSSION OF THE RESULTS:

As a logical extension of *in vitro* studies which demonstrated the extreme sensitivity of *M. tuberculosis* to inhibition by AABA the chemotherapeutic potential of the compound has been evaluated. In preliminary experiments it was determined that mice receiving a laboratory diet supplemented with 8% AABA showed normal weight gains and exhibited no overt signs of poor health. Plasma amino acids of animals receiving the supplement were somewhat elevated over controls. AABA was found to be present in concentrations as high as 3.5 μmoles/ml in serum, lungs and liver. By measuring the disappearance of AABA from serum after treatment with D-amino acid oxidase it was presumed to be present as the DL-isomer. Serum from animals receiving AABA were found to contain enhanced antimicrobial activity against *M. tuberculosis*.

With this background of information mice were challenged via aerosol with virulent *M. tuberculosis* and placed on control diet or diet supplemented with 8% AABA. The control diet contained 28% casein while the 8% AABA supplemented diet contained 20% casein. At 10 and 20 days, lungs from both groups were removed and examined for viable *M. tuberculosis*. At both intervals lungs from control animals contained more viable *M. tuberculosis* than lungs from animals receiving the supplement.

CONCLUSIONS:

AABA showed a slight *in vivo* effect in controlling the growth of *M. tuberculosis*. Based on the concentration of AABA found in serum and tissue much of the *in vitro* effectiveness of this compound was lost.

RECOMMENDATIONS:

It is recommended that structural analogs of DL-α-amino-n-butyric acid be tested against *M. tuberculosis*.

PUBLICATIONS:

O'Barr, T. P., D. J. Keith and E. B. Blair. An evaluation of the fluorometric determination of isonicotinic acid hydrazide. Manuscript cleared and submitted to the Amer. Rev. Resp. Dis. for possible publication.

STUDY NO. 4 — Biochemical transformation of ascorbic acid

PROBLEM:

To study intermediates in the metabolic conversion of ascorbic acid by *Escherichia coli*.

RESULTS AND DISCUSSION OF THE RESULTS:

Based on recent reports of the presence of ascorbate sulfate in man, trout, and brine shrimp, the initial objective of this investigation was to show the presence of ascorbate sulfate in microorganisms. An intestinal strain of *E. coli* was selected for study since it was found to metabolize ascorbic acid during growth in an ascorbate-peptone-mineral salts media. To minimize spontaneous oxidation of ascorbic acid, cultures were grown under anaerobic conditions. Experiments were also performed with washed cells obtained from growth in this media. These cells were found to have highly developed enzyme systems for oxidizing ascorbic acid.

The experimental technique consisted of incubating the cells with ascorbic acid and at various intervals adding trichloroacetic acid to stop metabolic activity and to extract the cell contents. Extracts of cells and aliquots of concentrated growth media were chromatographed on DEAE cellulose columns according to the technique employed for the

isolation of ascorbate sulfate in human studies. (E. M. Baker III, et al. Science 73: 826, 1971.)

The collected fractions were examined for absorbancy at 255 nm and further tested after reduction in volume for hydrazone formation with 2,4-dinitrophenylhydrazine. With this procedure only a hint of the presence of ascorbate sulfate was obtained.

More encouraging results have been obtained recently by omitting the extraction with trichloroacetic acid and shortening the time of incubation. The reaction was stopped and the cells disrupted by quick freezing in acetone-dry ice. When these extracts were chromatographed on DEAE cellulose, a compound was eluted in the area assigned to ascorbate sulfate which had absorbancy at 255 nm and reacted strongly with 2,4-dinitrophenylhydrazine. By thin layer chromatography this compound was found to have an Rf similar to authentic ascorbate sulfate.

Other work has shown that the first fraction eluted from DEAE cellulose could be further separated into three separate fractions by direct chromatography on Dowex 1. One acidic component was found to be specifically related to the metabolism of ascorbic acid.

CONCLUSIONS:

Recent evidence strongly suggests that ascorbate sulfate is present in extracts of E. coli actively metabolizing ascorbic acid.

RECOMMENDATIONS:

To attempt to label the presumed ascorbate sulfate with ^{35}S.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6374	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. /CODES.*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	02	065
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Precede with Security Classification Code)* (U) The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Status (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

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

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:* Not Applicable
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AM.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	2.0	85
	CURRENT 73	2.0	65

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Dsch & Nutr Lab
ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL
NAME: Canham, J. L., COL
TELEPHONE: 303 366-5311 X21108

21. GENERAL USE

Foreign Intelligence not Considered

20. PERFORMING ORGANIZATION

NAME:* US Army Med Dsch & Nutr Lab
Bioenergetics Division
ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Krzywicki, H. J.
TELEPHONE: 303 366-5311 X25222
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME: Consolazio, C. F.
NAME: Nelson, R. A. DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Diet and Work Performance of Soldiers; (U) Heat, Cold, Altitude Stress; (U) Nutrients and Body Composition; (U) Pulmonary Function

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

23. (U) The improvement of physical fitness and performance capacity are major concerns of the military. The evaluation of performance requires extensive testing during physical activity. Improvements are dependent upon training, adequate nutrition and may be influenced by electrolyte mixtures and dietary supplements. Problems to be investigated are the effects of various fluids containing minerals, carbohydrate and vitamins upon body composition, physical performance and fatigue and the effects of heat and environmental stress on the same parameters.

24. (U) Measurements of oxygen uptakes, heart rates, body temperatures, etc., will be made on men working on the treadmill, while consuming only water, water plus minerals, water plus glucose or water plus vitamins, under conditions of profuse sweating. Comparisons of blood acid-base parameters, pulmonary function and diffusion capacity, nitrogen, water and mineral balances and changes in body compartments (fluids, fat, protein, etc.) will be made.

25. (U) 71 07 - 72 06 Although body water and body protein losses are excessive during short term calorie restriction, submaximal, maximal or stamina work tests were not significantly impaired. The large body protein losses under these conditions would preclude the use of the calorie restricted diets. The whole body ^{40}K shadow-shield counter was modified by the addition of a stepping motor that increases the instrument's counting ability. Additional human subjects are required to calibrate this instrument. The computer system for collection, storage, calculation, etc. of data for measuring human energy expenditure has been highly successful. Gastro-intestinal gas was most effectively controlled with activated charcoal when compared with magnesium aluminum hydroxide or barium salts, however further testing of these and other anti-flatulents is warranted when high gas producing diets are fed.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498

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

Although hypohydration and protein catabolism occurred during calorie restriction (420 and 500 Cal/day), physiological work performance of submaximal, maximal or a stamina test was not impaired.

Body compartment data indicates that the total body water by D_2O dilution is significantly decreased during short term calorie restriction. In the 420 Cal/day study, the dry protein loss averaged 0.77 kg/man during the 10-day experimental period.

The whole body ^{40}K counter was modified by adding a stepping motor to increase the counting ability of the instrument. Permission to use ^{42}K in additional human subjects for calibrating the instrument is necessary.

BODY OF REPORT

WORK UNIT NO. 065 — The Effects of Nutrition and Environmental Stress Upon Work Capacity and Nutritional Status

STUDY NO. 1 — Performance, Calorie Restriction

STUDY NO. 2 — Body Composition

STUDY NO. 3 — Collaborative Studies

PROBLEM:

Physiological performance is of primary concern to the military services since a large part of the field military training program is devoted to physical conditioning for increased troop fitness. Any means of increasing physical training rates, particularly to develop and maintain physical fitness of troops, would be beneficial especially under conditions of nutritional, physiological or psychological stress.

Studies will be designed: a) to identify and quantify the various physiological and biochemical parameters of nutritional status and energy expenditure as reflected by body composition and respiratory function, and b) to prevent or alleviate any detrimental effects through dietary manipulation, physical conditioning and activity, and preconditioning to environmental stress.

Measurements will be made on troops of various ages and somatotypes while performing military duties of all types under conditions in which various nutrient levels will be utilized. In these studies, measurements will include work capacity, body compartment changes, anthropometric evaluation, blood acid-base parameters and pulmonary function. Interrelationships between these parameters can then be evaluated, especially with reference to diet.

Electronic and digital computer programming systems are required to handle the storage, retrieval, editing and processing of data derived from energy expenditure studies of subjects undergoing exercise on motor driven treadmills and bicycle ergometers. This need exists for the support of studies in the evaluation of physical performance of military personnel as influenced by multiple factors.

STUDY NO. 1 — Performance, Calorie Restriction

RESULTS AND DISCUSSION OF RESULTS:

a. Calorie Restriction. Performance was evaluated in two studies, with the subjects ingesting 420 or 500 Cal/day for 10 days. Calorie restriction did not produce any significant differences in performance during submaximal or maximal work on the treadmill. The same was also

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

true during a 60-min stamina test (either 4.0 or 4.5 mph on a 10% grade), where no differences were observed between the control or restriction phases. Although adverse problems such as hypohydration and protein catabolism may occur during calorie restriction, it appears that physical performance in young adults was not impaired during a 10-day period. A longer work period or a longer period of food restriction may be required to produce an impairment in work performance. However, the significant protein losses preclude the use of low calorie diets under any circumstances.

b. At high temperatures with increasing relative humidities (30-90%), one observes increases in pulmonary ventilation, $\dot{V}_E$, heart rates and body temperatures, $\dot{V}_{O_2}$ appears unchanged during submaximal work. The reasons for this are unclear at this time. Preliminary data indicates that $\dot{V}_{O_2}$, $\dot{V}_E$ and heart rates during standardized physical activities are significantly increased at 4,300 m. This indicates that energy requirements at this elevation may be increased.

c. In a study involving 110 men below the age of 25, the relationships of the various physiological work parameters were evaluated as predictors of submaximal $\dot{V}_{O_2}$. Although the correlation coefficients between heart rate and $\dot{V}_{O_2}$ ml/kg per minute were fair, the best relationships were observed between $\dot{V}_E$ l/min and $\dot{V}_{O_2}$ ml/kg per min at all work levels.

Although in one instance body weight greatly improved the correlation coefficients between heart rate and $\dot{V}_{O_2}$, this did not occur when the relationship between $\dot{V}_E$ and $\dot{V}_{O_2}$ ml/kg per min values was utilized. The maximal versus submaximal work data indicate that better correlation coefficients and regression equations are observed with the heaviest workloads.

d. In evaluating existing data, carbohydrate has been found to be 4-5% more efficient than fat as the energy source for the working muscles. This may appear to be minimal but it could make the difference between winning or losing a contest for an athlete. Although increased protein intakes for the athlete (for increasing muscle mass) have been the popular concept for coaches and trainers, scientific evidence indicates that protein is not utilized by the working muscle. It appears that protein requirements are not increased except in training where the energy expenditure is increased with a subsequent increase in calorie and protein intake to maintain body weight.

e. Work Unit 078, Metabolic Responses of Man to Nutrition and Disease. The Effects of IV Intralipid on Pulmonary Function of Normal Subjects (collaborative study with Metabolic Division and Pulmonary

Function Lab, FGH). The preliminary phase of this study with normal subjects was completed during the year. The effects of a 500 gm infusion of an 10% IV fat emulsion (Intralipid) was compared to a 500 gm infusion of saline during rest and at work levels of 25 and 50% of maximal on the treadmill. Ten Metabolic Ward subjects were studied (one/day) for the effects of the lipid infusion on pulmonary function (including pulmonary diffusion capacity), submaximal work at two levels, body temperature, arterial acid-base parameters and carbon monoxide levels in the blood. Four Bioenergetics Division personnel provided 100% of their time for a 2 and 1/2 week period. The data is now being processed by the Metabolic Division.

STUDY NO. 2 Body Composition

RESULTS AND DISCUSSION OF RESULTS:

a. Potassium ^{40} Whole Body Counter. The whole body counter was recently modified by adding a stepping motor to increase the counting capability since all pulses going into the multichannel analyzer are recorded even though a pulse is being processed when another comes in. Formerly, the pulses were lost in such situations. The potassium-40 whole-body shadow-shield counter data output has been further computerized so that the total gram of potassium, dry protein mass, fat free mass and the per cent that the fat free mass constitutes of the total body weight is calculated directly when a subject is measured. The counter was calibrated using only 5 subjects. An additional effort is required using ^{42}K to define the calibration curve as a linear function of weight and height. At present, work is at a standstill awaiting approval to use the radioactive isotope ^{42}K in normal humans for calibration purposes.

Whole body counting of ^{131}I are being continued to measure the residual retention of the isotope. Approximately 6 whole body counts were made for ^{131}I to determine iodine uptakes after thyroidectomy. Liaison with Fitzsimons General Hospital is being maintained.

b. Minimizing gastrointestinal gas production during body fat measurements by water displacement: Replicability of measuring body volumes is presently being studied in 6 subjects receiving Mylanta (an antacid that contains a defoaming agent), barium sulfate, and activated charcoal in an effort to minimize gastrointestinal gas production, while consuming a normal diet. The volume of GI gas is usually accepted as 125 ml at any one time in the gut, however flatulence greatly contributes errors in estimating body density, and subsequently body fat. Data will be analyzed statistically.

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

c. Body composition from densitometry was measured in 6 young adults consuming various mineral supplements and electrolytes, and receiving a nutritionally adequate diet while exercising strenuously each day. This study was completed in April, and the data is being readied for the computer permanent file prior to calculation of body compartments, etc.

d. Body composition changes were observed in eight healthy adult males, 19-21 years of age, subsisting on a 420-kcal/day, all carbohydrate liquid diet for 10 days. One-half of the subjects were denied essential mineral supplements (group I); the remainder received the supplement (group II). The mean loss in body weight of 5.7 and 4.1 kg in groups I and II, respectively, was partitioned into the following losses in body compartments: group I, 1.24 kg fat and 4.47 kg fat-free body mass (including 0.90 kg dry protein); group II, 0.98 kg fat and 3.13 kg fat-free body mass (including 0.64 kg of dry protein). The observed total body water (D_2O dilution) decreased significantly in both groups; however, a greater water loss was observed in group I. Predicted body water estimates were lower than the observed values. Estimates of the dry protein mass derived from urinary creatinine excretion and total body potassium were higher than observed values. Blood, plasma, and red cell volumes were significantly decreased in group I, whereas only blood and plasma volumes decreased in group II. Skin-fold thicknesses were decreased in both groups; however, those subjects of group II demonstrated lesser changes.

In the 500 Cal/day restriction study (when 160 Calories of protein and 340 of carbohydrate were added to the diet) protein losses by ^{40}K counting were slightly minimized and fat losses were accentuated. However, the sum total of deleterious body compartment losses precludes use of such low intakes under any circumstances.

e. (Under Work Unit 073) Progress in assembling body composition data for the permanent computer file is reflected in that the file now contains complete data on 137 males and 62 females studied at Ft. Huachuca, Arizona; 160 males at Ft. Campbell, Kentucky, and will eventually have data on approximately 185 additional male subjects. Particularly, body composition can be evaluated from three aspects: densitometry, potassium-40 counting and total body water. These factors can be compared to body compartments and various anthropometric measurements, i.e., height, weight and varied circumferences and diameters. This will allow for precise statistical analysis of the data using correlations coefficients and cluster analysis, and for describing various regression lines and predicting equations related to height, weight and skin fold thickness. All new data is

placed on coded data sheets for card punching and permits rapid calculation and reduction of the data. Most important, all computerized data and statistical procedures can be accepted for their accuracy.

The skin folds, heights and weights measured on subjects during the nutrition surveys at Ft. Lewis, Washington and Ft. Myer, Virginia are being readied for insertion into the computer file. Programs for computer development of histograms, etc. have been completed for previous body composition data and are ready to use. Ft. Myer data has yet to be key punched and filed.

A resume of the anthropometric data of Women Army Corps personnel studied at Ft. McClellan, Alabama in 1970 is shown in Table I. Of the three categories of personnel, it appears that the permanent party (mean age 25.3 years) outweigh the students and basic trainees by approximately 4 kg. The skin folds reflect the increase in body weight. The students and basic trainees are rather homogeneous, however the basic trainees show a small 0.3 kg difference in body weight over the students, but not so in skin fold thickness where the opposite is true.

TABLE I

ANTHROPOMETRY - WACS - FT. MCCLELLAN, ALABAMA, 1970

	All Subjects n=349		Students n=72		Basic Trainees n=188		Perm Party n=89	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Age	21.9	4.5	20.7	2.6	20.8	3.0	25.3	6.5
Weight (kg)	58.6	8.3	57.3	7.4	57.6	7.6	61.7	9.4
Height (cm)	162.8	6.4	162.0	7.3	162.6	6.0	164.0	6.4
Skin fold (mm)								
Rt Triceps	17.0	6.1	16.7	5.5	15.8	4.9	19.9	7.7
Lt "	17.8	6.3	17.8	5.8	16.3	5.2	20.8	7.7
Rt Scapula	12.9	6.3	13.2	6.0	11.7	4.7	15.1	8.5
Lt "	13.1	6.2	13.4	5.9	11.8	4.6	15.6	8.4

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

STUDY NO. 3 Collaborative Studies

RESULTS AND DISCUSSION OF RESULTS:

a. In a collaborative study with the Department of Physiology, University of Illinois, Urbana, Illinois, correlations between body volume and body mass in men were evaluated. A study was made of the body density of 979 U.S. Air Force enlisted men and associated personnel. The following measurements were made: a) body height, b) body weight, c) body volume by water displacement, and d) residual lung volume. Surface area, body volume, corrected for residual lung volume and gas in the intestinal tract and body weight divided by volume were then computed. There was a very close correlation between body weight and body volume, a poor correlation between body surface area and body volume. A linear equation was derived to compute volume from weight only, volume in liters = 1.015 weight (in kg) - 4.937. Prediction equations may be used to compute body fat, total body water, and dry fat-free mass from body weight alone. Two implications of this study are: a) among men of military age, individuals of equal body weight have remarkably similar body composition; and b) in this same population, the larger men tend to have the lower density.

b. Work Unit 072, Human Nutrition - Riboflavin Deprivation (collaborative study with Chemistry Division). Six subjects were deprived of riboflavin for 9 weeks and 1/2 of the subjects received 120 gm of protein in their 2,850 Cal/day intake, while the remainder received 60 gm of protein daily. Mean body weight decrease was 0.96 kg which reflected a 0.31 kg body fat gain, 0.26 kg dry protein loss, 0.92 kg body water loss and 0.09 kg mineral loss. Potassium-40 counting approximated the loss in the dry protein mass after the third and sixth week of deprivation, but over-estimated the dry-protein loss at the ninth week of the study. In this study, weekly whole-body counts showed a body potassium decrease over the 12-week period. However, this data is being further analyzed in light of similar densitometric data.

c. Human Nutrition - Scurvy II (collaborative study with Chemistry Division under Work Unit 072). Body composition was studied in adult males approximately 2, 3, or 4 weeks after being repleted with Vitamin C, after scurvy had been induced. Body weight was virtually regained and body fat was the greatest contribution to total body weight gain. Modest correlations were obtained between body pool, plasma or whole blood ascorbate and the dry protein mass of the body. No evidence of impaired protein utilization was noted with Vitamin C depletion with densitometric techniques, but unfortunately no measurements were made before or during depletion.

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

METHODOLOGY:

a. Determination of deuterium oxide in body fluid by gas chromatography: A series of experiments were conducted to test the reliability of the gas chromatographic method of detecting tracer deuterium oxide in biological fluids. Deuterium oxide-water standard peak heights were reproducible to within a 3% error. Recovery of deuterium in a series of two urine standards averaged 99.2%; recovery of deuterium in a series of two serum standards averaged 100.6%. Duplications of total body water determinations for two human subjects was within 0.5 liter. A preliminary study found deuterium to equilibrate at a similar rate and concentration in serum and saliva. The chromatograph technique was sufficiently reliable and accurate to be used routinely in determination of total body water in human subjects.

b. An 18-unit rodent activity treadmill cage and stimulation system was developed. The activity compartments measuring 34 x 8 x 11 cm are arranged six across in three adjoining sections and suspended over the belt that provides suitable running space. Sturdy copper grids are mounted at the rear of each compartment to motivate the animal to run. Numerous safety devices are described for protection of the animal while running. This apparatus is being satisfactorily used by this laboratory on work performance studies utilizing white rats.

CONCLUSIONS:

The data indicates large significant water and protein losses during 10 days of calorie restriction (420 and 500 Cal/day). The calculated dry protein loss by densitometry in the 420 Cal/day study averaged 0.77 kg for all of the subjects.

Additional subjects will be required to calibrate the ^{40}K shadow shield counter. This is a necessity. Modification of the ^{40}K counter with a stepping motor has resulted in an increased counting ability of the instrument.

The computer system for the collection, storage, calculation, etc. of the energy expenditure data has been highly successful.

Activated charcoal appears most effective at reducing gastric intestinal gas when subject men were fed normal diets. However, further testing of gas producing diets is warranted.

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

RECOMMENDATIONS:

For greater accuracy additional human subjects ingesting ^{42}K will be required to calibrate the ^{40}K shadow shield counter. An immediate decision should be made to permit the use of ^{42}K as a calibrating isotope as well as a diagnostic tool.

PUBLICATIONS:

1. Consolazio, C.F. Energy expenditure studies in military populations using Kofranyi-Michaelis respirometers. Am. J. Clin. Nutr. 24:1431, 1971.

2. Krzywicki, H.J., C.F. Consolazio, H.L. Johnson, and N.F. Witt. Metabolic aspects of caloric restriction (420 kcal), body composition changes. Am. J. Clin. Nutr. 25:67-73, 1972.

3. Consolazio, C.F., R.A. Nelson, R.A. Daws, H.J. Krzywicki, H.L. Johnson, and R.A. Barnhart. Body weight, heart rate and ventilatiory volume relationships to oxygen uptakes. Am. J. Clin. Nutr. 24:1180-1185, 1971.

4. Consolazio, C.F. and H.L. Johnson. Measurement of energy cost in humans. Fed. Proc. 30:1444-1453, 1971.

5. Nielsen, W.C. Jr., H.J. Krzywicki, H.L. Johnson, and C.F. Consolazio. Use and evaluation of gas chromatography for determination of deuterium in body fluids. J. Appl. Physiol. 31:957-961, 1971.

6. Bodenhausen, G.E. and H.L. Johnson. Design of an 18-unit rodent activity treadmill cage and stimulation system. J. Appl. Physiol. 31:954-956, 1971.

7. Wakat, D.K., R.E. Johnson, H.J. Krzywicki, and L.I. Gerber. Correlation between body volume and body mass in men. Am. J. Clin. Nutr. 24:1308, 1971.

8. Contribution of gastrointestinal gas to human body volume. Krzywicki, H.J., J. Gray, T. Ward, and C.F. Consolazio. Fed. Proc. 31:718, Apr 1972 (Abstract).

9. Consolazio, C.F. and H.L. Johnson. Dietary carbohydrate and work capacity. Am. J. Clin. Nutr. 25:85-90, 1972.

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

10. Krzywicki, H.J., C.F. Consolazio, H.L. Johnson, and N.F. Witt. Metabolic aspects of calorie restriction (500 Calories): Body composition changes. (Subritted for clearance)

11. Krzywicki, H.J. and J. Hood. Body composition in experimental human scurvy. (Submitted for clearance)

12. Krzywicki, H.J., C.F. Consolazio, and H.L. Johnson. Human body composition during riboflavin depletion in man. (Submitted for clearance)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6375	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	02	165
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Procede with Security Classification Code)

(U) Comparative Pathology of Animals Maintained and Utilized in Biomedical Research (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	CONT	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECED 72	6.5	164
	CURRENT 73	5.0	190

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366-5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Pathology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Trevino, G. S., LTC

TELEPHONE: 303 366-5311 X23230

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Whitmire, R. E., MAJ

NAME: Empson, R. N., CPT DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Comparative Pathology; (U) Experimental Animals; (U) Electron Microscopy; (U) Human Disease Analogues; (U) Military Research Support

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

23. (U) Much biomedical research depends upon the proper use of healthy laboratory animals. Investigations requiring the use of animals hinge upon the recognition and reliable interpretation of abnormal anatomic and physiologic data accruing from such studies. The broad objectives of this Work Unit are: (1) to maintain and provide experimental animals for biomedical research generated by any division within the Laboratory; (2) to provide pathology services, including gross, clinical, microscopic or special studies of diseases, whether spontaneous or induced, in animals; (3) to provide guidance and support to investigators utilizing animal models to study analogous human infirmities; and (4) to provide professional veterinary care to insure the health and well-being of the animals in the colony.

24. (U) Colonies of different animal species are housed and maintained for the use of biomedical investigators within the laboratory. Routine and special pathologic support services are furnished upon request and include necropsies, light and electron microscopy, autoradiography, serum and tissue enzyme studies and blood and urine analyses. All cases requiring histopathologic assessment are accessioned sequentially and appropriate reports are rendered. Material of special teaching value is utilized to supplement didactic seminars and conferences.

25. (U) 71 07 - 72 06 Approximately 6,417 animals, purchased from commercial sources or bred within the colony, were maintained for biomedical research in the interval covered by this report. These included 3,752 rats, 2,038 mice, 22 rabbits, 120 dogs, 50 hamsters, 401 guinea pigs, 4 monkeys, and 30 cats. Cases accessioned numbered 940; these produced 4,072 paraffin blocks, 8,335 H&E stained microslides, and 1,593 specially stained slides. Work performed under this work unit resulted in six publications and seven oral presentations.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498

ABSTRACT

PROJECT NO. 3A061102B71R Research in Biomedical Science

WORK UNIT NO. 165 Comparative Pathology of Animals Maintained and Utilized in Biomedical Research

During FY 72 eleven different projects generated by experimenters in other divisions were supported by this Work Unit.

Nine hundred and forty cases were accessioned, resulting in 4,072 paraffin blocks, from which there were 8,335 H and E microslides prepared. Additionally 1,593 slides requiring special stains were produced. Approximately 6,417 laboratory animals were maintained including 3,752 rats, 2,038 mice, 401 guinea pigs, 120 dogs, 50 hamsters, 30 cats, 22 rabbits, and 4 monkeys.

One microslide study set dealing with spontaneous skin tumors of animals has been prepared and work is progressing on the following: nutritional and metabolic diseases, granulomatous diseases, parasites of infrahuman primates, and spontaneous diseases of laboratory animals.

New numbered proposals are being written for implementation during FY 73.

BODY OF REPORT

WORK UNIT NO. 165

Comparative Pathology of Animals Maintained and Utilized in Biomedical Research

UNNUMBERED STUDIES

This Work Unit replaced 057 on 1 July 1971.

PROBLEM:

The maintenance and issuance of healthy laboratory animals destined for use in biomedical investigations conducted by researchers throughout the Laboratory was a major function of this Work Unit during FY 72. Within the Animal Service Branch the animals procured commercially or produced from breeding stock are housed, fed, handled and treated in accordance with experimenters' requirements. This Work Unit provides clinical, gross, microscopic, and special pathology services to investigators soliciting such support.

RESULTS AND DISCUSSION:

a. Maintenance of animals and cases accessioned during FY 71:

Loss of four primary investigators during calendar year 1971 and delay in assignment of replacements resulted in curtailment of primary research activities. However, there were eleven collaborative research projects in which members of this Division participated intimately during FY 72. To meet requirements of these investigations a total of 6,417 animals were procured commercially or bred from existing stock. These were represented by 3,752 rats, 2,038 mice, 401 guinea pigs, 120 dogs, 50 hamsters, 30 cats, 22 rabbits and 4 monkeys.

A total of 940 accessioned cases yielded 4,072 paraffin blocks from which there were 8,335 H and E stained microslides and 1,593 specially stained slides. Seventy-two biopsies requiring 288 histochemical stains were processed and 210 bone marrow preparations were examined.

b. Veterinary Pathology Preceptorship Program:

A function of this Division relates to the training of veterinary pathologists. Material used in slide seminars is derived almost exclusively from this Work Unit. Examples of cases which possess outstanding merit for teaching or diagnostic purposes are written for publication.

Comparative Pathology of Animals Maintained and Utilized in Bio-Medical Research (Cont'd)

During one month of FY 72 the Veterinary Pathology Preceptorship Program was augmented by the presence of a nationally renowned consultant in veterinary pathology. This training was in addition to regularly scheduled weekly seminars and conferences. The two officially appointed preceptees assigned to the Division, plus two other non-career veterinary officers, made notable progress in their study of pathology.

Each of the pathology trainees in the Division is preparing a different study set embracing such entities as laboratory animal diseases, granulomatous diseases, metabolic and nutritional infirmities, neoplastic diseases, and parasites of infrahuman primates. During FY 73 each officer presently assigned to the Division will engage in a primary research project designed and implemented by him.

c. Histochemistry:

This Division has greatly augmented its participation in investigative projects concerned with localization of tissue enzymes. In addition to intimate support of two ongoing projects, a study set has been prepared for investigators desirous of familiarizing themselves with tinctorial properties of such tissues as intestine, muscle, kidney, and liver stained with a variety of histochemical procedures. Although some of the stains are photolabile, lasting only a few weeks, preparation of new substitute microslides to maintain an example of each tissue is accomplished as required.

RECOMMENDATIONS:

This Work Unit should continue in operation until the relocation of USAMRNL to WRAIR forces revision or supplantation. Appropriate research proposals are being written by each of the veterinary officers currently assigned to the Pathology Division.

PUBLICATIONS:

1. Demaree, R. S., Jr., P. Shuster, W. R. Starke, and K. Alonso. 1972. Previously Undescribed Intraepithelial Deposits in Membranous Glomerulonephritis. Lancet 1 (7760): 1123,1972.

2. Demaree, R. S., Jr. and W. B. Nessmith. Ultrastructure of Hemobartonella felis from a Naturally Infected Cat. Amer. J. Vet. Res. 6:1303, 1972.

Comparative Pathology of Animals Maintained and Utilized in Biomedical Research (Cont'd)

3. Trevino, G. S. and Nessmith, W. B. Acrtic Body Tumor in a White Rat. Vet. Path. In press.

4. Trevino, G. S., Demaree, R. S., Jr., Sanders, B. V., and O'Donnell, T. A. Needle Biopsy of Canine Skeletal Muscle: A Useful Research Technique. I. Light and Microscopic Studies of Resting Muscle. Lab. An. Sci. In press.

5. Trevino, G. S. and Alden, C. L. Mucocytes in the Brain of a Llama. J. Wildlife Dis. In press.

6. Trevino, G. S. Cephalosporiosis in Three Caymans. J. Wildlife Dis. In press.

7. Mason, T. E., R. S. Demaree, Jr., and C. I. Margolis. Granulocytic Sarcoma (chloroma), Two Years Preceding Myelogenous Leukemia: with review of the literature. Cancer. In press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6377	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	02	166
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Precede with Security Classification Code)

(U) Design of Military Biomedical Research Information Systems (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

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

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	4.0	64
	CURRENT 73	4.0	69

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366-5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Computer Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Romero, R. S., MAJ

TELEPHONE: 303 366-5311 X25130

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Teplick, R. S., CPT

NAME: Nelson, R. A. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Digital Computers; (U) Military Research; (U) Data Files; (U) Biomedical Research Information; (U) Mathematics; (U) Statistics

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, develop, implement and utilize a digital computer-based information system capable of storing, selectively retrieving, displaying and statistically and mathematically analyzing military biomedical research information.

24. (U) The project will be developed in two areas: (1) development of generalized computer programs to store, selectively retrieve, display and analyze military biomedical research information, and (2) formatting, assimilation and analysis of military research information in the system. Division personnel participate as coinvestigators in various interdivisional research projects.

25. (U) 71 07 - 72 06 The basic system is operational. Information is being updated to the files and analyzed for the Administrative, Bioenergetics, Chemistry, Metabolic, Microbiology and Pathology Divisions, USAMRNL, and for the Department of OB/Gyn, FGH. In addition, a data file has been established as an interdivisional research project to support the USAMRNL's annual military nutrition survey. Data from past surveys have been updated to the file and computerized analysis of the data is being utilized to study the nutritional status of military troops.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498

ABSTRACT

PROJECT NO. 3A061102B71R Research in Bio-Medical Services

TASK NO. 02 Internal Medicine

WORK UNIT NO. 166 Design of Military Biomedical Research Information Systems

STUDY NO. 1 Data Processing Support for Biomedical Research

STUDY NO. 2 Direct Computer Support to the USAMRNL Divisions

This work unit has the objective of giving support to all users of computer data processing through the Nutrition Laboratory.

Study No. 1. The efforts of this study are general in nature, designing and/or remodeling programs that benefit all users of the computer facility.

Study No. 2. This study is specific in its approach of handling each USAMRNL division problems as to their requirements for computer data processing. Close coordination is attained by the computer programmer's active participation with the division in need of computer services so as to develop those programs and procedures that meet specific and individual needs of the investigators.

BODY OF REPORT

WORK UNIT NO. 166 — Design of Military Biomedical Research Information System

STUDY NO. 1 — Data Processing Support for Military Biomedical Research

PROBLEM:

There is a continuous need by military biomedical research personnel assigned to the USAMRNL to have the computer support in processing and analyzing data which could not be managed or that are cumbersome to be handled through manual procedures. Computer programs and procedures need to be developed and maintained to assist the researchers to meet their goals. The search for new avenues in computer processing is a constant requirement in order to improve the computer services to the researchers. Development of computer assistance to the research to be conducted must be implemented as early as possible in protocol development to insure maximum assistance and return of effort.

RESULTS AND DISCUSSION OF THE RESULTS:

Constant maintenance of existing computer programs have enhanced their usability in processing the data generated by the researching personnel. By adding new program routines, the availability of more computerized data, in turn, have assisted readily the researchers in reaching the scientific conclusion of their experiments. Improvement of the existing programs have affected positively efforts in Work Unit 082. The development of new programs, to replace in total old ones, have the same objectives of supporting the research endeavors assigned to USAMRNL.

The computer languages, RCA 301 Assembly and FORTRAN II, have been somewhat restrictive by the nature of the available ADP equipment. Nevertheless the programmers assigned to the Computer Division have been able to adapt new programming techniques thus optimizing the computer capabilities to its maximum.

A good example is the new retrieval system which in essence covered the engineering of the assembler compiler to meet new needs in the retrieval process of data already in storage. The new version obviates retrieving sequentially, going to specific portion of the store data to get a given information on a record. Needless to say that such an accomplishment speeded up the search procedure substantially; about half the time taken by the old retrieval system.

Design of Military Biomedical Research Information System (Cont)

CONCLUSIONS:

Continuous efforts in maintenance of existing programs, (adding new routines or writing entirely new programs) will be carried out so as to offer the computer as an additional tool to the investigators doing military biomedical research.

RECOMMENDATIONS:

With the availability of newer computers and considering such factors as economy of resources, et cetera, conversion of existing programs and systems should be highly considered. The investigators would appreciate more precise analytical manipulation, speedier results and confidence of factual evidence as they come to make scientific decisions.

PUBLICATIONS:

None.

STUDY NO. 2 Direct Computer Support to the USAMRNL Divisions

PROBLEM:

Each and every USAMRNL division can benefit immensely by using a digital computer whenever processing of data is of routine nature (repetitive) and when manual calculation becomes uneconomical in manipulating computation of research data. Tangible as well as intangible benefits can be attained firstly by savings in the overall operation of each division, secondly, the reduction of mechanical errors in data manipulation, and thirdly by the satisfaction of accomplishing a worthwhile research project in a more timely manner. The morale of the involved personnel obviously will be enhanced when tools are available which take care of the drudgery and routine aspects of the investigative efforts.

RESULTS AND DISCUSSION OF THE RESULTS:

a. Radioisotope Branch - Several divisions of the Nutrition Laboratory utilize the services provided by the Radioisotope Branch, Administrative Division. In turn, Radioisotope Branch uses the Computer Division services for computer programming and computer processing of data gathered during the counting of the disintegrations of the isotopes under study. To give Radioisotope Branch

continuous computer support, the Computer Division assigned an EM spending approximately 85% of his time writing computer programs to satisfy the needs of the investigators. The output in the form of a paper tape from teletype, which recorded scintillation gamma emissions via a Nuclear Chicago Mark I device were converted to paper tape readable by the RCA 301 computer. In addition several programs were written for the Packard 3375 and 3380 to assist in analyzing biochemical experiments such as assays of metabolic intermediates, vitamins, enzymes, and others.

b. Bioenergetics Division - Energy Expenditures and Work Performance - The original computer programming for the analysis of the data gathered on pulmonary functions, as affected by exercise, was accomplished with the direct assistance of the Computer Division. Programs have met the expectancies of the investigator and had been used whenever research in this area was performed.

c. Metabolic Division - Studies in Human Thermoregulation - The data file for this project is extensive. The ongoing temperature study required a computer programmer spending most of her time doing maintenance of files and adding needed subroutines to programs whenever the file data required more extensive analysis.

d. Chemistry Division - Extensive support has been given to this division in the filing of data pertaining to the Vitamin A Study. Accurate maintenance of this file, in addition to writing of subroutines to existing computer programs, have enhanced the data processing of the gathered information. In addition a new file has been initiated for the iron and liver study as related to Vitamin A.

e. Microbiology Division - Programming support, file maintenance and processing of the gathered information have continued uneventfully. (See report of Work Unit #065).

f. Pathology Division - Nutritional Aspects of Work Performance in Military Dogs - The Pathology fatigue files have been reactivated. In addition, a lesion data file has been developed. Computerized listings have assisted investigators in analyzing laboratory animal diseases. As this division gathers data, programs and procedures are available to computerize the processing of the gathered information.

g. Food Hygiene Division - Computer programs and procedures have been developed to support the activities of the Food Hygiene Division. A complete computer Food Hygiene standard operating procedure has been written and documented.

Design of Military Biomedical Research Information System (Cont)

h. Interdivisional Studies - Nutrition Surveys - Nutrient Intake, Laboratory Tests, Clinical Data - One of the main missions of the USAMRNL is in the assessment of the nutritional value of food ingested by the military personnel of the Armed Forces and the evaluation of the nutritional status of Armed Forces personnel. Consequently, intensive and extensive efforts by four of the USAMRNL divisions are oriented toward the scientific analysis of food consumed by the Armed Forces Personnel. The Computer Division has supported this effort in the collection and processing of all data amenable to computer processing. Several nutrition surveys have been conducted during this report year and the data are being analyzed. Survey data collected in previous years have been processed or are about to be processed by the Computer Division. In this regard, close cooperation and coordination with the Bioenergetics Division and Chemistry Division have enhanced achieving the results obtained in surveying food consumption by the troops.

Filing systems have been accomplished to store the collected data of the various nutrition surveys. New programs and subroutines to existing programs have been written to process the data thus gathered. Surveys now in file include the Ft. McClellan Study, Lowry AFB Study, Ft. Lewis Study, as well as Ft. Huachuca, Ft. Campbell, Ft. Irwin, Panama, and Ft. Myer studies. Statistical analysis on those surveys already on file has progressed as expected with the results made available to the investigators as rapidly as possible.

Table I indicates the type and quantity of data which have been stored primarily from Bioenergetics and Chemistry Divisions and the Office of the Commanding Officer. A message of data is a group of related items, usually about 80 characters in length. For example, the "Nutrition Clinical Examination" message collected on Nutrition Surveys is 78 characters in length and contains 49 items of information. In Table I, the data messages are tabulated into types of data messages ranging from basic descriptions of the individuals studied to data collected in the dining hall during the nutrient intake portion of nutrition surveys.

Data collected on human subjects and filed on the biomedical information system file are statistically evaluated. Data collected in dining halls, which measures the nutrient intake of individuals and composition of recipes served, are processed for a summary of intake by nutrients.

As individuals eat in a dining hall, they sign a meal sheet with a meal card number which they are issued on their particular post.

These meal card numbers for each meal in each dining hall studied are stored as headcount data on the data file. These items can then be retrieved in various combinations by their assigned unit, sex, etc., sorted, formatted for FORTRAN analysis, and analyzed for the pattern in which individuals ate in the dining hall. A number of summaries of eating patterns can be developed from this analysis. As an example some data from the Nutrition Survey conducted at Lowry Air Force Base in July 1971 are shown here on personnel eating in the dining facility surveyed. Table II is a summary of the number of people eating the various types of meals offered in the dining hall during the survey. Table III indicates the average rate at which the personnel utilized the dining hall over a number of days. In Table IV, the pattern of eating is indicated by the type of meals the individuals eat in an average day over a seven-day period. For example, over 7 days studied, 11.15% of the people surveyed ate dinner and supper and no other meal in the dining hall on the average day. Examples of other Computer Division contributions are contained in report of Bioenergetics Division Work Unit #073.

i. Dept. of OB/GYN, FGH. The Computer Division also has been supporting the FGH OB/GYN study. In order to process the data collected by the hospital in this study, several programs were written and documented. Outputs in the form of listings have been furnished to the investigators for further personalized scrutiny and analysis of results.

j. Administrative Division - Property Book - Until recently the Supply Branch of this Laboratory had a manual system of accounting for the property on hand, but the Computer Division assisted in developing a new property book system which is now handled by the computer. Extensive programming was required to adapt existing programs intended to handle scientific data manipulation to a business orientation. Nevertheless, the new system has provided the Supply Officer with a reliable up-to-date inventory account of the Laboratory property.

CONCLUSIONS:

Active participation in support to each and every division of the USAMRNL is the main goal of the Computer Division. This will be more so as the investigators come to realize the tremendous potentialities of the computer as an additional tool in research endeavors.

RECOMMENDATIONS:

The fact that the Computer Division has supported all the divisions as an active participant in multiple research studies should continue

Design of Military Biomedical Research Information System (Cont)

with full cooperation and understanding of the parties involved. The Computer Division assigned personnel should continue researching new computer techniques to improve the research program of USAMRNL.

PUBLICATIONS:

None.

TABLE I - TALLY OF DATA MESSAGES ON THE USAMRNL DATA FILE

	Messages of Data for Human Subjects								Messages of Mess Hall Data			
	# of Subjects	Descr. Info.	Performance	Pulmonary	Clinical	Anthropometric	Biochemical	Body Comp.	# of Meals	Nutrient Intake	Recipes	Headcount
Altitude Studies	83	147		275		83			131	1599		
Ft. Benning Survey	103	103		292								
Ft. Carson	501	610		838		234						
Ft. Campbell Survey	195	390	489	388		392			33	476	67	
Ft. Huachuca Survey	199	398	364	398		398		706	39	598	42	
Ft. Irwin Survey	246	487		967	269	486			51	795	194	
Ft. Lewis Survey	501	1002			501	1002	1503		357	2897	670	4658
Ft. McClellan Survey	351	1053			351	702	3510		36	648	241	
Ft. Myer Survey	348	696			348	696			106	2973	799	9384
Laboratory Survey	112	221		32								
Lowry AFB Survey									46	1230	426	1037
Malaysia Ration Study									350	3687		
Metabolic Type Studies	84	123	618	504		46						
Panama Ration Study	60	60		1084	114	304						
TOTAL (59,536 messages, 3,112,000 characters)	2782	5290	1471	4778	1583	4343	5013	706	1149	14903	2439	15079

TABLE II

LOWRY AIR FORCE BASE NUTRITION SURVEY

HEADCOUNT FOR PERSONNEL AUTHORIZED RATIONS

MEAL	DAY	1	2	3	4	5	6	7
		Wed	Thu	Fri	Sat	Sun	Mon	Tue
	JULY	14	15	16	17	18	19	20
Breakfast		338	405	270	103	64	388	340
Short Order Lunch		277	260	239	158	142	231	272
Dinner		415	406	355	296	309	434	369
Short Order Supper		431	244	206	173	229	314	279
Supper		305	361	360	209	303	392	302
Midnight		125	106	94	---	---	95	105
TOTAL		1891	1782	1524	939	1047	1854	1667

TABLE III

LOWRY AIR FORCE BASE NUTRITION SURVEY
MEAN OF DAILY DINING HALL UTILIZATION
BY PERSONNEL WITH MEAL CARD

	7 DAYS	5 DUTY DAYS	2 WEEKEND DAYS
Mean percent of 1 meal eaten per day	34.6	28.1	51.0
Mean percent of 2 meals eaten per day	43.5	42.7	45.4
Mean percent of 3 meals eaten per day	21.8	29.0	3.7
Mean percent of 4 meals eaten per day	0.1	0.2	----
Mean meals per man per day	1.9	2.0	1.5
Mean daily percent utilization of the dining hall	62.5	67.1	50.9

TABLE IV

LOWRY AIR FORCE BASE NUTRITION SURVEY
PERCENT OF PERSONNEL SURVEYED EATING VARIOUS MEAL COMBINATIONS
PERSONNEL AUTHORIZED RATIONS

MEAL COMBINATION	PERIOD OF TIME STUDIED		
	7 DAYS	5 DUTY DAYS	2 WEEKEND DAYS
Dinner, supper	11.2	9.5	16.9
Dinner	9.0	7.0	15.0
Supper	7.6	5.6	14.3
Dinner, Short order supper	7.3	6.1	11.4
Breakfast, Dinner, Supper	7.3	9.2	1.1
Short order lunch, Short order supper	6.0	5.9	6.8
Short order lunch	6.0	5.2	8.6
Short order supper	5.7	4.6	8.8
Short order lunch, supper	4.6	4.4	5.1
Breakfast, Dinner, Short order supper	4.4	5.4	0.9
Breakfast, Short order lunch, Short order supper	4.0	4.8	1.2
NUMBER OF MAN DAYS INCLUDED IN ANALYSIS	5452	4170	1275

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6312	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10 NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	05	080
b. CONTRIBUTING	61145011	3A014501B71R	05	
c. CONTRIBUTING	CDOG 114 (F)			

11. TITLE (Procede with Security Classification Code)

(U) High Altitude Bioenergetics - Determination of Mechanisms Responsible for Acute Mountain Sickness (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016200 Stress Physiology; 005900 Environmental Biology

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	2.2	65
	CURRENT 73	2.0	55

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Bioenergetics Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Johnson, H. L.

TELEPHONE: 303 366 5311 X25222

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Krzywicki, H. J.

NAME: Consolazio, C. F. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Hypoxia; (U) Altitude Stress; (U) Performance; (U) Balance-Metabolic; (U) Blood Gases; (U) Glucose Metabolism; (U) Respiratory Function

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

23. (U) To 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) Determine glucose metabolism and turnover rates in man at altitude utilizing glucose-^{14}C and measuring plasma and expired air radioactivity. Simultaneously measure insulin and growth hormone levels and administer glucagon to observe its effects upon plasma glucose and hormones. Determine carbohydrate-metabolizing enzymic activities, muscle and liver glycogen levels and serum metabolites in rats during the first 12 days of altitude exposure and study the effects of dietary carbohydrate levels and treadmill exercise upon these parameters.

25. (U) Fasting plasma glucose levels were reduced during altitude exposure and plasma radioactivity levels after infusion of glucose-^{14}C decreased faster in the 40-hour altitude exposed men compared to men studied immediately or 16 hours after exposure and the pair-fed men at sea level. Respiratory excretion of $^{14}CO_2$ was increased in men at altitude. Infusion of 60 micrograms of glucagon produced a higher elevation of plasma glucose in the "immediately after" exposure group, and an elevation of shorter duration in the 40-hour exposed group. Plasma human growth hormone levels were increased while insulin levels were unaffected by altitude. Three animal experiments on the effects of dietary carbohydrate levels, altitude exposure and exercise upon carbohydrate-metabolizing enzyme activities have been completed.

Available to contractors upon originator's approval.

FORM AR 66 1498

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 investigations have been conducted under this work unit.

STUDY NO.	12	Physiological and metabolic aspects of altering dietary carbohydrate-fat levels upon carbohydrate metabolism at altitude
STUDY NO.	13	(FY72) Effects of diet and altitude on carbohydrate metabolism and the metabolic responses to exercise (with labelled glucose)
STUDY NO.	14	Effects of high sublethal and lethal temperatures on hypoxia at the tissue level, and PART B. High temperature tolerance of mice.

STUDY NO. 12

Three animal experiments have been completed under this study. The first experiment established that an ad libitum feeding schedule and that three ratios of fat to carbohydrate were adequate to determine the effects of diet upon inducible enzyme systems. Through identification of non-inducible enzymatic or metabolic changes, the number of enzymatic activities and intermediates in energy metabolism being monitored was reduced. Glucose-6-phosphate dehydrogenase (G-6-PDH) was most influenced by dietary and exercise manipulation. The second experiment indicated that exercise enhanced some of the enzymatic changes, while altitude exposure of the rats in the third experiment moderated the alterations. Prolonging altitude exposure increased G-6-PDH activity and the water content of livers. These data indicate that the hexose monophosphate shunt's activity was increased at altitude.

STUDY NO. 13 (FY72)

A second study with the infusion of glucose-^{14}C was conducted in altitude exposed men and matched pair-fed men at sea level. Altitude exposure reduced fasting plasma glucose levels, increased disappearance of glucose-^{14}C from plasma, increased respiratory excretion of $^{14}CO_2$

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

and increased the respiratory quotient. The data obtained was consistent with the hypothesis that carbohydrate metabolism was increased and fat metabolism decreased at altitude.

STUDY NO. 14

Comparisons were made in mice of the physiological changes associated with thermal stress to those observed with hypoxia. Thermal stress increased tissue lactates and pyruvates indicative of hypoxia in brain and muscle; however, hypoxia did not appear to be severe enough to cause the fatalities observed.

BODY OF REPORT

WORK UNIT NO.	080	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 metabolism at altitude
STUDY NO. 13		(FY72) Effects of diet and altitude on carbohydrate metabolism and the metabolic responses to exercise (with labelled glucose)
STUDY NO. 14		Effects of high sublethal and lethal temperatures on hypoxia at the tissue level, and PART B. High temperature tolerance of mice.

PROBLEM:

Abrupt exposure of men and animals to hypoxia results in anorexia and other symptoms which could be disastrous to a combat operation unless adequate precautions or allowances had been made. Previous studies in this laboratory have shown that the detrimental effects of altitude exposure can be alleviated by the adequate consumption of carbohydrate and/or maintaining physical activity during exposure. These studies were designed to elucidate the mechanisms of the altitude effects in order to effectively reduce their influences upon the soldiers' performance and well being.

RESULTS AND DISCUSSION OF RESULTS:

STUDY NO. 12

Three of the five animal experiments have been completed. The first experiment was conducted at Denver to determine the best feeding schedule and to evaluate which metabolic parameters were affected by the dietary alterations. The data indicated that providing food for 8 out of every 48 hours (as reported in the literature to reduce variability between animals) did not affect the variability sufficiently to justify the additional work. An intermittant feeding schedule per se had a great effect upon liver glycogen levels depending upon the length of time between feeding and sacrifice of the animals. Six diets were used containing 8, 18, 28, 38, 48 and 58% fat calories, and 80, 70, 60, 50, 40 and 30% of the

calories from carbohydrate. The results obtained indicated that three diets would be sufficient for further studies, 8-80, 28-60 and 58-30% fat-carbohydrate calories. The number of biochemical parameters to be examined in further studies were reduced from 22 to 13, and included glucose-6-phosphate dehydrogenase in liver, glycogen in liver and muscle, phosphocreatine and phosphocreatine kinase (CPK) in muscle, serum glutamic-oxalacetic (SGOT) and glutamic-pyruvic transaminases (SGPT), blood glucose levels, pyruvic kinase and transketolase in liver and total nitrogen in blood, liver and muscle. The second experiment incorporated the effects of treadmill exercise upon diet and on changes in the biochemical parameters measured. G-6-PDHase showed the largest increases with diet and exercise. Pyruvic kinase and transketolase did not change significantly with diet but appeared to increase with exercise. Serum GOT, GPT, and CPK were increased by both increasing dietary carbohydrate content and exercise. Glycogen determinations were eliminated because they were greatly affected by the rapidity of sacrifice and tissue removal. Pre-exercise blood glucose levels were increased by both increased dietary carbohydrate and exercising of the rats. The third experiment was designed to study the effects of altitude exposure upon the diet-induced alterations. Altitude exposure reduced G-6-PDH activity in all groups of animals although the dietary effect was still present and the activity increased during altitude exposure. The other significant effect of altitude exposure was an increased water content of livers and spleens. The water content generally increased with increased duration at altitude with no change in dry weight of the tissues. Altitude exposure, diet and exercise appears to increase the role of the hexose monophosphate shunt in energy metabolism from carbohydrate.

STUDY NO. 13 (FY72)

A second study utilizing glucose-^{14}C infusion in men was conducted during August 1971. The study was designed for twelve pairs of subjects — one of each pair to be studied at altitude and his pair-fed mate at sea level. However, due to personal reasons, two of the altitude subjects were unable to participate so three men were studied within two hours, four after sixteen hours, and three after forty hours of altitude exposure. Each man received a 30-microcurie infusion of glucose-^{14}C and the blood and expired $^{14}CO_2$ was monitored for 210 minutes. Glucagon (70 micrograms) was infused 120 minutes after the glucose infusion. The sea level men were matched by age, height and weight, and were pair-fed and studied two weeks later. Altitude exposure reduced the fasting plasma glucose values about 10 mg%, although the values were not

consistently significant due to the small number and variability of the subjects. Neither altitude exposure nor length of exposure affected the specific activity of plasma glucose. The 40-hour exposed group had the fastest disappearance of the label from the plasma and this was significant by 90 minutes post injection. Combining the total $^{14}CO_2$ excretion data for all of the altitude subjects as compared to the combined data for sea level men, a significant increase at altitude from 70 to 140 minutes post injection of the tracer was shown. The respiratory quotient was also increased in the 40-hour exposed men. These data are consistent with, although not conclusive evidence for, the hypothesis that carbohydrate metabolism was increased at altitude with a concomitant decrease in fat metabolism. The glucagon infusion produced a significantly faster elevation of blood glucose in the immediate exposure group and a significantly shorter duration of effect in the 40-hour exposed group. This would indicate that the men had a greater sensitivity to glucagon during the initial period of altitude exposure. The low plasma gl values 50 minutes after glucagon infusion would indicate a de, ion of liver glycogen.

STUDY NO. 14

Two studies were conducted using mice to compare the physiological effects of thermal stress to those associated with hypoxia at altitude. Brain, muscle and liver pyruvate and lactate levels were determined in four groups of mice: (1) control, rectal temperature (T_R) = 37.2° C; (2) thermal stress, T_R = 40.8; (3) terminal thermal stress, T_R = 43.8; and (4) hypoxic death in nitrogen atmosphere. Group 2 had decreased muscle lactate, increased liver lactate with no change in brain lactate while pyruvate decreased 59% in muscle with no changes in brain and liver. Group 3 exhibited increases of 65 and 125% in brain and liver lactate, respectively, and an insignificant (12%) decrease in muscle lactate levels with concomitant pyruvate decreases of 57 and 74% in brain and muscle and unchanged liver pyruvate levels. The hypoxic Group 4 showed the largest increases in lactate levels: 130% in brain, 50% in muscle and 171% in liver, while pyruvate changes were not different from those of Group 3. These results indicate that hypoxia probably occurs in tissues of thermally stressed animals but did not appear to be the cause of death. The second study involved the changes associated with stressing the mice to their critical thermal maximum. Mortalities of 50 to 100% were observed in mice after stressing them to temperatures several degrees below that which causes thermoregulatory breakdown, suggesting that critical alterations occurred in tissues before the body lost its ability to regulate its temperature. Some of these alterations may occur during the period of hypothermia following heat stress.

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

CONCLUSIONS:

The data obtained from the animal studies indicate that increasing dietary carbohydrate content and/or exercising rats increased some of the rate limiting steps of carbohydrate metabolism, especially glucose-6-phosphate dehydrogenase which influences the hexose monophosphate shunt. Altitude exposure initially reduced the activity of these enzymes; however, their activities increased as exposure time to altitude was increased. Although hypoxia reduced these enzymatic activities, the dietary differences were still observed. These observations support the hypothesis that carbohydrates are the preferred energy source at altitude. High carbohydrate diets prior to altitude exposure and/or exercise increases the capacity of the carbohydrate metabolizing systems of the body. Further study is required before any conclusions can be advanced regarding the altitude induced hyperhydration of liver and spleen. The human study with the infusion of glucose ^{14}C indicated that carbohydrate metabolism was stimulated and/or fat metabolism inhibited by hypoxia since the respiratory quotient was increased after 40 hours of altitude exposure, disappearance rates of glucose -^{14}C from plasma was increased in the 40-hour exposed men and the $^{14}CO_2$ excretion was increased in all of the men at altitude. The altitude exposed men appeared to have an increased sensitivity to glucagon immediately after arriving at altitude.

Studies of thermal stress in mice indicated that this stress produced tissue hypoxia since lactate levels increased and pyruvate concentrations decreased; however, these changes were less than those associated with hypoxic deaths induced by a nitrogen atmosphere suggesting that hypoxia was not the primary cause of death of these animals. Mice stressed at temperatures below their point of thermoregulatory breakdown exhibited a period of hypothermia after return to a normal (25° C) environment followed by 50 to 100% mortalities. This suggested that tissue damage may have occurred during the hypothermia and was a contributing cause of the fatalities.

RECOMMENDATIONS:

1. Conduct experiments 4 and 5 of Study No. 12 to observe the effects of exercise during and prior to altitude exposure upon the diet induced and hypoxia reduced alterations in enzymatic activities in rats.

2. Study the hyperhydration of tissues at altitude and establish the time sequence of its onset and development. This is planned in conjunction with completeion of Study No. 12.

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

3. Complete all analyses and statistical evaluations of the altitude studies and publish the results.

PUBLICATIONS:

1. Wright, G.L., W.D. Lindsey, H.L. Johnson, and H.J. Krzywicki. The effect of high temperatures on tissue lactate, pyruvate and venous blood properties in the rat. Int. J. Biometeor. 16:71-77, 1972.

2. Johnson, H.L., C.F. Consolazio, R.F. Burk, T.A. Daws, and T.M. Ward. Glucose metabolism in man after abrupt exposure to altitude. Fed. Proc. 31:689 Abs, 1972 (Abstract)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6339	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	05	082
b. CONTRIBUTING	61145011	3A014501B71R	05	
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Precede with Security Classification Code)

(U) Metabolic, Physiological and Psychological Effects of Altitude (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	CONT	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	1.1	50
FISCAL YEAR CURRENT 73	1.1	85

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Physiology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Klain, G. J.

TELEPHONE: 303 366 5311 X26212

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Hannon, J. P.

NAME: Sullivan, F. J. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Altitude; (U) Adaptation; (U) Physiological; (U) Endocrine; (U) Biochemistry; (U) Physiology; (U) Military Stress

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

23. (U) Thirty to forty per cent 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 only partially be conducted 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 d) ascertaining the basic physiological and metabolic mechanisms which underlie the observed defects and adaptations.

24. (U) Laboratory animals will be subjected to actual and simulated high altitude environments. Various physiologic, metabolic and psychologic techniques will be used to determine the alterations caused by hypoxic exposure. Studies will be conducted at cellular, organ and organism levels. Efforts to ameliorate or eliminate the defects will be made with the use of drugs, special diets, training, etc. Two phase studies will be conducted: first, the acute response to hypoxia and second, the nature, extent and rate of adaptation to chronic exposure.

25. (U) 71 06 - 72 07 Computer software was developed to evaluate the "burst measures" of activity in rats subjected to hypoxia. The interrelationships of activity to food consumption, water consumption, and body weight were also studied. Studies of tyrosine metabolism and excretion indicate an increased turnover of catecholamines during the initial stages of adaptation to high altitude.

*Available to contractors upon originator's approval.

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

STUDY NO. 22 Effects of high altitude exposure and food consumption upon spontaneous activity of albino rats

STUDY NO. 26 Synthesis and degradation of catecholamines at high altitude

STUDY NO. 28 Effect of high altitude on selected catecholamine-synthesizing enzymes in the adrenal gland

The interrelationships between exposure duration, food intake and activity were studied in rats exposed to a simulated altitude of 4,300 meters for 7 days. Activity was found to be best described using "burst measures" based upon both intensity and duration factors. Computer software to evaluate burst measures has been developed.

Significant differences in tyrosine metabolism were observed between rats acutely exposed to high altitude and the controls maintained in Denver. Oxidation of tyrosine was markedly increased during the first three days of high altitude exposure and the pattern of urinary metabolites was different from that observed in the controls. This would indicate a shift in the degradative pathways of tyrosine at high altitude. Activities of specific enzymes participating in catecholamine biosynthesis were also increased during the initial exposure to altitude. The data indicate an increased turnover of catecholamines associated with an acute exposure to high terrestrial altitude.

BODY OF REPORT

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

STUDY NO. 22 — Effects of High Altitude Exposure and Food Consumption Upon Spontaneous Activity of Albino Rats

PROBLEM:

The purpose of this study was to assess selected behavioral measures as indices of high-altitude acclimatization in animals.

RESULTS AND DISCUSSION OF THE RESULTS:

In one experiment, following 3-day baseline measurements at a simulated altitude of 1,600 meters, rapid transition (≤ 1 hr) to 4,300 meters induced temporary decreases in food consumption, water consumption and body weight. These measures showed gradual return to baseline levels during the 7-days' exposure to 4,300 meters, with return to 1,600 meters alleviating all residual effects within 24 hrs. Alterations in feeding activity (time-in-foodwell) during the dark portions of the light cycle paralleled food intake shifts; whereas, no reliable changes in spontaneous activity as a function of hypobaric transition were noted. Theoretically, these results are of interest because: (a) the pattern of decreased food consumption, water consumption and body weight resembles previously described behavior patterns for animals with lateral hypothalmic lesions, and (b) the lack of observed decrements in spontaneous activity is compatible with much recent work disassociating locomotor and spontaneous forms of activity under specific stress conditions.

In two other experiments, certain data analyses concerning the effects of altitude exposure upon animal activity remain to be accomplished. However, considerable work using the low altitude data from these experiments was directed towards the development of improved measures of animal activity for future research. Since the daily activity of animals is characterized by intermittent periods (i.e., bursts) of intense activity and relative inactivity, changes in the duration and/or intensity of these "activity bursts" were viewed as offering useful measure of animal activity in studies involving environmental and/or nutritional manipulations. During the past year, computer software was devised to measure "burst" characteristics of activity. Results of preliminary analyses showed: (a) as the length of time or the amount of activity required to define a burst was increased, mean number of bursts declined according to a transitive function: (b) consistent diurnal effects were observed, with light-ON activity less than light-OFF activity. It is felt that the use of activity-burst measures, as opposed to per cent measures, may afford improved indices of animal behavior in experiments dealing with environmental and/or nutritional regimen.

CONCLUSIONS:

Data concerning the effects of hypobaric exposure upon animals indicates important differences in the behavioral effects are obtained as a function of activity measuring device. Burst measures of activity appear to afford improved indices of environmental- and/or nutritional-induced behavioral effects.

STUDY NO. 26 Synthesis and Degradation of Catecholamines at High Altitude

PROBLEM:

The role of catecholamines in cellular adaptation to stress is well recognized. Strong circumstantial evidence suggests that the biogenic amines are involved in the control of brain function and that the normal emotional behavior is the result of a biochemical balance in the central nervous system. The marked decrement in the mental performance of human subjects during the first few days of high altitude exposure thus could be a reflection of a derangement in the synthesis or degradation of some of the biogenic amines. In the present study, in vivo synthesis of tissue catecholamines and their urine metabolites from tyrosine-U-^{14}C were studied in high altitude exposed rats.

RESULTS AND DISCUSSION OF THE RESULTS:

After 1, 3, 6 and 13 days of exposure to 14,110 feet (summit of Pikes Peak) rats were injected with tyrosine-U-^{14}C, and the expired $^{14}CO_2$ was collected in a sodium hydroxide solution. Three and twenty-four hours after injection, the rats were killed, tissue samples were removed and frozen. Urine was collected for twenty-four hours. The control animals were kept in Denver (5,200 feet).

Compared to the controls, three hours after injection total counts expired as $^{14}CO_2$ were increased by approximately 49, 75, 20 and 12 per cent in rats exposed to altitude for 1, 3, 6 and 13 days, respectively. Total radioactivity in the urine followed a pattern similar to that observed in $^{14}CO_2$. A major urinary metabolite in the controls was 3, 4-dihydroxymandelic acid, followed by tyrosine, and small quantities of epinephrine. In altitude exposed rats the major metabolite excreted was 3,4-dihydroxyphenylglycol, indicating a shift in the metabolic degradation of epinephrine at altitude. Incorporation of tyrosine into adrenal and brain proteins was also increased in altitude exposed animal when compared with the controls. However, there was no difference in specific activities of liver proteins between the control and altitude groups of animals.

CONCLUSIONS:

An acute exposure to high altitude causes a shift of degradative pathways of epinephrine and norepinephrine. Reductive pathways apparently predominate at altitude, as indicated by the excretion of urinary metabolites. Turnover of catecholamines also appears to be elevated during the initial stages of altitude exposure.

RECOMMENDATIONS:

Determine the significance of the metabolic shifts observed in this study by further investigating degradation of specific catecholamines in animals exposed to high altitude.

STUDY NO. 28 — Effect of High Altitude on Selected Catecholamine-Synthesizing Enzymes in the Adrenal Gland

PROBLEM:

The role of the sympatho-adrenal system in the initial response to a wide variety of environmental stresses and stimuli is well recognized. Among these acute exposure to hypoxia has been shown to stimulate catecholamine secretion from the adrenal gland in experimental animals. An increase in the plasma and urine concentration of catecholamines in human subjects taken abruptly from low to high terrestrial altitudes has been reported. Recent data from this laboratory indicate an increased oxidation of tyrosine, a precursor of catecholamines, in rats exposed to high altitude. These data suggest an increased turnover of catecholamines during the initial stages of high altitude exposure. The investigation described here was designed to provide further information on the metabolism of catecholamines at high terrestrial altitude. Accordingly, activities of selected adrenal enzymes participating in the biosynthesis of catecholamines were examined.

RESULTS AND DISCUSSION OF RESULTS:

After 1, 3, 7 and 14 days of exposure to 14,110 feet (summit of Pikes Peak) groups of 5 - 7 rats each were decapitated and adrenal glands were assayed for dopamine--hydroxylase (DBH), tyrosine hydroxylase (TH) and phenylethanolamine-N-methyl transferase (PNMT). The control animals were kept in Denver (5,200 feet).

Daily gains in body weight and food intakes were significantly depressed after one-day exposure to altitude. Thereafter, altitude had no effect on weight gain or food intake. The adrenal weights were significantly increased only after seven or fourteen days of altitude exposure.

TH activity was elevated after one, three and seven days of exposure. After fourteen days at altitude, however, no significant increase in activity was observed. Levels of adrenal DBH were increased after the first day of exposure and remained elevated throughout the whole experimental period. The activity of PNMT was not affected on the first day at altitude, but an increase in activity was observed after three and seven days. At the end of the experimental period the activity was similar to that found in the controls.

The mechanisms controlling catecholamine synthesis by the adrenal gland are not fully understood at the present time. but it would appear that the hydroxylation of tyrosine is the rate-limiting step in the biosynthesis of epinephrine. In addition, an inhibition of TH activity by norepinephrine and other catechol derivatives has been observed, indicating a feedback control mechanism in epinephrine production.

CONCLUSIONS:

Activities of the key adrenal enzymes participating in catecholamine synthesis in rats were markedly increased during the initial stages of altitude exposure indicating an increased turnover of norepinephrine and epinephrine.

RECOMMENDATIONS:

Future studies in this particular aspect of the work unit should be directed toward the mechanisms controlling enzymatic activities at high altitudes.

PUBLICATIONS:

1. Klain, G. J. High altitude and tyrosine metabolism in the rat. Federation Proc. 31:390, 1972 (abstract).

2. Klain, G. J. Acute high altitude stress and enzyme activities in the rat adrenal medulla. Endocrinology, 1972 (in press)

3. Sterner, R. T. and G. D. Schwank. Hypobaric hypoxia: within-subject transition effects in albino rats. Perceptual and Motor Skills (submitted)

4. Krabill, L. F. and J. P. Hannon. Effects of high-altitude exposure on rate of ingesta passage in rats. Am. J. Physiol. 222: 458-461, 1972.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6356	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	05	085
b. CONTRIBUTING	61145011	3A014501B71R	05	
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Procode with Security Classification Code)

(U) Effects of Altitude on Myocardium of Animals (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

005900 Environmental Biology; 016200 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 04	CONT	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	3.0	12
FISCAL YEAR CURRENT 73	2.8	10

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Pathology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Demaree, R. S., CPT, MSC

TELEPHONE: 303 366 5311 X23230

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Empson, R. N., Jr., CPT, VC

NAME: Nessmith, W. B., CPT, VC DA

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Procode EACH with Security Classification Code) (U) Altitude; (U) Subhuman Primates; (U) Vertebrates; (U) Military Stress; (U) Cardiovascular System; (U) Biological Sciences

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

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 myocardial 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.

24. (U) Animals are housed in heated facilities at 14,110 ft. and at sea level, to 1) compare the physiologic and pathologic response of altitude-exposed animals, particularly subhuman primates, with observations from other species, including man, 2) investigate the changes in brain, cardiac and skeletal muscle after exposure, with biochemical measurements and light and electron microscopy, 3) quantitate cerebral blood flow, cerebrospinal fluid (CSF) pressure, brain water, and constituents of blood and CSF in subhuman primates during acute exposure to 14,110 ft.

25. (U) 71 07 - 72 06 In study 6 no changes detectable by either light or electron microscopy were seen at altitude exposure in monkeys given the diuretic furosemide. Femoral artery blood pressure increase with altitude exposure was significantly reduced in monkeys given the diuretic. In study 7 there were no statistically significant differences among preconditioned-exercised, preconditioned-non exercised, non conditioned non-exercised, and non-conditioned exercised rats with respect to cerebral edema, cardiac hypertrophy or daily food intake. There was a significant ($p<0.5$) weight loss in non conditioned-exercised rats but not in any other treatment groups.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3A061102B71R	Research in Biomedical Sciences
TASK NO.	05	Environmental Medicine
WORK UNIT NO.	085	Effects of Altitude on Myocardium of Animals

The following investigations have been conducted under this work unit.

STUDY NO. 6 Effects of Altitude on the Cebus Monkey (Cebus apella) with Emphasis on Alterations in Blood-Brain Barrier

STUDY NO. 7 Effects of Preconditioning by Exercise in Rats on Cardiac Hypertrophy, Food Intake, and Growth Rate at Altitude

The general objectives of Studies 6 and 7 are to characterize the effects of altitude on the myocardium and central nervous systems of animals and to correlate these where possible with physiological and subcellular functional changes.

In study 6 no changes detectable by either light or electron microscopy were seen at altitude exposure in monkeys given the diuretic furosemide. Femoral artery blood pressure increase with altitude exposure was significantly reduced in monkeys given the diuretic.

There were no statistically significant differences among preconditioned-exercised, preconditioned-non exercised, non conditioned non-exercised, and non-conditioned exercised rats with respect to cerebral edema, cardiac hypertrophy or daily food intake. There was a significant ($p<0.5$) weight loss in non conditioned-exercised rats but not in any other treatment groups.

BODY OF REPORT

WORK UNIT 085 — Effects of Altitude on Myocardium of Animals

STUDY NO. 6 — Effects of Altitude on the Cebus Monkey (Cebus apella) with Emphasis on Alterations in Blood-Brain Barrier

PROBLEM:

A mild perivascular edema was observed in Cebus apella monkeys with acute high altitude exposure in previous studies. And increased cerebral capillary permeability was demonstrated with acute altitude exposure in monkeys.

Since diuretics have been reported to have been useful in alleviating symptoms of "acute mountain sickness" in humans, the question still remained - what was the mechanism behind the amelioration of symptoms and to what extent is therapy effective in diminishing cerebral edema and altered capillary permeability, if any.

This study was designed to evaluate the clinical and morphologic effect of diuretic therapy on Cebus apella monkeys rapidly transported from sea level to 14,110 feet (PPLF).

RESULTS AND DISCUSSION:

Femoral artery blood pressures at sea level and then at altitude were tabulated for nine monkeys which survived the translocation to PPLF. One additional monkey was in shock and those data have been omitted. Mean arterial blood pressures at altitude were elevated 13 mm mercury or less in the nine monkeys given Furosemide. In contrast, mean arterial blood pressures at altitude were elevated 28-84 mm mercury in the monkeys not treated with Furosemide (FY 71).

All monkeys, sea level controls and those exposed to altitude, received 5.0 mg Furosemide daily for the duration of the experiment. Detailed light and electron microscopic examinations have been conducted on tissue from the medial temporal gyrus of three sea level controls and nine monkeys exposed to altitude for 1, 3 or 5 days. There were no detectable differences by either light and electron microscopy in any of these monkeys.

Effects of Altitude on Myocardium of Animals (Cont'd)

CONCLUSIONS

The diuretic Furosemide was apparently effective in reducing the increase in mean arterial blood pressure experienced with acute high altitude exposure. The diuretic apparently also eliminated the mild perivascular edema associated with acute altitude exposure.

RECOMMENDATIONS

It seems desirable to continue the study of diuretics as a possible therapy for acute mountain sickness, using human subjects.

STUDY NO. 7

The Effect of Preconditioning by Exercise in Rats on Cerebral Edema, Cardiac Hypertrophy, Food Intake and Growth Rate at Altitude

PROBLEM

Studies conducted by USAMRNL have demonstrated a mild cerebral edema in some monkeys rapidly transported to 14,110 feet (PPLF) from sea level. The extent of the edema was not determined.

The effect of altitude upon physically fit human subjects has been studied. Conflicting reports exist as to the effectiveness of physical fitness in reducing symptoms of "acute mountain sickness."

This study was proposed to determine if cerebral edema occurs in rats at high altitude and the extent of the edema. In addition, it was of interest to determine the effects of pre-conditioning by exercise on cerebral edema as well as its effect on cardiac hypertrophy, daily food intake and growth.

RESULTS AND DISCUSSION

Eighty Holtzman strain rats weighing approximately 250 grams were divided into two groups, exercised (A) and non-exercised (B). The forty exercised rats were forced to swim in a water tank 14 inches deep maintained at 33°C. The rats were exercised 1/2 hour the first day; the time was increased 1/2 hour each day until 6 hours of con-

tinuous swimming was reached. This continued at MRNL 6 days a week for 4 weeks prior to translocation to Pike's Peak Laboratory Facility (PPLF). Group B was not exercised. Food intake was recorded daily and weight was recorded weekly.

The eighty animals were translocated to PPLF. The exercised rats were divided into two equal groups, A1 which continued daily exercise, and A2 which did not exercise. Likewise the non-exercised group was divided into two groups, B1 which began the exercise regimen at altitude and B2 which continued as a non-exercised group. Daily food intake and body weights were measured at PPLF for the first 7 days and weekly thereafter.

Rats were sacrificed after 1, 3, 5 or 45 days at high altitude. One hour prior to sacrifice trypan blue, a vital dye, was administered intravascularly to check for cerebral edema. Brains were transected in five planes in accordance with standard MRNL procedures. Hearts were removed, weighted and atria trimmed from the ventricles. The right and left ventricles were separated from the septum, and the valves and peripheral fat removed. The right and left ventricles were weighed separately.

There were no differences detectable by light microscopy in brains or hearts among pre-conditioned exercised, preconditioned non-exercised, non-conditioned exercised and non-conditioned non-exercised rats. In addition, there was no leakage of the tracer trypan blue indicative of cerebral edema, although trypan blue did pass through capillaries in other body tissues as was expected.

There were no statistically significant differences among the experimental groups in either cardiac hypertrophy or daily food intake. There was a significant ($p<0.5$) weight loss in non-conditioned exercised rats, but not in any other treatment groups.

CONCLUSIONS

Based upon the above results it appears that rats are less susceptible to the effects of acute mountain sickness than primates.

RECOMMENDATIONS

Further research on acute mountain sickness should use primates rather than rats whenever possible.

Effects of Altitude on Myocardium of Animals (Cont'd)

PUBLICATIONS

1. Demaree, R. S., Jr., L. J. Ackerman and D. L. Anderson. "Permeability in the blood-brain barrier in monkeys following exposure to high altitude." In: 29th Ann. Proc. Electron Microscopy Soc. Amer., edited by C. J. Arceneaux, Boston, Mass., 1971.

2. Ackerman, L. J., R. S. Demaree, Jr., and D. L. Anderson. Sustained pressure perfusion method. Lab Animal Science 22:114, 1972.

3. Ackerman, L. J., R. S. Demaree, T. J. Bucci, G. A. Kennedy, C. L. Alden, T. S. Myers and R. Lazzara: Effects of Altitude on the Cebus Apella Monkey, USAMRNL Report No. 328.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6300	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H Termination	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	065
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Precede with Security Classification Code)

(U) Microbiological Research in Tuberculosis (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
59 08	30 June 1972	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	3.5	80
	CURRENT 72	2.0	58

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, John E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Microbiology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Blair, E. B., COL, MSC

TELEPHONE: 303 366 5311 X25223

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Tull, A. H.

NAME: DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Tuberculosis; (U) Mycobacteria; (U) Chemotherapy; (U) Drug Resistance; (U) Biological Assay; (U) Computer; (U) Laboratory Diagnosis

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

23. (U) To evaluate and/or design new methods which can be used in Army Medical Laboratories for isolation, identification and drug susceptability testing of pathogenic mycobacteria. To establish a computer data base of laboratory results on tuberculosis patients for correlation with clinical observations, treatment results, etc.

24. (U) Quantitative data pertaining to organism growth rates on various culture media, identification tests, drug susceptibilities, serum drug levels and serum inhibition titers are placed in computer files for correlation with patients' conversion to bacteriologic negativity as affected by therapy, dosage, etc. Analyses of computer data provide controls on laboratory methodology, procedure changes and on investigative procedures. Continuous improvement and updating of laboratory methodology and quantitation of results are imperative. The stability of antituberculosis drugs under varying conditions of storage or use are being tested.

25. (U) 71 07 - 72 06 (a) A computer file containing 38,000 messages of mycobacteriologic data on 1,690 Fitzsimons General Hospital tuberculosis patients is in routine operation and is periodically subjected to analyses. (b) Four additional modifications of the 7H10 and 7H11 agar media are being evaluated for optimal recovery of mycobacteria from clinical specimens. (c) Studies on effects of temperature and storage on activity loss of rifampin, ethambutol and kanamycin in 7H10 agar medium have been completed and the data are being analyzed. (d) Additional improvements have been made on the blue-light microscopy system for demonstrating auramine O-stained mycobacteria. Project terminated 30 June 1972 due to transfer of the Microbiology Division to Fitzsimons General Hospital and OMA funding.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine

WORK UNIT NO. 065 Microbiological Research in Tuberculosis

The following investigations have been conducted under this work unit:

STUDY NO. 1 To improve mycobacteriology laboratory methods

(a) A computer data bank containing five years of mycobacteriologic data on 1,690 Fitzsimons General Hospital tuberculosis patients is in routine operation. Pertinent analyses of these data are presented.

(b) Comparisons of growth-promoting qualities of four modifications of the Middlebrook 7H10 OA agar medium (7H10 OA-ph 6.6 no catalase, 7H11 OA-ph.6.8 no catalase, 7H11 OA-pH 6.8, catalase and 7H11 OA-pH 7.2, no catalase) revealed that the 7H11 OA-pH 7.2, no catalase medium was inferior to the other three; the latter were about equal in efficacy for recovery of mycobacteria from clinical specimens.

(c) Expanded capability of the blue light system for microscopic examination of auramine O-stained mycobacteria was achieved using an FITC interference primary filter with either of two secondary filters. Comparison of ultraviolet and blue light sources were equal in efficacy for detecting stained mycobacteria, although the blue light source was preferred because of less nonspecific fluorescence.

(d) Studies on inactivation of rifampin, ethambutol, and kanamycin in 7H10 OA agar plates stored or incubated at 5°, 24°, or 36°C revealed that rifampin and ethambutol are rapidly inactivated at 24° and 36°C but not at 5°C. Kanamycin was stable at all temperatures.

BODY OF REPORT

WORK UNIT NO. 065

Microbiological Research in Tuberculosis

STUDY NO. 1

To improve mycobacteriology laboratory methods

PROBLEM:

U. S. military personnel stationed in certain foreign countries are exposed to populations having a high incidence of active tuberculosis. In these countries the problem is increased by the high prevalence of drug-resistant tuberculosis strains in the population. Disease caused by mycobacteria other than M. tuberculosis further complicates the diagnosis and treatment of mycobacterioses. Definitive proof of mycobacterial disease is dependent on the laboratory successfully isolating and identifying the etiologic agent from clinical material. Intelligent and successful treatment depends on accurate drug susceptibility data on the infecting organism. Therefore, continuous examination and improvement of laboratory methodology is vital to provide this important information for the physician. Research includes evaluating new culture media formulations, diagnostic tests, methods for measuring serum drug level and serum inhibition titers, drug susceptibility tests, quality control methods, etc.

RESULTS AND DISCUSSION OF THE RESULTS:

(a) Mycobacteriologic data on tuberculosis patients has been input into computer files for approximately five years. The data bank now contains 38,000 messages on 1,690 patients. The file is constantly being revised to provide more information and to facilitate retrieval of information and analyses of patient data. A special printout, designed for inclusion in the patient's medical record, contains the patient's complete mycobacteriology history during his hospital confinement. Analyses on laboratory results from 409 sputum-positive patients on whom initial therapy data was input to the computer revealed that:

(1) A culture follow-up of at least 90 days on 218 bacteriologically-positive patients showed that 15% produced one to four positive cultures after initial sputum conversion. These patients were considered to have become bacteriologically-negative by virtue of having had a minimum of two negative cultures during a 30-day period. The range of appearance of these sporadic positive cultures varied between 31 and 337 days. Drug susceptibility studies on these mycobacterial isolates usually did not indicate increased

resistance, although occasionally single colony isolates at this stage of therapy did show increased resistance; the appearance of these "late positives" should be checked by additional cultures and drug susceptibility studies before changing the patients' drug regimen.

(2) Using the initial therapy data available, it was found that 48 patients had sputum conversions prior to initiation of therapy. Twenty-nine patients infected with susceptible M. tuberculosis had received no previous therapy, but became culture-negative between 0 and 59 days (median: 15 days, average: 8 days) prior to initiation of therapy, while six patients with drug-resistant M. tuberculosis strains: 0-34 day (median: 1 day, average: 8 days); and five infected with Group I or III strains: 0-34 days (median: 4 days, average: 11 days). Additionally, eight readmission patients became culture negative before therapy initiation. One explanation for the delay in initiation of therapy rests in the decision by the physician to await laboratory identification and susceptibility data before starting therapy. Another group of 24 patients became culture-negative within seven days of therapy initiation. Obviously, these interesting data must await correlation with clinical findings for explanation.

(3) The average sputum conversion time of 242 patients on therapy >7 days was determined. The patients were placed in four categories of therapy history: no prior therapy, prior therapy, on therapy when admitted to Fitzsimons General Hospital, and one or more previous courses of therapy and on therapy when admitted. The sputum conversion times for patients in these categories were as follows:

Therapy History	No. of Patients	Conversion Time (days) Average	Median	Range
No Prior Therapy	118	42	36	8-91
Prior Course of Therapy	10	43	34	22-88
On Therapy When Admitted	99	98	61	17-503
Prior Course and On Therapy When Admitted	15	165	101	37-677
Total	242	72	47	8-677

Studies are in progress to correlate the clinical parameters of patients in each of these groups.

(b) The following modified Middlebrook-Dubos 7H10 Oleic Acid Albumin Agar (7H10 OA) media were compared for their growth-promoting qualities for mycobacteria present in clinical specimens (1) 7H10 OA-pH 6.6, no catalase, (2) 7H11 OA-pH 6.8, no catalase, (3) 7H11 OA-pH 6.8 catalase, and (4) 7H11 OA-pH 7.2, no catalase. Contamination rates on the four media were equal. All media were compared using 117 specimens from 28 patients. Medium 7H11 OA-pH 7.2 was superior on only two patients, inferior on 25 patients, and was dropped from the study.

Media (1), (2), and (3) were compared using 130 specimens from 38 patients. The variation in strains encountered makes it desirable to use more than one culture medium, even though total results revealed the three media to be equal in recovery of mycobacteria from specimens. Only two of the 38 patients were infected with drug-resistant M. tuberculosis (one streptomycin-resistant and one isoniazid and p-aminosalicylic acid-resistant); growth response of the tubercle bacilli from these patients' specimens was best on 7H10 OA-pH 6.6. The reported need for catalase and casein hydrolysate in these media was not substantiated.

(c) Further modifications to the system using blue light excitation for microscopic demonstration of fluorochrome-stained mycobacteria have resulted in better visualization of these organisms:

(1) Modification of the Zeiss illuminator to accept a quartz-halogen bulb resulted in greater light intensity and more constant brightness and color temperature throughout the bulb life than was possible with tungsten bulbs.

(2) Use of an FITC (fluoroscein isothiocyanate) interference filter having a minimal or moderate red bypass as the primary filter in the system was found to provide excellent results. More light is transmitted by this filter, which has a sharp transmission cutoff at about 490 nm, than through the 3 mm BG 12 primary filter. If a bright field condenser is used, then it will be necessary, depending on the amount of red light transmitted through the FITC filter, to include a 1.0 mm or 1.5 mm thickness BG 12 filter to reduce red transmission. When primary FITC and 1.5 mm GS 12 filters are used, excellent demonstration of yellow, auramine O-stained mycobacteria is possible with either of two secondary (barrier) filters: Zeiss No. 50 (gray background), No. 53 (red background), or No. 50 superimposed on No. 65 (green background). Differences in transmission characteristics of FITC filters require experimentation to determine which combinations of filters give the best results. This system

can also be used for antinuclear antibody studies, or, by substituting a darkfield condenser, for fluorescent antibody studies. When the darkfield condenser is used only the FITC interference filter is employed as a primary filter.

(3) Using the same microscope, the bacilli in identical areas on 50 auramine O-stained smears from positive specimens were quantitated using a tungsten (blue) light illuminator and ultraviolet illumination. No significant differences in numbers of bacilli were found between the two illuminators. Blue light illumination was preferred, as ultraviolet excitation resulted in considerably more nonspecific fluorescence and more rapid fading of stained bacilli than did the blue light source.

(d) Studies to determine the rate of activity loss by rifampin, ethambutol, or kanamycin in 7H10 OA agar medium during storage at 5°, 24° and 36°C (USAMRNL Annual Report, 30 June 1971) were completed and the data are being analyzed to determine the half-life of each drug under stated conditions.

CONCLUSIONS:

(a) A data base of mycobacteriology laboratory results on 1,690 tuberculosis patients is used routinely for daily laboratory operations, analyses, laboratory quality control, to provide information on current or past patients, and is available for current or retrospective studies utilizing clinical data on these patients. Its use in prospective studies on patients with tuberculosis is to be encouraged.

(b) Longitudinal studies on four mofidications of the Middlebrook 7H10 and 7H11 culture media revealed three to be of equal efficacy, although individual strain variation sometimes favored different media. It is anticipated that at least two media should be routinely employed to achieve maximum recovery of mycobacteria from clinical specimens.

(c) Use of the FITC interference filter and quartz-halogen illumination has expanded and improved the system for blue-light fluorescence microscopy of tubercle bacilli. Comparison of ultraviolet with blue light as illumination sources revealed equal efficacy, however the blue light source was preferred because there was less nonspecific fluorescence than with the ultraviolet source. A report of the above studies is in preparation.

(d) The rates of activity loss of rifampin and ethambutol in 7H10 OA agar medium during storage at 24° and 36°C were sufficient to revise

reading times for cultures tested on these drugs. Data on drug inactivation rates in being analyzed.

RECOMMENDATIONS:

(a) Improvement of methods for computer storage and analysis of tuberculosis patients' laboratory data should be continued.

(b) The search for culture media and methodology for optimal recovery of mycobacteria from clinical specimens should be continued with the goal of shortening the growth time of these organisms.

(c) Continue to search for better and simpler methods for microscopic detection of mycobacteria, i.e. laser microscopy.

(d) No further studies are anticipated.

PUBLICATIONS:

Blair, E. B., O. L. Weiser and A. H. Tull. Mycobacteriology laboratory methods. Revised for 24th Annual Symposium on Pulmonary Diseases, Fitzsimons General Hospital, Denver, Colorado, pages 365-408, September 1971.

Blair, E. B., W. W. Bretherton and A. H. Tull. A method to render unstained mycobacterial smears safe for storage or shipment. Appl. Microbiol. 23: 826, 1972.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6301	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H Termination	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	066
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Procode with Security Classification Code)

(U) Microbiological Clinical Research in Military Medical Problems (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
64 10	30 June 1972	DA	C In-House

17. CONTRACT/GRANT

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.9	64
FISCAL YEAR CURRENT 72	1.5	36

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Microbiology Division
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Coan, R. M., MAJ, MC
TELEPHONE: 303 366 5311 X24234
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE

Foreign Intelligence not Considered

DA

22. KEYWORDS (Procode EACH with Security Classification Code) (U) Melioidosis; (U) Pseudomonas; (U) Serology; (U) L Forms; (U) BCG; (U) Mycoplasma; (U) Culture Media; (U) Agar Diffusion

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

23. (U) A number of fatal and other severe melioidosis infections (due to Pseudomonas pseudomallei) have occurred in U. S. troops in Vietnam. This disease can erupt months to years after personnel leave an endemic area with no previous sign or symptoms evidenced. A number of troops, especially the wounded, return from Southeast Asia with significant antibody titers to the organism. It is possible that some of these individuals with positive titers are carrying the organisms in a latent stage, and may later develop active disease. Wall defective variants (typically L phase variants, L forms) would provide a mechanism of prolonged persistence in a latent state. Wall defective variants of Mycobacterium tuberculosis might play a role in the up to decades long latency of tuberculosis. L phase variants of BCG might provide a mechanism of vaccinating against tuberculosis without causing the tuberculin skin test to become positive.
24. (U) A large number of healthy and wounded troops who have served in Southeast Asia will be screened for the presence of significant antibody titers for P. pseudomallei. Seropositive individuals will be cultured for the classical organisms and its wall defective variants, to include the use of hamster passage. Additional laboratory characterization of a reference strain of P. pseudomallei will continue as well as manipulation of induced wall defective variants. Stable wall defective variants of M. tuberculosis and BCG will be sought.
25. (U) 71 07 - 72 06 Anti-P. pseudomallei IHA titers among 279 SE Asian veterans and 203 stateside controls revealed 5 SE Asian returnees (4 wounded, 1 ill) to have significant (> 1:40) titers. Bacteriologic culture of these patients revealed a hitherto undetected P. pseudomallei infection of the hand. This project terminates 30 June 1972 due to transfer of the Microbiology Division to Fitzsimons General Hospital and OMA funding.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine

WORK UNIT NO. 066 Microbiological Clinical Research in Military Medical Problems

The following investigations have been conducted under this work unit:

STUDY NO. 1 The *in vitro* and *in vivo* characteristics of laboratory-induced L-forms of *Pseudomonas pseudomallei* and *Mycobacterium tuberculosis*

Experiment No. 1 Characterization of reference strain of *Pseudomonas pseudomallei* and its L-phase variant

STUDY NO. 2 Detection of latent melioidosis in U. S. military personnel exposed in Southeast Asia

(1) Melioidosis is a tropical disease which has recently been seen in U. S. veterans of the SE Asian conflict. Improved criteria are suggested for the laboratory recognition of the organism. Preliminary work *in vitro* and in mice with a new therapeutic combination of sulfamethrazole and trimethoprim was encouraging.

(2) A combined serological and culture survey was performed on 279 SE Asian conflict veterans and 203 controls to detect active or latent melioidosis. One active but previously undiagnosed case was discovered in which the use of hamster inoculation was helpful. Four other veterans of the SE Asian conflict had significant indirect hemagglutination titers indicating that they had at least subclinical infection at one time. Culture of tissue specimens from these four were negative. No clinically latent cases of melioidosis were discovered. It is recommended that the clinical features and the laboratory recognition of this infection be widely disseminated to the US medical community.

BODY OF REPORT

WORK UNIT NO. 066 — Microbiological Clinical Research in Military Medical Problems

STUDY NO. 1 — The *in vitro* and *in vivo* characteristics of laboratory-induced L-forms of *Pseudomonas pseudomallei* and *Mycobacterium tuberculosis*

Experiment No. 1 — Characterization of reference strain of *Pseudomonas pseudomallei* and its L-phase variant

PROBLEM:

Meliodosis is a severe tropical disease whose causative agent is the gram negative bacterium, *Pseudomonas pseudomallei*. Three of the problems with melioidosis are: the not infrequent difficulty in recognizing and isolating the organism from infected patients, the significant incidence of treatment failure on acute cases, and in a few instances the unexplained long latent period between exposure and onset of overt symptoms.

RESULTS AND DISCUSSION OF THE RESULTS:

After 12 to 24 hours' growth, *P. pseudomallei* colonies are minute in size even on optimal media. By 48 hours, the colonies achieve their typical wrinkled appearance. Because culture plates in the clinical laboratory are usually examined on the day after inoculation, such small colonies could easily be overlooked amidst the mixed flora of a chronic wound infection. In addition, gram-stained cells from the 24 hour colony will usually be more rod-like and have less bipolarity than "classical" *P. pseudomallei* cells. Another aid to the recognition of *P. pseudomallei* is the fluorescent antibody technique which was confirmed in the laboratory to be highly sensitive and specific.

In limited studies, the combination of sulfamethoxazole and trimethoprim was found to be effective *in vivo* and *in vitro* against *P. pseudomallei*. Mice showed no ill effects and were protected by this antibiotic combination against intraperitoneal challenge with 10^6 to 10^7 *P. pseudomallei* organisms of strain CA. Untreated mice died from 3 to 28 days after infection dependent on infective dose.

Another group of mice had been previously infected with a cycloserine-erythromycin-induced wall defective variant of *P. pseudomallei*. It

was possible to culture the organism from some of these apparently healthy mice for as long as seven months after inoculation, but later attempts at isolation were not successful. No attempt was made to reproduce this model of apparent latent melioidosis.

CONCLUSIONS:

The colonial and cellular morphology of P. pseudomallei is difficult to recognize until the second day of growth on culture media. The fluorescent antibody techniques is a sensitive laboratory tool in the recognition of P. pseudomallei. Sulfamethoxazole and trimethoprim are an effective combination in vitro and in vivo against P. pseudomallei and did not cause apparent harm to mice in vivo. Induction of wall defective variants of P. pseudomallei apparently attenuated virulence and allowed these organisms to persist symbiotically in mice for considerable periods of time.

RECOMMENDATIONS:

Laboratory personnel should be informed of the relatively prolonged period of time for P. pseudomallei to achieve its characteristic colony appearance. Further studies with the trimethoprim sulfamethoxazole combination should be encouraged to define its role in the therapy of melioidosis in animals and ultimately in patients. Additional investigation into the possible role of wall defective variants in latent melioidosis should be pursued. This project has been terminated effective 30 June 1972.

STUDY NO. 2 — Detection of latent melioidosis in U. S. military personnel exposed in Southeast Asia

PROBLEM:

Approximately 200 U. S. troops have developed melioidosis as a result of having served in SE Asia. Although there will now be far fewer acute cases in U. S. personnel, speculation has arisen as to the incidence of future recrudescent cases in veterans of the SE Asian conflict. Documented latency of months to years has been shown in some cases of melioidosis. Furthermore, there is a small percentage of returning troops who carry a significant serum IHA titer to P. pseudomallei. Explanations for the mechanism of latency in melioidosis might be in vivo persistence of the organism in indolent foci or for it to be in a different biological state, such as a wall defective variant.

RESULTS AND DISCUSSION OF THE RESULTS:

Four hundred and eighty-two individuals were interviewed and blood was taken for P. pseudomallei IHA testing. In this group were 279 veterans of the SE Asian conflict, including 173 wounded, 57 ill, and 49 who were well while there; there were 203 other subjects who had never been to SE Asia. Five individuals had significant IHA titers ($\geq$ 1:40): four of the wounded (2.3%) and one of the ill in SE Asia (1.8%). In the course of followup examinations and cultures of these five patients and 23 others in the study, a culture diagnosis of chronic melioidosis of the hand was made on one of the four wounded patients with a significant titer.

Bacteria other than P. pseudomallei had previously been cultured from this patient's draining hand wound. Recovery of the patient occurred after appropriate surgical and tetracycline therapy.

Significant IHA titers could not be attributed to infection with P. aeruginosa, to nonrecovery from wound, nor to a nonspecific effect of illness itself. The 28 patients mentioned above who exhibited significant or low IHA titers to P. pseudomallei were carefully examined and then tissue samples cultured on a variety of optimal media for both the classical and wall defective variants of P. pseudomallei. Twenty-seven samples from 11 patients were inoculated into hamsters. P. pseudomallei was recovered by culture and hamster inoculations from the one wounded patient mentioned above.

CONCULSIONS:

The only individuals with significant IHA titers for P. pseudomallei were five men wounded or ill in SE Asia, and these constituted 2.2% of such men tested. One of the five was diagnosed by culture and hamster inoculation as having previously unsuspected melioidosis of a chronically draining hand wound. Although the other four with significant titers probably had at least a past infection with the organism, neither they nor any of the others in the study could be shown to be harboring latent (i.e. not clinically apparent) P. pseudomallei.

RECOMMENDATIONS:

There has been no evidence to date that latent melioidosis can be detected by screening SE Asian conflict veterans. It is recommended that the effort to fully acquaint clinicians and laboratory personnel

on the characteristics of this alien disease be expanded. This project is terminated effective 30 June 1972.

PUBLICATIONS:

Coan, R. M., S. Foster, R. M. Marshall and R. A. Kishomoto. Chronic melioidosis in a U. S. soldier: Detection via serological survey. Submitted for publication in New England Journal of Medicine, 12 April 1972.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6303	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	H.Termination	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	068
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Precede with Security Classification Code)

(U) Computer Instrument Linkage (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 002400 Bioengineering; 009800 Med & Hosp Eq

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
63 12	CONT	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 71	2.0	13
SUMMARY FISCAL YEAR 72	1.5	19

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Computer Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Nelson, R. A.

TELEPHONE: 303 366 5311 X25130

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Bougie, M. DA

NAME:

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Computer; (U) Instrument; (U) Linkage; (U) Digital; (U) Conversion; (U) Bio-Medical;

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 and develop data recording, storage and analysis systems in order to automate various laboratory instruments at USAMRNL, specifically in support of approved laboratory research projects. The systems will minimize the need for hand calculations to transform raw data into a form easily understood by the research investigator. Consequently, the test results will be more timely, the possibility of human error will be reduced, and great saving in personnel time will be realized.

24. (U) A continuing survey to determine the feasibility and benefits of automating laboratory instruments at USAMRNL will be performed. Analog or digital output from laboratory instruments which are to be automated will be recorded on tape. Computer programs will be developed to (1) store the data in a computerized file, (2) retrieve and display the data and (3) transform the raw data into a form easily understood by research investigators. The resultant data will be formatted for further data reduction in statistical systems and/or for filing in a generalized data filing system for future reduction.

25. (U) 71 07 - 72 06 Using a DYMEC analog-digital converter machine in the analysis of oxygen consumption and by writing digital computer programs to accept the A-D output, the human intervention of handling data has been kept to a minimum. Thus, errors have been cut drastically in addition to allowing the investigators to have the results of their investigations much faster than by using manual methods. This system is operational and computer programs are in the maintenance stage. Occasional subroutines are written to satisfy new requirements of the researching personnel using this computerized system. Work Unit will be terminated due to lack of qualified personnel. Maintenance work for Study 1 will be conducted under Work Unit 166.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine

WORK UNIT NO. 068 Computer Instrument Linkage

STUDY NO. 1 Continuous Oxygen Consumption Measurement

A semi-automatic data handling system for the measurement of oxygen consumption has been operational during this reporting year. Subjects are exercised, analog recordings of the consumption of oxygen are converted to paper tape recordings through a DYMEC device which in turn are used by an RCA 301 computer as input media to update existing files.

BODY OF REPORT

WORK UNIT NO. 068 Computer Instrument Linkage

STUDY NO. 1 Continuous Oxygen Consumption Measurement

PROBLEM:

A need has existed for the support of studies in the evaluation of physical performance of military personnel as it is related to military nutrition. Data derived continuously from instruments monitoring subjects undergoing exercise on motor driven treadmills and bicycle ergometers is difficult and tedious to evaluate manually. The objective of this project has been to develop the electronic and digital computer programming systems required to handle the storage, retrieval, editing and processing of this energy expenditure data.

RESULTS AND DISCUSSION OF THE RESULTS:

The data handling system which has been developed for handling data derived from a "continuous oxygen consumption analysis system" (USAMRNL Report No. 318) has been in constant use during fiscal year 1972. The studies on which the system has been used follow:

STUDY	NO. OF SUBJECTS STUDIED	HOURS OF MEASUREMENT
Study using labeled glucose at altitude	24	24
Metabolic study on Intralipid infusion	6	24
Metabolic study on temperature regulation	3	42
Metabolic electrolyte utilization	12	265

This data represents about 46 hours of data processing time which, if it had been attempted by hand, would have taken about 3500 man hours without the automated data handling system.

CONCLUSIONS:

The use of the system for handling oxygen uptake measurement data over this reporting period has proven the feasibility and usefulness of off-line data handling of this type of physiological measurement.

Computer Instrument Linkage (Cont)

RECOMMENDATIONS:

1. Planning should include the incorporation of an on-line small computer to the gas analysis system for the real time analysis of data during experiments. This would allow monitoring the progress of an experiment as it is conducted and would allow the control of the experiment by the experiment itself.

2. Expanded effort must be made to utilize similar techniques in data acquisition, input manipulation, data display and output for multiple instruments throughout all divisions of the Laboratory in order to reach full potential of these instrumentation systems. This work unit can be considered to be in its infancy with one project having been brought to fruition in a dramatic, effective manner.

PUBLICATIONS:

Daws, T. A., C. F. Consolazio, S. L. Hilty, H. L. Johnson, H. J. Krzywicki, R. A. Nelson and N. F. Witt. Evaluation of cardiopulmonary function and work performance in man during caloric restriction. In press - J. of Applied Physiology.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6308	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	073
b. CONTRIBUTING	62156011	3A025601A822	00	[redacted]
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Precede with Security Classification Code)*

(U) Applied Nutrition Studies of Military Populations (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002300 Biochemistry; 012900 Physiology; 006500 Food Management

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

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:* Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	2.8	120
	CURRENT 73	3.0	115

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. F., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
Bioenergetics Division

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME:* Consolazio, C. F.

TELEPHONE: 303 366 5311 X25222

SOCIAL SECURITY ACCOUNT NUMBER [redacted]

ASSOCIATE INVESTIGATORS

NAME: Johnson, H. L.

NAME: Nelson, R. A. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nutrition Surveys; (U) Performance Evaluation; (U) Biochemical Evaluation; (U) Diet; (U) Rations; (U) Calorie Restriction; (U) Environ.

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

23. (U) To evaluate the nutritional status, nutrient intake, work performance, body composition and work capacity of the soldier in order to ensure that military performance is not impaired by improper nutrition.

24. (U) Annual nutrition surveys of military bases are designed to evaluate (a) nutrient intakes by preparing and analyzing average food composites, and computer calculation using handbook values; (b) individual's nutritional status through clinical examination and history; (c) biochemical status by blood and urine analyses; (d) man's physiological status by measuring body water, fat, protein and minerals; and (e) his physical status by treadmill and spirometry tests. Studies of caloric restriction and alteration of diet composition in humans are based upon the same protocol. All data is examined to determine correlations between the various parameters to develop predictive measures for improving performance.

25. (U) 71 07 - 72 06 Data processing and computations from the nutrition surveys at Lowry AFB, Colo., Ft. Lewis, Wash. and Ft. Myer, Va. are progressing. The dietary data indicates that food intakes at the short order and specialty houses per meal are considerably higher than the intakes at the regular dining halls. The Malaysian jungle data indicates that the two groups of men consumed 2,974 (I) and 1,750 (II) Cal/day during a 12-day field study. Under conditions of profuse sweating, both groups showed negative water, sodium and magnesium balances, and Group II also had negative potassium and nitrogen balances. These negative balances were due primarily to the high nutrient losses in sweat. During short term calorie restriction under conditions of fairly heavy physical activity, positive nitrogen balances are finally attained at the 1,350 Cal/day intake level.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO.	3A062110A822	Military Internal Medicine
WORK UNIT NO.	073	Applied Nutrition Studies of Military Populations

The following investigations have been conducted under this work unit.

STUDY NO. 1	Nutrition Surveys of Military Populations and Installations
	a. Lowry AFB, Denver, Colorado, 13-21 July 1971
	b. Ft. Lewis, Washington, 11 October-5 November 1971
	c. Ft. Myer, Virginia, 15-26 May 1972
	d. Ft. Huachuca, Arizona, 7 March-5 April 1966
	e. Ft. Campbell, Kentucky, 3-31 March 1967
STUDY NO. 2 (a-c)	Metabolic Aspects of Calorie Restriction (10 days)
STUDY NO. 2 d	Malaysia
STUDY NO. 3	The Effects of Ingesting Electrolyte and Sugar Upon Physical Training and Performance in Young Adults Under Conditions of Profuse Sweating

Nutrition surveys were completed at Lowry AFB, Colorado; a basic training mess hall, Ft. Lewis, Washington; the NLABS Experimental Feeding System at Ft. Lewis, Washington, and the civilian contracted catering service at the Tri-Service dining hall at Ft. Myer, Virginia. Preliminary data indicates that the food intakes at the short order and specialty houses are considerably higher than the intake/meal at the regular dining halls. The dietary intake data for the Ft. Myer survey is now being computer processed.

It is indicated that during short term calorie restriction positive nitrogen balances are attained at the 1,360 Cal/day intake level under conditions of fairly heavy physical activity.

During a field study in a hot humid environment, it was again indicated that the nutrient losses in sweat were high. No compensatory reductions in the urinary excretions of these nutrients were observed.

BODY OF REPORT

WORK UNIT NO. 073 — Applied Nutrition Studies of Military Populations

STUDY NO. 1 — Nutrition Surveys of Military Populations and Installations

PROBLEM:

Army post nutrition surveys are conducted to evaluate the adequacy of the Army diet in terms of established recommended dietary allowances under varied climatic conditions. Specifically, the adequacy of the ration actually consumed and the nutritional status of the average soldier eating in the military dining hall are being evaluated under varied climatic conditions and a variety of duty requirements. Longitudinal studies of the nutrient intake, body composition, work performance and respiratory function of the soldier is essential to insure that his effective military performance is not impaired by improper nutrition. Such impairment could limit the capability of the military at a time when instant readiness is mandatory.

In collaboration with the Computer Division a digital computer system has been devised to handle the filing and calculation of data involved in supporting military nutrition surveys.

RESULTS AND DISCUSSION OF RESULTS:

Nutrition surveys at military installations were continued. Three studies were completed during this period at Lowry AFB, Colorado, Ft. Lewis, Washington and Ft. Myer, Virginia. The Computer Division's "Nutrient Survey System" uses data derived from handbooks and laboratory analytical analysis together with observations from military dining halls during meal preparation and serving to determine the intake of nutrients by military populations. The system was used during this reporting period to determine the average nutrient consumption at the three military installations.

a. Lowry AFB, Colorado. The seventh in a series of nutrition surveys was conducted at Lowry AFB, Denver, Colorado to evaluate the nutrient intakes of Air Force personnel. The primary purpose of this study was to determine the daily food intake of men eating at the new "short order" meal system versus the regular conventional food system. Approximately 700-800 men were studied, and preliminary information indicates that 32-38% of the men were eating in the short order line. The "nutrition survey system" instituted by the Computer Division was utilized to determine the food intake. The intakes of men consuming the three regular meals averaged 872, 1,168 and 1,006 Calories/meal (total 3,046 Cal/day). The average

midnight meal was 1,278 Cal/day (Table I). The nutrient intake at the two short order meals was high, averaging 1,502 and 1,469 Cal/meal (Table II).

TABLE I

LOWRY AFB, COLORADO - 1971

FOOD INTAKES/DAY

	Breakfast	Dinner	Supper	Total	Midnight
Calories	872	1,168	1,006	3,046	1,278
Protein, gm	32.7	47.9	36.6	117.2	44.2
Fat, gm	43.2	58.0	44.7	145.9	64.7
Calcium, mg	500	522	473	1,495	622
Iron, mg	4.8	5.5	4.9	15.2	6.9
Vitamin A, IU	2,132	2,282	2,671	7,085	2,669
Thiamine, mg	0.50	0.62	0.56	1.68	0.83
Riboflavin, mg	0.96	1.07	0.89	2.92	1.24
Niacin, mg	3.3	9.7	7.3	20.3	6.2
Vitamin C, mg	40	26	28	94	58

TABLE II

LOWRY AFB, COLORADO - 1971

FOOD INTAKES/DAY - SHORT ORDER MEALS

	Dinner	Supper	Both
Calories	1,502	1,469	2,971
Protein, gm	59.7	53.5	113.2
Fat, gm	71.2	64.2	135.4
Calcium, mg	627	683	1,310
Iron, mg	8.3	8.0	16.3
Vitamin A, IU	1,694	1,696	3,390
Thiamine, mg	0.62	0.75	1.37
Riboflavin, mg	1.21	1.22	2.43
Niacin, mg	12.2	10.7	22.9
Vitamin C, mg	38	29	67

b. Ft. Lewis, Washington. The NLABS Experimental Feeding System (Café) at Ft. Lewis, Washington was designed to evaluate the concept of centralized food preparation and specialty dining halls. A survey was conducted at a short order house, a specialty house, one dining hall serving regular meals, and one serving a choice of regular meals and short order items. In addition, a dining facility, operated under conventional Army practices and serving the Master Menu food items was surveyed.

Food intakes at all of the dining halls were high, averaging 3,550 Cal/day for the three regular meals, and 3,750 Cal/day for the dining hall serving both the regular and short order meals (Table III). The intakes for men eating at the specialty and short order houses averaged 1,861 and 1,325 Cal/meal (Table IV).

TABLE III

FORT LEWIS, WASHINGTON SURVEY 1971* - HEAD COUNT 293

	GARRISON RATION DINING HALL (regular meals)		COMBINED SHORT ORDER and REGULAR DINING HALL
Head Count 293	Daily Intake/Man		Daily Intake/Man
Calories	3,550	Calories	3,750
Protein, gm	130.3	Protein, gm	139.6
Fat, gm	175.3	Fat, gm	181.4
Carbohydrate, gm	368.9	Carbohydrate, gm	395.2
Calcium, mg	1,263	Calcium, mg	1,355
Iron, mg	19.7	Iron, mg	20.1
Vitamin A, IU	9,651	Vitamin A, IU	7,873
Thiamine, mg	1.77	Thiamine, mg	1.83
Riboflavin, mg	2.83	Riboflavin, mg	2.90
Niacin, mg	25.3	Niacin, mg	25.4
Vitamin C, mg	113	Vitamin C, mg	95

* Average of men eating 3 meals at each dining hall.

TABLE IV

FORT LEWIS, WASHINGTON SURVEY - 1971

Specialty House		Short Order House	
Head Count 331	Intake/man/meal	Head Count 585	Intake/man/meal
Calories	1,861	Calories	1,325
Protein, gm	75.5	Protein, gm	48.6
Fat, gm	90.8	Fat, gm	59.9
Carbohydrate, gm	187.6	Carbohydrate, gm	150.3
Calcium, mg	730	Calcium, mg	443
Iron, mg	10.0	Iron, mg	6.7
Vitamin A, IU	2,925	Vitamin A, IU	1,107
Thiamine, mg	0.93	Thiamine, mg	0.52
Riboflavin, mg	2.08	Riboflavin, mg	1.10
Niacin, mg	15.8	Niacin, mg	9.9
Vitamin C, mg	49	Vitamin C, mg	25

In addition to the experimental feeding study, a 6-day survey was conducted on a group of basic trainees (essentially a captive clientele). These intakes averaged 3,588 Cal/day (Table V).

TABLE V

FORT LEWIS, WASHINGTON - 1971

FOOD INTAKE, BASIC TRAINEES, MEAN/MAN/DAY

	DAILY INTAKE
Calories	3,588
Protein, gm	121.2
Fat, gm	162.3
Carbohydrate, gm	419.2
Calcium, mg	1,433
Iron, mg	19.5
Vitamin A, IU	8,378
Thiamine, mg	1.85
Riboflavin, mg	2.81
Niacin, mg	22.4
Vitamin C, mg	126

The daily milk intakes were drastically reduced in comparison to previous military surveys, and due to the issue of the soft drinks. The soft drinks in the Basic Trainees' dining facility were purchased at the expense of milk (Table VI).

TABLE VI

FORT LEWIS, WASHINGTON SURVEY - 1971

GM/DAY CONSUMED

	Milk	Soft Drinks	Ice Cream
Specialty house*	333	208	165
Short order house*	225	351	199
Regular dining hall**	537	410	108
Combined dining hall**	603	545	122
Basic trainees**	603	---	---

* Average consumption/meal
** Average consumption each unit, 3 meals

It is important to realize that the nutrient intake data contained in Tables I-V represent calculated values and not chemically determined values. The calculations are based on actual consumption factors determined in the various dining facilities and on nutrient content data obtained from USDA Handbook #8 "Composition of Foods", and Bowes and Church "Food Values of Portions Commonly Used". Evidence is accumulating that the data contained in the two cited references are not correct in terms of foods currently available due to changes in the techniques of agriculture including harvesting, food processing, storage, transportation, distribution and food preparation. Menu nutrient planning is generally based on the references cited above. It should be again pointed out that the food served at the Lowry AFB and the Ft. Lewis basic trainee's dining halls was based on the Master Menu, while the food served in the four other dining facilities at Ft. Lewis was based on menus derived by the NLABS to conform with information obtained as a result of a food preference study. LaChance has recently shown that there is a marked discrepancy in the ascorbic acid content of food when chemically determined and compared with the data cited in the above references. Data on the chemically determined vitamin A content of food as compared to that calculated for the Ft. Lewis Survey is reported under Study 4, Work Unit 822-085 "Nutritional Requirements of Military Personnel."

Applied Nutrition Studies of Military Populations (Cont)

c. Ft. Myer, Virginia. The Surgeon, Military District of Washington, requested through DCSLOG, MDW and DCSLOG, DA that USAMRNL conduct a nutrition survey at the Tri-Service Dining Facility, North Post, Ft. Myer, Virginia. The feeding system has been implemented by the Department of the Army on a trial basis to evaluate a completely civilian-catered feeding system. The caterer is responsible for food procurement, preparation, serving, post-meal cleanup and waste disposal. The contract provides that the caterer serve up to 2,200 men/meal period in accordance with the Master Menu as modified by the Post Menu Board.

This nutrition survey was conducted between the period of 15 May to 26 May 1972, and included the evaluation of the dietary intakes and wastes, and the clinical and biochemical assessment of the nutritional status of troops subsisting in the dining facility. Evaluation of the data is now in progress.

d. Ft. Huachuca, Arizona. Body composition computer inputs of densitometry, potassium^{40} counting, total body waters, skin fold thickness and selected anthropometry have been developed. All programs have been completed for retrieval of the data, and have been tested for workability, allowing for rapid statistical analysis of the data (i.e., histograms, correlation coefficients, prediction equations, etc.) Data from the nutrition surveys will be treated in the same manner for future reports.

e. Ft. Campbell, Kentucky. This information is now being readied for key punching of data cards for computer insertion into the files. Data to be stored will include food intake data (to include food wastes and cooking losses), body composition to include skin fold thicknesses and anthropometry, pulmonary function and work performance.

STUDY NO. 2a-c — Metabolic Aspects of Calorie Restriction (10 days)

PROBLEM:

Recent emphasis on our military forces' mobility under difficult resupply conditions have created new problems in providing sufficient food and water for combat personnel to maintain adequate performance. The combat soldier may have to carry a heavy, bulky load consisting of his pack, radio equipment, weapons and a food and water supply adequate for periods of up to 10 days. The military has been concerned about the minimal food intake necessary to effectively maintain physical efficiency for varying periods where resupply is impossible. As a result, a series of studies have been initiated to determine

the minimum calories and nutrients required to maintain an individual's performance for periods up to 10 days.

RESULTS AND DISCUSSION OF RESULTS:

a. Niacin-Tryptophan Relationships During Caloric Restriction: Data indicates that during calorie restriction N^1MN excretions are increased 2-5 fold. This could be due to two factors: the synthesis of niacin from tryptophan liberated during the catabolism of body protein, and to the niacin utilized after its release from the catabolized tissue. The data suggests that during calorie restriction, the niacin requirements may be zero.

b. B_1, B_2 and B_6 excretion during acute starvation and calorie restriction: In the summary it was observed that daily excretion of thiamine and vitamin B_6 in micrograms per gram creatinine were in the low-to-deficient ranges established by the Interdepartmental Committee on Nutrition for National Defense during the calorie restriction period, indicating rapid depletion of body stores. Although riboflavin excretion decreased during the latter days of calorie restriction (starvation and 420 Cal/day), these values remained in the high range of acceptability. The elevated excretions of riboflavin are indicative of adequate reserves and catabolism of body protein for use as energy, or both.

These data suggest that under conditions of calorie restriction, the daily minimal allowances of vitamins B_1, B_2 niacin and B_6 are influenced by the calorie, vitamin, and protein intakes, the degree of negative nitrogen balance, and the subsequent protein catabolism.

c. Panama. A laboratory report on the various aspects of the Panama jungle study was completed and submitted to the Commanding Officer for review. In this study, the primary objective was to minimize the body nitrogen, water and mineral losses and to evaluate at what calorie intake level that positive nitrogen balances could be attained.

Four groups of heat acclimated men consumed 603, 947, 1,362 and 3,301 Cal/day for 10-day periods while on maneuvers in a jungle environment. Body weight losses were minimal in comparison to previous laboratory studies, averaging losses of 4.5, 4.0 and 3.7% of the initial body weight for the three restricted groups.

It appears that positive nitrogen balances were attained at approximately 1,362 Cal/day under these conditions. The daily urinary nitrogen losses of the restricted groups were gradually decreased during the experimental phase, and it appears that this may indicate some adaptation to the low calorie and protein intakes.

The data indicates the major body weight losses were due primarily to the calorie deficit and subsequent loss of body fat and some water in the three restricted groups for the 10-day period. The control groups did not show any significant change in body weight or body compartments. Body fat was the primary energy source in these three restricted groups, signifying the body fat loss that approximated 3% of the total body weight.

It appears that some body water was lost as plasma and blood volumes were significantly reduced and the total body waters by deuterium dilution were also decreased.

STUDY NO. 2 d Malaysia

PROBLEM:

The British Army study in Malaysia "Exercise Desire" was designed to evaluate the problems of caloric restriction as it would effect the military efficiency of troops working in a hot jungle environment. Physical activity was heavy during the 12 days of caloric restriction. Measurements of food and fluid intakes, energy expenditures, mineral and nitrogen balances were evaluated under severe heat stress conditions.

RESULTS AND DISCUSSION OF RESULTS:

Nutrient losses were evaluated in two groups of young adults during heavy physical activity in a hot, humid environment (Malaysia). During the experimental period, group I consumed 2,974 Calories and group II consumed 1,750 Cal/day for 12 days. The daily WBGT temperatures (maximal) ranged between 30-33C. Body weight losses averaged 2.57 and 4.20 kg, respectively, during the 12-day period. Some body dehydration occurred as total body waters (by D_2O dilution) were reduced by 1.07 and 2.37 kg for the same respective groups. Sweat rates during a 2-hour activity period averaged 3,508 and 3,061 grams, and daily sweats averaged 7,539 and 6,514 gm/day. Fluid intakes were high, averaging 8,124 and 7,124 gm/man/day. Nutrient losses in sweat were fairly high during the restriction period, with average daily losses of 7.13 and 4.61 gm of sodium/day, and 1.46 and 1.53 gm of potassium/day for groups I and II, respectively. Nitrogen losses in sweat averaged 2.68 and 2.78 gm/day for the same groups. No compensatory reductions in the urinary excretion of these nutrients during the restriction period were observed. Data again indicates that the nutrient losses, under conditions of profuse sweating, constitute an error that could seriously invalidate the accuracy of metabolic balance studies.

STUDY NO. 3

The Effects of Ingesting Electrolyte and Sugar Upon Physical Training and Performance in Young Adults Under Conditions of Profuse Sweating (formerly under Work Unit 061)

PROBLEM:

The primary objective was to evaluate the various food supplements ("energy" drinks with electrolytes and sugar) for maintenance of water and mineral balances under conditions of profuse sweating. Controlled studies were necessary to evaluate the "hearsay" reports of improved physiological work performance following ingestion of these beverages. These supplements were compared to the effects of water ingestion alone during fairly long periods of heavy physical activity and profuse sweating.

RESULTS AND DISCUSSION OF RESULTS:

A 12-week study to evaluate the various commercial nutrient supplements ("energy" or electrolyte drinks), under conditions specified by the producer, was completed during this period. These supplements are advertised to increase endurance and delay the onset of fatigue. Six young men from the Metabolic Division worked fairly strenuously for a 4-hour period daily on the bicycle ergometers and treadmills. The hot room for exercising was maintained at 90°F with 30% relative humidity so the men would sweat profusely and lose fairly large quantities of water and nutrients in sweat. The study was designed with each man consuming one of the supplements for 5 consecutive days, during the 4-hour exercise period.

Oxygen uptakes, heart rates and body temperatures were measured daily during the last hour on the treadmill, at two steady state work levels (3.4 mph at a 4% and 10% elevation), and maximal performance (using a modified Balke test) was evaluated on the last day of each phase. Four hour and 24-hour sweat rates were computed daily, and sweat samples were taken during the 4-hour exercise period. Complete nitrogen, mineral and water balances will be computed at weekly intervals on each subject. The data is now being computer processed for evaluation.

CONCLUSIONS:

Computation of the dietary intake data from the Lowry AFB and Ft. Lewis surveys are completed. The data indicates that the intakes in the short order line, LAFB, and at the specialty houses and the short order dining halls are considerably higher

per meal than those being served at the regular dining halls or in the regular feeding lines.

The dietary intake data for the Ft. Myer survey is now being processed.

A final (Malaysia) manuscript will be prepared this fall after a meeting with COL J. Crowdy, RAMC, and Dr. Allan Forbes, FDA. The data again indicates that under conditions of profuse sweating the nutrient losses in sweat are high.

During short-term calorie restriction under conditions of profuse sweating, positive nitrogen balances are attained at a calorie intake of 1,360 Cal/day.

RECOMMENDATIONS:

1. Continuation of the nutrition surveys to be done in an extremely cold environment and under other adverse conditions.

2. Initiation of protein metabolism studies to determine the effect of physical activity and extremely hot environments on protein requirements.

3. Initiate a study of the thiamine, riboflavin and niacin requirements as related to heavy energy expenditure.

4. Consolidate all of the calorie restriction results for a summary report.

5. Continue studies on the relationship of tryptophan and niacin during rapid growth to determine if conversion is more efficient under these conditions?

6. Study the degree that alteration of protein, fat and carbohydrate proportions in the diet affect the human vitamin requirements.

PUBLICATIONS:

1. Consolazio, C.F. Nutritional status and work capacity relationships. Proceedings of the First International Symposium on Work Performance and Food Intake, Vittel, France, 6-8 May 1971 (Abstract).

2. Johnson, H.L., C.F. Consolazio, H.J. Krzywicki, G.J. Isaac, and N.F. Witt. Metabolic aspects of calorie restriction: Nutrient balances with 500 Kilocalorie intakes. Am. J. Clin. Nutr. 24:913-923, 1971.

Applied Nutrition Studies of Military Populations (Cont)

3. Consolazio, C.F., A. Forbes, and J. Crowdy. Nutrient losses in humans during great water turnover in a hot humid environment (Malaysia). Western Hemisphere Nutrition Congress III, Bal Harbour, Miami, Florida, 2 Sep 71 (Abstract).

4. Consolazio, C.F., H.L. Johnson, H.J. Krzywicki, T.A. Daws, and R.A. Barnhart. Thiamine, riboflavin and pyridoxine excretion during acute starvation and calorie restriction. Am. J. Clin. Nutr. 24:1060-1067, 1971.

5. Consolazio, C.F., H.L. Johnson, H.J. Krzywicki, and T.A. Daws. Relationship of diet to the performance of the combat soldier. (Abstract) NAS, National Research Council IBP Rpt. #4, National Committee of the IBP, Washington, DC, 1971, pp. 107-108.

6. Consolazio, C.F. Chapter on "Nutrition and Athletic Performance" in Progress in Human Nutrition, Vol. I. Dr. S. Margen, editor, Avi Publishing Co., Westport, Conn., 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6345	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	074
b. CONTRIBUTING	62156011	3A025601A822	00	[redacted]
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Procede with Security Classification Code)

(U) Nutritional and Metabolic Aspects of Nutrients (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

002300 Biochemistry; 003500 Clin. Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	CONT	DA	C In-House

17. CONTRACT/GRANT

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	8.0	146
FISCAL YEAR CURRENT 73	7.5	117

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Chemistry Division
Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Raica, N., Jr.

TELEPHONE: 303 366 5311 X25206

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Sauberlich, H. E.

NAME: Dowdy, R. P. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Military rations; (U) Food Preservation; (U) Food Technology; (U) Nutritional requirements; (U) Metabolism; (U) Wholesomeness; (U) Malabsorption

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

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.

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.

25. (U) 71 07 - 72 06 Erythrocyte survival appears to be slightly reduced in the folacin-deficient rat. Ascorbic acid sulfate failed to enhance iron absorption in the rat to the degree that ascorbic acid did. The rainbow trout and Coho salmon were found to require ascorbic acid. Ascorbate-2-sulfate possessed vitamin C activity for these fish. In vitro stimulation coefficients (+pyridoxal PO_4 / - pyridoxal PO_4) obtained by optimized assay of plasma and erythrocyte GPT and GOT do not give an indication of vitamin B-6 nutrient status in the rat. The assay does, however, indicate a marked reduction in the activity of the enzymes in these samples. Urinary O-phosphorylethanolamine increased only in vitamin B-6 deficient rats fed a high protein diet. Retinol (^{14}C) metabolism is similar in vitamin A deficient and sufficient rats, but rate of ^{14}C-excretion is proportional to liver stores. Inhibition of lipase by fat extracted from heat or irradiation-processed meat is greater for ambient temperature than for frozen-stored meat.

*Available to contractors upon originator's approval

ABSTRACT

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

The following investigations have been conducted under this work unit:

STUDY NO. 1	Ascorbic Acid: Chemistry and Biological Functions
STUDY NO. 2	Irradiation Studies on Dietary Nutrients
STUDY NO. 3	Assistance to the Office of the Surgeon General on Irradiated Food Activities
STUDY NO. 4	Tissue and Blood Enzymes
STUDY NO. 8	Malabsorption, Diarrhea, Steatorrhea and Nutritional Deficiency Syndromes
STUDY NO. 9	Vitamin A and Carotene
STUDY NO. 13	Efficacy of Ascorbate-3-sulfate in Promoting Iron Absorption in Male, Young Adult Rats

Study No. 1. Additional studies were conducted to demonstrate that the Coho salmon and the rainbow trout metabolize ascorbic acid in a manner similar to man. Ascorbate-2-sulfate was found not only to be the major metabolite of ascorbic acid in the fish but also showed biological activity and reduced fish scurvy symptoms.

Study No. 2. Previously it was reported that fat extracted from ambient temperature-stored irradiated or thermally-processed beef inhibited lipase about equally when compared to fat from unprocessed frozen-stored beef. The effect of storage temperature was studied, and it was found that inhibition of lipase was markedly reduced after frozen storage of processed beef compared to ambient temperature storage. In comparing the effects of processing to non-processed meats, storage temperatures should be given due consideration.

Study No. 3. Technical support and consultation to U. S. Army Medical Research and Development Command has been continued for the irradiated beef studies. Quarterly reports from the contractor have been reviewed, abstracted and discussed with Contract Project Officer for Irradiated Foods.

Nutritional and Metabolic Aspects of Nutrients (Cont'd)

Study No. 4. Adult (270-320 gm) and early weanling rats were placed on high (60%) and low (20%) protein diets ± pyridoxine. Plasma and erythrocyte GPT and GOT activity, determined by an optimum spectrophotometric method, were not significantly affected when pyridoxal-PO_4 was added to the *in vitro* incubation medium. All deficient animals had reduced GPT and GOT activity by the second week of feeding and also had lower urinary excretion of Ca^{++} than control animals. Other vitamin B-6 symptoms were elevated Ca^{++} deposition in the kidneys and increased urinary excretion of xanthurenic acid and of O-phosphorylethanolamine (with the 60% protein diet).

Study No. 8. Secondary iron deficiency symptoms (increased absorption of ^{59}Fe and increased tissue uptake of the isotope, and reduced tissue iron concentration) have been observed in folacin-deficient rats regardless of whether the folacin deficiency was dietary or induced by sulfathiazole feeding. In addition, folacin deficiency appeared to slightly reduce erythrocyte survival time.

Study No. 9. Vitamin A-deficient and sufficient rats were administered retinol-15-^{14}C either orally or intravenously. Total excretion (% dose) after 7 days in the intubated group: 36% for the +A and 60% for the -A; for the IV group: 19% for the +A and 60% for the -A. Equilibrium between plasma and liver retinol specific activities tended toward equilibrium by the seventh day. Retinol utilization for the +A rats was 100 µg/day (high liver stores) and 2 µg/day (negligible liver stores) for the -A rats. It was significant that, of the administered dose, about 80% was stored in the livers of the +A rats but only about 7% in the -A rat livers.

Study No. 13. Ascorbic acid markedly increased the tissue uptake of ^{59}Fe from an *in situ* ligated intestinal segment in a normal rat. Tissues assayed were serum, RBC, liver, spleen, kidney and femur. Under similar conditions, ascorbic acid sulfate enhanced ^{59}Fe uptake in only the non-hematopoietic tissues (serum, liver and kidney).

BODY OF REPORT

WORK UNIT NO. 074 — Nutritional and Metabolic Aspects of Nutrients

STUDY NO. 1 — Ascorbic Acid: Chemistry and Biological Functions

PROBLEM:

A joint study on the metabolism of ascorbic acid in the rainbow trout and Coho salmon using ^{14}C, ^{3}H and ^{35}S labeled ascorbate-2-sulfate as well as iso-ascorbate-2-sulfate in conjunction with Dr. John Halver of the Western Fish Nutrition Laboratories has been completed. The reason for these studies is that the rainbow trout and Coho salmon require vitamin C and, further, appear to metabolize their ascorbate in a manner similar to man.

RESULTS AND DISCUSSION OF THE RESULTS:

Rainbow trout and Coho salmon fed diets without L-ascorbic acid (C_1) show lordosis, scoliosis, impaired collagen, cartilage and bone formation, hemorrhage in eye, and eventually die exhibiting the above symptoms of fish scurvy.

Radioautographs of fish intubated with ^{35}S-labeled ascorbate-2-sulfate (C_2) showed fixation of C_2 into collagen and support cartilage with the same distribution as ^{14}C and ^{3}H-labeled intubated ascorbic acid. Liver and head kidney had the greatest uptake of the labeled material. Chromatographic and radiometric assay of sagittal sections of specific tissues revealed $^{14}C/^{3}H$ ratios similar to the intubated dose. Isolation and co-chromatography of the tissue ascorbate indicated that the major component was vitamin C_2 using either ^{14}C or ^{35}S labeling.

When a similar experiment was performed using ^{14}C-labeled iso-ascorbic acid, it was found that the fish did not derivatize the iso-ascorbate to iso-ascorbate-2-sulfate but rather to an unidentified compound. Furthermore, 58% of the ^{14}C-labeled iso-ascorbic acid was excreted within the first 72 hours, whereas only 4% of ^{14}C L-ascorbic acid was excreted in the same time period.

Vitamin C-deficient rainbow trout and Coho salmon fed 72 mg of C_2 per 100 gm diet as sole source of vitamin C for 8 or 12 weeks showed no new deficiency symptoms; the fish began to feed avidly and grew. Ascorbic acid, when fed, reduced the incidence and extent of scurvy symptoms at the same rate as C_2, whereas fish maintained on a vitamin C-deficient diet became worse in numbers and severity of disease.

Hematology and routine histology failed to disclose toxicity or significant abnormality between C_1 or C_2 recovery fish groups. Therefore, vitamin C_2 showed biological activity and reduced fish scurvy symptoms.

CONCLUSIONS:

1. Rainbow trout and Coho salmon fed ^{14}C, ^{3}H-labeled ascorbic acid and ^{35}S-labeled ascorbate-2-sulfate showed urinary excretion patterns similar to man.

2. ^{14}C-labeled iso-ascorbic acid was more rapidly excreted than the ^{35}S-labeled ascorbate-2-sulfate or ^{14}C, ^{3}H-labeled ascorbic acid.

3. Ascorbate-2-sulfate (C_2) showed biological activity and reduced scurvy symptoms in the rainbow trout and Coho salmon.

PUBLICATIONS:

1. Halver, J. E., C. L. Johnson, R. R. Smith, B. M. Tolbert and E. M. Baker. Vitamin C_2 reduces fish scurvy. Fed. Proc. 31: 2764 Abs (1972).

STUDY NO. 2 Irradiation Studies on Dietary Nutrients

PROBLEM:

It was previously reported that fats extracted from ambient temperature-stored irradiated or thermally-processed beef were equally inhibitory toward lipase when tested in vitro against fat from frozen-stored unprocessed beef. Thin-layer separations, peroxide values or TBA numbers were not sufficiently different from the control sample to explain the reasons for the inhibition of lipase activity. These studies were continued in an attempt to define some of the factors contributing to the lipase inhibition.

RESULTS AND DISCUSSION OF THE RESULTS:

One obvious difference other than degree of processing was the storage temperature of the sample of beef. To study this effect, two cans of the frozen control beef were irradiated to 5 Mrads in the cobalt-60 source. One can was stored at ambient and the other at -20°C for several months. Fats were extracted and tested against lipase. Inhibition from the frozen-stored sample was 5% and from the ambient temperature sample was 16%.

Beef phospholipid oxidation products have been known to be responsible for the "off-flavors" that develop rapidly in roasted beef as well

as in irradiated beef. Crude fat extracts were separated into neutral and phospholipid fractions. Upon addition of the phospholipid to the test emulsions, the control sample was inhibited by 18% and the room temperature-stored irradiated sample was inhibited by 23%. Storage temperature effect on the phospholipid is in progress.

The significance of these results, which were not necessarily unexpected, points to the necessity for recognizing that some of the adverse effects attributed to irradiation (processing) may have been due in part to temperature-storage effects. Most comparisons were often made between a frozen-stored unprocessed control and an ambient-stored irradiated (processed) sample.

These studies will be completed by the end of this fiscal year, and this project will be terminated.

CONCLUSIONS:

In evaluating the effects of food processing, subsequent storage temperatures should be given due consideration in the evaluations.

STUDY NO. 3 — Assistance to the Office of the Surgeon General on Irradiated Food Activities

PROBLEM:

The contract that has been awarded by OTSG for the wholesomeness/toxicity evaluation of irradiated beef has been in progress for approximately one year. Progress reports have been received quarterly, and these reports have been reviewed and discussed in detail with Special Contract Officer, Irradiated Foods, Medical Research and Development Command. Although it is too soon for meaningful comparisons to be made between the several diet groups and test animal strains, no untoward findings have been observed in the irradiated diet groups.

STUDY NO. 4 — Tissue and Blood Enzymes

PROBLEM:

There is a need for an accurate, fast and reproducible method to determine the vitamin B_6 status in humans regardless of age, sex or dietary intake. Three approaches warrant detailed study because each shows a possible usefulness in providing the desired assay. O-phosphorylethanolamine (O-PE) has been shown to reach very high levels in the urine of rats on a high protein, pyridoxine-deficient diet (plus sulfathiazole to inhibit intestinal flora growth).

Stimulation coefficients (S.C. = +pyridoxal PO_4 ÷ -pyridoxal PO_4) for O-PE phospho-lyase increase with the progression of the vitamin B_6 deficiency state.

The other two assays also involve the use of pyridoxal PO_4 as a cofactor necessary for GPT and GOT activity. Erythrocyte S.C. for these two enzymes appear to give an indication that a deficiency state exists and to what degree it exists. Unlike urinary xanthurenic acid excretion from tryptophan loading, S.C.'s may remain high for several days after administration of adequate vitamin B_6.

RESULTS AND DISCUSSION OF THE RESULTS:

Adult and weanling rats on 20 and 60% protein diets ± pyridoxine were divided into groups of which some were utilized for weekly urine collections while others (4 to 6 animals per group) were sacrificed at 1, 2 and 4 weeks of the deficiency. O-PE phospho-lyase S.C.'s were not increased in deficient adult animals, and subsequent urinary O-PE excretion was negligible. Deficient weanling rats had a marked increase in urinary excretion of xanthurenic acid (deficient, 0.9 to 2.0 mg/16 hr collection; control 0.05 to 0.2 mg) and elevated urinary excretion O-PE (deficient, 800 to 900 nmoles/16 hr collection; control 100 to 200 nmoles) in animals receiving the 60% protein diet. Renal Ca^{++} was significantly increased in deficient animals on diets containing Bernhart-Tomarelli salt mix (BTSM). Calcium deposition was highest in the 20% protein + BTSM group; several of the deficient animals from this group were dead by the second week resulting from pyelonephritis and a copious quantity of calcium cast in the collecting tubules. Animals on a 60% protein diet in which Hawk-Oser salt mixture was added had renal calcium values eighteen times lower than the 20% protein + BTSM and four times lower than the 60% protein + BTSM. One should use a salt mixture other than BTSM since these effects appear to be somewhat extraneous to the rat's deficiency condition and can be avoided by the use of other salt mixes. Renal Mg^{++} was not significantly different in any of the groups.

CONCLUSIONS:

Stimulation coefficients using optimized GPT and GOT assays do not give an indication of vitamin B_6 nutrient status for the rat. One can obtain a marked reduction in activity for both enzymes in plasma and erythrocytes; however, this reduction is useless if the study is being conducted in the absence of controls. Urinary O-PE yields information about vitamin B_6 status only when employed in conjunction with high protein diets although it may be useful if a O-PE load is given before urine is collected. At present, one cannot apply either urinary O-PE or transaminase assay for an individual

vitamin B_6 status determination on the rat. The presence of isozymes has been reported for both transaminases. It may be imperative that special conditions exist before holo enzymes (S) can be formed. Apo-enzymes (S) may be very unstable (degraded, precipitated or inactivated by other means) in vitro.

RECOMMENDATIONS:

Studies should be continued with deficient animals to include O-PE loading tests, and, in vivo administration of pyridoxal-PO_4 and/or pyridoxine. Animals would then be sacrificed at various time intervals and a study made to compare these animals with deficient saline-injected animals. Methods should be developed to separate and identify various transaminase isozymes, both inactive and active forms, which may be present in the precipitated fraction of the hemolysate preparation. The difficulty in measuring in vitro apo-enzyme (S) forms in the rat does not appear to exist for erythrocyte GOT in the human.

PUBLICATIONS:

1. Fleshood, H. L., and R. P. Dowdy. Effect of moderate vitamin B_6 deficiency with low and high protein diets on alanine and asparatic aminotransferase and urinary O-phosphorylethanolamine in adult rats. Fed. Proc. 31: 2856 Abs (1972).

2. Tillotson, J. A., and H. E. Sauberlich. Effect of riboflavin depletion and repletion on the erythrocyte glutathione reductase in the rat. J. Nutr. 101: 1459, 1971.

3. Sauberlich, H. E., C. Rebouche and J. H. Judd, Jr. Influence of folic acid on ^{14}C-formate incorporation by blood and blood components. Fed. Proc. 31: 712 Abs (1972).

STUDY NO. 8 Malabsorption, Diarrhea, Steatorrhea and Nutritional Deficiency Syndromes

PROBLEM:

Anemia can result from either an iron deficiency or a folacin deficiency. Evidence concerning how these two nutrients may interact in the hematopoietic system is limited. Some data have been presented suggesting that folacin deficiency may result secondarily to iron deficiency. The reverse has not been studied to any great extent. The study reported here concerns the influence of folacin deficiency (either dietary or induced with sulfathiazole) on iron metabolism in the rat.

RESULTS AND DISCUSSION OF THE RESULTS:

Weanling rats were divided into four groups and each group placed on one of the following diets: control (+FA); folic acid deficient (-FA); control plus 0.5% succinyl sulfathiazole (+FA+S); and folic acid deficient plus 0.5% succinyl sulfathiazole (-FA+S). As a measure of red blood cell (RBC) turnover time (survival), five rats from each of the four dietary groups were each given an intraperitoneal injection of 30 μCi of ^{55}Fe after they had been consuming their respective diets for 5 weeks. At various time intervals (1-79 days) following isotope dosage, the rats were bled by the sinus orbital technique. Hemin was isolated and the specific activity of the iron determined. The reincorporation of ^{55}Fe into hemin (following the initial incorporation) began at day-66 in both of the folacin-deficient groups, whereas both control groups showed no reincorporation through the 79th day when the study was terminated. Sulfathiazole did not alter the ^{55}Fe specific activity patterns in either the deficient or the control rats.

Absorption and tissue distribution of iron was studied in groups of five rats from each dietary treatment group. The *in situ* ligated gut segment technique was used and tissue samples obtained 2 hours following dosage with 5 μCi of ^{59}Fe. Folacin deficiency resulted in greater ^{59}Fe uptake in the serum, liver and bone, with the same trend in the spleen.

CONCLUSIONS:

Folacin deficiency in the rat, whether dietary or induced by sulfathiazole, appears to slightly reduce RBC survival time thus increasing the turnover rate (and perhaps the requirement) for iron. Additional signs of a secondary iron deficiency in the folacin-deficient rat included increased uptake of radioiron in serum, liver and bone following dosage with the isotope in an isolated intestinal segment.

RECOMMENDATIONS:

Because of the possible influence on requirements for both iron and folacin, the metabolic interaction between these two nutrients should receive additional study. Especially important would be an understanding of the mechanism by which a deficiency of either iron or folacin might result in a secondary deficiency of the other.

PUBLICATIONS:

1. Lee, Y. C., R. M. McKenzie, R. K. Gholson and N. Raica. A comparative study of the metabolism of nicotinamide and nicotinic acid in normal and germ-free rats. Biochim. Biophys. Acta 264: 59-64, 1972.

STUDY NO. 9 Vitamin A and Carotene

PROBLEM:

Several reports over the past 15 years have appeared in which the metabolism of radiolabeled forms of vitamin A was studied. However, because the aims, forms of vitamin A, position of radiolabel and routes of dose administration were different in each of the studies, it was not possible to compare the reported results. The purpose of this study was to determine the rate of retinol-15-^{14}C utilization and tissue distribution in vitamin A-deficient and sufficient rats and to compare oral and parenteral supplementation.

RESULTS AND DISCUSSION OF THE RESULTS:

Vitamin A-sufficient and deficient male Fisher strain rats were supplemented orally or intravenously with 60 µg (3 µCi) of retinol-15-^{14}C. After supplementation, all rats were fed the vitamin A-deficient semipurified soy protein diet. Feces and urine were collected daily for 7 days. Expired air was monitored continuously for carbon-14 level for the first 24 hours and during the seventh day. Rats were sacrificed on day-7. Organs and tissues were collected, lyophilized and residual carbon-14 determined. Vitamin A levels as well as retinol carbon-14 specific activity were determined in liver and plasma.

Data in Table I show that, in the deficient rats, the single 60 µg dose of retinol was not adequate for detectable liver storage. Although not shown in this table, storage was detectable (3.0 µg/g liver) after a 120 µg dose of retinol. In the sufficient but not in the deficient rats, plasma and liver retinol specific activity approached equilibrium.

Tissue retentions of the retained dose are shown in Table II. There was fair agreement in retention between the two routes of supplementation in the deficient and sufficient rats, respectively. Recognizing that all of the tissue carbon-14 was not retinol carbon-14, these data give some indication of the degree of vitamin A depletion prior to supplementation. Of particular interest was that the livers of the deficient rats retained 6-9% of the dose whereas 75-87% was retained in the livers of the sufficient rats that contained very high amounts of liver vitamin A (5000 µg/liver). It should also be noted that, proportionate to organ weight, the eyes and adrenals of the deficient rats contained very high concentrations of the retained dose.

Calculations based on plasma retinol specific activity and total carbon-14 excreted showed that, on the seventh day, the deficient rats were utilizing retinol at a rate of 2.2 µg/day and that the sufficient rats were utilizing at the rate of 110 µg/day. The figure of 2 µg/day

TABLE I

Concentration and Specific Activity of Retinol in Plasma and Liver after Seven Days[1]

Diet	Weight g	Plasma Retinol[2] µg/100 ml	µCi/mg	Liver Retinol µg/g	µCi/mg
-A oral	204	12	35.9	0.3	8.1
-A IV	206	--	----	---	---
+A oral	283	38	0.2	739	0.32
+A IV	274	46	0.6	680	0.37

[1]Specific activity of administered retinol was 48 µCi/mg.

[2]Plasma retinol of unsupplemented deficient rats was 2.5 ± 1.6 µg/100 ml.

TABLE II

Percent Distribution of Retained Dose[1] after Seven Days

	-A oral	+A oral	-A vein	+A vein
Carcass	50.01	6.95	73.37	11.37
Kidneys	6.60	0.10	10.75	0.13
Liver	5.80	75.00	8.69	87.23
Blood	4.95	0.16	-----	0.13
Eyes	2.43	0.05	3.06	0.09
Adrenals	1.48	0.15	1.68	0.13
Other Tissues[2]	3.81	0.93	5.61	1.30
Total Recovery[3]	92.0	90.0	101.0	100.0

[1]Administered dose - excreted dose = retained dose.

[2]Includes: lungs, intestine, seminal vesicles, testes, colon, brain, heart and spleen.

[3](Excreted-^{14}C + Tissue-^{14}C) / administered dose x 100 = %.

is in agreement with other studies in which it was estimated that the minimal daily requirement for retinol in the rat is between 1-2 µg. The figure of 110 µg/day is also in agreement with other data that have suggested that the rate of retinol utilization is in some proportion to the liver vitamin A stores.

The total dose excreted over the seven days was about 60% in the deficient rats regardless of route of supplementation. In the sufficient rats, 19% of dose was excreted when intravenously supplemented and 36% when orally supplemented. Partition of the excreted dose between urine, feces and breath appeared to be related to the route of supplementation rather than to vitamin A status. Fecal excretion data, if it can be assumed that the enterohepatic circulation of retinol is independent of route of supplementation, indicated that the absorption efficiency of orally-administered retinol was about 85% in both the deficient and sufficient rats.

The patterns of daily carbon-14 excretions in urine, feces and breath were not too remarkable except that they suggested that the manner of utilization was comparable in deficient and sufficient rats but dependent in part on route of supplementation. For example, carbon-14 excretion peak rate was "instantaneous" in the intravenously-supplemented rats but, in the orally-supplemented rats, peak excretion rate occurred at about 5-6 hours after supplementation. Peak fecal excretion occurred on the second day for both the orally and intravenously-supplemented rats.

CONCLUSIONS:

Absorption and manner of utilization of retinol as measured by excretion patterns was comparable in vitamin A-deficient and sufficient rats. Rate of retinol utilization on the seventh day after supplementation was about 2 µg/day in the deficient rats but about 100 µg/day in the sufficient rats. In the deficient rats, a single dose of retained retinol was distributed throughout the body principally in eyes and adrenals and very little in the liver. In contrast, most of the retained retinol in sufficient rats was found in the liver.

PUBLICATIONS:

1. Raica, N., Jr., W. Nielsen, J. Scott and H. E. Sauberlich. The utilization and metabolism of retinol by retinol deficient and sufficient rats. Fed. Proc. 31: 685 Abs (1972).

2. Raica, N., W. Nielsen, J. Scott and H. E. Sauberlich. Retinol metabolism in the rat. J. Colo.-Wyo. Acad. Sci. VII: 43 Abs (1972).

STUDY NO. 13

Efficacy of Ascorbate-3-sulfate in Promoting Iron Absorption in Male, Young Adult Rats

PROBLEM:

Prevention of iron-deficiency anemia rests upon the ability to supply dietary iron in a metabolically-available form. Ascorbic acid (AA) has been reported to enhance iron absorption. Recently, ascorbic acid sulfate (AAS) has been reported to be a natural metabolite of AA. Thus, the present study was designed to determine whether or not AAS would also increase the absorption of iron from the gastrointestinal tract.

RESULTS AND DISCUSSION OF THE RESULTS:

Adult rats (weighing approximately 300 g) were used in these studies. The rats were fed a commercial laboratory chow until the absorption studies were performed. The rats were divided into three groups and, following an overnight fast, were treated as follows: Group 1 received 5 μCi of ^{59}Fe in 25 μg Fe^{++}; Group 2 was given the same quantities of isotopic and stable iron as Group 1 plus 57 μmoles of AA; and Group 3 received the same quantities of isotopic and stable iron as Group 1 plus 57 μmoles of AA as AAS. Iron absorption was measured in each group by the amount of ^{59}Fe taken up by various tissues during 2 hours following dosage of the treatment solution into an *in situ* ligated intestinal segment. AA significantly enhanced the incorporation of radioiron into all tissues studied (plasma, RBC's, bone, spleen, kidney and liver) compared to either the control or AAS rats. Conversely, AAS treatment significantly increased ^{59}Fe incorporation into only the kidney, liver and plasma (78%, 41% and 82%, respectively) compared with the control rats. The residual isotope in the isolated gut segment substantiated the tissue uptake data; being greater in the control group (76.3%), least in the AA group (53.2%), and intermediate (slightly less than the control group) in the AAS group (74.0%). Thus, AA markedly increased iron absorption in the normal rat. Conversely, AAS was not as effective as AA in promoting iron absorption, especially into those tissues directly related to the hematopoietic process (RBC, bone and spleen).

CONCLUSIONS:

These data confirm literature reports that ascorbic acid significantly increases the gastrointestinal absorption of iron. Ascorbic acid sulfate was significantly effective in increasing the amount of radioiron taken up by the kidney, liver and in the plasma when compared with iron-treated controls. The fact that AAS was not as effective as AA in

promoting iron absorption may be related to the loss, by AAS, of the AA reducing properties or to some difference in chelation. Concerning chelation, it is possible that AAS chelates ferric iron, whereas AA very likely chelates ferrous iron.

RECOMMENDATIONS:

Further studies seem warranted concerning the form in which the AAS iron is absorbed. If AAS promotes the absorption of the ferric iron, then the concern for supplying all iron in the ferrous state might not be so important.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6337	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS?	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	076
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Precede with Security Classification Code)*

(U) Analytical Biochemistry (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002300 Biochemistry; 003500 Clin. Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	CONT	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:* Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	7.2	227
FISCAL YEAR CURRENT 73	7.5	349

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
Chemistry Division

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME:* Skala, J. H.

TELEPHONE: 303 366 5311 X21133

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Sauberlich, H. E.

NAME: DA

21. GENERAL USE

Foreign Intelligence not Considered

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 (Furnish individual paragraphs identified by number. Precede text 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, USAMRNL, 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 assay 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) 71 07 - 72 06 Analytical support was provided to 87 laboratory research project and subproject requests in military medicine and nutrition of military personnel and working animals. Significant improvements in nitrogen analysis of urines, stools and diets, and in data processing were accomplished. Service was expanded by implementation of an isotope-ratio mass spectrometer and the development of sample preparation systems for stable isotopes of carbon and hydrogen.

*Available to contractors upon originator's approval.

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 Biochemical Procedures

Analytical support was provided to 87 laboratory research project and subproject requests in military medicine and nutrition of military personnel and working animals. Significant improvements in nitrogen analysis of urine, stools and diets, and in data processing were accomplished. Service was expanded by implementation of an isotope-ratio mass spectrometer and the development of sample preparation systems for stable isotopes of carbon and hydrogen.

BODY OF REPORT

WORK UNIT NO. 076 Analytical Biochemistry

STUDY NO. 1 Analytical Support and Services

PROBLEM:

The expanding requirements of current research impose a considerable demand on the Analytical Biochemistry Branch for existent services and lead to the need for implementation of additional methods and instrumentation.

RESULTS AND DISCUSSION OF THE RESULTS:

A total of 87 project and subproject requests were supported originating from studies in military medicine and nutrition of military personnel and working animals. This included comprehensive support to two military nutrition surveys (Ft. Lewis and Ft. Myer) requiring Branch logistical service, field staffing and administration for the biochemical portion of the surveys. Home-based Branch personnel were dedicated to analyzing daily sample shipments on 17 constituents of the blood and urine specimens from each of 500 subjects at Ft. Lewis. The specimens from each of the 346 subjects at Ft. Myer are currently being analyzed for 18 constituents by Branch personnel.

An isotope-ratio mass spectrometer, which is a 60^{o} 6-inch magnetic sector instrument, has been acquired. It is a high-sensitivity system for ratio determinations of individual isotopic species including carbon, hydrogen, oxygen, nitrogen and sulfur isotopes. The instrument can also be operated in a scanning mode, but application is limited to simple gas mixture analysis. Organic scanning with the isotope analyzer tube would produce excessive fouling which would demur isotope-ratio work.

A sensitive micro-sample spectrophotometer and sampler-timing system, both of which are compatible with existent automatic analyzers, has been acquired and is being evaluated for speedup and accuracy improvement in certain automated systems.

The alternate output of the central integrator phase of the automated analyzer systems has been converted to a punch tape output compatible with the Branch data processor (PDP-8). Working prototype forms for complete data processing and automatic summary statements have been prepared for evaluation.

CONCLUSIONS:

In order to provide pertinent support to the investigators of the various divisions, the Branch must accept samples for analyses which

are beyond the investigator's capabilities because of numbers or technology. At the same time, service must expand to accommodate requirements for new services. Coincidental is an increase in the workload on the personnel and equipment.

RECOMMENDATIONS:

Continued effort must be made to meet the critical needs of the Branch in the areas of automatic data reduction and summarization and achievement of operational efficiency through use of automated methods.

STUDY NO. 2 Development of Analytical Biochemistry Procedures

PROBLEM:

With increased workloads and expanded service, manual methodologies and manual data handling (even from automatic analyzers) have a deleterious effect on operational efficiency. Consequently, continued efforts must be exercised to modify existent methodologies or innovate new techniques to meet added analytical demands.

RESULTS AND DISCUSSION OF RESULTS:

Nitrogen determinations in urine, stools and diets can now be satisfactorily performed on an automated system. The majority of urine specimens can be processed with full automation due to incorporation of on-line dilution which brings most urines within range of the normally used standards. Manual Kjeldahl methodology is used as a reference method on random unknowns for quality control. Mean percent recoveries ± the standard error of the mean of manual Kjeldahl nitrogen on reference samples analyzed thus far are as follows: urine with manual dilution, 99.95 ± 0.25%; urine with on-line dilution, 99.73 ± 0.32%; stools, 99.68 ± 0.48%; and diets, 100.0[illegible] ± 0.79%.

The Branch experienced a marked increase in demand for blood pyruvate analyses which were performed manually at first. A study has been initiated to automate this procedure, as available automated methods did not prove satisfactory. Lactic acid determination is almost always required on the same protein-free filtrate. An automated adaptation of a manual analysis for lactic acid which has been used in this laboratory for quite some time is the subject of another laboratory report.

Sample preparation systems have been developed for isotopes of carbon and hydrogen and are being evaluated for completeness of isolation of the gaseous derivatives. Systems for other isotopes are planned.

Analytical Biochemistry (Cont'd)

Data processing was augmented by development of software programming for computation and summarization of serum lipid and electrophoretic analysis results.

Certain problems arising in supporting military medicine studies of carbohydrate metabolism abnormalities were solved by modifying an automated glucose procedure to facilitate analysis of extremely hypoglycemic specimens and by the implementation of an automated micro-glucose procedure.

CONCLUSIONS:

The automated nitrogen system performs in a very satisfactory manner on all specimens normally submitted for this analysis including sweat samples. This is a marked contribution to operational efficiency for this often-requested analysis.

Automated blood lactic acid analysis results in considerable time saving for this enzymatic determination.

The data processing improvements eliminate a significant portion of the manual computation and summarization of results.

RECOMMENDATIONS:

Optimization of laboratory operations should be achieved by elimination of manual processing in analyses, minimizing the amounts of specimen (particularly blood components) required, and introduction of machine computation and summarization wherever possible.

Immediate objectives should be in the areas of (a) mineral analyses utilizing the digest from the automatic nitrogen analyzer, and (b) the complete development of an automated pyruvic acid analysis. A subsequent improvement to be attempted would be the combining of lactic and pyruvic acid assays into a simultaneous system.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6324	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	077
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 114(F)			

11. TITLE (Precede with Security Classification Code)

(U) Nutritional Physiology (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

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

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	6.0	65
FISCAL YEAR CURRENT 73	6.0	117

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Physiology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Klain, G. J.

TELEPHONE: 303 366 5311 X22119

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Meikle, A. W., MAJ, MC

NAME: Sullivan, F. J. DA

21. GENERAL USE

Foreign Intelligence not Considered

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, the nutritional requirements and the nutritional-physiological characteristics of the military population may be quite different, particularly under combat conditions, than those of the civilian population. The troops in the field may be subjected to numerous stresses, and yet, they may be required to complete successfully a variety of missions. The purpose of these investigations is to 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: 1) simultaneous stresses in animals and humans; 2) responses common to one, two or more stresses; 3) time sequences of the onset of the response to particular stresses; and 4) the duration of these responses after removal of the stressing factor. Specific techniques will be: 1) measurement of growth and/or food consumption; 2) assay of enzyme activities; 3) determination of levels of tissue and urinary metabolites; 4) determination of metabolic pathways; and 5) clinical observations.

25. (U) 71 06 - 72 07 Thiamin-deficient rats have significantly lower rises in plasma corticosterone in response to ACTH injection. Adrenals from thiamin-deficient rats produced significantly less corticosterone in response to ACTH added to the incubation media than adrenals from pair-fed controls. Thiamin deficiency may result in diminished NADPH and cyclic AMP formation and thereby interfere with the steroidogenic action of ACTH. Cyclic AMP, glucagon and mannoheptulose inhibit hepatic fatty acid synthesis from glucose.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498

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. 6 Metabolic effects of starvation-refeeding

STUDY NO. 12 The effect of thiamin deficiency on adrenal cortical function

Cyclic AMP, glucagon, mannoheptulose and epinephrine inhibit fatty acid synthesis from glucose in hepatic slices from rats refed after a 48-hour period of food deprivation. Glucose oxidation is also inhibited by these compounds. In contrast, lipogenesis was stimulated when ACTH was included in the incubation media.

Marked decreases in transketolase and pyruvate decarboxylase activities were observed in adrenals from thiamin deficient (TD) rats. ACTH produced negligible stimulation of 1- and 6-^{14}C-glucose oxidation in quartered adrenals from TD animals, whereas a significant increase was observed in pair-fed controls. The activity of HMP dehydrogenase and of cytosol malic enzyme was not significantly affected by thiamin deficiency as compared to pair-fed controls. It is concluded that adrenal glucose catabolism and steroidogenesis in response to ACTH are impaired in thiamin deficiency. This effect of thiamin deficiency on adrenal glucose catabolism may result in diminished NADPH and cyclic AMP formation and thereby interfere with the steroidogenic action of ACTH.

BODY OF REPORT

WORK UNIT NO. 077 Nutritional Physiology

STUDY NO. 6 Metabolic Effects of Starvation-Refeeding

PROBLEM:

The importance of dietary glucose in enhancing hepatic lipogenesis, the apparent decrease in blood glucose level and the significant increase in blood free fatty acid concentration in response to a short term fast suggest that glucagon and insulin may be involved in the control of hepatic lipogenesis. Since many of the functions of these, as well as other hormones, are mediated through cyclic-3',5'-adenosine monophosphate (cAMP), a series of experiments were conducted to determine the effect of cAMP and other hormones on glucose-U-^{14}C oxidation and incorporation into fatty acids of liver slices.

RESULTS AND DISCUSSION OF THE RESULT :

Male, Holtzman rats, weighing 180 - 200 gm were fed a casein-sucrose diet for ten days. After this period of dietary adjustment the effect of various hormones on in vitro incorporation of glucose-U-^{14}C into hepatic fatty acids was studied.

Lipogenesis in liver slices from both fed and fasted-refed rats was inhibited when cAMP was included in the incubation media. Glucose oxidation was also markedly reduced by cAMP. Fatty acid synthesis was also decreased by glucagon and epinephrine. In contrast, inclusion of ACTH in the incubation medium increased glucose incorporation into fatty acids. Mannoheptulose inhibited fatty acid synthesis both in vivo and in vitro. Inhibition of fatty acid synthesis in adipose tissue was overcome by insulin. It appears that mannoheptulose interferes with normal pancreatic secretion of insulin. Cortisone had no effect upon fatty acid synthesis in hepatic tissue preparations.

CONCLUSIONS:

The results demonstrate that hepatic lipogenesis is inhibited by cAMP and suggest that the concentration of cAMP in liver may be involved in the control of lipid synthesis in vivo. Hepatic cAMP content is known to be under hormonal control, in that its concentration is increased by glucagon and decreased by insulin. It would appear that a short-term fast may increase circulating glucagon levels which in turn could increase hepatic cAMP concentration and thereby inhibit lipogenesis. The opposite would be true in animals refed after starvation.

RECOMMENDATIONS:

Determine the mechanism whereby cAMP inhibits liver lipid synthesis. Further studies should be conducted to determine metabolic effect of mannoheptulose.

STUDY NO. 12 The Effect of Thiamin Deficiency on Adrenal Cortical Function

PROBLEM:

The steroidogenic action of ACTH is apparently supported by glucose oxidation in the rat adrenal. Glucose also facilitates the formation of cyclic AMP in response to ACTH and facilitates the action of cyclic AMP. It may be anticipated, therefore, that factors which decrease the glucose oxidation could result in an impairment of the steroidogenic action of ACTH. Thiamin pyrophosphate is a coenzyme for transketolase, pyruvate decarboxylase and α-ketoglutarate decarboxylase. Thus, thiamin deficiency may alter glucose metabolism in such a way as to decrease NADPH, acetyl CoA and cyclic AMP formation and thereby impair adrenal steroidogenesis. Accordingly, the effect of severe thiamin deficiency on rat adrenal steroidogenesis in response to ACTH was investigated. An attempt was made to ascertain the effects of thiamin deficiency on glucose metabolism and steroidogenesis.

RESULTS AND DISCUSSION OF RESULTS:

Male Holtzman rats, 160 - 180 gm in weight, were fed a thiamin-deficient (TD) diet for 32 - 35 days. At this time neurological dysfunction signs (incoordination, ataxia and drowsiness) appeared. Normal behavior was observed in the pair-fed and ad libitum-fed controls.

When compared to the pair-fed controls, TD rats exhibited attenuated rises in plasma corticosterone in response to ether vapor and to ACTH injection. The half-time of intravenously injected radioactive corticosterone was similar in both groups. Quartered adrenals from TD rats produced significantly less corticosterone in response to ACTH added to the incubation media than adrenals from pair-fed controls. Severe thiamin deficiency was confirmed by markedly decreased *in vitro* transketolase activity in adrenals and red blood cells and by low activity of pyruvate decarboxylase in adrenals. A marked *in vitro* thiamin pyrophosphate effect was observed only in the activities of red blood cell transketolase and adrenal pyruvate decarboxylase of TD rats. The effect was trivial in red blood cells and adrenals from pair-fed controls. ACTH produced negligible stimulation of 1-^{14} and 6-^{14}C-glucose oxidation in quartered adrenals from TD animals whereas a significant increase was observed in pair-fed controls. The activity of hexose monophosphate pathway dehydrogenase and of cytosol malic enzyme was not significantly affected by thiamin deficiency as compared to pair-fed controls.

CONCLUSIONS:

Adrenal glucose catabolism and steroidogenesis in response to ACTH are impaired in thiamin deficiency. This effect of thiamin deficiency on adrenal glucose catabolism may result in diminished NADPH and cyclic AMP formation and thereby interfere with the steroidogenic action of ACTH.

RECOMMENDATIONS:

Future studies in this particular aspect of the work unit should be directed towards the effects of thiamin deficiency on the synthesis of cofactors and cyclic AMP in response to ACTH

PUBLICATIONS:

1. Meikle, A. W., P. J. Wittek and G. J. Klain. Adrenal steroidogenesis in thiamin-deficient rats. Federation Proc. 31:284, 1972 (Abstract)

2. Meikle, A. W. and G. J. Klain. Effect of fasting and fasting-refeeding on the conversion of leucine into CO_2 and lipids in rats. Amer. J. Physiology 222:1246, 1972.

3. Meikle, A. W., P. J. Wittek and G. J. Klain. An aberration of glucose metabolism and steroidogenesis in adrenals of thiamin deficient rats. Endocrinology 1972 (in press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6343	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	078
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Precede with Security Classification Code)*

(U) Metabolic Response of Man to Nutrition or Disease (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	CONT	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:* Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	5.5	84
	CURRENT 73	6.8	183

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
Metabolic Division

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME:* Herman, R. H., COL, MC

TELEPHONE: 303 366 5311 X25193

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Hagler, L., LTC, MC

NAME: Stifel, F. B., DAC DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Gastrointestinal Disease in Combat Soldiers; (U) Environmental Stress; (U) Jejunum; (U) Jejunal Enzymes; (U) Glycolytic Enzymes

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

23. (U) Acute and chronic gastrointestinal disease in combat soldiers cause a large proportion of military ineffectiveness. If latent defects of the gastrointestinal tract become manifest with environmental stress it is necessary to identify the defects, the stressful conditions and the susceptible individuals. To uncover gastrointestinal tract defects it is necessary to study normal gastrointestinal metabolic processes. Our studies suggest that much disabling gastrointestinal disease is related to a failure of gastrointestinal enzyme adaptation to diet. The regulation of blood glucose and lipid levels are related to the type of diet, the functions of the intestine, liver and pancreas and the utilization of glucose and lipid by muscle and adipose tissue.
24. (U) The response of gastrointestinal enzymes of normal human volunteer subjects and selected patients with specific gastrointestinal disease to specific dietary substances and certain oral drugs is under study. The regulation of blood lipids and glucose in normal human volunteer subjects and selected patients with abnormalities of lipid and glucose metabolism is under study. Basic studies are in work unit 059.
25. (U) 71 07 - 72 07 Autoclaved folate deficient diets in rats produce a folate deficient state and obviates the use of sulfathiazole. Folate deficiency decreases the activities of jejunal glycolytic enzymes. Various hormones affect the enzyme activities of rat and human intestine. Glucagon and insulin affect jejunal glycolytic enzymes so rapidly that the mechanism must involve enzyme activation-inactivation rather than protein synthesis. L-DOPA causes growth hormone secretion in man. Some patients with maladaptation of jejunal enzymes to carbohydrate also are intolerant to dietary protein. Other metabolic diseases and systems have been studied: fructose-diphosphatase deficiency, glycogen storage disease, platelet response to and tolbutamide effects on cAMP, effects of hormones on jejunal adenyl cyclase and formiminotransferase deficiency.

*Available to contractors upon originator's approval.

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 on gastrointestinal enzymes of the jejunal mucosa.

STUDY NO. 5. The effect of alpha and beta blockade upon the release of growth hormone, insulin and ACTH.

STUDY NO. 6. The effect of clomiphene on serum FSH and LH levels and sperm production in male hypogonadism.

STUDY NO. 7. The effect of testosterone on jejunal glycolytic enzyme activities in male hypogonadism.

STUDY NO. 12. Lack of jejunal glycolytic enzyme adaptation in patients with chronic gastrointestinal disease: the gastrointestinal maladaptation syndrome.

STUDY NO. 20. Effect of hormones, diet and drugs on jejunal cyclic AMP.

STUDY NO. 21. Study of formiminotransferase deficiency.

STUDY NO. 22. Study of a patient with glucose-6-phosphatase deficiency.

STUDY NO. 24. Hypoglycemia syndromes.

Study No. 1. An autoclaved folate deficient diet is as effective as a sulfathiazole folate deficient diet in causing a folate deficient state in the rat. Formiminoglutamic acid inhibits the activities of certain folate-metabolizing enzymes. Different hormones given in vivo with different dietary regimens affects jejunal enzymes in a complex fashion. Insulin given in vivo and glucagon alter hepatic enzyme activities very rapidly (within minutes) and in a reciprocal fashion.

Study No. 5. L-DOPA administration causes growth hormone secretion. This effect is inhibited by phentolamine but not by propranolol.

Study No. 6. Clomiphene seems to be a useful therapy for oligospermia of unknown etiology.

Study No. 7. Hypogonadal patients have a poor adaptation of jejunal enzymes to diet. Testosterone therapy restored pyruvate kinase adaptation only.

Study No. 12. Many patients with maladaptation of jejunal enzymes improved on a carbohydrate restricted diet. Other of these patients appear to have protein intolerance also.

Study No. 20. Clofibrate seems to increase platelet cyclic AMP concentrations, decrease pyruvate kinase activity and decrease urinary cyclic AMP. Tolbutamide alters jejunal glycolytic enzyme activities but does not change cyclic AMP concentrations. The cyclic AMP of human jejunum is affected in vitro by theophylline, prostaglandin E_1 and cholera toxin.

Study No. 21. A patient with formiminotransferase (FIT) deficiency had a deficiency of FIT in the jejunum, red cells and liver and formiminoglutamic aciduria. She is intolerant of carbohydrate and carbohydrate plus various proteins which cause hepatomegaly (due to a fatty infiltrate) and various G.I. symptoms. On an elemental diet (Vivonex) the patient gained weight and became asymptomatic. Her diet is severely restricted due to her intolerance of many proteins and vegetables. Nevertheless, good nutrition can be maintained. Her metabolic problem is quite complex and the nature of the protein intolerance is not yet clear.

Study No. 22. Treatment of a patient with glucose-6-phosphatase deficiency with clofibrate decreased her hepatomegaly, hypertriglyceridemia, lactic acidemia and improved her acidosis. Clofibrate therapy together with other therapeutic modalities (hyperalimentation, portocaval shunt, frequent glucose feedings, and dietary elimination of fructose, galactose and glycerol) offers these patients clinical improvement that otherwise has not been attainable.

Study No. 24. We have found several patients with fructose-diphosphatase deficiency (FDPase). In a child with the severe form a chronic hypoglycemia is present and in a child with a milder form "ketotic" hypoglycemia associated with infection, fasting and alcohol is present. Treatment with folic acid increased the levels of the hepatic fructose diphosphatase and in the severe chronic hypoglycemia child raised blood glucose levels from about 20 mg% to 35-40 mg%. In the child with the "ketotic" hypoglycemia folate therapy prevented hypoglycemia due to fasting and made the child more resistant to glycerol. An adult with reactive hypoglycemia was found to have FDPase deficiency and one of her daughters also had FDPase deficiency. Folate therapy improved both of these patients. It would seem that FDPase deficiency may manifest in a variety of ways and may represent different types of enzymatic defects.

BODY OF REPORT

WORK UNIT NO. 078 — Metabolic Response of Man to Nutrition or Disease

STUDY NO. 1. — The effect of diet, drugs and sex steroids on gastrointestinal enzymes of the jejunal mucosa.

PROBLEM:

Various substances (diet, drugs and steroid and non-steroid hormones) produce adaptive changes in the activities of intestinal enzymes. Different dietary regimens and hormonal states, exert profound effects upon the adaptive responses of intestinal enzymes. Thus, studies have been conducted to further elucidate the complex interactions which are involved in the overall regulation of gastrointestinal enzyme function.

RESULTS AND DISCUSSION OF THE RESULTS:

Previous studies have demonstrated that an autoclaved folic acid-deficient diet produces a marked decrease in the activities of certain jejunal and hepatic glycolytic enzymes when compared with rats fed a folic acid repleted diet. These enzyme changes are indicative of a folate-deficient state, despite the fact that megaloblastic changes were not observed in the bone marrow. The enzyme changes were restored to normal levels with the addition of folic acid to the diet. Changes in the enzyme levels correlated quite well with the plasma and erythrocyte folate levels. However, the folate levels in the rats receiving the autoclaved folate-deficient diet, although markedly depressed when compared with the rats receiving folate in the diet, were considerably higher than those reported in rats receiving sulfathiazole. Thus, a study was designed to compare the effect of an autoclaved folate-deficient diet or sulfathiazole on numerous parameters used to assess the folate status of the male rat. These parameters included plasma, red blood cell and liver folate levels, urinary formimino glutamic acid (FIGLU) levels, bone marrow smears and various jejunal and hepatic glycolytic enzyme activities. In both the autoclaved folate-deficient group and the sulfathiazole folate-deficient groups the urinary FIGLU excretion was elevated within two weeks following the initiation of the experimental diets. Throughout the 12 week study, the urinary FIGLU levels remained elevated and comparable. The urinary FIGLU levels were also statistically significantly higher in the group of rats receiving folate plus sulfathiazole when compared with the group receiving only folate. As noted in the literature and in our previous studies, the folate levels were the lowest in the folate-deficient rats receiving sulfathiazole. However, the enzyme changes were similar in both the autoclaved folate-deficient group and the sulfathiazole folate-deficient group. These data suggest that both diets are effective in producing the folate-deficient state and that sulfathiazole may have some antagonistic

role in folate metabolism as demonstrated by the effect on urinary FIGLU levels.

We have previously demonstrated that oral folic acid produces marked increases in jejunal glycolytic enzyme activities in normal individuals. We have also identified a large group of patients who lack the normal adaptive response to diet (see Study #12). In some instances, glucose produces the apparent maladaptation, while in others both glucose and fructose produce a maladaptation. Most of these individuals also fail to respond to oral folate while on a carbohydrate-free diet. In another patient who has an adult form of formiminotransferase (FIT) deficiency (see Study #21) in the RBCs, jejunum and liver, no response to folate was seen on any dietary regimen. Since FIGLU levels are markedly elevated in this latter patient in both the urine and jejunum, we investigated the effect of FIGLU in vitro upon the folate-metabolizing enzymes which control the alternate pathways of folate metabolism (methylene tetrahydrofolate (THF) dehydrogenase, formyl THF synthetase and serine hydroxymethyltransferase). Studies have demonstrated that FIGLU, at concentrations as low as .05 mM, markedly inhibit each of these enzyme activities in both human and rat jejunum and liver. The patient with adult form of formiminotransferase deficiency does have low levels of the folate-metabolizing enzymes necessary for the alternate pathway of folate metabolism. However, the FIGLU inhibition of the enzymes involved in the alternate pathways of THF utilization which bypass the FIT reaction could explain why these alternate pathways do not appear to compensate adequately for the FIT deficiency.

Adrenalectomy was shown to decrease the activity of three glycolytic enzymes and one jejunal gluconeogenetic enzyme, fructose diphosphatase (FDPase). However, responses to dietary fructose, glucose and casein were qualitatively the same in the adrenalectomized rats as in the normal rats. The administration of corticosterone restored the levels of the enzymes in the adrenalectomized rats to those of the normal non-adrenalectomized rats.

Thyroidectomy decreased the activities of rat jejunal pyruvate kinase (PK), when compared to normal non-thyroidectomized rats. The repletion of thyroidectomized rats with thyroxine produced a marked increase in the activities of this enzyme in the jejunum. These changes were noted in fasted rats as well as rats receiving a high casein, a high glucose or a high fructose diet. The addition of thyroxine to normal rats produced a significant increase in PK activity in the fasted rats and those on the high casein or high glucose diets. However, thyroxine had no effect upon PK activity in normal rats on the fructose diet. Changes in PK activities on the different diets were not the same in the liver as in the jejunum. In thyroidectomized rats, the addition of thyroxine increased the activity of hepatic PK in the

fasted group and those receiving the high-casein or high-glucose diets. However, on the fructose diet, thyroxine had no such effect. In the liver, thyroidectomy and thyroxine had little or no effect upon fructose diphosphate (FDP) and fructose-1-phosphate (F1P) aldolase activities, while in the jejunum, thyroidectomy produced a marked increased in F1P aldolase activity on each of the diets when compared to the normal non-thyroidectomized animals.

In previous studies, we demonstrated that oral and intramuscular testosterone produced adaptive increases in jejunal PK activity in hypogonadal rats. These rats were maintained on a Purina chow diet which is high in casein. In recent studies, we demonstrated that the oral testosterone response occurs only in rats which have been fasted or maintained on a high-casein diet. The testosterone-repleted normal and castrated rats failed to show an adaptive response of jejunal pyruvate kinase to testosterone when fed a high-glucose or high-fructose diet. This is in contrast to hypogonadal human males who respond to oral testosterone on a high-casein, high-glucose or high-fructose diet. Interestingly, castration in rats produced a decrease in jejunal PK activity only on the high-glucose or high-fructose diets. Jejunal PK decreased in hypophysectomized rats - partial restoration was accomplished by growth hormone and a combination of other pituitary hormones.

Due to the apparent maladaptation of jejunal enzymes to diet in diabetics and the ability of glucagon to rapidly increase the activity of hepatic FDPase in several patients with FDPase deficiency, we focused our attention on the reciprocal roles of insulin and glucagon on the regulation of hepatic glycolysis and gluconeogenesis. Within 4 minutes after the injection of glucagon into the inferior vena cava, a significant decrease occurred in the activities of two key glycolytic enzymes, hepatic PK and phosphofructokinase (PFK). On the other hand, FDPase, a key gluconeogenetic enzyme, increased significantly. The activity of FDP aldolase, an enzyme involved in both glycolysis and gluconeogenesis, did not change. The glucagon effect persisted for at least 15 minutes. The changes in these key enzyme activities were associated with a marked increase in hepatic cyclic adenosine 3',5'-monophosphate (cyclic AMP) concentration. Insulin produced reciprocal changes to those seen with glucagon. PFK and PK activities increased significantly within 4 minutes of insulin injection and persisted for at least 15 minutes. FDPase activities declined significantly and FDPA activity did not change. Cyclic AMP levels were not significantly altered at 4 or 15 minutes after insulin injection. Our data indicate that glucagon and insulin rapidly change the activities of two key enzymes unique to the glycolytic pathway and one key enzyme unique to the gluconeogenetic pathway.

CONCLUSIONS:

Based upon urinary FIGLU levels and hepatic and jejunal glycolytic enzyme activities, an autoclaved folate-deficient diet produces a folate-deficient state which is comparable to that seen in rats receiving sulfathiazole. The autoclaved diet also obviates the possible antagonism which sulfathiazole seems to produce in the metabolism of folate. FIGLU, an important metabolite of folate metabolism, decreases the activities of other folate-metabolizing enzymes which control the alternate pathways of folate metabolism. The hormonal status of the rat, as well as man, appears to play a prominent role in ability of the individual to respond to different dietary stimuli. This was demonstrated in studies of hypothyroid, hypoadrenal and hypogonadal rats. The reciprocal roles of glucagon and insulin were explored in the rat liver demonstrating very rapid responses of hepatic PK, PFK and FDPase activities and cyclic AMP concentrations. Our data suggests that these two hormones exert a rapid activation or deactivation of these enzymes.

RECOMMENDATIONS:

It is apparent that the intestine provides a very convenient model for the study of enzyme regulation in man. It is also apparent that numerous factors, including diet, oral folic acid, steroid and non-steroid hormones, exert a profound influence upon the regulation of jejunal and hepatic glycolytic enzymes. Since a large population of patients with an apparent gastrointestinal maladaptation syndrome have been discovered and are apparently-helped by dietary control and oral folic acid (see study #12), it is important to pursue the mechanism by which jejunal glycolytic enzymes are regulated in normal individuals and patients with gastrointestinal problems. Hopefully, this will lead to a better understanding of the biochemical basis of the maladaptation syndrome.

PUBLICATIONS:

1. Stifel, F. B. and R. H. Herman. Effects of L-histidine on human jejunal pyruvate kinase activity. Canad. J. Biochem. 49: 1105, 1971.

2. Stifel, F. B., H. L. Greene and R. H. Herman. Effect of oral contraceptive steroids on jejunal pyruvate kinase and adenyl cyclase activities. Endocrinol. 89: 896, 1971.

3. Stifel, F. B. and R. H. Herman. Is histidine an essential amino acid in man? Amer. J. Clin. Nutr. 25: 182, 1972.

4. Stifel, F. B., N. S. Rosensweig and R. H. Herman. Inhibitory effects of formimino-L-glutamic acid in vitro on rat jejunal folate-metabolizing and glycolytic enzymes. Fed. Proc. 31: 712, 1972 (Abstr.).

Metabolic Response of Man to Nutrition or Disease (Cont'd)

5. Herman, R. H., Y. F. Herman and F. B. Stifel. Oral folic acid requirement for the normal adaptive response of jejunal pyruvate kinase activity to sex steroids in rats. Fed. Proc. 31: 712, 1972 (Abstr.).

6. Herman, R. H. Mannose metabolism, I. Amer. J. Clin. Nutr. 24: 488, 1971.

7. Herman, R. H. Mannose metabolism, II. Amer. J. Clin. Nutr. 24: 556, 1971.

8. Anderson, J. W., R. H. Herman, J. B. Tyrrell and R. M. Cohn. Hexokinase: A compartmented enzyme. Amer. J. Clin. Nutr. 24: 642, 1971.

9. Gozansky, D. M. and R. H. Herman. Water and sodium retention in the fasted and refed human. Amer. J. Clin. Nutr. 24: 869, 1971.

10. Herman, R. H. The metabolic consequences of insulin deficiency in: Juvenile-Type Diabetes and Its Complications, Theoretical and Practical Considerations. Ed. by Karl E. Sussman. Charles C. Thomas, Springfield, Ill, 1971, Chap. III, pp. 19-55.

11. Greene, H. L., F. B. Stifel and R. H. Herman. Dietary stimulation of sucrase in a patient with sucrase-isomaltase deficiency. Biochem. Med., In press.

12. Herman, R. H., F. B. Stifel, H. L. Greene and Y. F. Herman. Intestinal metabolism of fructose. Acta Med. Scand., Suppl., In press.

13. Lufkin, E. G., F. H. Katz and R. H. Herman. Primary aldosteronism due to hyperplasia of zona glomerulosa: Failure of suppression by DOCA or stimulation by angiotensin. Amer. J. Med. Sci. Accepted for publication.

Study No. 5. The effect of alpha and beta blockade upon the release of growth hormone, insulin and ACTH.

PROBLEM:

The release of ACTH and growth hormone from the pituitary in humans is modified by adrenergic influences. We have extended our studies to learn whether growth hormone and insulin secretion can be altered by L-DOPA (dihydroxyphenylalanine), a drug which is metabolized in the hypothalamus (and perhaps in the pancreas) to norepinephrine. Preliminary studies in 2 normal volunteer subjects were performed. On

separate days they received an insulin tolerance test, a control infusion of normal saline, L-DOPA orally, L-DOPA plus propranolol infusion, L-DOPA plus phentolamine infusion, glucose, and glucose plus L-DOPA.

RESULTS AND DISCUSSION OF THE RESULTS:

L-DOPA administration in low dosage caused the secretion of growth hormone, similar in magnitude to that following insulin-induced hypoglycemia. This effect was abolished by phentolamine but was unaffected by propranolol. Glucose-induced insulin release was unaffected by L-DOPA.

CONCLUSIONS:

These preliminary results are consistent with the postulate that adrenergic mechanisms influence growth hormone release, and that L-DOPA stimulates growth hormone release by conversion to the alpha-agonist, norepinephrine.

RECOMMENDATIONS:

These studies should be continued.

PUBLICATIONS:

1. Lufkin, E. G., H. L. Greene, J. R. Meek and R. H. Herman. Adrenergic control of hormone secretion. J. Lab. Clin. Med. 78: 820, 1971 (Abstr.).

2. Lufkin, E. G., H. L. Greene, J. R. Meek and R. H. Herman. Adrenergic control of hormone, thyroid stimulating hormone, adrenocorticotrophic hormone and insulin secretion in man. Submitted for publication and presented before the Regional Meeting of the American College of Physicians, Colorado Springs, Colorado, 18 January 1972.

Study No. 6. The effect of clomiphene on serum FSH and LH levels and sperm production in male hypogonadism.

PROBLEM:

The commonest form of male infertility is oligospermia of unknown etiology. We have performed studies in 4 additional men with this disorder. Following control studies and testicular biopsy, clomiphene citrate, 50 mg daily, was given for 90 days. Serial plasma and urine collections were made for later determination of testosterone, hydroxy- and ketosteroids, and serial semen analyses were made.

Metabolic Response of Man to Nutrition or Disease (Cont'd)

RESULTS AND DISCUSSION OF THE RESULTS:

A total of 7 men have been studied. The full 90-day course of clomiphene has been completed in only 5. Pregnancy has occurred in the wives of 3 of the 7 males studied.

CONCLUSIONS:

It is of interest that pregnancy occurred in 3 wives related to the use of clomiphene citrate by the husbands. The data is consistent so far with the hypothesis that clomiphene stimulates the production and/or release of gonatropins. Blood typing excluded non-paternity in one couple but was not feasible in the other 2 couples.

RECOMMENDATIONS:

We recommend that the study be continued until at least 10 men have been studied.

PUBLICATIONS: None.

Study No. 7. The effect of testosterone on jejunal glycolytic enzyme activities in male hypogonadism.

PROBLEM:

In 7 patients with hypogonadism and testosterone deficiency the activities of certain glycolytic enzymes in the jejunal mucosa were decreased. Activity of pyruvate kinase (PK) was increased by oral and intramuscular testosterone as previously shown in the rat. Adaptation of jejunal glycolytic enzyme activities to dietary changes was reduced in these men. In order to extend these observations and to learn whether the dietary maladaptation was related to the testosterone deficiency, 5 additional patients with Klinefelter's syndrome and testosterone deficiency were studied. These men were hospitalized on the metabolic ward for 7 week periods. Liquid synthetic diets were consumed and jejunal glycolytic enzyme activities were measured serially.

RESULTS AND DISCUSSION OF THE RESULTS:

Enzyme assays have been completed in 3 of the 5 patients. The results confirm that the activities of pyruvate kinase (PK) and fructose-1-phosphate aldolase (F1PA) and fructose diphosphate aldolase (FDPA) were reduced and adapted poorly to dietary changes. Treatment with oral testosterone restored to normal the adaptive response of PK to changes in diet, but had little effect on the responses of hexokinase (HK), F1PA or FDPA.

CONCLUSIONS:

Testosterone deficient men have a decreased adaptive response of jejunal PK to dietary changes, which are restored to normal by oral testosterone. Testosterone seems to play a permissive role in the dietary adaptive responses of jejunal PK.

RECOMMENDATIONS:

The differential effect of testosterone on human jejunal enzymes should be investigated. It would be of interest to do similar studies in estrogen-deficient women.

PUBLICATIONS:

1. Lufkin, E. G., F. B. Stifel, R. H. Herman and N. S. Rosensweig. Effect of testosterone on jejunal pyruvate kinase activities in normal and hypogonadal males. J. Clin. Endocr. 34: 586, 1972.

Study No. 12. Lack of jejunal glycolytic enzyme adaptation in patients with chronic gastrointestinal disease: the gastrointestinal maladaptation syndrome.

PROBLEM:

Previous work in this laboratory has shown that jejunal enzyme activity is regulated by dietary sugar, folic acid and a variety of drugs. Patients with a variety of chronic gastrointestinal symptoms have been studied by comparing the effects of different diets, folic acid and several drugs on jejunal glycolytic enzyme activity in these patients as compared to normal. Study of the abnormal adaptive responses found in these patients when compared to normal individuals may clarify the mechanism of the adaptive failure and the pathogenesis of symptoms as well as providing for diagnosis and therapy of these patients.

RESULTS AND DISCUSSION OF THE RESULTS:

We have studied 10 patients with "functional gastrointestinal" disease, four of whom were previously studied. The histories and types of evaluation of these patients was similar to that reported in the FY 71 Annual Progress Report. All of these patients showed varying degrees of failure of glycolytic enzyme adaptation to diet and/or folic acid. In all there has been improvement with oral folic acid and dietary carbohydrate restriction. In some of these patients prolonged carbohydrate restriction has provided continuing freedom from diarrhea and diminution in other gastrointestinal complaints. In others, symptoms, although decreased in severity and frequency, have continued to occur despite strict dietary adherence. In these patients, there seems to be a clear

historical relationship with the ingestion of dietary protein. This suggests that dietary protein as well as dietary carbohydrate may cause symptoms. Thus, we have initiated studies to assess the function and the response of intestinal dipeptidases to various diets and folic acid.

CONCLUSIONS:

1) Chronic gastrointestinal symptoms including chronic diarrhea may be the result of jejunal mucosal enzyme abnormalities which renders the patient sensitive to dietary carbohydrate. 2) Elimination of dietary carbohydrate in these patients usually results in improvement of the diarrhea and other symptoms. 3) In some patients the ingestion of dietary protein also leads to gastrointestinal symptoms. Evaluation of jejunal dipeptidase function which plays a terminal role in protein digestion is currently underway.

RECOMMENDATIONS:

The relationships between the intake of food and chronic gastrointestinal symptoms require further study. Further evaluation of the influence of diet and drugs on gastrointestinal enzyme function and acute and chronic gastrointestinal symptoms should be conducted. Studies such as this may help to explain peculiar dietary avoidance patterns which is seen in some patients, the puzzling persistence of chronic gastrointestinal symptomatology in certain patients despite strict adherence to a "therapeutic diet", and may help to establish the physical, organic nature of some of the so-called functional gastrointestinal diseases, thereby allowing the application of rational and objective diagnostic and therapeutic methods.

PUBLICATIONS:

1. Rosensweig, N. S., R. H. Herman, F. B. Stifel, L. Hagler, H. L. Greene, and Y. F. Herman. Gastrointestinal disease associated with a failure of adaptation of jejunal glycolytic enzymes. Presented at the Amer. Gastroenterology Assoc., Gastroenterology Research Group, Dallas, Texas May 24-27, 1972 (Abstr.).

STUDY NO. 20. Effect of hormones, diet and drugs on jejunal cyclic AMP.

Experiment 1.

PROBLEM:

Clofibrate has been shown to decrease adenyl cyclase activity in rat and human jejunum and fat. This study was done to determine the effect

of this drug on cyclic AMP levels in human tissues and urine in an effort to determine if the mechanism of action of this drug is related to alterations in cyclic AMP metabolism.

RESULTS AND DISCUSSION OF THE RESULTS:

There was no consistent change in cyclic AMP levels in jejunum and fat. However in 3 of 4 subjects, cyclic AMP levels increased following clofibrate in platelets and decreased in the urine. Platelet pyruvate kinase decreased 35% while on treatment, while fructose-1,6-diphosphate aldolase did not change.

CONCLUSIONS:

Clofibrate seems to increase platelet cyclic AMP concentrations, decreases platelet pyruvate kinase activity and decreases the urinary excretion of cyclic AMP.

RECOMMENDATIONS:

Similar studies need to be done in more subjects and platelet glycolytic enzymes, cyclic AMP and urinary cyclic AMP measured. This would be done in conjunction with work unit 062.

PUBLICATIONS: None.

STUDY NO. 20. Effect of hormones, diet and drugs on jejunal cyclic AMP.

Experiment 2.

PROBLEM:

Tolbutamide has been reported to decrease phosphodiesterase and increase adenyl cyclase activity *in vitro*. This would be expected to increase cyclic AMP concentrations and would be antagonistic to insulin. This study was done to evaluate the effect of this drug on human jejunal cyclic AMP concentrations, glycolytic enzyme activities and the urinary excretion of cyclic AMP.

RESULTS AND DISCUSSION OF THE RESULTS:

Tolbutamide treatment caused no significant change in cyclic AMP concentrations in jejunum or in the urinary excretion of cyclic AMP. However, jejunal pyruvate kinase and FDP aldolase activities increased significantly, FDPase decreased significantly, and F-1-P aldolase did not change. These effects could be due to insulin release.

CONCLUSIONS:

Tolbutamide significantly alters human jejunal glycolytic enzymes and FDPase without changing cyclic AMP concentrations.

RECOMMENDATIONS:

The effect of tolbutamide, insulin and insulin plus tolbutamide needs to be studied in more normal subjects.

PUBLICATIONS: None.

STUDY NO. 20. Effect of hormones, diet and drugs on jejunal cyclic AMP.

Experiment 3.

PROBLEM:

The role of cyclic AMP in gastrointestinal function is unknown. Since it is possible that some of the defects causing the maladaptation syndrome in response to diet could be mediated through cyclic AMP, and since hormones are known to alter cyclic AMP levels in other tissues, the effect of a number of hormones and cholera toxin on jejunal cAMP was investigated.

RESULTS AND DISCUSSION OF THE RESULTS:

The following substances did not alter cyclic AMP levels in human jejunum *in vitro*: epinephrine, glucagon, insulin, vasopressin, parathormone, serotonin, histamine, acetylcholine, bradykinin and dopamine. Theophylline, prostaglandin E_1 (PGE_1) and cholera toxin did cause a marked increase in cyclic AMP levels.

CONCLUSIONS:

Theophylline, prostaglandin E_1 cholera toxin increased cyclic AMP levels in human jejunum *in vitro* while a variety of other substances had no effect.

RECOMMENDATIONS:

The time and dose response to PGE_1 and cholera toxin should be investigated. In addition the effect of these substances on adenyl cyclase and phosphodiesterase should be investigated.

PUBLICATIONS: None.

STUDY NO. 21. Study of formiminotransferase deficiency

PROBLEM:

Formiminotransferase deficiency has been described in several Japanese children all of whom manifested severe mental and motor retardation. Two adult patients with formiminotransferase (FIT) deficiency were previously discovered in this laboratory. Both of these patients have normal to superior intellect. Both of them manifest severe dietary intolerance to both protein and carbohydrate.

RESULTS AND DISCUSSION OF THE RESULTS:

Continued treatment of the first adult patient with FIT deficiency with pharmacological doses of folic acid and a carbohydrate-free diet was only partially successful. The patient continued to have intermittent symptoms. However, later diarrhea, profound weakness, severe weight loss, and anorexia intensified and the patient became quite ill. Parenteral hyperalimentation was instituted with Aminosol(R) (fibrin hydrolysate) which improved the patient's debilitated state and poor nutrition and increased her weight from 90 to 115 lbs. However, attempts to feed the patient even small amounts orally caused abdominal pain, nausea and diarrhea. Continued use of Aminosol despite weight gain resulted in hyperglycemia, hepatomegaly due to a fatty infiltration, abdominal pain and nausea. Use of a synthetic formulated amino acid solution led to resolution of these adverse clinical manifestations. It became apparent that the patient was intolerant to various peptides present in the Aminosol solution. Because of the success with an "elemental" amino-acid solution by parenteral hyperalimentation oral Vivonex was substituted. With Vivonex all symptoms disappeared, nutrition was greatly improved and body weight was maintained at 120 lbs. The use of single protein test diets showed that the patient was intolerant of beef, pork, tuna fish, soy bean, egg, milk, and peanut protein. These substances caused abdominal pain, hepatomegaly, nausea, diarrhea and a hepatic fatty infiltrate. Avoidance of these substances caused resolution of the hepatomegaly and disappearance of the hepatic fatty infiltrate. The patient was able to tolerate chicken, gluten, butter, and various fruits and vegetables. With a judicious mixture of Vivonex and the tolerated foods the patient has regained her strength, maintained her weight and has become asymptomatic. It has also been found that she is intolerant of vegetables belonging to the cruciferae class of plants (cabbage, onion, turnip, radish, etc.). These plants are characterized by a high content of various pungent substances including nitriles. The present hypothesis is that the Fe FIT deficiency has secondarily led to a failure of hepatic detoxification mechanisms which may also account for the patient's intolerance to the usual adult dose of various medications (demerol, codeine, anti-histamines, etc.).

CONCLUSIONS:

Formiminotransferase deficiency is related to the occurrence of chronic gastrointestinal symptoms. Since formiminotransferase is an enzyme in the pathway of folate metabolism it appears that folic acid is involved in the normal G.I. enzyme adaptive responses and abnormalities of folate metabolism can cause chronic gastrointestinal symptoms. Study of the first patient has clearly demonstrated that certain proteins may be toxic for certain individuals and has added a new dimension of inquiry to our larger class of patients with G.I. adaptive failure.

RECOMMENDATIONS:

This area should receive further study in conjunction with our larger group of patients with the gastrointestinal maladaptation syndrome.

PUBLICATIONS: None.

STUDY NO. 22. Study of a patient with glucose-6-phosphatase deficiency.

PROBLEM:

A child with glucose-6-phosphatase deficiency, massive hepatomegaly, lactic acidosis, hyperuricemia, hyperlipemia, retarded growth and skeletal deformities has been studied. Clofibrate administration decreased the hypertriglyceridemia and the liver size. On the basis of the presumed mechanism of action of clofibrate it was postulated that chronic hypoglycemia caused increased levels of hepatic cyclic AMP which caused glycogenolysis and gluconeogenesis. Because glucose-6-phosphate levels would activate the dependent form of glycogen synthetase glycogen synthesis would continue leading to continued glycogen deposition and hepatomegaly. Similarly, chronic hypoglycemia would decrease blood insulin, elevate adipose tissue cyclic AMP which would enhance lipolysis and inhibit lipoprotein lipase activity. Hence, free fatty acids from adipose tissue would recirculate to the liver, be incorporated into lipoprotein which would accumulate in the circulation giving rise to hyperlipemia. This hypothesis and the effect of clofibrate was studied further.

RESULTS AND DISCUSSION OF THE RESULTS:

The patient was given clofibrate, 2 gms/day, for 9 months. During this period her weight and growth improved and serum triglycerides and lactate decreased. Prior to therapy glucagon caused hyperpyrexia, acidosis and increased blood lactic acid. After clofibrate therapy glucagon had less effect upon serum lactate and blood pH did not change. Hepatic glycogen content was decreased as well. Parenteral hyperalimentation caused further shrinkage of the liver size probably by decreasing

the fatty infiltrate that contributed to the hepatomegaly. After the parenteral hyperalimentation a portocaval shunt was done at the University of Colorado Medical School. This procedure has been beneficial in patients of this type. The patient was improved and has been sent home on a regimen of clofibrate, frequent glucose feeding and elimination of fructose, galactose and glycerol from her diet.

CONCLUSIONS:

The inability of this patient to maintain proper blood glucose levels set in motion gluconeogenetic, glycogenolytic and lipolytic mechanisms which resulted in rapid glycogen re-synthesis, fatty infiltration of the liver, hypertriglyceridemia, acidosis, elevated blood lactic acid, hyperuricemia, fever, retarded growth and development and skeletal deformities. Treatment with clofibrate interrupted this sequence of events. The beneficial results were augmented by parenteral hyperalimentation, portocaval shunt, frequent glucose feedings and dietary elimination of fructose, galactose and glycerol.

RECOMMENDATIONS:

Metabolic studies of patients with inborn errors of metabolism not only may benefit the patient but will enhance our understanding of the mechanism of metabolic control and complement the work discussed in studies #1, 20 and 24. Therefore, it is vital that these types of studies should be continued.

PUBLICATIONS:

1. Greene, H. L., R. H. Herman, F. B. Stifel and O. D. Taunton. Glycogen storage disease due to glucose-6-phosphatase deficiency: Treatment with clofibrate. Ped. Res. 6: 398/138, 1972, (Abstr.).

STUDY NO. 24. Hypoglycemia syndromes.

PROBLEM:

Hypoglycemia may result from a variety of metabolic aberrations and its cause is not understood in many patients. Recent reports indicate that chronic hypoglycemia may be due to FDPase deficiency and because of our finding two such pediatric patients we have looked for FDPase deficiency in adults with hypoglycemia of unknown etiology.

RESULTS AND DISCUSSION OF THE RESULTS:

In one adult patient with reactive hypoglycemia FDPase was found to be lower than 50% of normal in the jejunum, liver and platelets. The patient's glucose response to glycerol ingestion was abnormal. Fasting produced symptomatic hypoglycemia in 48-72 hours. Folic acid treatment, 30 mg/day, produced approximately a 100% increase in her FDPase level

in jejunum, liver and platelets and relieved all her symptoms. Investigation of her children disclosed a deficiency in her 18 months old daughter while her 7 year old son was normal. In the daughter there was FDPase deficiency in jejunum and liver, hypoglycemia within 12 hours of fasting, and hypoglycemia after ingesting glycerol. Folic acid, 15 mg/day, increased the daughter's FDPase level and increased her ability to withstand fasting. FDPase activity was increased rapidly in the liver in both patients by glucagon. Electrophoretic mobility of the patient's FDPase was not different than normal.

Another patient was found to have reactive hypoglycemia without excess insulin. His jejunal FDPase activity was normal. However, his response to arginine infusion was abnormal in that he did not show the expected rise in glucose. This suggests a partial glucagon deficiency. Plasma samples will be obtained and glucagon levels will be measured.

It would appear from our studies in children and adults that the degree and type of FDPase deficiency determines the specific clinical manifestations of the deficiency. In children the severe FDPase deficiency results in chronic hypoglycemia while the milder form results in the so-called ketotic hypoglycemia only after fasting. In adults a defect in activation might only be manifest after eating, resulting in reactive hypoglycemia, secondary to insulin inactivation of an already deficient FDPase (see study #1, this report).

CONCLUSIONS:

1. Hypoglycemia associated with FDPase deficiency has been documented.

2. Folic acid improved clinical symptoms and increased FDPase activity.

3. FDPase deficiency is probably due to a deficient activating system although an amino acid substitution in the FDPase which does not alter the charge of the protein molecule has not been rigorously excluded.

4. Partial glucagon deficiency is also a possibility that must be considered in studying hypoglycemia.

RECOMMENDATIONS:

These patients must be followed and other patients should be investigated. The studies should include investigation of hormonal and enzyme mechanisms in animals as well as man and should include *in vitro* as well as *in vivo* work.

Metabolic Response of Man to Nutrition or Disease (Cont'd)

PUBLICATIONS:

1. Greene, H. L., F. B. Stifel, R. H. Herman. Hypoglycemia due to fructose-1,6-diphosphatase deficiency and the treatment of two patients with folate. Ped. Res. 6: 432/172, 1972 (Abstr.).

2. Greene, H. L., F. B. Stifel, and R. Herman. "Ketotic" hypoglycemia due to a deficiency of hepatic fructose-1,6-diphosphatase. Treatment with folic acid. Am. J. Dis. Child., In press.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6357	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	079
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Procede with Security Classification Code)

(U) Radioisotope Support for Military Medical Research (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

008500 Isotopes; 013900 Radioactivity; 011000 Nuclear Instrumentation

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
64 05	CONT	DA	C In-house

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 72	.25	97
CURRENT FISCAL YEAR 73	.25	76

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Administrative Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Morrissey, R. L., CPT, VC

TELEPHONE: 303 366 5311 X26111

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

Foreign Intelligence not Considered

DA

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Military Research Projects; (U) Radioisotopes; (U) Instrumentation; (U) Data Acquisition

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

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 USAMRNL mission.

24. (U) Methodology research is conducted as required to improve existing procedures. Nine automatic sample changing radiation detection instruments are maintained for detection of beta and gamma radiation. All aspects of the radiological protection program as required by AEC licensure are conducted.

25. (U) 71 07 - 72 06 Two research protocols (St-1 and St-2 of this work unit) have been initiated, completed, and the results used to determine optimum liquid scintillation counting solutions for various radioisotopes and applications at USAMRNL. As a result, counting stability and thus reliability of results have been markedly improved for all isotopes counted and particular improvement is apparent in ^{45}Ca, ^{32}P, and ^{35}S counting procedures. Computer programs for disintegrations per minute (DPM) calculations are being utilized more extensively with all USAMRNL investigations now utilizing computer programs for these basic calculations. Direct computer calculations beyond DPM calculations are also being accomplished for specific studies, and procedures are being developed for generalized programs to calculate the following as requested by investigators: 1. DPM X (constant factor provided by the investigator); 2. DPM X (average of standards for an assay run X constant factor provided by the investigator); 3. Y when X = DPM, samples 2 through A (card imput) are standards with known conc. of Y, and the respective concentrations of Y are entered on a card with the program.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3A062110A822	Military Internal Medicine
WORK UNIT NO.	079	Radioisotope Support for Military Medical Research

Radioisotope support functions as defined in MRNL Reg. 40-14 have been maintained. Use of computer technology for calculations related to assays utilizing radiosotopes has continued to expand. Two methodology research protocols were initiated, completed, and the results used to modify and improve the radioisotope counting procedures employed at USAMRNL.

BODY OF REPORT

WORK UNIT NO. 079 — Radioisotope Support for Military Medical Research

STUDY NO. 1 — Counting Stability of Five Radioisotopes in Seven Different Liquid Scintillation Counting Solutions

PROBLEM:

Brays counting solution was being used extensively within USAMRNL, including counting of ^{35}S, ^{32}P, ^{45}Ca, ^{3}H and ^{14}C. Visual observation of the counting procedures revealed that some samples developed color changes with time. Also, it was observed that when dual label $^{3}H/^{35}S$ samples were counted, refrigerated for a period of time (1-2 weeks) and recounted, the DPM results were different. A review of the literature failed to produce data on relative stability and counting efficiency for the isotopes and counting solutions available.

RESULTS AND DISCUSSION OF THE RESULTS:

The subject samples were prepared in replicas of five, counted, and graphs were prepared illustrating counting efficiency versus time for each of the five isotopes (^{14}C, ^{3}H, ^{35}S, ^{45}Ca, and ^{32}P) in each of the seven counting solutions (10 percent BBS-3 in toluene, Instagel, Aquasol, Brays, Cellosolve, 23 percent ethanol in toluene and Packard Oxidizer Formula II). The detailed results are being prepared for publication. The major impact of the study has been the nearly complete curtailment of the use of Brays and replacement with 10 percent BBS-3 in toluene or Aquasol, depending on the counting situation. Recommendations for specific counting situations are given below.

STUDY NO. 2 — Counting Stability of ^{45}Ca, ^{32}P and ^{35}S in Aquasol plus Water and Instagel plus Water

PROBLEM:

The results of Study No. 1 of this work unit failed to reveal counting methods that were adequate for all ^{35}S, ^{45}Ca, and ^{32}P counting requirements. Sample precipitation and adsorption to the glass counting vials were suspected as part of the problem. Thus, it was expected that gelling the sample might prevent these effects.

RESULTS AND DISCUSSION OF THE RESULTS:

More than 30.0 percent increase in count rate had occurred within 28 days when ^{32}P was counted in any of the seven counting solutions listed in Study No. 1. Similar results were obtained when Aquasol was gelled by adding 5 ml of water to 10 ml of Aquasol (33% H_2O). However, when samples were gelled by adding 3 ml of water to 10 ml of Aquasol (23% H_2O), less than 2.5 percent change in efficiency had occurred after 28 days and less than 1.0 percent change had occurred at 8 days. Both ^{35}S and ^{45}Ca were also stable (less than 1.5% change in efficiency) in 10 ml Aquasol plus 3 ml of water. Use of polyethylene vials did not significantly improve stability over that obtained with borosilicate glass vials.

CONCLUSIONS:

Selection of a liquid scintillation counting solution and method that is optimum for the isotope and sample form is extremely important. Within three days, as much as 15, 10 and 7 percent change in count rate was observed for ^{32}P, ^{35}S, and ^{45}Ca respectively. When appropriate methods revealed by the above studies were employed, this error could be reduced to less than 1 percent.

RECOMMENDATIONS:

Recommendations for various counting requirements are indicated below.

1. ^{14}Carbon can be counted at efficiencies of 87 to 90 percent in 10-percent BBS-3 in toluene, Instagel, and Aquasol with good stability (< 1.2 percent change) for at least 21 days.

2. ^{3}Hydrogen can be counted at efficiencies above 45 percent in 10 percent BBS-3 in toluene, Instagel, Packard Oxidizer Formula 2, and Aquasol with good stability (< 1.0 percent change) for at least 21 days.

3. ^{35}Sulfur can be counted in 10 percent BBS-3 in toluene; or Instagel or Aquasol can be used but the sample must be gelled by adding 3.0 ml of water to 10.0 ml of counting solution (23% H_2O). Samples are stable (< 1.5 percent change) for at least 23 days when treated in this manner.

4. ^{45}Calcium can be counted in 10 ml of Aquasol or Instagel, but it must be gelled with 3 ml of H_2O prior to counting in order to prevent loss of counting efficiency with time. Such samples are stable (< 2.0 percent change) for 23 days.

Radioisotope Support for Military Medical Research (Cont)

5. ^{32}Phosphorus can be counted in 10 ml of Aquasol gelled with 3 ml of H_2O for up to 8 days with less than 1.0 percent change in counting efficiency.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6364	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	082
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Precede with Security Classification Code)

(U) Mathematical and Computer Support of Military Bio-Medical Research

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

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

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 72	4	86
CURRENT FISCAL YEAR 73	4	120

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Computer Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Teplick, R. S., CPT, MC

TELEPHONE: 303 366 5311 X25130

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Goforth, H. H.

NAME: Lowell, J., PFC DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Mathematics; (U) Statistics; (U) Research Data; (U) Processing and Analysis; (U) Support of Military Bio-Medical Research

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

23. (U) To provide mathematical, statistical and computer support for Military Biomedical Research at USAMRNL.

24. (U) An RCA 301/355 Electronic Digital Computer and a GE 600 Remote teletype terminal Digital Computer are available for the information processing requirements of laboratory investigators. In addition, mathematical and statistical consultation and services are available. Investigative support will be provided for projects involving the development of mathematical models. Study No. 1 will be concerned with organizing, documenting and maintaining a library of computer programs related to the areas of mathematics, statistics, file manipulation and data reduction. It also will be concerned with development of a computer program to perform the calculations required to determine the nutrient content of military rations determined during Military Nutrition Surveys. In addition it will make mathematical and statistical services and consultations available to laboratory investigators.

25. (U) 71 07 - 72 06 Study No. 4 - The library of mathematical, statistical and utility routines developed in a previous study using the FORTRAN language has been modified and/or expanded to suit the investigators' needs in analyzing the gathered data. The FORTRAN compiler also has been modified so as to increase its capabilities. New mathematical models have been tested to facilitate the statistical analyses required by the investigators.

*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO.	3A062110A822	Military Internal Medicine
WORK UNIT NO.	082	Mathematical and Computer Support of Military Biomedical Research

STUDY NO. 4 Computerized Mathematical Applications

The goal of this work unit is to support the Nutrition Laboratory investigators with up-to-date techniques utilizing a digital computer as the instrument that handles the statistical and mathematical computations of the researched investigations.

BODY OF REPORT

WORK UNIT NO. 082 — Mathematical and Computer Support of Bio-Medical Research

STUDY NO. 4 — Computerized Mathematical Applications

PROBLEM:

USAMRNL investigators have been generating a large amount of data which should be processed by a computer for its complete analysis. Use of desk calculators should be kept to a minimum as the Laboratory has a computer facility. Using a computer, programs may be developed to handle with greater precision and greater speed, the computation of the data collected by the investigators.

RESULTS AND DISCUSSION OF THE RESULTS:

Taking advantage of the FORTRAN language capability of the existing computer, a Generalized Research Analysis Statistical System (GRASS) was developed. Computer programs were written and the mechanics of how to utilize the system have been explained to the researching personnel as that data to be statistically analyzed would be set up by the interested investigator who need not know the intricacies of computer programming. This statistical system has continued to grow through the year, adding subroutines and improving methodology of handling various statistical analyses.

The FORTRAN compiler also has been modified numerous times in order to optimize the existing computer capabilities. It also has expanded the potentialities of mathematical manipulation and therefore increased its usability in processing the statistical analyses required by the investigators. Other accomplishments are as follows:

a. Compartment Modeling for Vitamin A. This involves three independent projects:

(1) Non-Linear Curve Fitting. This has involved the development of a conjugate gradient curve fitting program with a modified search to run on the RCA 301. This routine has been implemented and is being utilized in conjunction with another fitting routine to fit the vitamin data.

(2) Creation and Solution of a Compartment Model. A 4 compartment model was developed to simplistically represent vitamin A distribution. An analytic solution was obtained.

Mathematical and Computer Support of Bio-Medical Research (Cont)

(3) Parameter Estimation. The first attempts to completely solve the model for the various parameters was partially unsuccessful, yielding contradictory values. Nonetheless rates of utilization for 4 compartments were theoretically derived. Data refinements and novel curve fitting techniques developed will hopefully facilitate a complete solution for all eight subjects.

b. Simulation. Multicompartment models have simulated a LEANS (Lehigh Analogue Simulator) in an effort to determine the effects of parameter variation and to compare equilibrium with non-equilibtium situations. These simulations will be randomized and the ability to solve compartment models as a function of the errors in the method will be estimated.

c. Classification and Discrimination. Work has continued in the theoretical aspects and ramifications of a method proposed to maximally discriminate groups with a minimal number of variables which define the groups. To this end a generalized model of disease is being developed to quantitatively test this method.

CONCLUSIONS:

This work unit has continued to progress at an exemplary rate. The products obtained so far have been of great help to the investigating teams working at the USAMRNL.

RECOMMENDATIONS:

Search for inovating methodology must continue so as to facilitate the mathematical support of biomedical research. The utilization of the computer, as a researching tool, already has been accepted by the investigators assigned to USAMRNL. The new mathematical computerize techniques will be made available to the investigators once these approaches have been tested and found reliable and feasible by using a digital computer as the data handling instrument.

PUBLICATIONS:

1. Messa, C. J., J. R. zumBrunnen, K. C. Stuart and H. Goforth. Generalized Research Analysis Statistical System, USAMRNL Laboratory Report No. 329, 1971.

2. Wallace, D. L., E. M. Baker, J. E. Canham, N. Raica, H. E. Sauberlich, R. S. Teplick and R. E. Hodges. Vitamin A depletion in the human male adult. Federation Proc. 31: 672, Abs, 1972.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6370	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8. DISP'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	083
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG	114 (f)		

11. TITLE (Procede with Security Classification Code)*

(U) Military Food Hygiene (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

006500 Food, 007800 Hyg. & Sanitation, 016800 Toxicology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 04	CONT	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:* Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	1.2	60
FISCAL YEAR CURRENT 73	2.4	73

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J.E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
Food Hygiene Division

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME:* Fowler, James L., COL, VC

TELEPHONE: 303 366 5311 X22223

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: DA

NAME:

21. GENERAL USE

Foreign Intelligence Not Considered

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Military Subsistence; (U) Wholesomeness; (U) Freeze-dehydration; (U) Safety; (U) Foodborne Diseases; (U) Food Contaminants

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

23. (U) Evaluate food hygiene aspects of military subsistence, present and future supplies; conduct research in established wholesomeness problem areas; utilize findings in recommending procurement specifications of new or existing military subsistence items.

24. (U) Maintain liaison with military preventive medicine, Public Health Svc, Fd & Drug Admin. and food science and technology depts of Universities to keep abreast with problem areas of food hygiene and to prevent duplication of on-going research. Conduct research in-house or collaborates on research contracts with other food research organizations. Maintain current reference information in the fields of microbiology, mycology, virology, toxicology, chemistry and radiobiology as related to food research and processing.

25. (U) 71 07 - 72 06 -- The first study performed was the microbiological evaluation of the Food Packet, Long Range Patrol, after storage under simulated field conditions. Analyses, which were completed in Dec. 1972, tend to indicate poor quality control during processing though no pathogenic organisms were identified. Personnel were trained, and research in the study of staphylococci and salmonellae in military foods and rations begun but data is too preliminary to permit conclusions.

During the period covered by this report specification reviews were performed by the division, and appropriate comments in the area of food hygiene made. The area of specification reviews is considered most important, since much of the methodology investigated or developed by the division will eventually be offered for adoption through this medium.

*Available to contractors upon originator's approval.

DD, FORM 1 MAR 68 1498

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine

WORK UNIT NO. 083 Military Food Hygiene

The following investigations have been conducted under this work unit:

STUDY NO. 1 Identification and Quantitation of the Microbiological Flora of the Food Packet, Long Range Patrol Under Simulated Field Conditions

STUDY NO. 2 Survival Time of Experimentally Inoculated Staphylococcus aureus In Military Freeze-Dehydrated Products

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

STUDY NO. 4 A Survey for Salmonellae In Beef

Interest has recently been focused by governmental and consumer agencies alike on insuring safety in food manufacture, technology, handling, storage, and serving. The Food Hygiene Division was established to provide a nucleus for a continuing research program in military food hygiene. Emphasis has been placed on food microbiology with its attendant problems. Research in the area of methodology in the detection and enumeration of foodborne pathogens has been conducted, with the aim of developing a data base for recommending bacterial standards for military subsistence and the techniques for performing the analyses.

Liaison has been established with the military testing laboratories, Food and Drug Administration Laboratory officials, U.S. Army Natick Laboratory personnel, and various food science departments of recognized universities. Current problems in the area of food hygiene are being investigated.

BODY OF REPORT

WORK UNIT NO. 083 — Military Food Hygiene

STUDY NO. 1 — Identification and Quantitation of the Microbiological Flora of the Food Packet, Long Range Patrol Under Simulated Field Conditions

PROBLEM:

Military operational rations are designed as special purpose food items, and often incorporate new and advanced techniques or procedures. An example of this was the development of the Food Packet, Long Range Patrol, which was found to be highly acceptable by troops in the field. Unfortunately, a backlog of information, usually available from commercial sources, was not available on this product since it was newly developed and used freeze-dehydration techniques in its production. The microbiological specifications under which these products were procured was suspected of being lax. The Food Packet, Long Range Patrol was therefore reexaminated for its microbiological characteristics. Particular emphasis was placed on the detection and enumeration of pathogenic species.

RESULTS AND DISCUSSION OF THE RESULTS:

Representative menus were stored under three conditions simulating field conditions, and subsequently examined for microbiological flora. The storage conditions were: Phase I, room temperature storage simulating ambient temperature in conventional dry storage warehouses; Phase II, high altitude storage at the Pikes Peak Laboratory Facility simulating storage in mountainous areas where radical extremes of temperature and internal packaging pressure were likely to occur; and Phase III, refrigerated storage at 38°F simulating more ideal storage conditions. Packets were held in these storage conditions for varying periods of time, and were returned to 3°C storage until analyzed.

The varied storage conditions did not appear to greatly influence the microbiological flora. Standard plate counts were found to be acceptable in all cases except for one food packet which was found to be 400,000/gm or twice the allowable specification limit. The flora was found to be the same under the three storage conditions, and were identified as gram positive aerobic bacilli, micrococci, enterococci, yeasts, and molds. Of particular significance was the extreme variability of the microflora within different

packets of the same meal, indicating poor quality control during processing. No pathogenic organisms were isolated from any of the packets in the three phases of testing.

CONCLUSIONS AND RECOMMENDATIONS:

On the basis of data generated in this investigation, the following suggestions were submitted:

a. The microbiological limits of the Food Packet, Long Range Patrol should be changed to:

Standard Plate Count	-	NMT 100,000/gm
Coliform organisms	-	NMT 10/gm
E. coli	-	NMT 1/gm
Fecal streptococci	-	NMT 1/gm
Clostridium perfringens	-	NMT 1/gm
Coagulase-positive staphylococci	-	NMT 1/gm
Salmonellae	-	NMT 1/gm
Yeast & Mold	-	NMT 100/gm

NMT = Not more than

b. The microbiological test procedure should be modified to include analyses for organisms listed in (a) above.

c. The methods of detecting coagulase-positive staphylococci should be investigated, and suitable procedures developed and media recommended for adequate methods of detection and enumeration of this organism.

d. Notification of proper authority that the following statement be printed on each Food Packet, Long Range Patrol: "To be eaten within two hours of rehydration."

PUBLICATIONS:

Henderson, J. E., B. D. Nelson, and J. L. Fowler. Identification and quantitation of the microbiological flora of the Food Packet,

Long Range Patrol under simulated field conditions. USAMRNL Laboratory Report No. 333, April 1972.

STUDY NO. 2

Survival Time of Experimentally Inoculated Staphylococcus aureus in Military Freeze-Dehydrated Products

PROBLEM:

The military services have food requirements which are unique to the food industry in many ways. Freeze-dehydrated products appear to be admirably suited for use in military applications due to their high stability, prolonged shelf life, and low weight. The freeze-dehydrated process, however, is fairly new, and little information appears to be available on the survival time of Staphylococcus aureus in military freeze-dehydrated foods. Experimentally inoculated food packets were prepared, and the survival rate of Staphylococcus aureus through the freeze-dehydration process determined. The rate of post-dehydration bacterial death is being determined by periodic analyses of samples stored under refrigeration and at ambient room temperature.

RESULTS AND DISCUSSION OF THE RESULTS:

Analyses of samples before and after freeze-dehydration indicates a slight reduction in the number of viable Staphylococcus aureus due to the freeze-dehydration process, however, it does not appear to be of practical value in reducing bacterial population. Samples stored at ambient room temperature have shown a marked reduction in numbers (from 5×10^4 to 6×10^2 in seven months) while identical samples stored at -20°F have shown little reduction. Analyses of samples at periodic intervals will be continued for a total period of 18 months, at which time final evaluation of the results will be accomplished.

CONCLUSIONS:

Data generated indicates a slight reduction in numbers of viable Staphylococcus aureus due to the freeze-dehydration process. A significant reduction in numbers has been detected in samples of military food stored at ambient room temperature, while little reduction in numbers has been seen in identical samples stored at -20°F.

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

PROBLEM:

The danger of food poisoning from the enterotoxin produced by certain strains of Staphylococcus aureus is well known. Modern food practices and methods, with mass serving techniques increasing, make it imperative that the food be free of this bacterial toxin. Reliance is placed on the freedom of the product from viable coagulase-positive staphylococci as determined by cultural methods.

The techniques of detecting and enumerating these organisms are, however, not standardized among the regulatory laboratories. Many of the media and laboratory techniques available to the food microbiologist are modifications of media developed for clinical use, and have inherent drawbacks in their use. One of the primary aims in food microbiology is accurate enumeration; many of the media available today give widely varying results.

Investigations into this subject are being made to determine the most reliable media, in terms of highest recovery of organisms, among six commercially available preparations. Comparison of direct plating techniques, pre-enrichment most probable number techniques, and selective enrichment most probable number procedures are being made. Statistical evaluation of both media and techniques will be performed.

RESULTS AND DISCUSSION OF THE RESULTS:

Preliminary results indicate that a wide range of counts are obtained from an experimentally inoculated freeze-dehydrated food sample with the six commercially available prepared media. Counts have consistently indicated that one media, officially recognized as a medium of choice for enumeration, gives a lower count than other media. Extreme difficulty has been encountered with one medium (containing a high percentage of sodium chloride) in making proper pour plates. This problem is due to the high temperature at which the medium solidifies, thereby possibly destroying part of the organisms because of the high temperature required to keep the media in a liquid state.

Preliminary comparison of the two most-probable-number techniques indicates a considerable difference in the pre-enrichment technique and the selective enrichment technique. Examination of the raw data does not indicate a high degree of difference between confirmatory plating media used; however, statistical analyses will be performed within techniques and between techniques when the study is completed.

CONCLUSIONS:

No conclusions have yet been reached, since the study is not completed.

STUDY NO. 4 A Survey for Salmonellae in Beef

PROBLEM:

Meat and meat products play a major role in reported Salmonella outbreaks in humans. Even though there are numerous reports of Salmonella isolations from calves and adult cattle, limited data is available concerning the potential infectiveness and/or contamination of beef and beef products. Several reports in the literature, however, have demonstrated that contaminated beef can be a source of Salmonella food poisoning in man.

Since the Army is a large consumer of beef, a safe, wholesome product is desirable to maintain the health of the soldier and his dependents. Isolation and screening procedures would hopefully help to determine if potentially pathogenic Salmonella exist in beef and beef products as they are handled by the present day system and as they are exposed to a military environment. Efficient accelerated screening procedures are needed to readily detect Salmonella in the field and to decrease the time necessary to diagnose food poisoning outbreaks.

RESULTS AND DISCUSSION OF THE RESULTS:

Preliminary results indicate an undetectable level of Salmonella from commissary beef carcass swabs. The isolation procedure used was that recommended by the National Communicable Disease Center (CDC). Either this procedure has been unable to detect low levels of the bacterium or no viable Salmonella have been present.

Attempts to decrease the time required for isolation by using a shaker bath technique have not had any advantages over the recommended procedure.

Additional preliminary results have not detected Salmonella from commissary ground beef. Evaluation of screening procedures for Salmonella are being carried out by seeding the sampled ground beef with different salmonella sertotypes, freezing them, and then sampling at periodic intervals. The screening techniques being employed include plate agglutination (polyvalent O and polyvalent H antisera), tube agglutination (polyvalent H antiserum), and the fluorescent antibody tagging method. In all instances, the isolation procedures recommended by CDC are carried out to check the efficiency of the screening techniques.

Enough data has not been collected at this time to allow adequate evaluation of the screening methods.

CONCLUSIONS:

The inability to detect Salmonella in commissary carcass beef and ground beef does not necessarily indicate their absence, but does possibly indicate low numbers if they are present at all.

Serological screening procedures are considerably less time consuming compared to the recommended isolation procedures for Salmonella but their efficiency and accuracy remain to be evaluated.

RECOMMENDATIONS:

1. Continue evaluation of serological screening procedures for Salmonella.

2. Continue further isolation attempts for Salmonella from commissary carcass beef and ground beef.

PUBLICATIONS:

RESEARCH WORK COMPLETED ELSEWHERE BUT MANUSCRIPTS PUBLISHED OR COMPLETED IN FY 1972.

1. Ruff, M.D., J.L. Fowler, K. Matsuda and R.C. Fernau. Babesia gibsoni: Influence of infection on serum enzymes of dogs. S. E. Asia J. of Trop. Med. and Pub. Hlth. 2:297, 1971.

2. Scott, M.V., J.L. Fowler and M.D. Ruff. Babesia gibsoni: Infection of a dog in Korea. JAVMA, 159:1122, 1971.

3. Fowler, J.L., Y. Furusho and R.C. Fernau. Further testing of fenthion for prophylactic effects against the developing stages

of Dirofilaria immitis. S. E. Asia J. of Trop. Med. and Pub. Hlth. 2:466, 1971.

4. Fowler, J.L., J.L. Young, R.C. Fernau and D.E. Ferguson. Laboratory technique for producing microfilariae of Dirofilaria immitis in mosquitoes. USAMRNL Laboratory Report No. 331, February 1972.

5. Fowler, J.L., K. Matsuda and R.C. Fernau. Experimental infection of the domestic cat with Dirofilaria immitis. JAAHA, 8:79, 1972.

6. Fowler, J.L., M.D. Ruff, R.C. Fernau and Y. Furusho. Babesia gibsoni: Chemotherapy in dogs. AJVR 33:1109, 1972.

7. Fowler, J.L., M.D. Ruff, R.C. Fernau and D.E. Ferguson. Biochemical parameters of dogs infected with Babesia gibsoni. Cornell Vet. (In Press)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6371	72 06 30	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	II Termination	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	034
b. CONTRIBUTING				
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Procede with Security Classification Code)

(U) Immunologic Research in Tuberculosis (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	30 June 1972	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER: Not Applicable

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	2.1	30
FISCAL YEAR CURRENT 72	2.5	35

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Microbiology Division

ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME: Brown, G. L., LTC, MSC

TELEPHONE: 303 366 5311 X24234

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Rothlauf, Mary V.

NAME: DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Immunity; (U) Tuberculosis; (U) Vaccine; (U) Gamma Globulin; (U) Immunochemistry

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

23. (U) There is an annual turnover and exposure of approximately 500,000 U. S. troops in areas where incidence of active tuberculosis is between 10-25% (15-20 million infectious cases annually); no acceptable vaccine is available. To meet the objectives for an adequate T.B. control program there exists requirements for: (1) rapid serodiagnostic tests to evaluate cellular and humoral antibody responses following tuberculosis infection and immunization; (2) development of a vaccine against tuberculosis which protects without eliciting positive tuberculin skin test; (3) evaluation of immune responses as affected by physiological extrinsic stress factors.

24. (U) Strains of M. tuberculosis are used for preparation of vaccines, bacterial fractions, and for animal challenge experiments. Sequence and kinetics of molecular species of antibody response to mycobacterial antigens, rapid serodiagnostic tests, and relationship of delayed hypersensitivity to acquired antituberculous immunity are studied. Resistance to infection and cellular antibodies brought about by the environmental extrinsic factors are evaluated.

25. (U) 71 07 - 72 06 (1) Mice vaccinated with a purified glycoprotein fraction, isolated from BCG culture filtrate, developed 80% acquired resistance to tuberculosis with no evidence of delayed hypersensitivity. (2) A total of 568 human sera samples were assayed for antibodies to tuberculosis with the Latex Flocculation test; test proved to be sensitive yet nonspecific in titration against humoral antibodies to tuberculosis. (3) Development of delayed hypersensitivity to viable BCG was significantly reduced in mice maintained in Vitamin E depleted chow, this immune response was restored when animals were placed on diet containing N,N-diphenyl-phenylene diamine. (4) Project termined due to transfer of the Microbiology Division to Fitzsimons General Hospital and OMA funding.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498

ABSTRACT

PROJECT NO.	3A062110A822	Military Internal Medicine
WORK UNIT NO.	084	Immunologic Research in Tuberculosis

The following investigations have been conducted under this work unit:

STUDY NO. 4 Immunizing capacity of viable and nonliving components of BCG against experimental tuberculous infection

STUDY NO. 5 Effect of extrinsic stress factors on the immune response to BCG vaccination

Study No. 4. The relationship of nonviable Bacillus Calmette-Guérin (BCG) components for usage as immunogenic agents for the development of acquired resistance to experimental tuberculous infection was evaluated in mice. Results showed that the protection developed with a purified glycoprotein fraction, isolated from BCG culture filtrate, was of the same magnitude as with viable BCG vaccination. In addition, these animals did not develop positive skin test reaction when tested with purified tubercular protein derivative of M. tuberculosis. A total of 568 human serum samples were assayed for humoral antibodies to tuberculosis with the Latex Flocculation Test (LFT). The latex particles were sensitized with purified protein derivative extracted from M. tuberculosis culture filtrate. The LFT proved to be a sensitive test yet serologically was shown to be nonspecific in titration against humoral tuberculosis antibodies.

Study No. 5. Investigative studies revealed: (a) Mice exposed to various extrinsic and intrinsic stress factors--altitude, starvation, thymectomy, diet with excess or depleted alpha tocopherol--when vaccinated with viable BCG developed identical degrees of acquired resistance to tuberculosis on challenge with virulent strains: 100% survival rate 30 days post challenge infection; (b) the humoral immunoglobulin response to sheep erythrocyte was enhanced in mice maintained a high levels of Vitamin E (2.0 IU/gram chow): three-fold increase of circulating anti-sheep erythrocyte hemagglutinating titer and plaque forming units from splenic lymphoid cells; (c) delayed hypersensitivity to viable BCG, as measured by foot-pad swelling 24 hours post injection with purified protein derivative, was significantly reduced in mice maintained on Vitamin E deficient chow. These animals regained the delayed

hypersensitivity response to tuberculoprotein constituents when placed on diet containing N,N-diphenyl-phenylene diamine (100 mg/lb chow); (d) thymectomy followed with viable BCG vaccination did not affect the development of delayed hypersensitivity or acquired resistance to tuberculosis in mice.

BODY OF REPORT

WORK UNIT NO. 084 — Immunologic Research in Tuberculosis

STUDY NO. 4 — Immunizing capacity of viable and components of BCG against experimental tuberculous infection

PROBLEM:

Vaccination against tuberculosis with living and nonliving M. tuberculosis preparations date from the discovery of the tubercle bacillus. Contradictory results have been reported as to the protection afforded with components extracted from nonliving Bacillus Calmette-Guérin (BCG) preparations. Presently, there are still many reasons for continuing research in the development of a nonliving antituberculosis vaccine: of significance, safety of a nonliving bacterial preparation, reduced allergenicity to tuberculin sensitization and storage stability. Exposure of U. S. troops in world areas where there is a high incidence of active tuberculosis necessitates an active vaccine evaluation program. An adequate T. B. control program also requires the development of a single, noncomplicated, serologic test to supplement existing criteria for detecting active tuberculosis.

RESULTS AND DISCUSSION OF THE RESULTS:

Mice vaccinated with various forms of BCG preparations (viable, formalin killed, cytoplasmic or culture filtrate components) developed various degrees of acquired resistance to tuberculosis and delayed hypersensitivity to tuberculoproteins. Animals immunized with viable BCG cells developed 100% survival rate 30 days post challenge with virulent M. tuberculosis. This group of mice also developed maximum delayed type hypersensitivity to tuberculoproteins. Minimal protection, 68% survival rate 30 days post challenge, was seen in animals vaccinated with formalin killed cells. No protection was observed in the group vaccinated with cytoplasmic BCG components. Of significance was the observation that animals vaccinated with intact concentrated BCG culture filtrate not only failed to develop acquired resistance to tuberculosis but such treatment resulted in increased mortality on challenge. Histologic evaluation of splenic tissues from these animals showed marked lymphoid depletion. This histologic observation was not observed in animals nonvaccinated or immunized with viable BCG when challenged with virulent M. tuberculosis.

Mice vaccinated with a purified BCG culture filtrate component (gel filtration chromatographic peak 1, Bio-Rad P-2, 1.5 x 90 cm column) developed 80% acquired resistance to tuberculosis and no evidence of delayed hypersensitivity. Chemical and UV spectral evaluation of this filtrate component identified this substance as a glycoprotein. Amino acid analysis showed the presence of a predominance of nonpolar amino acids, except lysine (12.9%); lysine probably adds to the antigenicity of this fraction. Four purified components isolated from BCG cell cytoplasm, when tested individually in mice, were shown to have no antigenic properties for the development of acquired resistance to tuberculosis.

A total of 568 human serum samples were serologically evaluated for tuberculosis humoral antibodies with Latex Flocculation Test (LFT). The latex particles were suspended in Borate-Saline buffer (0.1 M, pH 8.5) and were sensitized with either tuberculoproteins or with tuberculopolysaccharides. Only the latex particles sensitized with tuberculoproteins formed visible aggregates in serum containing tubercular antibodies. Total serum samples tested included: (a) 490 representing 55 tuberculosis patients; (b) 51 representing 28 skin negative individuals; and (c) 27 representing 7 skin positive nontubercular individuals. The LFT proved to be extremely sensitive yet serologically nonspecific in titration against circulating antibodies to tuberculosis. Recorded titers of $\geq$1:160 were as follows: 28.16%, clinically diagnosed tubercular patients; 5.4%, skin test positive nontubercular individuals; 3.92%, skin test negative individuals. Titers of 1:2560 were observed only in two samples from tuberculosis patients.

CONCLUSIONS:

Acquired resistance in mice against experimental tuberculous infections does not require vaccination with living BCG cells. It appears that a glycoprotein component from BCG culture filtrate is equally immunogenic as viable BCG preparations. Furthermore, this filtrate component does not elicit delayed type hypersensitivity to tuberculoproteins. The Latex Flocculation Test proved to be sensitive yet serologically nonspecific in titration of humoral antibodies to tuberculosis.

RECOMMENDATIONS:

Studies to evaluate BCG culture filtrate component which evokes acquired resistance to tuberculosis with no allergic properties should be continued. Related investigations should concentrate

on the characterization of the filtrate components(s) which appear to block immune response to tuberculosis infection.

PUBLICATIONS:

None

STUDY NO. 5 — Effect of extrinsic stress factors on the immune response to BCG vaccination

PROBLEM:

The adverse effect(s) of nutritional and environmental extrinsic factors on native or acquired immunity have been observed over many years. Limited investigations have examined lymphoid tissue depletion and its effect on the immunologic apparatus. The conclusions available from these studies suggest that the total information on the subject of stress factors and immunity is very far from being complete. The importance of defining the precise role of how such a factor alters host response to infection is self-evident.

RESULTS AND DISCUSSION OF THE RESULTS:

The effect of altitude stress, starvation, thymectomy, and Vitamin E on the immune response to M. tuberculosis was evaluated in mice vaccinated with viable BCG. Of significance was the observation that regardless of the experimental variable applied, the response of the immunization with viable BCG was the same: 100% survival rate 30 days post challenge infection (%, S-30). Animals vaccinated with nonviable BCG cells and maintained at an altitude of 14,100 feet developed less protection to tuberculosis as compared with animals similarly treated but maintained at 5330 feet: S-30, 37% and 52%, respectively.

Immunologic response in mice sensitized with sheep erythrocytes and maintained on high dietary levels of alpha-tocopherol (2.0 IU/gm chow) was enhanced. A three-fold increase of circulating anti-sheep erythrocyte hemagglutinin and plaque forming units from splenic tissue was observed. Mice vaccinated with viable BCG and maintained for 30 days on high levels of alpha tocopherol chow or an alpha tocopheral-depleted diet developed high degrees of acquired resistance to tuberculosis: 100% S-30. On the other hand, delayed hypersensitivity to tuberculo-proteins was not observed in animals

maintained on Vitamin E depleted chow. The group of Vitamin E deficient mice when treated with N,N,diphenyl-phenylene diamine developed delayed hypersensitivity.

Partial lymphoid tissue depletion, affected by thymectomy, and subsequent maintenance of this group of animals on chow with normal, depleted or high levels of Vitamin E did not affect the development of delayed hypersensitivity or acquired resistance to tuberculosis; these mice were challenged with viable H37Rv M. tuberculosis 30 days post immunization. The data suggest that the development of delayed hypersensitivity is not dependent on a continuous availability of thymic derived lymphocytes. It appears that circulating thymic derived lymphocytes (T-lymphocytes) or bone marrow derived lymphocytes (B-lymphocytes) can provide the necessary stem cells for cellular immunity. Additional studies using sublethal radiation with thymectomy are indicated to resolve the question as to the origin of cellular immunity (i.e., thymus or bone marrow). In the present investigation it would appear that thymectomy does not eliminate all of the available T-lymphocytes. The data further shows that in mice Vitamin E is required for the development of delayed hypersensitivity to tuberculosis only in a system in which the thymus tissue is intact. Vitamin E depletion seems to affect only the development of thymocytes within the intact thymic tissue with no apparent effect on formed and circulating T-lymphocytes.

CONCLUSIONS:

Stress of high altitude has an inhibitory effect on the cellular immune response to BCG vaccination with no apparent effect to humoral response. This finding suggests that acquired resistance to tuberculosis is mediated primarily by a humoral immune response mechanism. Increased intake of Vitamin E during the vaccination process stimulates humoral immune response with no increase to resulting acquired immunity. On the other hand, in the same system depletion of Vitamin E blocks the development of cellular immune mechanism; reversal of this block occurs when the test animals are fed chow containing N,N, diphenyl-phenylene diamine. Thymectomy does not impair cellular or humoral immune response to BCG vaccination.

RECOMMENDATIONS:

Studies to characterize effects and role of Vitamin E on the regulation of the immune response should be continued.

PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6376	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A822	00	085
b. CONTRIBUTING				[redacted]
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Precede with Security Classification Code)*

(U) Nutritional Requirements of Military Personnel

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

00800 Agri. 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

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

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING 72	7.6	146
CURRENT 73	8.0	155

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

TELEPHONE: 303 366-5311 X21108

20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

ADDRESS:* Chemistry Division
Fitzsimons General Hospital
Denver, Colorado 80240

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

NAME:* Baker, E. M., COL

TELEPHONE: 303 366-5311 X24214

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Sauberlich, H. E.

NAME: Raica, N., Jr. DA

21. GENERAL USE

Foreign Intelligence not Considered

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 (Furnish 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) 71 07 - 72 06 Data from the human vitamin A study have been placed in the computer file for evaluation and statistical analysis. Chromatographic and radiometric analyses of urine from human subjects administered 1-^{14}C-4-^{3}H labeled ascorbic acid were observed to contain several labeled compounds. One of the compounds, ascorbate-2-sulfate, is excreted at a level of 30-60 mg/day. With the onset of scurvy, this level decreases. The pool size of ascorbate sulfate in the human is approximately 10 to 20 mM. An optimized spectrophotometric method has been utilized for the assay of plasma and erythrocyte GPT and GOT samples obtained from military nutrition surveys. Extensive analytical biochemical support was provided the military nutrition surveys conducted at Lowry AFB, Denver; Ft. Lewis, WA; and Ft. Myer, VA.

*Available to contractors upon originator's approval.

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	Experimental Vitamin A Deficiency in Humans
STUDY NO. 3	Vitamin B-6 Metabolism and Requirement in Man
STUDY NO. 4	Military Nutrition Surveys

Study No. 1. Chromatographic and radiometric assays of urine from subjects receiving ascorbate-1-^{14}C-4-^{3}H have shown a variety of labeled products. A chromatographic and colorimetric assay for ascorbate-2-sulfate in biological samples has been developed, and the application of this assay has shown that ascorbate-2-sulfate is excreted at 30-60 mg/day when calculated as the dipotassium salt. (Earlier reports have referred to this metabolite as ascorbate-3-sulfate, but a recent X-ray crystallographic study shows it is the 2-sulfate ester.) These levels decrease with the onset of scurvy. Cumulative excretion or specific activity calculations show an approximate pool size of ascorbate-2-sulfate of 10 to 20 mM.

Study No. 2. The data from the adult human vitamin A study have been committed to the computer file for evaluation. The evaluation and statistical analysis is in progress.

Study No. 3. An optimized spectrophotometric method has been utilized for the assay of plasma and erythrocyte GPT and GOT involving stimulation coefficients that are calculated from the effects of <u>in vitro</u> additions of pyridoxal phosphate in the assay. The procedure was applied to human samples obtained from military nutrition surveys. The assay, employing stimulation coefficients, provides an assessment of an individual's vitamin B-6 nutritional status.

Study No. 4. Extensive personnel and analytical services were provided in support of military nutrition surveys conducted at Lowry Air Force Base, Denver; Ft. Lewis, Washington; and Ft. Myer, Virginia. An intensive biochemical and nutrient analysis was performed on the blood, urine and/or diet samples obtained from these surveys.

BODY OF REPORT

WORK UNIT NO. 085 — Nutritional Requirements of Military Personnel

STUDY NO. 1 — Vitamin C Metabolism and Requirement in Man

PROBLEM:

This report described studies to identify ascorbic acid metabolites in man, to study their physiological role and to show their relationship to the nutritional needs for ascorbic acid.

RESULTS AND DISCUSSION OF THE RESULTS:

This group has synthesized and characterized ascorbate-2-sulfate, ascorbic-6-sulfate and iso-ascorbate-2-sulfate as reference compounds to identify the form of ascorbate sulfate excreted by man, rainbow trout, rats and guinea pigs.

Chromatographic and radiometric assays of urine from subjects receiving ascorbate-1-^{14}C-4-^{3}H have shown a variety of labeled products. A chromatographic and colorimetric assay for ascorbate-2-sulfate in biological samples has been developed, and the use of this assay has shown that ascorbate-2-sulfate is excreted at a level of 30 to 60 mg/day (calculated as the dipotassium salt), and these levels decrease with the onset of scurvy. Cumulative excretion or specific activity calculations show a pool size of ascorbate-2-sulfate of 10 to 20 mM. The ascorbate-2-sulfate pool equilibrates only slowly, if at all, with the reduced ascorbate pool. On zero ascorbate intake, the urinary ascorbate-2-sulfate specific activity decreased several fold indicating a third ascorbate pool. Thus, there appears to be at least three major ascorbate pools in man: reduced ascorbate, ascorbate-2-sulfate and a bound pool with a very long turnover time.

It was further found that ascorbate-2-sulfate represents 30% of the excreted ^{14}C and ^{3}H ingested as ascorbate-1-^{14}C-4-^{3}H. In addition, a derivative form, with a molecular weight of ~ 10,000, represents approximately 40% of the ^{14}C and ^{3}H found in human urine after ingesting ascorbate-1-^{14}C-4-^{3}H. This fraction may be cell membrane degradation products. Numerous other metabolites are present in small amounts, some containing ^{14}C only and others ^{3}H only.

CONCLUSIONS:

1. It would appear that there are at least three major ascorbate pools in man: reduced ascorbate, ascorbate-2-sulfate and a derivative pool with a very long turnover time.

2. Ascorbate-2-sulfate represents 30% of the excreted ^{14}C and ^{3}H ingested as ascorbate-1-^{14}C-^{3}H.

3. A derivative form with a molecular weight of approximately 10,000 and containing approximately 40% of ^{14}C and ^{3}H found in human urine was observed after ingesting ascorbate-1-^{14}C-4-^{3}H.

4. Numerous other metabolites are present, some containing ^{14}C only and others ^{3}H only.

5. Data indicate that the etiology of scurvy in man is as closely related to the body stores of ascorbate-2-sulfate and the bound ascorbate derivative as it is to the body stores of free ascorbate.

RECOMMENDATIONS:

A patent for the synthesis and use of ascorbate sulfate is being applied for through the U. S. Army Medical Research and Development Command and D.O.D. It is recommended that further work be done to ascertain if ascorbate sulfate can be converted back to ascorbate in animals and humans. Studies should be continued on elucidating the physiological role of both ascorbate and ascorbate sulfate.

PUBLICATIONS:

1. Baker, E. M., D. Hammer, S. C. March, B. M. Tolbert and J. E. Canham. Ascorbate sulfate a urinary metabolite of ascorbic acid in man. Science 173: 826, 1971.

2. Baker, E. M., J. E. Kennedy, B. M. Tolbert and J. E. Canham. Excretion and pool size of ascorbate sulfate and other ascorbate derivatives in man. Fed. Proc. 31: 2760 Abs 1972.

3. Tolbert, B. M., A. M. Spears, D. J. Isherwood, R. W. Atchley and E. M. Baker. Chemistry of the ascorbate sulfates. Fed. Proc. 31: 2761 Abs 1972.

4. March, S. C. A quantitative procedure for the assay of ascorbate-3-sulfate in biological samples. Fed. Proc. 31: 2762 Abs 1972.

5. Campeau, J. D., and S. C. March. Distribution of sulfur-35 in subcellular fractions from rat tissues following incubation with ascorbate-3-$^{35}SO_4$. Fed. Proc. 31: 2763 Abs 1972.

STUDY NO. 2

Experimental Vitamin A Deficiency in Humans

PROBLEM:

The objectives of this study with male human volunteers were: (1) to measure, by labeling with retinyl-^{14}C-acetate, the body pool(s) of vitamin A and to study its rate of depletion during vitamin A and β-carotene deprivation, (2) to induce deficiency of vitamin A in healthy men, (3) to observe the relationship between the size of the body pool(s) of vitamin A and clinical signs and symptoms of deficiency, (4) to estimate the minimal requirements for vitamin A, (5) to determine the amount of vitamin A necessary to replete the body pool(s) and alleviate clinical signs and symptoms of deficiency, and (6) to study vitamin A metabolites excreted via the urine.

RESULTS AND DISCUSSION OF THE RESULTS:

The data from the human vitamin A study have continued to become available throughout this fiscal year. As the data are received, they are committed to computer file for storage and analysis.

Isolation and identification of urinary metabolites of vitamin A progressed along the lines of refinement of methodology in chromatographic techniques. Some purified fractions have been obtained, but no conclusive identifications have been made.

CONCLUSIONS:

Interpretation of the results await the completion of the computer analysis of the data.

PUBLICATIONS:

1. Wallace, D. L., E. M. Baker, J. E. Canham, N. Raica, H. E. Sauberlich, R. S. Teplick and R. E. Hodges. Vitamin A depletion in the human male adult. Fed. Proc. 31: 2575 Abs (1972).

2. Hodges, R. E. Experimental vitamin A deficiency in man (abstract). Proc. Western Hemisphere Nutrition Congress III, 1971, p. 67. Futura Pub. Co., Inc., Mount Kisco, N.Y. 10549.

3. Sauberlich, H. E. Vitamin A and carotenoid content of tissues. Proc. Workshop on Biochemical and Clinical Criteria for Determining Human Vitamin A Nutriture. National Academy of Sciences, Washington, D.C., 1971, p. 32.

4. Raica, N., Jr., J. Scott, L. Lowry and H. E. Sauberlich. Vitamin A concentration in human tissues collected from five areas in the United States. Am. J. Clin. Nutr. 25: 291, 1972.

STUDY NO. 3 — Vitamin B-6 Metabolism and Requirement in Man

PROBLEM:

A survey of the literature revealed the following facts concerning vitamin B_6 nutrient status assessment: (1) Most methods of assessment involve procedures that are not practical for routine or large-scale analysis. As a result, no one test has been accepted by hospital clinical laboratories. This is unfortunate in the light of numerous human studies demonstrating the relatively short period (1-3 weeks) for vitamin B_6 deficiency findings to appear along with numerous metabolic problems associated with vitamin B_6 deficiency. (2) Serum or plasma GPT and GOT are not useful for human assessment of vitamin B_6. (3) Erythrocyte GPT and GOT stimulation coefficients (S.C.) appear to give an early indication of vitamin B_6 deficiency. It is questionable as to which of the two enzymes show the greatest and earliest response to the deficiency. (4) The assay method most frequently used for EGPT and EGOT is a colorimetric procedure which has several deficiencies.

RESULTS AND DISCUSSION OF THE RESULTS:

Plasma and erythrocyte transaminase measurements were performed in two surveys: Ft. Lewis, Washington, involving 500 subjects and U. S. Army Medical Research and Nutrition Laboratory, Denver, with 112 subjects.

Plasma GPT and GOT did not give significant stimulation coefficients. The stimulation coefficient (S.C.) when applied to either plasma or erythrocyte (RBC) GPT or GOT measurements is defined as follows:

$$\text{Stimulation Coefficient} = \frac{\text{enzyme activity with in vitro added pyridoxal } PO_4}{\text{enzyme activity without in vitro added pyridoxal } PO_4}$$

For erythrocyte transaminase measurements of the stimulation coefficients, a 15-minute preincubation with pyridoxal phosphate (PLP) was found to be sufficient. Accurate measurements of erythrocyte GPT are difficult because of the high absorbance of the substrate-hemolysate mixture plus the fact that GPT activity is much less than erythrocyte GOT. Significant erythrocyte GPT stimulation was not observed; however, erythrocyte GOT-S.C.'s may be very helpful in evaluating vitamin B_6 status. Erythrocyte GOT activity units (expressed as moles of NADH oxidized/RBC content of

1 ml whole blood/minute) ranged from 248 to 1299. The average S.C. was 1.60 for Ft. Lewis subjects and 1.45 for MRNL personnel. Subjects were asked to indicate whether or not they took vitamin supplements; 4.8% and 35.7% of the subjects at Ft. Lewis and MRNL took vitamin supplements, respectively. From the Ft. Lewis survey, twenty-five subjects had S.C.'s greater than 1.7, five greater than 1.8 and one greater than 1.9. A stimulation coefficient of 1.8 or higher may be cause for concern, and a value of 2.0 or greater would most probably be indicative of an advanced state of vitamin B_6 deficiency. A full validation of this statement will require an evaluation of the procedure in a controlled human vitamin B_6 deficiency-repletion study.

CONCLUSIONS:

The optimized spectrophotometric method employed for the assay of erythrocyte GOT activity appears to be a reliable, relatively simple, fast (40-50 samples/8-hour period) and reproducible method for vitamin B_6 nutrient assessment in individual human subjects.

RECOMMENDATIONS:

A controlled human deficiency-repletion study is required where this method as well as other methods for evaluating vitamin B_6 status could be compared. If the method proves satisfactory, then it should be utilized for all military nutrition surveys conducted by the laboratory.

PUBLICATIONS:

1. Sauberlich, H. E., J. E. Canham, E. M. Baker, N. Raica and Y. F. Herman. Biochemical assessment of the nutritional status of vitamin B_6 in the human. Am. J. Clin. Nutr. 25: 629, 1972.

STUDY NO. 4 Military Nutrition Surveys

PROBLEM:

The objectives of this study are to provide continuing support to the military nutrition surveys conducted to evaluate the adequacy of military diets in terms of established recommended dietary allowances under varied climatic conditions.

RESULTS AND DISCUSSION OF THE RESULTS:

Personnel and analytical support were provided the military nutrition surveys conducted at Lowry Air Force Base, Denver; Ft. Lewis, Washington; and Ft. Myer, Virginia. Blood, urine and/or diet samples were obtained from these surveys. The samples are analyzed in detail for biochemical

components and essential nutrients to provide information concerning nutritional status and nutrient intakes. Data obtained are transferred to computer file for storage and detailed analysis.

In the determination of nutrient intake during nutrition surveys, accurate measurement is made of the recipes used in food preparation and of the amount of specific food items consumed by the soldiers. An average intake per man is derived based upon an accurate head count and food composites for the specific meals are prepared for later chemical analysis. Nutrient intake data are calculated by computer programs which are based upon data from USDA Handbook 8 and other supplementary sources providing nutrient intake values.

At Ft. Lewis, Washington, five different feeding facilities were surveyed: (1) basic training dining facility utilizing standard Army practices and food items prescribed by the Master Menu; and (2) four of the dining facilities under the CAFE feeding system. CAFE was an experimental program run by Natick Laboratories which utilized a central food preparation. The regular CAFE feeding lines provided food based upon a menu derived from a food preference study previously conducted by NLabs at Ft. Lewis and not upon the Master Menu. In Table I, the calculated content of vitamin A as determined by the computer is compared to the chemically-determined content of vitamin A or its biological equivalent from beta carotene. CAFE Regular indicates a dining facility which provided only a regular serving line. CAFE Combination indicates a dining facility with a regular serving line but, during the noon meal, a short order line was also present. The Specialty House provided principally seafood, Mexican foods, Italian foods, hamburgers, hot dogs, et al. The Short Order House principally served pizzas, hamburgers, cheeseburgers, hot dogs, et al. It can be seen that the chemically-determined vitamin A content of the meals served was considerably below that predicted by the calculated value. During the 8 days of survey of the CAFE Combination facility, only on one day did the chemically-determined vitamin A intake exceed 5,000 IU's. the amount recommended as the daily intake by AR 40-25. In general, based on the chemical determinations, it can be stated that the personnel using the basic training dining facility were receiving a more adequate supply of vitamin A than those dining at the other facilities.

Table II describes the distribution of the 89 meals upon which chemical determinations for vitamin A were made. It can be seen that over 60% of the meals contained less than 50% of the vitamin A content predicted by calculation. The above suggests that the values cited by Handbook 8 are not accurate and that there is need for analytical studies to determine the nutrient content of individual food items in order to compensate for the many changes that have occurred in the agriculture and food industry since derivation of many of the values contained in Handbook 8.

TABLE I

Vitamin A Content of Foods - Ft. Lewis Survey - 1971

Dining Facility or Meal	Calculated Content	Chemically Determined*	% Diff.**	# Days Intake >5,000 IU (Determined)
Basic Trainee	8,379 IU/day	5,727 IU/day	68.3	3 of 6
CAFE Regular	9,651 IU/day	4,443 IU/day	46.1	2 of 8
CAFE Combination	7,875 IU/day	3,476 IU/day	44.1	1 of 8
Specialty House	2,925 IU/meal	1,485 IU/meal	50.8	
Short Order House	1,107 IU/meal	457 IU/meal	41.3	
Breakfast***	2,230 IU	920 IU	41.2	
Dinner***	3,493 IU	1,568 IU	44.9	
Supper***	3,229 IU	2,150 IU	66.6	

* Homogenates were saponified, extracted with ether and chromatographed on magnesia-Hyflosupercel columns. Eluates were assayed for carotene (absorption at 450 nm) and vitamin A (trifluoroacetic acid procedure).

** % Diff. = Chemically determined/calculated content.

*** Based on Basic Trainees, CAFE Regular and CAFE Combination dining facilities only.

TABLE II

Distribution of 89 Meals - Vitamin A Content - Chemical-Calculated Difference

All Meals

Percent* Diff.	Percent of Total Meals	Percent Total Calculated IU-A	Percent Total Determined** IU-A	Ave/Meal (IU of Vit.A) Calculated	Ave/Meal (IU of Vit.A) Determined
10 - 19	4.5	4.9	1.5	2,896	440
20 - 29	14.6	14.6	7.4	2,660	689
30 - 39	15.7	11.0	7.4	1,850	639
40 - 49	27.0	30.0	26.6	2,959	1,334
50 - 59	19.1	17.9	19.1	2,487	1,355
60 - 69	6.7	7.9	10.1	3,119	2,037
70 - 79	5.6	5.6	7.9	2,653	1,903
80 - 89	2.2	3.4	5.7	4,070	3,410
90 - 99	1.1	0.6	1.2	1,513	1,456
≥ 100	3.4	4.0	12.2	3,114	4,913

* Percent Diff. = Chemically determined content/calculated content.

** Homogenates were saponified, extracted with ether and chromatographed on magnesia-Hyflosupercel columns. Eluates were assayed for carotene (absorption at 450 nm) and vitamin A (trifluoroacetic acid procedure).

CONCLUSIONS:

Extensive personnel and analytical support were provided the military nutrition surveys conducted at Lowry Air Force Base, Denver; Ft. Lewis, Washington; and Ft. Myer, Virginia. Analytical data derived from these surveys are transferred to a computer file for storage and detailed evaluations.

PUBLICATIONS:

1. Canham, J. E. The nutritional status of the modern military male and female. Fed. Proc. 31: 704 Abs (1972).

OTHER ANCILLARY STUDIES

Cooperative efforts were maintained with various agencies as a part of Medical Research and Nutrition Laboratory's continuing interest in the nutritional status of military and civilian populations as it reflects on the overall defense preparation, planning and capabilities. Participation in the Ten-State Nutrition Survey has been completed. Assistance has been provided in drafting the final report. The report will be completed and available after 1 July 1972. Results of other activities are available from the manuscripts published or prepared for publication.

PUBLICATIONS:

1. Tillotson, J. A., and E. M. Baker. An enzymatic measurement of the riboflavin status in man. Am. J. Clin. Nutr. 25: 425, 1972.

2. Sauberlich, H. E. Problems of assessment of nutritional status: An overview of biochemical methodologies. In: Problems of Assessment and Alleviation of Malnutrition in the United States (proceedings of a workshop sponsored by Vanderbilt University, HSMHA and NIH held at Nashville, TN, January 13-14, 1970). Edited by R. G. Hansen and H. N. Munro, Washington, D.C., U. S. Government Printing Office, Pub. #916.086, 1971, p. 9-34.

3. Chase, H. Peter, V. Kumar, J. M. Dodds, H. E. Sauberlich, R. W. Hunter, R. S. Burton and V. Spalding. Nutritional status of preschool Mexican-American migrant farm children. Am. J. Diseases Children 122: 316, 1971.

4. Sauberlich, H. E., W. Goad, Y. F. Herman, F. Hiland and P. Jamison. Biochemical assessment of the nutritional status of the Eskimos of Wainwright, Alaska. Am. J. Clin. Nutr. 25: 437, 1972.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6347	72 07 01	DD-R&E (AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A827	00	070
b. CONTRIBUTING	61256011	3A025601A827	00	[redacted]
c. CONTRIBUTING	CDOG 114 (f)			

11. TITLE (Precede with Security Classification Code): (U) High Altitude Bioenergetics - The Physiological Consequences of Altitude Exposure Upon the Soldier (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
016200 Stress Physiology; 005900 Environmental Biology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	CONT	DA	C In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	2.0	28
FISCAL YEAR CURRENT 73	1.8	70

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240
RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Bioenergetics Division
Fitzsimons General Hospital
Denver, Colorado 80240
PRINCIPAL INVESTIGATOR (Enter SSAN if U.S. Academic Institution)
NAME: Consolazio, C. F.
TELEPHONE: 303 366 5311 X25222
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]
ASSOCIATE INVESTIGATORS
NAME: Johnson, H. L.
NAME: Krzywicki, H. J. DA

21. GENERAL USE
Foreign Intelligence not Considered

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 are 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) Study sea level soldiers at a sea level site, while manipulating their dietary habits, physical condition and/or physical activity. Then repeat the measurements at altitude after abrupt exposure and again upon return to sea level. Parameters measured include: (a) clinical symptomology; (b) food intake and nutrient balances; (c) pulmonary, cardiovascular and metabolic changes during rest, various treadmill work levels and recovery, and (d) changes in body fluids and other compartments. Animal studies include enzymatic and tissue changes.

25. (U) 71 07 - 72 06 Studies indicate that the loss of body water during acute altitude exposure may be an adaptive mechanism in preventing or reducing the severity of acute mountain sickness. Although anorexia is common during acute altitude exposure, the daily intake can be maintained providing that the men are highly motivated and in good physical condition. In the summary of three studies, oxygen uptakes were significantly increased during submaximal work indicating an increase of energy requirements at high altitudes.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498-1

ABSTRACT

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

Two additional studies were completed at altitude (4,300 m) during the past year, and were designed to obtain additional information on respiratory function (including pulmonary diffusing capacity D_LCO) and acid-base parameters.

In the first study, four men were evaluated at 1,600 m (Denver), 4,300 m and on return to 1,600 m. In the second study, six sea level natives were evaluated at sea level (Ft. Sam Houston, Texas), 4,300 m, and again on return to sea level. Information on respiratory function (including FRC and D_LCO) and blood acid-base changes are now being evaluated

BODY OF REPORT

WORK UNIT NO. 070	High Altitude Bioenergetics - The Physiological Consequences of Altitude Exposure Upon the Soldier
STUDY NO. 7	Physiological and nutritional effects of high altitude. V. Mineral and water balances at altitude.
STUDY NO. 12, PART C	Effects of dietary intake upon metabolic responses to exercise. Part C. Measurement of pulmonary function and blood acid-base parameters at altitude (pulmonary diffusing capacity at rest)

PROBLEM:

The military necessity for physiological studies at high terrestrial altitude became apparent with the Chinese invasion of India. With support from the Advanced Research Project Agency and the Medical Research and Development Command, investigators at this laboratory began research in this area in 1963. Since the work capacity of the soldier is of prime concern to the military, any impairment of performance would result in a decreased efficiency. Information obtained from these studies should provide data to commanders for planning operations during maneuvers or combat situations. Our objective continues to be: to locate and quantitate the performance decrements to be expected in soldiers during military operations at 10,000 to 14,100 feet; to measure the extent and rate of acclimatization; to investigate the physiology, biochemistry and pharmacology of the affected organ systems by selection, conditioning, previous environmental exposure, nutrition, drugs, or other variables. We plan to measure and correlate pulmonary function (including the blood acid-base parameters) at rest, at various work levels and recovery in healthy populations at both low and high altitude.

Investigations under these work units have aimed at delineating the influence of dietary manipulations and heavy physical conditioning on the symptoms of acute mountain sickness in human subjects exposed to high altitude.

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

STUDY NO. 7 — Physiological and nutritional effects of high altitude: Phase V. Mineral and water balances at altitude.

RESULTS AND DISCUSSION OF RESULTS:

It has been indicated that the loss of body water is beneficial in preventing or reducing the severity of acute mountain sickness symptoms. During 6 days of altitude exposure at 4,300 m, the following changes in body water compartments were observed: a) total body water was significantly decreased by 2.25 kg during the 6-day altitude exposure, b) extracellular water appeared to increase by 1.27 kg at altitude, although not significantly, c) intracellular water, in turn, was significantly decreased by 3.52 kg at altitude, which is contrary to some previous reports. Under the conditions of this study, with heavy physical activity prior to and during altitude exposure, and with fairly high food intakes (above 3,400 kcal/day), it appeared that hypohydration and a diuresis still occurred during acute altitude exposure. This suggested that body water loss may have been an adaptive mechanism in acute altitude exposure.

STUDY NO. 12, PART C — Measurement of Pulmonary Function and Blood Acid-Base Parameters

RESULTS AND DISCUSSION OF RESULTS:

Two high altitude studies were completed during the past year. In the first study pulmonary diffusion capacities, FRC's, the various lung compartments and blood acid-base parameters were compared in 4 men at 1,600 m twice daily for 5 days during acute altitude exposure (4,300 m) and again on days 2, 7 and 14 after return to 1,600 m. Data is now being statistically analyzed.

A second high altitude study at Pike's Peak was completed in August 1971. Six sea level residents (from Ft. Sam Houston, Texas) were acutely exposed to 4,300 m for 6 days. Control measurements were made at sea level, on immediate arrival at 4,300 m, and then daily for 5 days, and again daily during the return to sea level phase. The measurements included resting pulmonary diffusion capacities, functional residual capacities, inspiratory reserve volume, expiratory reserve volume, residual lung volumes, total lung and vital capacities. The blood acid-base parameters included pH, pCO_2, pO_2, bicarbonate, etc. The data is now being statistically analyzed.

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

CONCLUSIONS:

Respiratory function including D_LCO is being evaluated in two studies: a) 1,600 vs 4,300 m, and b) sea level to 4,300 m and return to sea level. Data is being statistically evaluated.

The severity of acute mountain sickness can be reduced by physical conditioning prior to altitude exposure and by the ingestion of high carbohydrate diets. Although anorexia occurs during altitude exposure, a normal daily intake can be maintained. This results in normal biochemical values. Hypohydration occurs during acute altitude exposure and appears to be a compensating effect for adaptation to the hypoxia of high altitude.

RECOMMENDATIONS:

Further studies on the effects of a normal food intake at altitude as they relate to blood acid-base parameters (pH, pCO_2, bicarbonate, etc.) and respiratory function changes, especially FRC and pulmonary diffusion capacities during rest and standardized exercise will be studied.

PUBLICATIONS:

1. Johnson, H.L., C.F. Consolazio, T.A. Daws, and H.J. Krzywicki. Increased energy requirements of man after abrupt altitude exposure. Nutr. Rpts. Intl. 4:77-82, 1971.

2. Krzywicki, H.J., C.F. Consolazio, H.L. Johnson, W.C. Nielsen, Jr., and R.A. Barnhart. Water metabolism in humans during acute high altitude exposure (4,300 m). J. Appl. Physiol. 30:800-809, 1971.

3. Consolazio, C.F., H.L. Johnson, H.J. Krzywicki, and T.A. Daws. Adaptation to high altitude (4,300 m). J. de Physiol. (Paris) 63:232-235, 1971.

4. Consolazio, C.F., H.L. Johnson, H.J. Krzywicki, and T.A. Daws. Metabolic aspects of acute altitude exposure (4,300 m) in adequately nourished humans. Am. J. Clin. Nutr. 25: 23-29, 1972.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6350	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A827	00	073
b. CONTRIBUTING	62156011	3A025601A827	00	
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Precede with Security Classification Code): (U) Physiological, Metabolic and Psychological Aspects of High Altitude Exposure (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 013400 Psychology; 012600 Pharmacology; 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	CONT	DA	C In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	6.2	96
FISCAL YEAR CURRENT 73	4.8	80

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
Physiology Division
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Hannon, J. P.
TELEPHONE: 303 366 5311 X22119
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME: Sullivan, F. J.
NAME: Sterner, R. T., CPT, MSC DA

21. GENERAL USE
Foreign Intelligence not Considered

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

23. (U) Acute mountain sickness (AMS) can be a severe and incapacitating illness. At 14,100 ft. 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 is designed to study various aspects and correlates of symptomatology in humans at high altitude. Efforts will be 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.

24. (U) Human volunteers will be subjected to actual and simulated high altitude environments for periods of short (days) and long (weeks) duration. Various physiological, biochemical and psychological measures will be applied to describe the alterations - defects and adaptation - caused by exposure. Valid estimates of symptom severity will be 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. Thus, a sounder scientific basis for therapeutic or other procedures directed at the relief of AMS will be available.

25. (U) 71 07 - 72 06 Further evaluation of the General High Altitude Questionnaire revealed significant diurnal variations in the four symptomatology subscales: Arousal Level, Somatic Discomfort, Tired and Mood. The effects of drugs on AMS symptomatology are superimposed on these diurnal variations. A long-term study of altitude acclimatization has revealed transient increments in basal metabolic and related cardiopulmonary functions. It has also delineated the time-course changes in acid-base balance, body composition, cardiopulmonary function and physical work capacity.

* 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 or initiated under this work unit during the past year:

STUDY NO. 5 Acute mountain sickness symptomatology scale

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

Man exposed to high altitude manifests various biochemical, physiological and psychological alterations which change with the length of exposure. During the first few days at altitude, the phenomenon of acute mountain sickness becomes manifest with most of the symptoms disappearing within a week. Physical, psychomotor, mental and sensory functions decrement during acute exposure; however with prolonged exposure, performance improves.

Further analysis of the General High Altitude Questionnaire has revealed the subscales of Arousal Level, Somatic Discomfort, Tired and Mood show a distinct diurnal variation with higher values being observed in the morning than in the evening. Alterations in AMS symptomatology with drugs (Phenformin - placebo) are superimposed on these diurnal effects.

Depending on the function being studied significant altitude acclimatization can occur as early as 1 - 2 weeks after the onset of exposure while complete acclimatization may require 2.5 months or longer. Loss of physical work capacity is particularly resistant to acclimatization with only a small minority of subjects showing sizable recoveries after 2.5 months. Reduced physical work capacity seems largely due to compromised cardiovascular function, particularly during prolonged, exhaustive exercise. The responsible hemodynamic mechanisms are unknown but are currently being investigated.

BODY OF REPORT

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

STUDY NO. 5 — Acute Mountain Sickness Symptomatology Scale

PROBLEM:

Acute Mountain Sickness (AMS), a debilitating condition that can be observed in unacclimatized individuals following rapid transport from low to high altitude, is characterized by such symptoms as nausea, dizziness and fatigue. The original General High Altitude Questionnaire (GHAQ) is a 22-item, self report questionnaire developed to evaluate the symptoms associated with rapid high altitude exposure. Previously, it has been shown that a selected 16-item, four dimension breakdown of the GHAQ into subscales (i. e., Arousal Level, Somatic Discomfort, Tired and Mood) offers improved estimates of AMS symptomatology. In the current report, data from an earlier study (Stamper et al., Perceptual and Motor Skills 33:735-742, 1971) were reanalyzed in an attempt to assess potential diurnal variation associated with these subscales.

RESULTS AND DISCUSSION OF THE RESULTS:

Analyses of variance of the Arousal Level, Somatic Discomfort, Tired and Mood subscales indicated that symptom scores were significantly higher for morning than evening response sessions, and high altitude reports were more severe than those obtained at low altitude. In addition, Time-of-GHAQ by Altitude interaction, as well as, an effect due to drugs (e.g., Phenformin - placebo) were also significant for the Somatic Discomfort subscale. These findings suggest that Somatic Discomfort reports are differentially affected by high altitude and that the items comprising the subscales can reflect symptomatic effects due to conditions other than high altitude (e.g., drugs).

CONCLUSIONS:

The major findings of this study is that the Time-of-GHAQ administration measured by the GHAQ subscales, affects the self-reported severity of AMS symptomatology.

RECOMMENDATIONS:

Experiments to assess AMS symptom changes across time should control for within-day sampling variation. Moreover, when AMS symptom measures are obtained to validate behavioral and physiological changes at high altitude, time between measurement of symptomatology and the experimental variables used must be minimized.

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

STUDY NO. 9

The Interrelationships of Cardiopulmonary Function and Performance During Prolonged Altitude Exposure In Humans

PROBLEM:

Most studies of human performance at high altitude have been of short duration, lasting a few hours or days, occasionally two or three weeks, but rarely for periods longer than one month. Such studies have consistently shown maximum work capacity is reduced at elevations greater than 10,000 feet. They have also shown that other aspects of human performance including psychomotor function and sensory function are seriously decremented during acute exposure. Yet, little is known about the recovery rate of various performance functions as the interval of altitude exposure is prolonged and acclimatization is achieved. Similarly, little is known about the physiological alterations or adaptations that are responsible for the improvement in performance during prolonged altitude exposure. At present we only know that the long-term sojourner performs considerably better than the acutely-exposed individual.

During the last quarter of FY 71 and the first quarter of FY 72 a long-term, 78 day, study of human acclimatization to high altitude was conducted on the summit of Pikes Peak (14,110 feet). Emphasis was given to the alterations in cardiopulmonary, acid-base and metabolic functions which underly the acclimatization process, particularly in terms of physical work capacity.

RESULTS AND DISCUSSION OF RESULTS:

Eight female volunteer subjects were recruited at the University of Oregon, Eugene, Oregon and low altitude control measurements were conducted over a two-week period in early June. Thereafter, the subjects journeyed by air and surface transportation to the summit of Pikes Peak where they remained until early September. During the first two weeks at high altitude the subjects were housed in the Laboratory facility for intensive study of their acute responses to hypoxia. Subsequently, they transferred residence to the Pikes Peak Summit House where they lived and worked for the remainder of the summer. The adaptations associated with chronic exposure were examined after exposure intervals of 28 and 78 days. At these times the subjects returned to the Pikes Peak Laboratory Facility for 24-hour periods for testing. Two subjects withdrew from the project during the latter part of the summer; however, their loss had only minimal effect on the study since in most measures only three data points out of 64 were lost. To date, analyses of basal metabolic and cardiopulmonary function, acid-base balance and body composition

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

changes have been completed. Cardiopulmonary and metabolic changes associated with mild, moderate and heavy exercise have been partially evaluated.

Measurements of basal metabolic and cardiopulmonary function revealed an average end-tidal P_{O_2} decrease from 99.7 to 46.6 mm Hg during the first 12 hours at altitude. Subsequently, a slight but progressive recovery was observed over the course of the summer. Thus, an end-tidal P_{O_2} of 54.6 mm Hg was obtained after 78 days on Pikes Peak. End-tidal P_{CO_2} decreased progressively from a sea level value of 37.7 to 28.5 mm after 7 days and to 24.5 mm after 78 days at altitude. O_2 consumption and CO_2 production increased transiently with a maxima of about +28% being achieved at 60 hours. An oral temperature increment of 0.7° C accompanied these metabolic alterations. The respiratory quotient, on the other hand, was unaffected by altitude. $\dot{V}_E$ rose very rapidly during the first 60 hours at altitude and reached stable values (+13%) at 28 days. Respiratory rate increased (40%) and tidal volume decreased (18%) transiently during the first 60 hours of exposure. Subsequently, rate returned to normal after 14 - 28 days while tidal volume stabilized at supranormal values (34%) after 28 - 78 days. O_2 and CO_2 ventilatory equivalents did not change during the first 60 hours of exposure but increased progressively thereafter. Systolic pressure and heart rate rose transiently, achieving maxima of +15 to +50%, respectively, at 60 hours while diastolic pressure exhibited a sustained increase throughout the altitude sojourn.

The transient increments in basal metabolic rate would appear to be attributable to several factors. Accordingly, the rise in body temperature could account for approximately 24% of the metabolic increment observed on Pikes Peak. Increased myocardial work (due to rate and pressure elevations) could account for an additional 28% while the metabolic cost of hyperventilation could account for 2% or less. The remaining 45% would appear to be largely associated with hypocapnia and its effect on general tissue metabolism. Such hypocapneic effects were indicated by sizable increments in resting serum lactate and pyruvate concentrations during the early stages of the sojourn. Elevated catecholamine secretion during this period probably potentiated or supported the foregoing metabolic changes.

Acid-base studies were conducted on resting (not basal) arterial blood samples and 24-hour urine samples. Arterial P_{O_2} averaged 89 mm Hg in Oregon and fell to 46 mm Hg after 2 days on Pikes Peak. It recovered to 54 mm Hg at 7 days and thereafter remained constant. Arterial O_2 saturation decreased from 96% to 83% on the second day but recovered to 91% on the 78th day. Arterial P_{CO_2} fell from 39 to 25 mm Hg after 2 days at which point it remained stable. Arterial pH increased from 7.36 to 7.42 after 2 days on the Peak and to 7.44 after 78 days. Bicarbonate levels were reduced from 21.6 meq./l to 16.3 meq./l after 2 days and showed little or no change subsequently. Metabolic acidosis increased from 3.0 meq./l to 6.2 meq./l on the second day of exposure

after which the values remained constant. Twenty-four hour urine volumes were measured during a 5 day period at low altitude and during the first two weeks at high altitude. They were consistently reduced during the altitude sojourn with the most marked reductions (30 to 40%) being observed during the first week of exposure. Mineral and ammonia excretion were similarly reduced at high altitude although there was a significant retention of potassium. Thus, the urinary Na/K ratio was elevated during the first two weeks on Pikes Peak. Urinary titratable acidity decreased from 268 to 121 ml after one day but recovered to 212 ml after 3 days. Urinary pH rose from 5.93 to 6.09 after one day and then fell to an average value of 5.67 during the remainder of the sojourn. On the basis of these data it is concluded that only partial compensation for altitude respiratory alkalosis is achieved during a 78-day period of acclimatization.

Alterations in body composition were studied by Miss Dorothea Sudman using conventional body volumeter techniques. Body densitometry measurements, body weight changes, nitrogen washout measurements were made. In addition, extracellular space was estimated by the dilution (extrapolation procedure) of intravenously injected sucrose. Functional residual volumes increased markedly during the first two weeks at altitude and more slowly thereafter. After 78 days an increment of 265 ml (about 25%) was observed. Body weights decremented sharply during the first two weeks of exposure with an average loss of 1.88 kg being observed. A small recovery (about 0.25 kg) occurred during the latter stages of the sojourn. At the end of the second week on Pikes Peak densitometry measurements revealed a 0.94 kg loss of body fat and a 0.93 kg loss of lean body mass. The decrement in body fat was associated with a marked (about 50%) increase in serum ketone levels. The loss in lean body mass included a 0.68 kg loss of body water, a 0.19 kg loss of body protein and a 0.06 kg loss of body mineral. Measurements of extracellular (sucrose) space revealed marked decrements during the first two weeks on Pikes Peak. These data, along with the densitometric measurements of total body water, indicated a sizable, about 2.0 liter, water shift from the extra- to the intracellular fluid compartment. At the end of the high altitude sojourn the major changes in body composition included a 1.84 kg loss of body fat but no change in lean body mass, total body water, mineral or protein. Because of procedural difficulties and limitations no attempt was made to assess the state of body hydration by balance techniques.

Partial analysis of cardiopulmonary function during mild, moderate and heavy exercise revealed a sizable, expected, decrement in physical work capacity during the acute stages of altitude exposure. As exposure was extended some recovery was observed and in two of the subjects work capacity at the end of the summer approximated that observed at the outset in Oregon. In the others, however, a moderate decrement remained.

An unusual, and unexpected type of cardiovascular deterioration was observed during the final stages of exhaustive, maximal exercise on

Pikes Peak. It occurred in two of the subjects during exercise tests conducted on the second day of exposure and in all of the subjects during tests conducted on the 7th, 14th, 28th and 78th days of exposure. It was characterized by a progressive decrease in systolic and in some instances a disappearance of diastolic pressure, tingling or numbness in the fingers, hands and arms, dizziness, tunnel vision and frequently syncope. The hemodynamic factors responsible for this phenomenon are unknown. The possibilities include pulmonary hypertension and resultant right heart failure which in turn would lead to a reduction in left ventricular filling. Alternatively, as the subject nears exhaustion generalized vasodilation could occur and this in turn could lead to a peripheral pooling of blood and inadequate venous return. Finally, a decrement in myocardial contractility may have occurred during maximal exercise at altitude and this could result in compromised left ventricular pump capacity. This problem is currently being investigated in follow-up study involving 8 male subjects exposed to high altitude for 2 weeks.

CONCLUSIONS:

Altitude acclimatization in terms of basal or resting cardiopulmonary function requires at least 4 weeks. In terms of body composition two months or more are required to re-establish low altitude characteristics. Recovery of physical work capacity occurs in some individuals as early as 2.5 months but in most individuals a considerably longer period is required. Acid-base balance is largely re-established after 2 weeks at high altitude, although minor changes may persist thereafter.

RECOMMENDATIONS:

To complete this study the remaining data on cardiopulmonary function during exhaustive exercise needs to be reduced and analyzed. Other data analyses to be completed include serum metabolite changes associated with exercise, urinary catecholamine analyses, measurements of caloric and nitrogen balance and measurements of 24-hour urinary steroid excretion. In addition, the follow-up study to ascertain the hemodynamic factors responsible for cardiovascular deterioration during prolonged, exhaustive exercise needs to be completed and the data analyzed.

PUBLICATIONS:

1. Stamper, D. A., R. T. Sterner and R. A. Kinsman. Symptomatology subscales for the measurement of acute mountain sickness. Perceptual and Motor Skills 33:735, 1971

2. Hannon, J. P., K. S. K. Chinn and J. L. Shields. Alterations in serum and extracellular electrolytes during high-altitude exposure. J. Appl. Physiol. 31:266-273, 1971

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

3. Stamper, D. A. and R. T. Sterner. Acute mountain sickness: A note on within-day symptomatology effects. Perceptual and Motor Skills (submitted)

4. Hannon, J. P., E. L. Gibbs, T. E. Paxson and D. M. Sudman. Basal metabolic and cardiopulmonary function of college women during altitude acclimatization. Federation Proc. 31:389, 1972.

5. Sudman, D. and J. P. Hannon. Acid-base balance of college women during altitude acclimatization. Colo.-Wyoming Acad. of Science 22:1972.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6358	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D Change	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A830	00	061
b. CONTRIBUTING	6215601A	3A025601A830	00	
c. CONTRIBUTING	CDOG 114(f)			

11. TITLE (Precede with Security Classification Code)

(U) Nutritional Aspects of Military Dog Performance (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

006500 Food; 016700 Stress Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 06	CONT	DA	C In-House

17. CONTRACT/GRANT

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

18. RESOURCES ESTIMATE

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING 72	4.0	35
CURRENT 73	4.0	40

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Pathology Division
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Trevino, G. S., LTC
TELEPHONE: 303 366 5311 X23230
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME: Miller, J. G., CPT
NAME: Plopper, C. G., CPT DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Working Military Dogs; (U) Rations; (U) Enforced Exercise; (U) Work Performance; (U) Stamina; (U) Palatability; (U) Nutrient Requirements

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 attempt to establish objective criteria for assessing physical capacity of dogs to work under conditions of enforced exercise, similar to that required in a combat zone, (2) to correlate differences in endurance and stamina with variations in diet, and (3) to assess the adequacy of a 3 test rations in the sustenance of military working dogs subjected to enforced exercise.

24. (U) Seventeen German Shepherd dogs will be studied. Three groups will be fed different rations. All are subjected to equal periods of enforced exercise. Capacity to perform is assessed by physical examination, time required to induce fatigue, maintenance of body weight, and recording of other physiologic parameters. Food consumption will be recorded and correlated with clinical and biochemical indices of nutritional status. Diets will be chemically analyzed for selected nutrients.

25. (U) 71 07 - 72 06 During the first 11 months of FY 72 daily food consumption of 6 dogs fed Purina Dog Chow (PDC) averaged 1.265 lbs; 6 dogs fed Pooch Tasty Nuggets (PTN) averaged 1.557 lbs; and 5 dogs on Maximum Stress Diet (MSD) averaged 1.096 lbs. Average body weight of the dogs on PDC was 58 lbs; those on PTN averaged 63.5 lbs; and those on MSD, 58.2 lbs. Gross daily caloric intake of the PDC group, per pound of body weight, amounted to 43.184 Cal.; for the PTN group, 41.242 Cal.; and for the MSD group, 46.024 Cal. Since the digestibility of the diets is 78% for PDC, 64% for PTN, and 94% for MSD, the net caloric daily intake for each group was as follows: PDC, 33.683 Cal./lb.; PTN, 26.394 Cal./lb; and MSD, 43.385 Cal./lb. Results of pre- and post-exercise biochemical parameters were tabulated and statistically analyzed.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 88 1498

ABSTRACT

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

The following investigation has been conducted under this work unit.

STUDY NO. 2 To establish objective criteria for evaluating the nutritional adequacy of three test rations for military dogs at rest and under induced physical stress.

Study No. 2 has been in progress since April 1970. Daily individual food consumption has been recorded since that time in 3 groups of military working dogs subsisting on three different rations. Body weights have been taken at monthly intervals.

Because of serious personnel shortages and a protracted delay in the delivery of a treadmill, the 15 German Shepherds were exercised to fatigue only during the latter six months of FY 72. Deep body temperature was continuously monitored throughout each exercise period. Blood samples were collected immediately before and after each exercise. One resting biopsy and 3 fatigue muscle biopsies were taken from the triceps of each animal. Samples of fatigued muscle were compared by both light and electron microscopy using special stains.

The large volume of data obtained from this study has been subjected to statistical analysis. Those data derived from the last 3 months of FY 72 will be reported as soon as analyses are complete. The remainder is recorded in the text.

A report describing the muscle biopsy technique, the results of fiber type size and distribution in resting muscle, and the light and microscopic assessment of myofiber morphology has been submitted for publication.

BODY OF REPORT

WORK UNIT NO. 061

Nutritional Aspects of Military Dog Performance

STUDY NO. 2

To establish objective criteria for evaluating the nutritional adequacy of three test rations for military dogs at rest and under induced physical stress

PROBLEM

The development of purely objective criteria in assessing the adequacy of diets in working dogs is difficult. Clinical parameters, such as weight gains, appearance, and condition of hair coat, indices which have been used in some studies, may not be wholly reliable or relevant to adequate nutrition.

In recent years it has become apparent that caloric requirements for dogs differ as widely as the physical, metabolic, and environmental conditions under which the animals are maintained. Thus, not only are caloric requirements of a subject dog influenced by hot and cold climates, but also by rate of growth, pregnancy, lactation, and physical activity. Moreover, whereas the composition of a specific diet may be caloricall y adequate for a dog, the failure of the animal to ingest it in the amounts necessary to sustain itself in positive energy and nitrogen balance could lead to manifestations of nutritional deficiencies. A diet must not only be calorically adequate but must also be palatable if the animal is to be properly nourished.

Field reports from Vietnam have indicated that some scout dogs and dogs trained in mine and tunnel detection have shown insufficient endurance under strenuous working conditions. Weight loss in government-owned dogs has also been reported. Nutritional influence upon the onset of fatigue is of vital importance to those personnel whose lives depend on the maximal performance of a trained military dog.

This project was designed as a long-range study to establish objective criteria for determining the dietary adequacy of 3 test rations in a random population of German shepherds subjected to enforced, controlled exercise. The dogs were divided into 3 groups of 5 each (with one substitute back up dog for each of two groups if necessary).

Group A received a high caloric, high fat diet; Group B was fed a diet used in sustenance of dogs on long-term studies; and Group C received an inexpensive ration with a lower digestibility coefficient. All 3 diets are commercially available.* A daily record of the food intake of each animal has been maintained since May of 1970. For 7 weeks the animals were maintained at rest while they adjusted to their environment and their specific diets. Monthly weights have been obtained.

Each animal was exercised to fatigue once weekly between June and December, 1970. The precise methodology of inducing fatigue, collection of specimens, and parameters assayed was presented in the Annual Research Progress Report for FY 1971.

The animals were maintained on their respective diets but were not exercised during calendar year 1971.

A treadmill adequate for exercising large dogs was ordered 4 March 1971 and installed 1 December 1971. During December the animals were placed on the treadmill a few minutes each day to condition and prepare them for the exhaustive exercises to follow. Baseline specimens were drawn during this preliminary period.

From 1 January to 31 March 1972 eight of the dogs were exercised to fatigue on a treadmill 5 days a week. Between 1 April and 30 June 1972 the other seven were similarly fatigued. Three baseline blood specimens and one muscle biopsy were drawn from each subject prior to the onset of the treadmill runs. Three additional blood samples were drawn immediately before and after exercise and three muscle biopsies were obtained just after fatigue. The following parameters were assayed on blood, plasma, or serum: serum glutamic-oxalacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), transketolase, plasma ascorbate, Vitamin A, serum iron, iron-binding capacity, calcium, phosphorus, sodium, magnesium, potassium, chloride, and serum cholesterol. Results of these findings are currently being compiled and analyzed and will be documented in a subsequent report.

* Group A was fed MSD 198 (Hill's Division of Riviana Foods, Topeka, KA); Group B received Purina Dog Chow (Ralston Purina Co., Checkerboard Square, St. Louis, MO); Group C was feed Pooch Tasty Nuggets (Perk Foods Co., Chicago, Ill).

Nutritional Aspects of Military Dog Performance (Cont'd)

RESULTS:

a. Food Consumption. Table 1 presents the average daily food intake of each animal during the first 11 months of FY 72.

TABLE 1
DAILY AVERAGE FOOD CONSUMPTION OF ADULT GERMAN SHEPHERDS ON THREE TEST RATIONS

Ration	Dog No.	Average Body Weight Pounds	Average Daily Food Consumed Pounds
A	2	56.5	1.133
	3	66.8	1.321
	8	71.7	1.282
	11	45.9	.763
	12	50.4	.983
Mean		58.2	1.096
B	6	70.3	1.447
	9	60.0	1.361
	10*	48.4	1.148
	13	47.7	1.155
	14	60.6	1.281
	15	61.2	1.199
Mean		58.0	1.265
C	1	58.8	1.485
	4	61.4	1.718
	5	64.0	1.522
	7	66.0	1.346
	16	63.8	1.530
	17*	66.9	1.745
Mean		63.5	1.557

* Supernumerary; not exercised

Table 2 presents a comparison of food intake and body weight of each group for FY 71 and FY 72.

TABLE 2
SUMMARY AND COMPARISON OF FOOD INTAKE AND BODY WEIGHTS OF MILITARY GERMAN SHEPHERDS DURING FY 71 AND FY 72

Group	Monthly Average Body Weight, lbs		Average Daily Food Consumed **	
	FY-71	FY-72*	FY-71	FY-72
A	56.00	58.20	.976	1.096
B	55.50	58.00	1.304	1.265
C	61.83	63.50	1.502	1.557

* Only first 11 months of FY-72

** Pounds per dog.

Proximate analyses of each of the diets were provided by the manufacturers on request. One of the diets has been analyzed by the Chemistry Division of USAMRNL, and the results were in close agreement with those submitted by the manufacturer. Table 3 presents proximate analyses of each diet as furnished by the manufacturer.

TABLE 3

PROXIMATE ANALYSES OF THE RATIONS USED IN THE STUDY*

Diet	Protein	Fat	Ash	Fiber	Moisture	NFE**
A	29.0	26.0	4.8	5.0	11.0	25.0
B	23.0	8.9	7.4	3.7	9.0	47.5
C	24.3	9.6	7.6	3.5	7.5	47.5

* All values expressed in per cent.

** NFE = Sum of the percentages of protein, fat, ash, fiber, and moisture subtracted from 100%. Because moisture varies slightly, and NFE is not calculated chemically, this is only an approximation.

Digestibility coefficients and gross energy content of each of the diets were provided by the manufacturers on request. Diet A provides approximately 2444 Cal./lb., and is 94% digestible; Diet B furnishes about 1980 Cal./lb. and has a digestibility of about 78%; while Diet C contains about 1682 Cal./lb and a digestibility coefficient of 64%. From these figures and the average body weight of each animal, it was possible to determine the gross and net caloric intake of each animal. The average daily intake for each group was calculated and the results are presented in Table 4.

TABLE 4

DAILY GROSS AND NET CALORIC INTAKE OF MILITARY DOGS ON THREE TEST RATIONS DURING FY 72

Diet	Average Daily Food Intake, lbs	Average Daily Gross Calories Ingested	Average Daily Digestible Calories	Digestible Daily Cal. per lb of body wt
A	1.096	2678.62	2525.02	43.385
B	1.265	2504.70	1953.66	33.683
C	1.557	2618.87	1676.08	26.394

b. Pre- and post-exercise whole blood, plasma, and serum findings.

The results of these parameters represent the analyses of samples drawn during FY 70 and compiled during FY 71.

For the purpose of statistical evaluation the following parameters were considered different entities: free fatty acids, hematocrit, total white blood cell count, neutrophils, bands, lymphocytes, eosinophils, monocytes, basophils, total protein, calcium, inorganic phosphates, cholesterol, uric acid, creatinine, bilirubin, alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase, SGOT, chlorides, carbon dioxide (CO_2), potassium, sodium, blood urea nitrogen (BUN), and glucose.

For each of these values and for each animal a mean was obtained for pre-swimming, pre-treadmill, post-swimming, and post-treadmill blood specimens.

An array was developed for statistical analysis to account for the singular effects of diet, exercise, and conditioning, and then

interaction among these. Analysis of variance appropriate for repetitive measurements in the same animal was employed. The results revealed only 2 parameters were statistically significant ($P < .025$) for dietary effects: lymphocytes and neutrophils.

The following 13 parameters were significantly affected by exercise-conditioning: lymphocytes ($P<.001$), neutrophils ($P<.01$), LDH ($P<.01$), free fatty acids ($P<.001$), hematocrit ($P<.01$), total white blood cell count ($P<.001$), glucose ($P<.005$), CO_2 ($P<.01$), total bilirubin ($P<.01$), alkaline phosphatase ($P .05$), Chlorides ($P<.01$), potassium ($P<.005$), and uric acid ($P<.025$).

The following values showed statistically significant interaction: neutrophils ($P<.05$), alkaline phosphatase ($P<.01$), uric acid ($P<.05$), basophils ($P<.001$), and BUN ($P<.005$).

c. Muscle biopsies.

The technique of biopsy of canine triceps, fiber size, fiber type population, and light and electron microscopic assessment of morphologic changes in resting muscle have been described and submitted for publication. To date no statistically significant changes produced by exercise or diet have been found in muscle examined by light microscopy and histochemistry. Greatly enlarged mitochondria were observed by electron microscopy in myofibers of fatigued muscle of non-conditioned dogs, but this effect was only transient, and was not observed after the first post-exercise biopsy. Because of the small volume of muscle harvested by needle biopsy, it is necessary to sort and count a large number of myofibers from these specimens to fulfill statistical analytical requirements. Statistical evaluation of the results is still underway.

d. Additional observations.

No difference can be detected in the appearance of the 3 groups of dogs. Two of the animals on Diet C have shown coprophagy. The Vitamin A blood level in all dogs in Group C is approximately one-fourth of that in the other groups. The animals in Group A have an obviously higher serum cholesterol level. However, the clinical appearance and treadmill performance of the animals has not been adversely affected by these findings to date. Analysis of the other values measured during the last 6 months of FY 72 is not yet completed.

Nutritional Aspects of Military Dog Performance (Cont'd)

CONCLUSIONS:

To date no significant difference has been noted in the clinical appearance or performance among these 3 groups of dogs. Differences in physiologic biochemical parameters are observed mainly as effects of exercise. Effects of lowered Vitamin A level in Group C and of increased cholesterol levels in Group A will be monitored closely in FY 73. The other sensitive indices of nutritional status, as exemplified by transketolase, SGOT, SGPT, plasma ascorbate, serum iron, iron-binding capacity, cholesterol, and electrolytes are being closely scrutinized.

RECOMMENDATIONS:

Effects of high serum cholesterol levels on the cardiovascular system should be monitored closely in Group A dogs. Ophthalmoscopic examinations for retinal angiopathy will be performed, and blood pressures will be recorded.

Toward the end of FY 73 the animals in this study should be killed. Complete pathologic studies, to include clinical, gross and microscopic observations, should be undertaken at that time with particular emphasis paid to possible atherosclerotic changes.

PUBLICATIONS:

1. Trevino, G. S., R. S. Demaree, Jr., and B. V. Sanders. Nutrition of the Military Dog. (Abstract) Proceedings of IX World Veterinary Congress, 3:1077, Aug 1971.

2. Trevino, G. S., R. S. Demaree, Jr., B. V. Sanders, and T. A. O'Donnell. Needle Biopsy of Canine Skeletal Muscle: A Useful Research Technique. I. Light and Electron Microscopic Findings in Resting Muscle. Lab. Animal Sci., 1972. In press.

3. Bucci, T. J., and C. R. Pope. Comparison of three rations in military scout dogs under moderate thermal stress, USA NEL Laboratory Report No. 327, Sep 1971.

4. Trevino, G. S. Nutrition and Feeding of the Military Dog. Ch. 12. TM 8-450. 1971.

APPENDIX A

PUBLICATIONS - FISCAL YEAR 1972

LABORATORY REPORTS

327. Bucci, T. J., and C. R. Pope. Comparison of three rations in military scout dogs under moderate thermal stress Ft. Benning field study, dated September 1971.

328. Ackerman, L.J., Demaree, R.S., Bucci, T.J., Kennedy, G.A., Alden, C.L., and Myers, T., Effects of Altitude on the cebus apella monkey, dated September, 1971.

329. Messa, C. J., J. R. zumBrunnen, K. C. Stuart and H. H. Goforth. Generalized research analysis statistical system, dated September 1971.

330. Scaief, C. C., III, and H. J. Krzywicki. Development and application of a whole-body radiation system, dated November 1971.

331. Fowler, J.L., J.L. Young, R.C. Fernau, D.E. Ferguson. Laboratory Technique for Producing Microfilariae of Dirofilaria Immitis in Mosquitoes

332. Demaree, R.S., P.L. Senger, M.B. Bischoff, L.D. Jones. A Sequential Ultrastructural Study of the Effects of an Intravenous Lipid Emulsion

333. Henderson, J.E., B.D. Nelson, J.L. Fowler. Identification and Quantitation of the microbiological Flora of the Food Packet, Long Range Patrol Under Simulated Field Conditions

MEDICAL AND SCIENTIFIC JOURNAL PUBLICATIONS

* 767. Herman, R. H. Book Review - Modern nutrition in health and disease. 4th ed., edited by M. G. Wohl and R. S. Goodhart. Philadelphia: Lea and Febiger, 1968. In: Am. J. Med. Sci. 257: 153, 1969.

768. Krzywicki, H. J., C. F. Consolazio, H. L. Johnson, W. C. Nielsen and R. A. Barnhart. Water metabolism in humans during acute high altitude exposure (4,300 m). J. Appl. Physiol. 30: 806, 1971.

769. Consolazio, C. F., H. L. Johnson, H. J. Krzywicki and T. A. Daws. Adaptation to high altitude (4,300 m). J. Physiol. (Paris) 63: 232, 1971.

*770. Sauberlich, H. E. Problems of assessment of Nutritional status: An overview of biochemical methodologies. In: Problems of Assessment and Alleviation of Malnutrition in the United States (proceedings of a workshop sponsored by Vanderbilt University, HSMHA and NIH held at Nashville, Tennessee, January 13-14, 1970) edited by R. G. Hansen and H. N. Munro. Washington, DC, US Government Printing Office, Pub. #916.086, 1971.

*771. Hodges, R. E., and J. E. Canham. Vitamin deficiencies: Studies of experimental vitamin C deficiency and experimental vitamin A deficiency in man. In: Problems of Assessment and Alleviation of Malnutrition in the United States (proceedings of a workshop sponsored by Vanderbilt University, HSMHA and NIH held at Nashville, Tennessee, January 13-14, 1970). Edited by R. G. Hansen and H. N. Munro. Washington, DC, US Government Printing Office, Pub. #916.086, 1971.

772. Gozansky, D. M., and R. H. Herman. Water and sodium retention in the fasted and refed human. Am. J. Clin. Nutr. 24: 869, 1971.

773. Hannon, J. P., K. S. K. Chinn and J. L. Shields. Alterations in serum and extracellular electrolytes during high altitude exposure. J. Appl. Physiol. 31: 266, 1971.

774. Consolazio, C. F., and H. L. Johnson. Measurement of energy cost in humans. Federation Proc. 30: 1444, 1971.

775. Johnson, H. L., C. F. Consolazio, T. A. Daws and H. J. Krzywicki. Increased energy requirements of man after abrupt altitude exposure. Nutrition Reports International 4: 77, 1971.

*776. Demaree, R. S., Jr., and W. C. Marquardt. Avian trypanosome division: A light and electron microcope study. J. Protozoology 18: 388, 1971.

777. Johnson, H. L., C. F. Consolazio, H. J. Krzywicki, G. J. Isaac and N. F. Witt. Metabolic aspects of caloric restriction: Nutrient balances with 500-kilocalorie intakes. Am. J. Clin. Nutrition 24: 913, 1971.

778. Baker, E. M., III, D. C. Hammer, S. C. March, B. M. Tolbert and J. E. Canham. Ascorbate sulfate: A urinary metabolite of ascorbic acid in man. Science 173: 326, 1971.

*779. Adcock, E., and H. Greene. Poliovirus antibodies in breast-fed infants. Lancet, 18 Sep 71.

780. Kishimoto, R. A., G. L. Brown, E. B. Blair and D. Wenkheimer. Melioidosis: Serologic studies on U. S. Army personnel returning from Southeast Asia. Military Med. 136: 694, 1971.

781. Stifel, F. B., R. H. Herman and N. S. Rosensweig. Dietary regulation of glycolytic enzymes. XI. Effects of inhibitors of protein synthesis on the adaptation of certain jejunal glycolytic and folate-metabolizing enzymes to diet and sex steroids. Biochim. Biophys. Acta 237: 484, 1971.

782. Demaree, R. S., Jr., L. J. Ackerman and D. L. Anderson. "Permeability in the blood-brain barrier in monkeys following exposure to high altitude." In: 29th Ann. Proc. Electron Microscopy Soc. Amer., edited by C. J. Arceneaux. Boston, Mass., 1971.

783. Consolazio, C. F., H. L. Johnson, H. J. Krzywicki, T. A. Daws and R. A. Barnhart. Thiamin, riboflavin, and pyridoxine excretion during acute starvation and calorie restriction. Am. J. Clin. Nutrition 24: 1060, 1971.

784. Consolazio, C. F., R. A. Nelson, T. A. Daws, H. J. Krzywicki, H. L. Johnson and R. A. Barnhart. Body weight, heart rate, and ventilatory volume relationships to oxygen uptakes. Am. J. Clin. Nutrition 24: 1180, 1971.

785. Greene, H. L., R. H. Herman and S. Kraemer. Stimulation of jejunal adenyl cyclase by ethanol. J. Lab. Clin. Med. 78: 336, 1971.

786. Stifel, F. B., and R. H. Herman. Effect of L-histidine on human and rat jejunal pyruvate kinase activity. Can. J. Biochem. 49: 1105, 1971.

787. Stamper, D. A., R. T. Sterner and R. A. Kinsman. Symptomatology subscales for the measurement of acute mountain sickness. Perceptual & Motor Skills 33: 735, 1971.

*788. Chase, H. Peter, V. Kumar, J. M. Dodds, H. E. Sauberlich, R. W. Hunter, R. S. Burton and V. Spalding. Nutritional status of preschool Mexican-American migrant farm children. Am. J. Diseases Children 122: 316, 1971.

789. Stifel, F. B., H. L. Greene and R. H. Herman. Effect of oral contraceptive steroids on jejunal pyruvate kinase and adenyl cyclase activities. Endocrinology 89: 896, 1971.

790. Tillotson, J. A., and H. E. Sauberlich. Effect of riboflavin depletion and repletion on the erythrocyte glutathione reductase in the rat. J. Nutrition 101: 1459, 1971.

791. Weiser, P. C. "Alteration in enzyme activity in work and fatigue," In: Physiology of Work Capacity and Fatigue, edited by Ernst Simonson. Springfield: C. C. Thomas, 1971.

*792. Wakat, D. K., R. E. Johnson, H. J. Krzywicki and L. I. Gerber. Correlation between body volume and body mass in men. Am. J. Clin. Nutrition 24: 1308, 1971.

*793. Levine. R. A. Effects of exogenous adenosine 3',5'-monophosphate in man. II. Glucose, nonesterified fatty acid and cortisol responses. Metabolism 17: 34, 1968.

*794. Levine, R. A., L. H. Dixon and R. B. Franklin. Effects of exogenous adenosine 3',5'-monophosphate in man. I. Cardiovascular responses. Clin. Pharmacol. Therapeutics 9: 168, 1968.

795. Consolazio, C. F. Energy expenditure studies in military populations using Kofranyi-Michaelis respirometers. Am. J. Clin. Nutrition 24: 1431, 1971.

796. Nielsen, W. C., Jr., H. J. Krzywicki, H. L. Johnson and C. F. Consolazio. Use and evaluation of gas chromatography for determination of deuterium in body fluids. J. Appl. Physiol. 31: 957, 1971.

797. Bodenhausen, G. E., and H. L. Johnson. Design of an 13-unit rodent activity treadmill cage and stimulation system. J. Appl. Physiol. 31: 954, 1971.

798. Vogel, J. A., R. L. Genovese, T. L. Powell, G. W. Bishop, T. J. Bucci and C. M. Harris. Cardiac size and pulmonary hypertension in dogs exposed to high altitude. Am. J. Vet. Research 32: 2059, 1971.

799. Greene, H. L., and R. H. Herman. Human jejunal adenyl cyclase and phosphodiesterase. Biochem. Med. 6: 19, 1972.

800. Consolazio, C. F., H. L. Johnson, H. J. Krzywicki and T. A. Daws. Metabolic aspects of acute altitude exposure (4,300 meters) in adequately nourished humans. Am. J. Clin. Nutrition 25: 23, 1972.

801. Krzywicki, H. J., C. F. Consolazio, H. L. Johnson and N. F. Witt. Metabolic aspects of caloric restriction (420 Kcal): Body composition changes. Am. J. Clin. Nutrition 25: 67, 1972.

802. Consolazio, C. F., and H. L. Johnson. Dietary carbohydrate and work capacity. Am. J. Clin. Nutrition 25: 85, 1972.

803. Dowdy, R. P., and G. L. Dohm. Effects of training and exercise on serum ceruloplasmin in rats. Proc. Soc. Exper. Biol. Med. 139: 489, 1972.

*804. Meikle, A. W., P. J. Wittek and G. J. Klain. Adrenal steroidogenesis in thiamin-deficient (TD) rats. Federation Proc. 31: 285 abs, 1972.

*805. Hannon, J. P., E. L. Gibbs, T. E. Paxson and D. M. Sudman. Basal metabolic and cardiopulmonary function of college women during altitude acclimatization. Federation Proc. 31: 389 Abs, 1972.

*806. Klain, G. J. High altitude and tyrosine metabolism in the rat. Federation Proc. 31: 390 Abs, 1972.

*807. Sullivan, F. J., and N. J. Corneil. The relationship between serum low molecular weight dextran (LMD) and peripheral blood flow. Federation Proc. 31: 392 Abs, 1972.

*808. Dowdy, R. P., and F. H. Nielsen. Effect of histidine, histamine, and aspirin on sulfur-35 metabolism in zinc-deficient chick bone. Federation Proc. 31: 668 Abs, 1972.

*809. Wallace, D. L., E. M. Baker, J. E. Canham, M. Raica, H. E. Sauberlich, R. S. Teplick and R. E. Hodges. Vitamin A depletion in the human male adult. Federation Proc. 31: 672 Abs, 1972.

*810. Raica, M., F. Neilsen, J. Scott and H. E. Sauberlich. The utilization and metabolism of retinol by retinol deficient and sufficient rats. Federation Proc. 31: 685 Abs, 1972.

*811. Johnson, H. L., C. F. Consolazio, R. F. Burk, T. A. Daws and T. M. Ward. Glucose metabolism in man after abrupt exposure to altitude. Federation Proc. 31: 689 Abs, 1972.

*812. Brown, D. G., and R. F. Burk. Selenium retention in tissues and sperm of rats fed a torula yeast diet. Federation Proc. 31: 692 Abs, 1972.

*813. Burk, R. F., and C. F. Consolazio. Selenium-containing rat plasma proteins. Federation Proc. 31: 692 Abs, 1972.

*814. Canham , J. E. The nutritional status of the modern military male and female. Federation Proc. 31: 704 Abs, 1972.

*815. Baker, E. M., J. E. Kennedy, B. M. Tolbert and J. E. Canham. Excretion and pool size of ascorbate sulfate and other ascorbate derivatives in man. Federation Proc. 31: 705 Abs, 1972.

*816. Tolbert, B. M., A. M. Spears, D. J. Isherwood, R. W. Atchley and E. M. Baker. Chemistry of the ascorbate sulfates. Federation Proc. 31: 705 Abs, 1972.

*817. Halver, J. E., C. L. Johnson, R. R. Smith, B. M. Tolbert and E. M. Baker. Vitamin C_3 reduces fish scurvy. Federation Proc. 31: 705 Abs, 1971.

*818. Ziporin, Z. Z., S. T. Amimoto and P. P. Waring. Acute effects from shifting rats on a low phosphorus diet to one containing adequate phosphorus. Federation Proc. 31: 707 Abs, 1972.

*819. Sauberlich. H. E., C. Rebouch and J. H. Judd, Jr. Influence of folic acid on ^{14}C-formate incorporation by blood and blood components of the rat and human. Federation Proc. 31: 712 Abs, 1972.

*820. Herman, R. H., Y. F. Herman and F. B. Stifel. Oral folic acid requirement for the normal adaptive response of jejunal pyruvate kinase (PK) activity to sex steroids in rats. Federation Proc 31: 712 Abs, 1972.

*821. Stifel, F. B., N. S. Rosensweig and R. H. Herman. Inhibitory effects of formimino-L-glutamic acid in vitro on rat jejunal folate-metabolizing and glycolytic enzymes. Federation Proc. 31: 712 Abs, 1972.

*822. Krzywicki, H. J., J. Gray, T. Ward, C. F. Consolazio and H. L. Johnson. Contribution of gastrointestinal gas to human body volume. Federation Proc. 31: 718 Abs, 1972.

*823. Huston, R. L., G. L. Dohm, P. W. Weiser, J. Boyd and E. W. Askew. The effect of diet, exercise and training on skeletal muscle glycolytic and liver gluconeogenic enzymes of the rat. Federation Proc. 31: 719 Abs, 1972.

*824. Dohm, G. L., R. L. Huston, E. W. Askew and H. L. Fleshood. The effects of diet, exercise and training on Krebs cycle enzymes in skeletal muscle of the rat. Federation Proc. 31: 719 Abs, 1972.

*825. Askew, E. W. Dohm, G. L., R. L. Huston and W. H. Doub, Jr. Effects of diet, exercise, and physical training on lipid metabolism in the rat. Federation Proc. 31: 719 Abs, 1972.

Publications - Fiscal Year 1972 (Cont)

*826. Fleshood, H. L., and R. P. Dowdy. Effect of moderate vitamin B_6 deficiency with low and high protein diets on alanine and aspartic aminotransferance and urinary O-phosphorylethanolamine in adult rats. Federation Proc. 31: 723 Abs, 1972.

827. Fowler, J. L., Y. Furusho and R. C. Fernau. Further testing of Fenthion for prophylactic effects against the developing stages of Dirofilaria Immitis. Southeast Asian J. Trop. Med. Public Health 2: 466, 1971.

828. Ackerman, L. J., R. S. Demaree, Jr., and D. L. Anderson. Sustained pressure perfusion method. Lab. Animal Science 22: 114, 1972.

829. Stifel, F. B., and R. H. Herman. Is histidine an essential amino acid in man? Am. J. Clin Nutrition 25: 182, 1972.

830. Krabill, L. F., and J. P. Hannon. Effects of high-altitude exposure on rate of ingesta passage in rats. J. Appl. Physiol. 222: 458, 1972.

*831. Anderson, J. W., and R. H. Herman. Effect of fasting, caloric restriction, and refeeding on glucose tolerance of normal men. Am. J. Clin. Nutrition 25: 41, 1972.

*832. Consolazio, C. F., H. L. Johnson, H. J. Krzywicki and T. A. Daws. Relationship of diet to the performance of the combat soldier. In: Research Programs Constituting U. S. Participation in the International Biological Program (Report No. 4 of the U. S. National Committee for the International Biological Program). Washington, D. C.: National Academy of Sciences, 1971.

*833. Consolazio. C. F. Nutrition and athletic performance. In: Progress in Human Nutrition, edited by Sheldon Margen, M. D. Westport, Conn.: The AVI Publishing Company, Inc., 1971, Vol. 1.

834. Raica, N., J. Scott, L. Lowry and H. E. Sauberlich, Vitamin A concentration in human tissues collected from five areas in the United States. Am. J. Clin. Nutr. 25: 291-296, March 1972.

835. Dowdy, R. P. and F. H. Nielsen, Effect of histidine, Histamine and aspirin on sulfur-35 metabolism in zinc-deficient chick bone. J. Nutrition 102: #4, 529-534, April 1972.

*836. DeVillez, R. L., E. G. Lufkin, and J. J. Bergin, Symmetrical enlargement of breasts and testes due to leukemic infiltration. Southern Med. J. 65: 341-343, March 1972.

Publications - Fiscal Year 1972 (Cont)

*837. Greene, H. L., J. E. Canham, R. H. Herman and Y. Herman, Vitamins. AMA Symposium on Total Parenteral Nutrition, Nashville, Tenn., Vol. I, 78-88, Jan 14-16, 1972.

*838. Taylor, R. F., T. J. Bucci and C. H. Garvin, Oligodendroglioma in a dog. J. Small Animal Practice 13: 41-46, 1972.

839. Tillot on, J. A. and E. M. Baker, An enzymatic measurement of the riboflavin status in man. Am. J. Clin. Nutr. 25: 425-431, April 1972.

840. Sauberlich, H. E., W. Goad, Y. F. Herman, F. Milan and P. Jamison, Biochemical assessment of the nutritional status of the Eskimos of Wainwright, Alaska. Am. J. Clin. Nutr 25: 437-445, April 1972.

*841. Anderson, J. W., R. H. Herman, J. B. Tyrrell, R. M. Cohn, Hexokinase: A compartmented enzyme. Am. J. Clin. Nutr. 24: 642-650, June 1971.

842. Fowler, J. L., K. Marsuda, and R. C. Fernau. Experimental infection of the domestic cat with Dirofilaria Immitis. J. Am. Animal Hosp. Assoc. 8: #2, 79-80, March 1972.

843. Blair, E. B., W. W. Bretherton, and A. H. Tull, A method to render unstained mycobacterial smears safe for storage or shipment. Applied Microbiol 23: #4, p 826, April 1972.

844. Demaree, R. S. and W. B. Nessmith, Ultrastructure of Haemobartonella felis from a naturally infected cat. Am. J. Vet. Rsch. 33: #6, 1303-1308, June 1972.

845. Demaree, Jr., R. S., P. Suster, W. R. Starke, and K. Alonso, Previously undescribed intraepithelial deposits in membranous glomerulonephritis. Lancet 1: (#7760) 1123-1124, 20 May 1972.

*846. Greene, H. L., D. R. Hazlett, J. G. Dramise, R. S. Demaree and R. H. Herman, Effects of intralipid on pulmonary function. Clin. Research 20: 274, 1972, (Abstract).

*847. Trevino, G. S., R. S. Demaree, and B. V. Sanders, Nutrition of the military dog. Proc. XIX Cong. Mundial de Medicina veterinaria zootechnia, 3: 1077, 1971, (Abstract).

848. Hannon, J. P., R. E. Beyer, R. F. Burlington, G. N. Somero, and J. H. Bland, "A guide for future studies on low temperature metabolic function," in Hibernation and Hypothermia: Perspectives and Challenges edited by F. E. South, Amsterdam, Holland, Elsevier Pub. Co , 1971.

*849. Fowler, J. L., M. D. Ruff, R. C. Fernau, and Y. Furusho, Babesia gibsoni: Chemotherapy in dogs, Am. J. Vet. Rsch. 33: #6, 1109-1114, June 1972.

850. Meikle, A. W. and G. J. Klain, Effect of fasting and fasting-refeeding on conversion of leucine into CO_2 and lipids in rats. Am. J. Physiol. 222: #5, 1246-1250, May 1972.

851. Lufkin, E. G., F. B. Stifel, R. H. Herman and N. S. Rosenweig, Effect of testosterone on jejunal pyruvate kinase activities in normal and hypogonadal males. J. Clin. Endocrinol. Metabolism 34: #3, 586-591, March 1972.

*Indicates that reprints of this paper are NOT available.

APPENDIX B

DIRECTORY OF OFFICER AND SENIOR PROFESSIONAL STAFF

Commanding Officer and Director	John E. Canham, COL, MC M.D. (Col. of P&S, Columbia Univ.)
Administrative Division Chief	John Lada, COL, MSC B.S. (Univ. of Maryland)
	Gerald Fisher, MAJ, MSC B.S. (Univ. of Notre Dame)
	Robert L. Morrissey, CPT, VC D.V.M. (Univ. of Ill.) Ph.D. (Cornell Univ.)
	Ronald Dutton, 1LT, MSC M. S. (Univ. of Minn.)
Computer Division Chief	Roque S. Romero, MAJ, MSC M.A. (Middle Tenn. State College)
Ass't Chief	Richard S. Teplick, CPT, MC M.D. (Univ. of Penn. School of Med,)
	Richard A. Nelson, GS-12 A.B. (Univ. of Calif.)
Metabolic Division Chief	Robert H. Herman, COL, MC M.D. (Univ. of Illinois) Biochemistry (Univ. of Penn.)
Ass't Chief	Louis Hagler, LTC, MC M.D. (U. of Colo. Med. School)
	Harry L. Greene, MAJ, MC M.D. (Emory Med. Sch.)

Directory of Officer and Senior Professional Staff (Cont)

Clara L. Miller, MAJ, AMSC
B.S. (Florida A&M)

Oscar D. Taunton, MAJ, MC
M.D. (Univ. of Alabama)

Janis C. Mullinnix, CPT, ANC
B.S. (Ft. Hays Kansas State College)

Frederick B. Stifel, GS-13
Ph.D. (Iowa State Univ.)

Bioenergetics Division

Chief — C. Frank Consolazio, GS-15
(Harvard Univ.)

Ass't Chief — Herman L. Johnson, GS-13
Ph.D. (VPI)

Raymond F. Burk, Jr., MAJ, MC
M.D. (Vanderbilt)

Harry J. Krzywicki, GS-12
M.S. (Northwestern Univ. Grad. Sch.)

Physiology Division

Chief — John P. Hannon, GS-15
Ph.D. (Univ. of Calif.)

Ass't Chief — Francis J. Sullivan, GS-13
Ph.D. (Jefferson Medical Coll.)

Edward G. Lufkin, LTC, MC
M.D. (Northwestern Univ. Med. Sch.)

Alfred W. Meikle, MAJ, MC
M.D. (Vanderbilt)

Philip C. Weiser, MAJ, MSC
Ph.D. (Univ. of Minn.)

Ray T. Sterner, CPT, MSC
Ph.D. (Univ. of Wisc.)

George J. Klain, GS-14
Ph.D. (Univ. of Ill.)

Directory of Officer and Senior Professional Staff (Cont)

Chemistry Division	
Chief	Howerde E. Sauberlich, PL-313 Ph.D. (Univ. of Wisc.)
Ass't Chief	Eugene M. Baker, III, COL, MSC Ph.D. (Georgetown Univ.)
	Eldon W. Askew, CPT, MSC Ph.D. (Michigan State)
	Harold H. Fleshood, CPT, MSC Ph.D. (Univ. of Wisc.)
	Donald L. Wallace, CPT, MSC Ph.D. (Michigan State)
	Zigmund Z. Ziporin, GS-14 Ph.D. (Georgetown Univ.)
	Richard P. Dowdy, GS-13 Ph.D. (North Carolina State)
	Nicholas Raica, Jr., GS-13 Ph.D. (Univ. of Ariz.)
	James H. Skala, GS-13 Ph.D. (Univ. of Minn.)
	Yaye F. Herman, GS-12 M.S. (Ill. Institute of Tech.)
	Richard L. Huston, GS-12 Ph.D. (Univ. of Ill.)
	Jerry Ann Tillotson, GS-12 M.S. (Univ. of Minn.)
Microbiology Division	
Chief	Eugene B. Blair, COL, MSC Ph.D. (Univ. of Texas)
Ass't Chief	George L. Brown, LTC, MSC Ph.D. (Univ. of Rhode Island)
	Ralph M. Coan, MAJ, MC M.D. (Georgetown Univ.)
	Thomas P. O'Barr, GS-13 Ph.D. (Univ. of Texas)

Directory and Officer and Senior Professional Staff (Cont)

Pathology Division	
Chief	Gilberto S. Trevino, LTC, VC D.V.M. (Texas A&M) Ph.D. (Michigan State)
Ass't Chief	Richard E. Whitmire, MAJ, VC D.V.M. (Texas A&M)
	Richard S. Demaree, CPT, MSC Ph.D. (Colorado State Univ.)
	Richard M. Empson, Jr., CPT, VC D.V.M. (Washington State Univ. School of Veterinary Medicine)
	Patrick R. Gavin, CPT, VC D.V.M. (Washington State Univ. School of Veterinary Medicine)
	John G. Miller, CPT, VC D.V.M. (Ohio State Univ.)
	Billy V. Sanders, CPT, VC D.V.M. (Texas A&M)
Food Hygiene Division	
Chief	James L. Fowler, COL, VC D.V.M. (Univ. of Georgia)
Ass't Chief	Warren C. Ladiges, CPT, VC D.V.M. (Washington State Univ. School of Veterinary Medicine)