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U. S. ARMY MEDICAL RESEARCH & NUTRITION LABORATORY

FITZSIMONS GENERAL HOSPITAL
DENVER, COLORADO 80240



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ANNUAL RESEARCH PROGRESS REPORT

(REPORTS CONTROL SYMBOL MEDDH 288 (R1))

30 JUNE 1971

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Fitzsimons General Hospital
Denver, Colorado 80240

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13. ABSTRACT

During Fiscal Year 1971 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-six work units presented.

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UNCLASSIFIED

Security Classification

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Reports Control Symbol MEDDH 288 (R1)

US Army Medical Research
and Development Technical Report

FISCAL YEAR 1971

30 June 1971

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 71 under the following projects and Task Area:

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 objectives 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 three USAMRNL Laboratory Reports, sixty papers published in the open scientific literature and thirty-seven published abstracts. This does not represent the entire productivity of the Laboratory for FY 71. 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, dietetic interns, graduate students, medical students on research clerkships, and

Forward (Cont'd)

reserve officers is conducted in-house.

Credit and congratulations for the assemblage and publication of this report are given to Mr. Walter O. Keinsley, C, Management Services Branch, USAMRNL.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6346	71 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. DOD'N INSTR'N	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
70 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	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	CONT	DA	C In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER: Not Applicable
c. TYPE: d. AMOUNT:
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18. RESOURCES ESTIMATE

	PRECEDING	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	71	1.1	40
	CURRENT		
	72	1.5	44

19a. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
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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 ov various therapeutic agents on fever induced by pyrogenic substances in normal human volunteers will be studied. Selected patients with thermoregulation defects will be investigated as to ways of returning temperature regulation to normal and in determining the site of the defect in the regulating mechanism.

25. (U) 70 07 - 71 06 Three patients with 3 different types of fever have been studied on a continuing basis. One patient responds to dexamethasone, one develops fever with exercise and the third, now refractory to uronic acid therapy, is made worse by prednisone but responds to an oral contraceptive estrogen. Hypopituitary patients do not respond to parenterally administered etiocholanolone. Hypophysectomized rats develop decreased levels of hepatic beta-glucuronidase which is an enzyme important in the metabolism of etiocholanolone. Growth hormone treatment does not restore enzyme activity. Computer programs have been developed for storing, plotting and analyzing human body temperature data.

1

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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. 3. The effect of hypophysectomy on beta glucuronidase activity in rat liver.

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

Study No. 2. Three patients with 3 apparently different types of periodic fever have been intensively evaluated and have been found to develop fever (and other abnormalities) by different maneuvers that do not provoke fever in normal subjects. These 3 patients develop fever by spontaneous mechanisms which are not well understood. These patients manifest paradoxical responses to medication and have shown equivocal or transient responses to uronic acids. The hypopituitary patients have provided clues regarding the relationships between pituitary function and thermogenic agents. These studies all support the concept that the periodic fevers are a heterogeneous group of diseases perhaps due to different defects along a final common pathway necessary for temperature regulation. The apparent participation of pituitary function in etiocholanolone induced fever adds a new dimension of consideration to temperature regulation in normal subjects and to the mechanisms of fever production.

Study No. 3. We studied the effect of hypophysectomy on liver beta-glucuronidase activity in control and hypophysectomized rats. A clear decrease in activity of beta-glucuronidase was apparent after 6 days and continued throughout the study. The addition of 0.5 units of porcine growth hormone on day 16 had no effect on beta-glucuronidase activity. These results indicate that a pituitary factor is involved in the regulation of liver beta-glucuronidase activity.

Study No. 4. All human body temperature data obtained on the Metabolic ward in various studies has been recorded on special forms and has been stored in the computer. All such data has been plotted and tabulated by the Computer Division. Data is now in the proper form for inspection and analysis.

BODY OF REPORT

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

STUDY NO. 2. — Studies in patients with fever.

PROBLEM:

As a result of previous studies we have postulated that human temperature in part may be regulated by the ratio of conjugated to free etiocholanolone in the liver. We have continued to study selected patients with periodic fever and other disorders of temperature regulation as well as normal individuals to further evaluate this postulate. The present studies represent a continuing effort to delineate rational categories of classification for these diseases which appear to be heterogeneous in nature. The studies are also directed at increasing our understanding of mechanisms of temperature regulation in the normal.

RESULTS AND DISCUSSION OF THE RESULTS:

Patients with periodic fever-like abnormalities in temperature regulation have been studied. Two patients have undergone continued investigation and a third patient who has previously been evaluated was re-evaluated due to a severe exacerbation in her disease.

The first patient has a periodic fever-like illness which began in 1966. He has been studied on several occasions since that time. He initially manifested polyserositis and fever and has subsequently developed a polyarthropathy with fever but without involvement of other serous membranes. Thrombocytosis and hyperfibrinogenemia have complicated the clinical picture. Indomethacin and dexamethasone provided objective resolution of his problems, but an allergic reaction to the indocin precluded it further use. His responses to uronic acids have been inconsistent. The polyarthritis which is an unusually severe but nondeforming type has been well controlled on dexamethasone therapy, as has the fever, thrombocytosis and hyperfibrinogenemia. Further study of this patient is planned.

The second patient has previously undergone study on the Metabolic ward. He has recurring episodes of fever, and was found to have associated hepatic granulomata. Liver function studies usually become modestly abnormal during febrile periods. Despite the presence of the hepatic granulomata, liver function studies during afebrile periods are normal. This patient develops striking fever with minimal to moderate amounts of exercise, and his temperature remains elevated long after exercise is discontinued. These findings are very reproducible with treadmill walking. Various antipyretics seem to have little effect on this exercise-induced fever. A recent completed series of studies on this patient measured the temperature of leg

muscle during various graded levels of treadmill walking. Muscle temperature rises during exercise and remains elevated for a prolonged period following the cessation of exercise, and thus parallels gross body temperature. A dose-response type of effect is seen with muscle temperature during exercise in that increasingly difficult exercise causes an increase in muscle temperature.

The third patient has undergone study on the Metabolic ward. She has a 20+ year history of recurring episodes of fever and abdominal and/or flank pain. Following extensive study she was treated with glucuronic and glucaric acids, both of which seemed to be beneficial. After a time, however, neither medication helped, and she had daily fever and continuous pain. Re-evaluations of her responses to uronic acids indicated that none helped and steroids made her worse (i.e., continued fever and intensification of pain). This paradoxical response to steroid medication has been seen before in some patients with periodic fever and serves to underscore the underlying heterogeneity of this group of patients. During 2 pregnancies she was completely asymptomatic. We, therefore, initiated treatment with oral contraceptive medication and after 4-6 weeks she began to experience dramatic improvement with cessation of fever and absence of pain. This therapy will be continued so long as it controls symptoms without complications.

The study of the mechanisms of action of etiocholanolone and piromen, both of which will cause fever when given parenterally has continued. Previously it was demonstrated that patients with hypopituitarism did not develop fever when given etiocholanolone, but did respond with fever when given piromen. Piromen causes growth hormone release (as well as fever) when given to normal subjects. Etiocholanolone does not appear to have any effect on growth hormone, despite provoking high fever in normal subjects. Initially it appeared that adrenal steroid replacement was the necessary factor in the genesis of etiocholanolone fever in hypopituitary patients. It appears that this is not true, and that the necessary factor is growth hormone. Further studies of hypopituitary patients have disclosed that those with low but detectable levels of growth hormone will respond to etiocholanolone by developing fever.

CONCLUSIONS:

Three patients with 3 apparently different types of periodic fever have been intensively evaluated and have been found to develop fever (and other abnormalities) by different maneuvers that ordinarily do not provoke fever in normal subjects. These 3 patients, in addition, develop fever by spontaneous mechanisms which are not well understood. These patients manifest paradoxical responses to medication and have shown equivocal or transient responses to uronic acids. The hypopituitary patients have provided clues regarding the relationships between pituitary function and thermogenic agents. These studies all support

the concept that the periodic fevers are a heterogeneous group of diseases perhaps due to different defects along a final common pathway necessary for temperature regulation. The apparent participation of pituitary function in etiocholanolone induced fever adds a new dimension of consideration to temperature regulation in normal subjects and to the mechanisms of fever production.

RECOMMENDATIONS:

The mechanisms which provoke and prevent fever should be evaluated on a continuing basis. These studies give promise of elucidating normal mechanisms of temperature regulation and of delineating disorders of temperature regulation into a rational classification. These studies have been conducted on an in-house basis. The in-house support for these studies will terminate in FY72.

PUBLICATIONS: None.

STUDY NO. 3. The effect of hypophysectomy on beta glucuronidase activity in rat liver.

PROBLEM:

A mechanism appears to exist in human liver whereby the ratio of conjugated to free etiocholanolone is regulated through a beta glucuronidase enzyme system. Since patients with hypopituitarism may fail to develop fever from etiocholanolone a pituitary hormone may regulate hepatic beta-glucuronidase activity. Therefore, the level of beta-glucuronidase in hypophysectomized rats was determined.

RESULTS AND DISCUSSION OF THE RESULTS:

Beta-glucuronidase activities in the liver of control, hypophysectomized and hypophysectomized - growth hormone (0.5 units, pig GH) treated rats are seen in the following table.

Days post hypophysectomy	Control	Hypophysectomy	Hypophysectomy plus growth horome
2	26.21 ± 4.15*	25.6 ± 6.24	--
6	35.78 ± 5.87	10.67 ± 1.32	
9	28.19 ± 7.72	18.52 ± 0.789	
13	38.34 ± 13.98	11.78 ± 3.21	
16	28.1 ± 4.84	11.41 ± 1.27	
20	54.42 ± 29.05	30.86 ± 4.46**	24.7 ± .473
23	44.35 ± 14.13	28.07 **	11.88 ± 3.22
27	39.875± 25.58**	14.12 **	10.705± .822**

The Mechanism of Body Temperature Control (Cont'd)

Beta-glucuronidase activity expressed as µg phenolphthalein split/hr/mg liver protein 37°.

* Mean ± Standard error of mean for 3 rats.
** Other than 3 rats used.

A clear difference in the activities of beta-glucuronidase is apparent after the 6th post-hypophysectomy day which is not corrected by the addition of growth hormone on day 16. These results are compatible with our clinical impression that a pituitary factor is involved in the regulation of beta-glucuonidase and perhaps in fever regulation.

CONCLUSIONS:

A preliminary study of 50 control and hypophysectomized rats showed a decrease in beta-glucuronidase activity in the hypophysectomized animal not corrected by the addition of growth hormone.

RECOMMENDATIONS:

These studies should be continued. Hormonal replacement studies should be performed utilizing the various trophic hormones in combination (ACTH, TSH, GH and gonadotropin) to see if these correct the defect in beta-glucuronidase activity. Selective removal of each agent can be done to see which is responsible for the decline in beta-glucuronidase activity. If the trophic hormones are not effective than a search for a new hormone is indicated.

PUBLICATIONS: None.

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

PROBLEM:

The studies conducted on the mechanisms of body temperature regulation in normal subjects and on selected patients with periodic fever and other disorders of temperature regulation have provided large amounts of human temperature data both in normal subjects and in the patients. The amount and type of data lends itself particularly well to computer analysis. We have developed methods to enter, store, analyze, compare and retrieve all human temperature data obtained on the metabolic ward since these studies were initiated.

RESULTS AND DISCUSSION OF THE RESULTS:

The application of computer analysis of temperature data has been

coordinated with personnel of the Computer Division. Two temperature worksheets were devised to provide information for entry into the computer. The first identifies the individual by name and SSAN and denotes whether he is a patient or normal subject. It further characterizes his daily condition (asymptomatic, better, worse, etc.) and his test status (off study, control, test, illness, etc). It then indicates whether fever inducing or supressing agents were utilized and the dose. The second sheet provides a tabulation of temperature measurements by time, characterizes the type of temperature measured (oral, rectal, tympanic, muscle, etc.), any other events (vital signs, injections, etc.) and allows for nursing personnel comments. These two worksheets are then provided to the Computer Division where the data is placed on punch-tape or cards and entered into the computer. They in turn will provide us two separate computer print-outs. One will be a graph of the temperature curve for the data provided and the second will be a running tabular account of the temperatures, other events and nurses' comments. These two separate sheets have been printed for each patient-day for all temperature studies conducted and have been returned to us for analysis. Fourier analysis of temperature curves, analysis and comparison of temperature curves from patients and normal subjects, comparison of mathematical model temperature curves and real curves obtained from normal subjects and patients are all being initiated. Further analytical methods will be applied after these preliminary studies have been completed.

CONCLUSIONS:

The appropriate administrative action and forms have been established to store, analyze, compare and retrieve human temperature data obtained on the metabolic ward. All of our temperature data has been processed and future temperature data will be similarly handled in an ongoing fashion as it is accumulated. The amount and type of data lends itself particularly well to computer analysis.

RECOMMENDATIONS:

This is an important adjunct to the studies conducted in work unit 049. It appears that this type of application has great potential for the more detailed analysis of temperature data and may provide insights into controlling mechanisms.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6362	71 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
70 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	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 71	2.4	21
FISCAL YEAR CURRENT 72	3.0	15

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
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NAME: Trevino, G. S., LTC 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) 70 07 - 71 06 Study 1: Rabbits received 20cc/kg of intravenous lipid emulsion and were sacrificed from 2 hours to 8 weeks post-infusion. Lipid was assimilated into hepatocytes 2 hours post infusion. Pigment became prominent at 6 days. Pigment was still present in liver at 8 weeks. Lipid was surrounded by pigment. At 8 weeks both splenic lipid and pigment were still present. The pigment was morphologically identical to secondary lysosomes. These results suggest that current lipid emulsions are incorporated into lysosomes in liver and spleen, possibly reducing their availability for energy production, and are stored in the liver and spleen. Final reports are in preparation for Study 1.

*Available to contractors upon originator's approval

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

ABSTRACT

PROJECT NO.	3A061101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	054	Ultrastructure of Normal and Diseased Animal Tissue
	STUDY NO. 1	A Sequential Study of the Ultracellular Changes Within the Hepatic and Reticuloendothelial Cells Following Administration of Parenteral Lipid Emulsions

A pigment morphologically identical to secondary lysosomes was produced in rabbit liver and spleen by intravenous administration of an egg yolk phosphatide/ cottonseed oil emulsion. The incorporation and assimilation of the lipid emulsion by reticuloendothelial and hepatic parenchymal cells was studied by electron microscopy after the following post-injection intervals: 2 and 24 hours; 6 days; and 2, 4, and 8 weeks. Lysosomal pigment and lipid remained in liver and in spleen at 8 weeks.

BODY OF REPORT

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

STUDY NO. 1 — A Sequential Study of the Ultracellular 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.

RESULTS AND DISCUSSION OF RESULTS:

Liver and spleen from rabbits which received 20cc/kg of a cottonseed oil emulsion (SSRL) were examined by electron microscopy. Control liver and spleen had ultrastructural morphology typical of the respective mammalian tissues.

Two hours after injection, lipid was present in sinusoids and in the periphery of hepatocytes. None was seen in Kupffer cells. No indication of endocytosis of lipid was noted in hepatocytes.

At 24 hours more lipid was incorporated into hepatocytes and some into Kupffer cells. At 6 days post injection, lysosome-like pigment became prominent and was usually associated with lipid droplets in both Kupffer cells and hepatocytes. This pigment increased at 2 and 4 weeks post-injection and had not diminished at 8 weeks.

Two hours after injection, most lipid droplets in spleens were extracellular, although numerous examples of endocytosis of intact lipid droplets were seen in macrophages. Some lysosome-like pigment was seen. By 24 hours, most lipid was intracellular.

At 6 days after injection, lysosome-like pigment was prominent in splenic macrophages and usually was associated with lipid droplets.

At 2 weeks after injection, the size and number of lysosomes was reduced. This continued until 8 weeks, when no lipid or associated lysosome-like material was seen by electron microscopy. The pigment was still present however in light microscope sections.

A unique paracrystalline orientation of lipid within splenic macrophages was observed both by light and electron microscopy. This was most prominent at 6 days after injection.

Pigment deposition in rabbit livers was similar to that reported by other investigators. However, no other organelle alterations (dilatation of rough-surfaced endoplasmic reticulum, central core formation in mitochondria, and ribosomal detachment from rough-surfaced endoplasmic reticulum) were seen. This lipid emulsion (cottonseed oil with egg lecithin emulsifier) apparently produced fewer and more mild ultrastructural alterations in liver than other emulsions previously tested.

Pigment deposition in rabbit spleens was located apparently only in macrophages. The pigment was morphologically identical to lysosomes and was usually associated with lipid droplets. Evidence of endocytosis of lipid into macrophages was similar to that reported for other reticuloendothelial cells.

Electron microscopy in support of chemistry Work Unit 058 has been performed. Results are reported in that Work Unit. A manuscript describing a technique for the isolation of skeletal muscle mitochondria is in preparation.

CONCLUSIONS:

The results of this study suggest that much of the lipid seen in liver and spleen was incorporated into lysosomes, probably rendering that lipid unavailable for energy production. The lipid and associated pigment-complex were stored in the liver and spleen at least for the duration of this study.

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

A laboratory report is being prepared and an additional protocol is in preparation.

PUBLICATIONS:

1. Bucci, T. J., Dean, W. D., Bishop, G. W., Frics, L.A. and Bischoff, M.B. Species comparisons of cardiac hypertrophy in animals chronically exposed to high altitude. USAMRNL Lab. Rpt. No. 326, Jan 1971.

2. Demaree, R. S., Jr., and Marquardt, W. C. Avian trypanosome division; a light and electron microscope study. J. Protozool. 18 (3), 1971, (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6369	71 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
70 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	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 71	1.6	21
	CURRENT 72	2.5	18

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

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Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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

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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) 70 07 - 71 06 Experiments, infusing low molecular weight dextran into dogs, have shown that cardiac output and peripheral blood flow have been doubled, principally by increasing stroke volume. About 10% of the augmented flow following dextran is associated with a lowering of whole blood viscosity. Studies in military surgical patients at Fitzsimons General Hospital, currently in progress, indicate the patients are significantly aided by the dextran-increased peripheral blood flow during the important post-operative period when blood flow to ischemic tissues should be maximized.

* Available to contractors upon originator's approval

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

ABSTRACT

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

The following investigation was conducted under this work unit during the past year.

Study No. 1	The effects of low molecular weight dextran on the cardiovascular system of dogs

During the past year experiments were completed showing that low molecular weight dextran (LMD) infused at a rate of 1 g LMD/kg per hour doubled cardiac output and peripheral blood flow. One hour after infusion peripheral blood flow was still 50% above control. During this time reactive hyperemic flow was correlated most closely with hematocrit. Vasoactive drugs, thus far, have resulted in variable responses of cardiac output, heart rate and blood pressure. Studies have been started at Fitzsimons General Hospital to evaluate the cardiovascular system in two series of patients: those in which an aorto-coronary artery bypass has been constructed, and those in which an aorto-iliac artery bypass has been constructed. Present information indicates that LMD substantially increases coronary blood flow following surgical reconstruction.

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 the injury. Reversal of this ischemia is dependent upon reestablishment of occluded blood flow or augmentation of reduced flow to the involved area(s) of the body.

In recent years considerable interest has been generated in the use of low molecular weight dextran (LMD) for the improvement of blood flow in various ischemic states. The mechanism for this is still controversial. In an attempt to explore this mechanism three phenomena were examined: The degree and duration of enhanced limb flow following infusion, the relationship of increased cardiac output to increased limb flow and the relation of the level of circulating LMD to increased limb flow. Other experiments investigated the relationship of hematocrit to the magnitude of reactive hyperemia. Lastly, in military patients, the enhancement of peripheral blood flow by LMD is being evaluated. In conjunction with this, the effects of clinically used vasoactive drugs are also being monitored.

RESULTS AND DISCUSSION OF RESULTS

A study in which LMD was infused (1 g LMD/kg per hr) into ten dogs was completed. At the end of one hour's infusion, cardiac index had doubled. Concomitantly, arterial blood pressure remained constant whereas heart rate and stroke volume had increased 18% and 56%, respectively. Simultaneously, limb blood flow was increased 114% while limb vascular resistance was decreased to 46% of control. In comparison to the studies reported last year, a slow infusion of LMD increases peripheral blood flow to a greater extent and for a longer period of time than a rapid injection of the same quantity of LMD. Some of the efficacy of LMD to augment peripheral blood flow seems to be related to an increased venous return, as indicated by a decreased hematocrit, maintenance of pulmonary arterial pressure and increased left ventricular dp/dt.

In a study of five intact and five splenectomized dogs, plasma volume was measured with T-1824 dye prior to LMD infusion, at the end of a 60-minute infusion of LMD (1 g LMD/kg per hour) and 60

Peripheral Blood Flow Studies (cont)

minutes after the termination of infusion. In the intact preparation, plasma volume was 0.95 L, 1.43 L and 1.1 L at these intervals. However, in the splenectomized preparation the values were 1.1 L, 1.2 L and 1.2 L respectively. Studies are planned to determine the influence of splenectomy on this difference in plasma volume expansion in dogs during LMD infusion.

Reactive, hyperemic blood flow was studied to ascertain the effect of hematocrit on flow. Experiments have shown that regardless of the level of preocclusive flow, the magnitude of reactive hyperemic flow was related only to changes in hematocrit, demonstrating that less than 10% of the increased flow following LMD can be explained on the basis of a decreased viscosity of whole blood.

Presently, we are investigating regimes to chronically increase peripheral blood flow in man without significantly affecting cardiac work. Intra-arterial injections of papaverine caused peripheral vasodilatation and a transient increase in blood flow (200-300%) that returned to control values within 5 minutes. Intravenous injections of papaverine have caused only a slight and transient hypotension with an elevated heart rate. Low molecular weight dextran (500 ml 10% LMD-in-saline) has consistently doubled cardiac output within 15 minutes after administration. At the same time, however, flow through aorto-coronary artery by-passes is increased but not proportionately to the enhanced cardiac output. LMD significantly potentiated the papaverine induced vasodilatation increasing peripheral blood flow up to 8 times control values. The onset of the increased flow was not affected, however, maximal flow lasted for at least 10 minutes. It returned to control values within 25 minutes. Patients with reconstructed A-V fistulas (in the leg) have been studied also with the same regime. Similar results with papaverine and LMD have been observed. Intravenous infusions of isoproteranol up to 16 µg/min have given conflicting results: peripheral vasoconstriction, tachycardia and hypertension have been observed in all patients. However, in only one patient was cardiac output and peripheral blood flow slightly elevated. In two others cardiac output and peripheral flow significantly decreased.

In both these groups of patients, the longevity of LMD in plasma, its relationship to enhanced cardiac output and the metabolism of LMD is being studied for 24 hours. Analysis of the data is incomplete at present because 5 more patients from each group should be studied. In patients with A-V fistulas the cardiovascular studies have been consistent preoperatively, intra-operatively, except for the responses to isoproteranol, and postoperatively. The usually high cardiac output has been decreased by the repair of the fistula to about 6 liters/min. LMD doubled cardiac output intra-operatively. This high value continues for at least 6 hours. At the end of 24 hours

Peripheral Blood Flow Studies (cont)

it has remained about 10 liters/min. Presently, venous plethysmography is being used to monitor limb flow following surgery. In the two patients studied, limb flow was increased (75%) following fistula repair and remained high 24 hours after surgery.

CONCLUSIONS:

1. The increase in plasma volume consequent to LMD infusion is 30% greater than can be accounted for on the basis of dose. This fluid must have entered the vasculature from pericapillary tissues.

2. Augmentation of limb blood flow appears maximized when a dose of LMD of approximately 0.5 g/kg/hr has been administered.

3. Increased limb flow continues for a longer period than the increased cardiac output. This is probably the result of capillary bed dilatation by the dextran molecule, an increase in plasma volume, and a decrease in hematocrit and blood viscosity.

4. The relationship between hematocrit and hyperemic blood flow indicates that about 10% of the increased peripheral blood flow is dependent upon hematocrit.

5. Studies on military surgical patients are presently being conducted. These studies are directed at regimes to increase cardiac output and peripheral blood flow to affected organs, acutely and chronically to reverse tissue ischemia. Preliminary data from LMD studies suggest it is effective in augmenting flow during the postoperative period when specific organs require as great a flow as possible to reverse ischemia.

6. The use of vasoactive agents has given conflicting results. Isoproteranol caused tachycardia, hypertension and peripheral vasoconstriction. In only one case was cardiac output elevated. Papaverine caused only a transient vasodilatation which was significantly potentiated by LMD.

RECOMMENDATIONS:

1. Continue studies in humans to delineate the effects and metabolism of dextran. Expand studies to administer 500 ml LMD at one time, one half the dose twice daily and one quarter the dose four times daily to ascertain the regime which will give the greatest and longest lasting enhanced cardiac output.

2. Continue studies on vasoactive drugs to determine their effects on normal and diseased cardiovascular systems.

3. Continue studies of peripheral flow following vascular surgery

Peripheral Blood Flow Studies (cont)

utilizing venous occlusion plethysmography to ascertain long-term effects of dextran and the adequacy of blood flow.

PUBLICATIONS:

1. Sullivan, F. J. and N. J. Corneil. Low molecular weight dextran and reactive hyperemia. Federation Proc. 30:322, 1971 (abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6372	71 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
70 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 (Procode 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 71	1.2	17
FISCAL YEAR CURRENT 72	2.0	18

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

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20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
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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 (Procode 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. Procode 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) 70 07 - 71 06 In a study of physical exhaustion, 57 subjects rode a bicycle at about 56% aerobic capacity and had an average quitting time (QT) of 34 min (range 1-1/2 to 98 min). Part of these QT differences can be explained by the endurance capacity of their cardiovascular system and by the degree of discomfort that each subject permitted himself to endure before quitting. A 27-item Physical Fatigue Scale was simultaneously developed and is being compared to QT. Pilot testing for a study on changes in regional blood flow and regional energy metabolism of military patrol dogs running to exhaustion is nearly complete. The effect of gait on treadmill walking in above-knee amputees is being studied. In addition, performance assessment data obtained in the course of two vitamin depletion studies (i.e., vitamin A and riboflavin) are being analyzed.

*Available to contractors upon originator's approval.

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

ABSTRACT

PROJECT NO.	3A061101A91C	In-House Laboratory Independent Research
WORK UNIT NO.	059	Performance, Fatigue, and Exhaustion

The following investigations have been conducted under this work unit:

Study No. 1	Effect 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 of 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 riboflavin deficiency

Pilot testing experiments to assess the changes in regional blood flow and regional energy metabolism of patrol dogs running to exhaustion is nearly complete. During a bicycle ride to exhaustion at greater than 50% aerobic capacity, a greater heart rate increment from about 4 to 12 minutes is associated with shorter quitting times. A 27-item Physical Fatigue Scale (PFS) for humans was developed and demonstrated highly significant increases in feelings of leg fatigue, general fatigue, and task aversion. A time course study of heart rate, blood metabolites, PFS, and reaction time walking to exhaustion at 50% aerobic capacity is in progress. The influence of gait on oxygen consumption, heart rate, and PFS score of above-knee amputees while walking at 1.5 mph, 5% grade is being studied. Behavioral data collected during eight test sessions of a two and one-half year vitamin A study (see Work Unit 072: Study No. 2) and four main test sessions of a 12-week riboflavin study (see Work Unit 072; Study No. 12) were prepared for statistical analysis. Analysis of the vitamin A data is currently in progress. Analysis of the riboflavin data showed: (1) urinary riboflavin values were related to certain personality variables (2) significant changes in select physical performance tests occurred during depletion, and (3) practice effects obscured certain physical and psychomotor performance effects.

BODY OF REPORT

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

STUDY NO. 1 — Effect 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. This study will focus on describing alterations in the control of energy metabolism during prolonged, exhaustive exercise.

RESULTS AND DISCUSSION OF THE RESULTS:

Pilot testing for Experiment 1 (Ketone Body Metabolism in Trained and Untrained Patrol Dogs During Treadmill Running) is nearly complete. Catheterization techniques for measuring cardiac output and injection techniques for quantitating regional blood flow with isotopically-labeled microspheres have been developed. A method for simultaneously measuring four gamma-emitting radionuclides contained in such microspheres has been evaluated. The determination of the average quitting times for four dogs at 6 km/hr and 15% or 20% grade and 8 km/hr at 20% is in progress. Nonavailability of a large dog treadmill between September 1970 and May 1971 prevented substantial progress in this study.

RECOMMENDATIONS:

1. With the recent availability of a suitable large dog treadmill and the provision of patrol dogs from Edgewood Arsenal, this study should be completed as soon as possible.

2. If the results demonstrate a significant ketone body production by working muscles additional experiments should be directed at the factors responsible for incomplete fatty acid oxidation by muscle tissue.

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 the exhaustion point for 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) is to 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 tolerance for discomfort relative to their capacity to do physical work.

RESULTS AND DISCUSSION OF THE RESULTS:

Phase I. A Perceived Discomfort Questionnaire (PDQ) has been developed to assess the relationship of discomfort tolerance to physical fatigue. Preliminary analysis of PDQ scores indicates a distinct clustering of preferred activities. This clustering suggests that a subject's activity preferences will predict his discomfort tolerance as well as his capacity for sustained physical work. The reliability of this prediction is highly dependent, as would be anticipated, on an accurate measure of physical condition. Further statistical testing of this hypothesis is in progress.

Phase II. In 53 male subjects riding a bicycle ergometer at 56% aerobic capacity under neutral motivational conditions, quitting time ranged from 1.5 to 98 minutes with a mean ($\pm$ SD) of 34 $\pm$ 22 minutes. The mean final heart rate of 146 $\pm$ 12 was independent of quitting time. The heart rate increase per minute from 5 to 13 minutes in general indicated ($r = -.38$) that larger heart rate increments are associated with earlier quitting times. Under conditions of higher motivation and work load (75% aerobic capacity), 18 of the same riders showed an increase in quitting time predictability (to $r = -.59$) from the heart rate increment between 4 to 12 minutes.

Before and at the end of each ride, an Initial Fatigue List of 63 items each having a 5-point Likert-like scaling was given. Through item analysis, 27 well-spaced and discriminating items were chosen from the (PFS). Three clusters of items comprise almost all the common variance of the PFS: "general fatigue" (worn out, physically tired, out of gas, weary); "leg fatigue" (weak legs, leg cramps, aching muscles, shaky legs); and "task aversion" (rather do something else, uncomfortable, bored, perspiring).

Performance, Fatigue, and Exhaustion (cont)

Phase III. Pilot testing to determine the time course changes in heart rate, blood metabolites, PFS, and reaction time in a walk to exhaustion at 50% aerobic capacity is in progress.

CONCLUSIONS:

Endurance fitness, in contrast to cardiopulmonary fitness, appears related to the work pulse increment from 4 to 12 minutes. Subjective symptoms of fatigue from prolonged strenuous exercise can be quantitated.

RECOMMENDATIONS:

1. Further analysis of personality factors, heart rate increment, and subjective symptomatology collected from Phase II should be completed.

2. The Phase III time course studies of these and other factors of physical fatigue 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 gait pattern, leaning toward their prosthetic side with legs slightly abducted. If the therapist's assumption that the cosmetic gait is the most efficient, why does the amputee revert to a gait pattern which is assumed to be more difficult for him energetically and is uncosmetic for him as well?

RESULTS AND DISCUSSION OF THE RESULTS:

Both normal subjects and above-knee amputees are being studied while walking at 1.5 mph, 5% grade for twelve minutes. Oxygen consumption and heart rate are measured intermittently, while a modified PFS will be given before and at the end of the walk.

RECOMMENDATIONS:

Work should be completed promptly to enable the Physical Therapy Section, Fitzsimons General Hospital, to evaluate its amputee rehabilitation programs.

Performance, Fatigue and Exhaustion (cont)

Study No. 4 — Vitamin A requirement of the adult human: relationship to performance

PROBLEM:

The purpose of the current study was to detail the potential effects of avitaminosis A upon a set of selected Behavioral Measures in humans. The study was conducted in cooperation with Chemistry Division, USAMRNL (see Work Unit 072; Study No. 2).

RESULTS AND DISCUSSION OF THE RESULTS:

The Behavioral Measures included were classified under the following headings: Personality (Minnesota Multiphasic Personality Inventory), Physical (Fleishman Physical Fitness Battery, Reaction Time, Minnesota Turning Test, Crawford Pins and Collars Test), and Perceptual Measures (Brightness Discrimination, High Frequency Audiometry, Orthorater). Considerable effort was devoted to reducing and transcribing data onto IBM cards. To date, only preliminary correlational analyses of these data have been completed. Such evidence indicates: (1) strong inter-test correlations for each measurement category occurred (r's $\geq$.80) (2) few measures correlated linearly with blood level values of vitamin A.

CONCLUSIONS

Additional, highly specific analyses of the data are planned. Final conclusions regarding behavioral effects of vitamin A depletion are reserved, pending further examination and analysis of the data.

Study No. 5 — Performance and psychological effects of riboflavin deficiency

PROBLEM:

The current study was an attempt to delineate the potential effects of ariboflavinosis upon a set of selected Behavioral Measures in humans. This study was conducted in cooperation with Chemistry Division, USAMRNL (see Work Unit 072; Study No. 12).

RESULTS AND DISCUSSION OF THE RESULTS

Six male volunteers from the Metabolic Ward, mean age - 22 years, were administered a battery of behavioral tests during four 2-day test sessions spaced throughout Control, Depletion, and Repletion phases of the experiment. Specifically, the measures were: WAIS Blocks, WAIS Vocabulary, WAIS Memory for Digits, Orthorater, Minnesota Multiphasic Personality Inventory, Rorschach, Pursuit Rotor, Dynamometer, Hand Dexterity, Brightness Discrimination.

Performance, Fatigue, and Exhaustion (cont)

Correlational analyses indicated that urinary riboflavin values were significantly related to personality subscales scores of the MMPI measuring Hypochondriasis (-.66), Depression (-.71), Hysteria (-.47), Psychopathic Personality (-.59), and Psychasthenia (-.46). Regarding physical performance changes, hand-grip strength as measured with a Dynamometer was found to decrement as a function of test periods ($F = 10.05$; $df = 3, 35$: $P \leq .01$), a change reflecting riboflavin depletion. Although additional tests of physical and psychomotor performance yielded significant F values as a function of test periods, interpretation of these results is difficult due to certain initial improvements during the first three testings, a result probably indicative of practice.

CONCLUSIONS:

Results are viewed as suggesting that riboflavin depletion is related to certain personality and performance changes in man. However, lack of separate control subjects, coupled with the occurrence of an initial increase in certain performance measures coincident with riboflavin depletion, requires cautious interpretation of the obtained findings.

RECOMMENDATIONS:

Future studies of behavioral effects induced by vitamin depletions require separate control subjects for purposes of isolating potential ward and treatment-related effects from those of the depletion regima, per se.

PUBLICATIONS:

1. Weiser, P. C. Alterations in enzyme activity in work and fatigue. In: Theory of Work Capacity and Fatigue, edited by E. Simonson. Charles C. Thomas: Springfield, Ill., 1971, 97-131.

2. Kinsman, R. A. and J. Hood. Some behavioral effects of ascorbic acid deficiency. Am. J. Clin. Nutr. 24:455, 1971.

3. Weiser, P. C., D. A. Stamper, and R. A. Kinsman. Development of a Physical Fatigue Scale. Med. Sci. Sports 3:501, 1971 (abstract).

4. Weiser, P. C., R. A. Kinsman, D. A. Stamper, and J. P. Hannon. Relationship of heart rate increment during exercise to rate of exhaustion. Fed. Proc. 30:372, 1971 (abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6373	71 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 04 09	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 (Precede 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 71	.1	1
FISCAL YEAR CURRENT 72	.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
Administrative Division

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

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

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TELEPHONE: 303 366 5311 X26111

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: DA

NAME:

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Combat Bone Injuries; (U) Vitamin D; (U) Calcium; (U) Phosphorus; (U) Mineral Metabolism

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

23. (U) 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. From Jan 1965 through Sep 70, 2,347,464 man days have been lost due to hospitalization of 59,472 soldiers in Viet Nam with bone and joint injuries. It is hoped that 25-HCC might prove effective in the prevention and/or treatment of the demineralization of bones which accompanies disuse during the prolonged convalescence involved in extensive wounds. Ultimate aim would be to decrease the period of convalescence. Demineralization also occurs naturally with advancing age (senile osteoporosis). This study is designed to determine the relative toxicity, tissue distribution sites and biological effects of vitamin D_3 and 25-HCC.

24. (U) The sites of tissue localization and the dynamics of metabolism of vitamin D_3 and 25-HCC will be studied in chicks by injection of ^{14}carbon vitamin D_3 and ^{3}H-25-HCC and sampling tissues over a period from 15 minutes to 72 hours. The relative vitamin D_3 and 25-HCC toxicity and metabolic effects will be studied by feeding 6 graded levels of vitamin D_3 and 6 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 ^{45}Ca binding activity, electrophoretic pattern, ^{45}Ca and ^{32}P uptake, 3',5' cyclic AMP conc., and membrane ATPase activity will be assayed in duodenal mucosa. Calcium conc. in kidney and per cent bone ash will also be determined at those times.

25. (U) 71 04 - 71 06 The tissue samples have been collected at the appropriate times as indicated in the protocol and are currently being assayed by the various investigators.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A06110A91C	In-House Laboratory Independent Research
WORK UNIT NO.	060	Vitamin D, Calcium and Phosphorus Metabolism

Study No. 1, Toxicity and tissue localization of vitamin D_3 and 25-hydroxycholecalciferol (25-HCC) has been initiated under this work unit. The study is designed to elucidate factors involved in the control of mineralization and determine the safe limitations of 25-HCC as a therapeutic agent to prevent and/or treat the bone demineralization which occurs during prolonged convalescence from extensive wounds. The sites of tissue localization and the dynamics of metabolism of vitamin D_3 and 25-HCC are being studied in chicks by injection of ^{14}Carbon vitamin D_3 and ^{3}H-25-HCC and sampling tissues over a period from 1.0 minute to 72 hours. Samples have been collected but have not yet been assayed. The relative toxicity and metabolic effectiveness of vitamin D_3 and 25-HCC are being evaluated by feeding 6 graded levels of each and observing gross and microscopic pathology as well as several metabolic parameters of calcium metabolism. Preliminary mortality rates indicate that 25-HCC is more toxic than vitamin D_3.

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 Viet Nam 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 are being studied by feeding 6 graded levels of vitamin D_3 and 6 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 phospate and alkaline phosphatase concentrations were also determined. Samples have been collected for determination of calcium concentration in kidney and percent bone ash but assays have not been performed as yet.

The sites of tissue localization and the dynamics of metabolism of vitamin D_3 and 25-HCC are being 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 will be 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 results have not been assimilated and statistically evaluated as yet. However, the mortality data gives preliminary indication that 25-HCC is more toxic than vitamin D_3. Remaining results and discussion will be deferred until the analysis of data is completed.

CONCLUSIONS:

None

RECOMMENDATIONS:

Completion of this initial study.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6335	71 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
70 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	059
b. CONTRIBUTING	61145011	3A014501B71P	01	
c. CONTRIBUTING	CDOG 1412A	(2)		

11. TITLE (Procede 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: 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	1.2	40
FISCAL YEAR CURRENT 72	1.5	50

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
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20. PERFORMING ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
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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 perfomance 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) Rats will be fed dietary lipid and carbohydrate to determine mechanisms by which carbohydrate is transformed into lipid, is circulated to adipose tissue, and is utilized by muscle. Bile steroid metabolism will be studied. The effect of vitamin D, clofibrate, and other substances on jejunal, adipose and hepatic tissue enzymes will be studied. These studies provide basic support for parallel human studies covered in work unit 078.

25. (U) 70 07 - 71 06 Vitamin D doesn't alter adenyl cyclase activity in rat duodenum, liver and renal cortex nor cation-sensitive membrane-ATPases in rat duodenum. Clofibrate and probucol inhibit adenyl cyclase, pyruvate kinase and phosphofructokinase in rats. Both drugs decreased serum triblyceride levels. Probucol was less inhibitory than clofibrate. Enzyme activities returned toward normal despite continued treatment with probucol. No change in cAMP levels were found with clofibrate or probucol.

*Available to contractors upon originator's approval

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

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. 1. The effect of vitamin D on rat duodenal, liver and renal cortical adenyl cyclase activity.

STUDY NO. 2. The effect of vitamin D on duodenal cation sensitive membrane ATPases.

STUDY NO. 3. The effect of clofibrate and probucol on rat tissue adenyl cyclase, PK, PFK and serum lipid concentrations.

STUDY NO. 5. Effect of clofibrate and probucol on cyclic AMP concentrations in rat duodenum, liver and fat.

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

Study No. 1. The effect of vitamin D on adenyl cyclase activity in rat duodenum, liver and renal cortex was measured. No effect of vitamin D was demonstrated.

Study No. 2. Vitamin D deficient and replete male Charles River rats given varying doses of vitamin D were studied at various times with regard to the activity of duodenal cation-sensitive membrane ATPase activity. A brush border (450 x g) fraction was assayed for calcium, magnesium, calcium plus magnesium, and calcium plus potassium stimulated activity. No significant differences in ATPase activity under the various conditions employed were seen among the different groups of rats. No significant hypocalcemia was encountered in the deficient animals. It appears that rats from a low vitamin D colony must be employed to elicit a response of the cation sensitive membrane ATPases in rats.

Study No. 3. The drugs clofibrate and probucol can be used to treat patients with hypertriglyceridemia. Clofibrate is most effective in lowering serum triglyceride concentrations in patients with endogenous hypertriglyceridemia (type IV hypertriglyceridemia) whereas probucol is more effective in lowering serum cholesterol in patients with type II hypertriglyceridemia. Administration of clofibrate to rats caused a decrease in adenyl cyclase activity in rat adipose tissue,

liver and jejunum. Since clofibrate and probucol presumably have different sites of action in lowering serum lipids, these studies were repeated and several parameters were measured simultaneously. It was found that adenyl cyclase, pyruvate kinase and phosphofructokinase were inhibited by both agents but the enzyme inhibition with probucol was only about 40% of that seen with clofibrate. Similarly, the serum lipids decreased proportionately the same as the enzymes. The cholesterol was affected more by probucol than by clofibrate.

Study No. 5. Cyclic AMP concentrations were measured in rat epididymal fat, liver and duodenum in normal rats and rats given clofibrate or probucol. No change in cyclic AMP levels was found in the drug treated group. Technical problems may have masked changes in the cAMP levels so the study will be repeated using changes in experimental technique.

Study No. 6. Adenyl cyclase activity was measured in rat duodenum and liver in normal, adrenalectomized and adrenalectomized corticosterone-treated rats on different diets and after fasting for 24 hours. Adenyl cyclase activity was the same in each group of animals on the diets but was higher in the adrenalectomized group after fasting. The cyclic AMP results are pending.

BODY OF REPORT

WORK UNIT NO. 059 Basic Studies in Lipids

STUDY NO. 1 The effect of vitamin D on rat duodenal liver and renal corticol adenyl cyclase activity.

PROBLEM:

Vitamin D alters the absorption and metabolism of calcium and phosphate in animals and man. Parathormone, which also alters calcium metabolism in man, seems to exert its effect through changes in adenyl cyclase activity in renal cortex and skeletal tissue. This study was done to evaluate the possible role of adenyl cyclase activity in vitamin D action. A previous study in rats suggested an effect of vitamin D on duodenal and renal cortical adenyl cyclase activity in the rat.

RESULTS AND DISCUSSION OF THE RESULTS:

No significant change in adenyl cyclase activity was found in rats deficient in vitamin D as compared to rats treated with a low and high dose of vitamin D.

CONCLUSIONS:

Vitamin D does not alter adenyl cyclase activity in rat duodenum, liver and renal cortex.

RECOMMENDATIONS:

This study does not rule out the possibility that vitamin D affects tissue levels of cyclic AMP. This study should be repeated and cyclic AMP levels measured.

PUBLICATIONS: None.

STUDY NO. 2 The effect of vitamin D on duodenal cation sensitive membrane ATPases.

PROBLEM:

In a previous study vitamin D-deficient and -replete male Holtzman rats given varying doses of vitamin D were studied at various times with regard to the activity of duodenal cation-sensitive membrane ATPase activity. Assay of the gut beyond 15 cm from the pylorus gave considerable variation. This was eliminated by employing the first 15 cm of the duodenum only. Under the latter conditions decreased calcium-sensitive membrane-ATPase activity was seen in the vitamin D

deficient animals with a return to normal or greater levels in animals given Vitamin D replacement. Since this study suggested the possibility of a direct relationship between vitamin D and calcium-stimulated membrane-ATPase activity in the duodenum these studies were then repeated to confirm the findings.

RESULTS AND DISCUSSION OF THE RESULTS:

In the present study 60 male Charles River weanling rats weighing 30-60 grams were placed on a vitamin D deficient diet with adequate calcium and phosphorus. The animals were divided into four groups: I) vitamin D deficient, II) vitamin D deficient plus 12.5 I.U. units vitamin D_2 twice per week, weeks 4-6; III) normal rats receiving 12.5 I.U. twice per week; IV) rats fed high vitamin D 250 I.U. twice per week.

A brush border (450 x g) plasma membrane fraction was employed and calcium-sensitive ATPase activity was assayed at calcium concentrations ranging from 1 to 30 mM. Magnesium stimulated activity and combinations of calcium with magnesium or potassium were also assayed. No significant differences in ATPase activity under the various conditions employed was seen among the different groups of rats. Moreover, the apparent K_m for calcium and V_{max} values were very similar among the different groups.

In the Holtzman rats but not in the Charles River rats the vitamin D deficient group developed significant hypocalcemia.

CONCLUSIONS:

1. It appears that to elicit a response of the cation-sensitive membrane-ATPase in rats to vitamin D one must employ weanling rats obtained from a low vitamin D colony (Holtzman or Sprague-Dawley).

2. Inasmuch as the rat is refractory to developing vitamin D deficiency it does not appear to be a suitable model for studying the effects of vitamin D on the jejunum.

RECOMMENDATIONS:

Since the chick is much more sensitive to the effects of vitamin D deficiency it is proposed that certain parameters of vitamin D action be studied in chick jejunum as a preparation for later study in human subjects.

PUBLICATIONS: None.

Basic Studies in Lipids (Cont'd)

STUDY NO. 3 — The effect of clofibrate and probucol on rat tissue adenyl cyclase, pyruvate kinase, phosphofructokinase and serum lipid concentrations.

PROBLEM:

Patients with endogenous hypertriglyceridemia (type IV hyperlipoproteinemia) have a defect in the regulation of serum triglyceride (pre-beta-lipoproteins) as related to dietary carbohydrate. Patients with hypercholesterolemia (type II hyperlipoproteinemia) have a defect in the regulation of serum cholesterol or beta-lipoproteins. Clofibrate has been a useful drug in lowering the triglyceride levels in patients with type IV hyperlipoproteinemia, whereas probucol appears to be more useful in lowering serum cholesterol levels in the type II hyperlipoproteinemias and has little effect on serum triglyceride levels. The mechanism of action of the two drugs would appear to be different. We postulated that the primary mechanism by which clofibrate lowered serum triglycerides was by inhibiting liver and fat adenyl cyclase. This enzyme was found to be decreased in rats as well as in normal human gut and fat tissue after clofibrate administration. Since probucol seemed to have a different mechanism of action than clofibrate, the effects of the two drugs on a number of enzymes (adenyl cyclase, pyruvate kinase and phosphofructokinase) and serum lipid concentrations were compared.

RESULTS AND DISCUSSION OF THE RESULTS:

The results showed that probucol and clofibrate had similar inhibitory effects on all the enzymes measured although the inhibition with probucol was only 40-60% of that seen with clofibrate. After 4 weeks of treatment the enzymatic activity of all the enzymes returned to control levels in the probucol treated animals, whereas the clofibrate treated animals showed no presumed compensatory response in the enzymes. Similar qualitative changes were seen in the serum lipid concentrations although the serum cholesterol concentrations were decreased slightly more in the probucol treated group as compared to the control or clofibrate treated groups. Animals were made hypertriglyceridemic by feeding diets high in fructose for a period of 5 weeks. The control animals received no other treatment (group I) whereas the treatment groups (group II and III) received 1% clofibrate and 0.5% probucol in their diets, respectively. The animals were pair-fed since the clofibrate treated animals ate less than the control or probucol treated animals.

CONCLUSIONS:

1. Clofibrate and probucol decrease rat liver and intestinal adenyl cyclase, pyruvate kinase, and phosphofructokinase although probucol

is less effective in decreasing the enzymes. There is a return toward normal activity after 4 weeks of treatment with probucol.

2. Both clofibrate and probucol cause a decrease in serum triglyceride concentrations. However, probucol is less effective than clofibrate.

3. Probucol caused a decrease in serum cholesterol concentrations whereas clofibrate had no effect on serum cholesterol concentrations.

4. The effect of clofibrate and probucol an adenyl cyclase may be the primary mechanism whereby they exert their triglyceride lowering action.

RECOMMENDATIONS:

Similar studies should be extended to man, measuring the actual changes that take place in tissue cyclic AMP concentrations in an effort to further characterize the mechanism of action of clofibrate in the human.

PUBLICATIONS: None.

STUDY NO. 5 — Effect of clofibrate and probucol on cyclic AMP concentrations in rat duodenum, liver and fat.

PROBLEM:

Clofibrate lowers fluoride-stimulated adenyl cyclase activity in rat and human tissues. If this effect on adenyl cyclase is important in the mechanism of action of the drug, one should be able to demonstrate decreased tissue levels of cyclic AMP in animals given this drug. This study was done to test this hypothesis. At the same time a new cholesterol lowering agent, probucol was also evaluated.

RESULTS AND DISCUSSION OF THE RESULTS:

Cyclic AMP levels in rat epididymal fat, liver and duodenum was not significantly different from that in control animals. Probucol also had no effect on cyclic AMP levels in these tissues. However, the animals were killed by cervical fracture before the tissue was removed for cyclic AMP determination and this may have obliterated any drug induced change in cyclic AMP levels since it has been recently shown that cyclic AMP levels change extremely rapidly.

CONCLUSIONS:

No conclusions can be drawn because of technical difficulties in the method used.

RECOMMENDATIONS:

The study should be repeated and tissue removed for cyclic AMP measurement after pentobarbital anesthesia prior to sacrifice of the animals.

PUBLICATIONS: None.

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 in the adrenalectomized animals was greater than in the normal or adrenalectomized animals given corticosterone after fasting for 24 hours. Cyclic AMP concentrations are being measured.

CONCLUSIONS:

Corticosterone does not affect adenyl cyclase activity in rat duodenum and liver after feeding different diets. However, the data suggests that adenyl cyclase activity is increased during fasting in adrenalectomized animals.

RECOMMENDATIONS:

The effect of corticosterone deficiency during fasting on adenyl cyclase activity in rat duodenum and liver should be evaluated in more animals to determine if this is a consistent finding.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6344	71 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
70 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	
c. CONTRIBUTING	CDOG 1412A	(2)		

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 71	3.2	143
FISCAL YEAR CURRENT 72	5.0	103

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

21. GENERAL USE

Foreign Intelligence not Considered

ASSOCIATE INVESTIGATORS

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NAME: Dowdy, R. P. DA

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) 70 07 - 71 06 Incorporation and turnover of sulfate sulfur is reduced in bone-forming regions of zinc-deficient chicks. Some antiarthritic drugs partially correct this abnormality. Sulfate metabolism in bone and soft tissues appears to be altered in zinc-deficient rats. In vitro cation concentration and incubation time greatly affects the rate of ^{14}C amino acid incorporation and integrity of liver fat polyribosomes. Factors other than parathormone and calcitonin appear to participate in calcium and phosphorus homeostasis.

*Available to contractors upon originator's approval

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

ABSTRACT

PROJECT NO.	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. 5	A Study of the Effects of Amino Acid Deficiency, Hormones and Divalent Cations on Rat Liver Polysomes and Amino Acid Incorporations

Study No. 2. Experiment 2. The experiments required to answer remaining problems have been performed. As before, the main finding is the failure of actinomycin D to inhibit the effect of vitamin D. This work is concluded with the preparation of a manuscript.

Experiment 3. Cyclic AMP and cyclic CMP depress the uptake of calcium by intestinal slices in vitro, while cyclic GMP, cyclic IMP and theophylline had no effect. In the presence of all the cyclic nucleotides used, vitamin D significantly increased the uptake of calcium.
Blood levels of calcium and phosphorus are profoundly influenced by the ratio of those minerals in the diet. Using purified components, the calcium and phosphorus content of the diet may be varied at the will of the investigator. With two basic diets (1.2% Ca: 0.06% P, and 0.02% Ca: 0.3% P) for rearing the animals during a 3-week post-weaning period, followed by a change to diets with extremes in calcium and phosphorus content, the blood levels of those minerals were assessed as were weight changes, food consumption, sodium and potassium excretion etc. When animals reared on a 1.2% Ca: 0.06% P diet for 3 weeks were changed to a < 0.02% Ca: 0.3% P diet for 24-48 hours, their food consumption decreased and death ensued in 12 of 15 animals at 48 hours after the change in diet.

Study No. 3. Histidine, histamine, or aspirin was fed to Zn-deficient or control chicks for 4 weeks, at which time the birds were intubated with $Na_2{}^{35}SO_4$. These drugs appeared to only partially correct the depression of sulfate incorporation into bone-forming regions caused

by Zn deficiency. Similarly, the drugs seemed to enhance sulfate incorporation into the tibia epiphyseal plate and primary spongiosa of chicks receiving adequate Zn. Of the three drugs tested, histamine was generally the most effective for increasing the incorporation of radiosulfur into the bone-forming regions.

Zinc deficiency in the rat appears to enhance sulfate absorption as evidenced by greater amounts of sulfur-35 in the serum, liver and brain six hours following an oral dose of the isotope. The epiphyseal cap of the femur from Zn-deficient rats also contained a greater concentration of the isotope than the control rats at the 6-hour interval. This is the reverse of the pattern observed in Zn-deficient chicks.

Study No. 5. In vitro amino acid incorporation by rat liver polyribosomes results in their degradation to oligosomes and free ribosomes within the first 20 seconds of incubation (37°). ^{14}C amino acid incorporation remains linear however for 2 minutes. The proportion of monomer, dimer, and trimer constitutes 55% of the total ribosomal material following an 8-minute incubation, the time when ^{14}C amino acid incorporation rate has greatly diminished and was no longer linear.

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. Same as stated for this study in FY 70 report.

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. As part of this study, it was deemed desirable to standardize the conditions so that the experimental animal would not be so variable. For that purpose, animals were reared on 1.2% Ca: 0.06% P or 0.02% Ca: 0.3% P diets for 20 days. Following this, they were placed on a < 0.02% Ca: < 0.02% P diet for 24 hours. By this procedure, it was expected that one could substitute this treatment for the 24-hour fast commonly used in experiments involving calcium and phosphorus metabolism.

RESULTS AND DISCUSSION OF THE RESULTS:

EX-2. In comparing the uptake of calcium by intestinal slices in vitro, the tissue from animals reared on a 1.2% Ca: 0.06% P had much higher uptakes than did animals reared on a 0.02% Ca: 0.3% P diet. Following a 15 or 24-hour fast, both animal groups exhibited a marked decrease in calcium uptake, with those from 1.2% Ca: 0.06% P reaching levels that were lower than those on the 0.02% Ca: 0.3% P diet.

EX-3. Following incorporation of various cyclic nucleotides in the incubation media, intestinal slices from vitamin D-deficient and vitamin D-repleted rats were assayed for their ability to accumulate ^{45}Ca and ^{32}P from the medium. By incubating the tissues under oxygen or nitrogen it was possible to distinguish any effects of cyclic AMP on intestinal accumulation due to energy-requiring processes vs. those due to anaerobic processes. In general, intestinal slices from vitamin D-deficient rats showed little difference in calcium uptake when incubated in the presence of oxygen vs. nitrogen. On the other hand, tissues from vitamin D-treated animals demonstrated a decrease in uptake when incubated in the presence of cyclic AMP, whether under oxygen or nitrogen. Yet, the vitamin D-fed animals showed greater accumulations of calcium than did the vitamin D-

deficient animals. In comparing the various cyclic nucleotides with regard to their effect on calcium uptake by intestinal slices, all tissues were taken from vitamin D-repleted animals and incubated under oxygen. It was found that cyclic AMP and cyclic CMP depressed the accumulation of calcium, while cyclic GMP, cyclic IMP and theophylline had no effect. Since literature reports deal only with cyclic AMP, calcium and vitamin D, only our results with cyclic AMP may be compared. Where we find a decreased uptake of calcium by intestinal slices of vitamin D-repleted rats when incubated in the presence of 2 μM/ml of cyclic AMP, others report an increased uptake of calcium by everted sacs of rat intestine incubated in the presence of 10 μM/ml of cyclic AMP. The basis for the different findings will be investigated.

In our previous work we reported that fasting a rat prior to experimental tests markedly alters the calcium and phosphorus metabolism of the test animal (Fed. Proc. 29: 367, 1970). We proposed that standard conditions should be applied to the test animals so that all investigators would start with the same animal preparation. Since the value of fasting the animal lay in the depletion of calcium and phosphorus in target organs, the same purpose could be accomplished by transferring the animals to a <0.02% Ca: <0.02% P diet which contained all the nutrients except for calcium and phosphorus. By this means the nutritional stress would be limited, and the emotional stress of hunger with attendant metabolic alterations eliminated. The following results were obtained:

Blood calcium and phosphorus levels (mg/100 ml blood) of animals reared on stated diet for 21 days before indicated treatment

Treatment	Diet			
	1.2% Ca:0.06% P		0.02% Ca:0.3% P	
	Ca	P	Ca	P
No fast	10.5	1.0	4.9	8.3
Fast 24 hrs	4.2	15.5	4.4	10.2
0 Ca: 0 P 24 hrs	8.9	1.7	7.2	3.0

The elevation of blood calcium in the 0.02% Ca: 0.3% P animals changed over to <0.02% Ca: <0.02% P for 24 hours cannot be explained on the basis of present knowledge regarding mechanisms for maintaining calcium homeostasis. Further investigations of the effects produced by altering the dietary ratios of calcium and phosphorus have revealed that animals reared on a 1.2% Ca: 0.06% P diet for 21 days and then changed to a <0.02% Ca: 0.3% P diet for 24-48 hours show signs of deterioration in 18-24 hours with some deaths, resulting in 12 of 15 dead in 48

hours. Again, current knowledge of mechanisms for maintaining calcium homeostasis do not explain our findings.

CONCLUSIONS:

EX-2. These data point to the existence of a calcium transport mechanism in the duodenum of the small intestine, whose activity is determined, in part, by the availability of calcium. This study may be used as the basis for future investigations.

EX-3. None.

STUDY NO. 3 Studies on Mineral Metabolism and Interactions

PROBLEM:

A. Effect of some antiarthritic drugs on sulfur-35 incorporation into bone-forming regions of zinc-deficient chicks. Zinc deficiency in chicks results in joint swelling which can be corrected by antiarthritic drugs. Also associated with the deficiency is a marked alteration in sulfate metabolism in the epiphyseal plate and primary spongiosa of the tibia. The study to be reported here was designed to determine the effect of histidine, histamine, and aspirin on sulfate metabolism in these bone-forming regions in Zn-deficient chicks.

B. Effect of zinc deficiency on sulfate metabolism in the rat. Zinc deficiency in the chick has been shown to alter sulfate metabolism in bone-forming regions. A study was designed to determine whether or not this effect was species specific by using the rat as the experimental animal, and to determine whether or not the effect of Zn deficiency on sulfate metabolism was a general effect, or if it was limited to bone-forming tissue.

RESULTS AND DISCUSSION OF THE RESULTS:

A. Day-old chicks were divided into 8 groups. Four of the groups were fed a soy protein diet supplemented with 5 ppm Zn (Zn-deficient); the remaining four groups were fed the same diet supplemented with 80 ppm Zn (controls). The diets of 3 of the control and 3 of the Zn-deficient groups were also supplemented with either 1.0% histidine, 0.2% histamine, or 0.75% aspirin. Following 4 weeks on this regimen, the birds were orally dosed with 10 μCi of $Na_2{}^{35}SO_4$. At each of 6, 12, and 24 hours following isotope dosage, at least 5 birds from each group were sacrificed and a tibia from each bird removed for radiosulfur assay of the epiphyseal plate (EP) and primary spongiosa (PS). Zinc deficiency alone reduced initial ^{35}S incorporation into both EP and PS by approximately 25%. A

similar trend was observed at the 12-hour interval, but not at 24 hours. This depression of ^{35}S incorporation in the Zn-deficient birds was partially corrected by histidine and aspirin, and completely corrected by histamine. The drugs also enhanced the incorporation of ^{35}S into bone-forming regions of the control chicks, again with histamine being the most effective.

B. Weanling rats were used in a study to ascertain the effect of Zn deficiency on sulfate metabolism in bone and soft tissue. The rats were grown on either a control (20 ppm Zn) or a Zn-deficient (6 ppm Zn) diet for 8 weeks at which time they were intubated with 10 μCi of $^{35}SO_4$. At each of 6, 12, and 24 hours following isotope dosage 9 rats from each group were sacrificed. Serum, liver, brain, and femur were removed from each rat for isotopic assay. Significantly greater amounts of ^{35}S were found in the serum, liver, and brain of the Zn-deficient group 6 hours following dosage, and in the serum and liver at the 12-hour period. Thus, it appears that Zn-deficiency results in an enhancement of sulfate absorption. Only the epiphyseal cap from the femur was assayed. The Zn-deficient group, again, showed enhanced incorporation of the isotope at the 6-hour interval following ^{35}S administration. Thus, the pattern of ^{35}S incorporation into the bone-forming region in the rat is opposite that observed in chicks.

CONCLUSIONS:

A. The study reported here supports previous data suggesting that Zn deficiency in chicks reduces both incorporation and turnover of sulfur in bone-forming regions. Additionally, this study suggests that the antiarthritic drugs tested (histidine, histamine, and aspirin) are partially effective in correcting this abnormality in Zn-deficient birds.

B. Zinc deficiency in the rat appears to increase the absorption of sulfate. This deficiency in rats also appears to enhance the incorporation of sulfate into bone forming tissue. Since the latter observation is the reverse of patterns seen in Zn-deficient chicks, the Zn-sulfate interaction with reference to bone formation is difficult to evaluate at this time.

RECOMMENDATIONS:

A. Further studies should be performed concerning metabolic interactions between zinc, sulfate, and some of the general, commonly used drugs. An understanding of the mechanisms involved could possibly provide improved rationale for treatment of wounds and bone fractures.

Basic Studies of Nutrition and Metabolism (Cont'd)

B. Continue to study the nutritional and metabolic interaction between zinc and inorganic sulfate in an attempt to better understand the role of Zn in bone formation and repair.

PUBLICATIONS:

1. Nielsen, F. H., R. P. Dowdy and Z. Z. Ziporin. Effect of zinc deficiency on sulfur-35 and hexosamine metabolism in the epiphyseal plate and primary spongiosa of the chick. J. Nutr. 100: 903, 1970.

2. Nielsen, F. H. and H. E. Sauberlich. Evidence of a possible requirement for nickel by the chick. Proc. Soc. Exptl. Biol. and Med. 134: 845, 1970.

STUDY NO. 5 — A Study of the Effects of Amino Acid Deficiency, Hormones and Divalent Cations on Rat Liver Polysomes and Amino Acid Incorporation

PROBLEM:

An investigation into conditions required for optimal *in vitro* ^{14}C amino acid incorporation into polyribosomal protein has led to the establishment of procedures optimizing rat liver polyribosomal isolation as well. *In vitro* incubations (37^{o}) of rat liver polyribosomes for 2 minutes results in linear rates of ^{14}C amino acid incorporation into polyribosomal protein, however, the incorporation rate ceases to be linear between 2 and 3 minutes of incubation until the rate plateaus after 16-32 minutes. A failure in the recombination of ribosomes to form polyribosomes after an *in vitro* incubation will result in lowered rates of incorporation. Studies designed to investigate the integrity and size of polyribosomal aggregates via profile analysis after various periods of *in vitro* incubation have been completed.

RESULTS AND DISCUSSION OF THE RESULTS:

Polyribosomes isolated from a pooled liver sample of 12 rats weighing 200 ± 15 gms were incubated in the presence of ^{14}C (UL) leucine and quickly frozen in a dry ice-ethanol mixture after an incubation period ranging from zero to 32 minutes. Polyribosomes reisolated from reaction mixtures after a 60 second incubation revealed that approximately 36% of the polyribosomal profile tracing was made up of light molecular weight material, i.e., monomer, dimer and trimer compared to only 21% from reactions that were kept cold and frozen immediately following the addition of polyribosomes and cell sap enzymes. Allowing the reactions to proceed for 2 and 8 minutes resulted in 48 and 55% light molecular weight material respectively, hence the greatest amount of degradation takes place in the early stages of incubation. The control polyribosomal

preparation which had not been added to any reaction mixture prior to profile analysis contained approximately 18-20% of light molecular weight material. Even though an adequate supply of amino acids is available for *in vitro* protein synthesis to take place, the system apparently lacks a chain initiation factor required for an interaction between messenger RNA and free ribosomes which is responsible for maintaining large polyribosomal aggregates responsible for active *in vitro* protein synthesis.

CONCLUSIONS:

Rat liver polyribosomes quickly become degraded to smaller ribosomal aggregates *in vitro* resulting in a gradually lower rate of ^{14}C amino acid incorporation with increasing incubation time. Factors required for the recombination of ribosomes with messenger RNA following the completion of a peptide chain are lacking in this *in vitro* system.

PUBLICATIONS:

Whitten, B. K., L. E. Schrader, R. L. Huston and G. R. Honold. Hepatic polyribosomes and protein synthesis: Seasonal changes in a hibernator. *Internat. J. Biochem.* 1: 406, 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6341	71 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
70 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	061
b. CONTRIBUTING	61145011	3A014501B71P	01	
c. CONTRIBUTING	CDOG 1412A (2)			

11. TITLE (Precede 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 71	1.8	38
FISCAL YEAR CURRENT 72	2.5	37

19. RESPONSIBLE DOD ORGANIZATION
NAME:* US Army Med Rsch & Nutr Lab
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RESPONSIBLE INDIVIDUAL
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TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION
NAME:* US Army Med Rsch & Nutr Lab
Bioenergetics Division
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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 (Precede 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. Precede text of each with Security Classification Code.)

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

24. (U) Complete mineral balances are being conducted on soldier volunteers and conscientious objector subjects during studies of the effects of altering diets or environmental conditions upon physical performance, physiological function and body composition. Mineral balances are correlated with body fluid changes and other nutrient balances. Interrelationships of selenium and dietary sulfur amino acids, vitamin E and macro nutrients and the selenium distribution in the body are being investigated in animals. Further studies in the distribution and metabolism of selenium are progressing.

25. (U) 70 07 - 71 06 A calcium-magnesium deficiency was inadvertently produced in men within 30 days on a liquid diet. Evaluation of mineral status during studies of dietary alterations and physical performance in man are continuing. Utilization of ^{75}Se in animals have shown (a) an inverse relationship of ^{75}Se retention to dietary Se levels and to carrier doses; (b) that a serum protein increases specific activity after the ^{75}Se has been taken up in the liver, and (c) that several organs, especially the testes, concentrates the label. An alphatocopherol assay has been developed and is being evaluated.

*Available to contractors upon originator's approval.

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

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

The following investigation has been conducted under this work unit:

STUDY NO. 06 Selenium Metabolism

Complete balance studies and serum levels of sodium, potassium, calcium and magnesium are performed during most human studies conducted by this Division. Observed changes can be correlated with alterations in body fluid compartments to provide information on the mechanisms of the changes. A calcium-magnesium deficiency developed in normal young men during an extended treadmill study due to the low dietary levels. Urinary excretion of the two minerals decreased rapidly while the deficient diet was fed and increased to normal within two days of diet supplementation. Fecal excretion was slower to respond to decreased intakes with output exceeding intake for the first week. Mineral balances, exclusive of sweat losses, were near equilibrium after two weeks on the deficient diet, indicative of rapid body adjustment.

Four experiments on selenium metabolism have been initiated. The biological half-life of ^{75}Se was 60-70 days in deficient animals. Increasing dietary levels of Se decreases organ retention of a tracer dose. The testes appears to concentrate Se. Isolation of a serum protein which binds 75Se is continuing.

BODY OF REPORT

WORK UNIT NO. 061 Mineral Metabolism - The Requirement of Trace Minerals in Man Under Various Stresses

STUDY NO. 06 Selenium Metabolism

PROBLEMS:

Fluid and electrolyte balances are affected by the various stresses (nutritional, physical and/or environmental) imposed upon our experimental subjects. Total measurement of intake and excretion of sodium, potassium, calcium and magnesium provide data for calculation of balance which are then correlated with fluid balance and fluid compartment shifts. These results provide information on the mechanisms for these changes. Large weight losses, partially attributable to hypohydration resulting from a relative diuresis, have been consistently observed during abrupt altitude exposure. The reason for this diuresis has not been ascertained as yet, but continued studies of mineral and water balances in future studies should provide further information on this phenomenon.

Additional essential nutrients, especially in the area of trace minerals, are being found. The essentiality of several of these have been established only in animals using special diets under well controlled environments to prevent airborne contamination. Selenium is one of these elements which has been shown to prevent nutritional hepatic necrosis in rats and other degenerative diseases in several species of animals. Reduced serum selenium levels have been found in some kwashiorkor cases and it is difficult to attain positive nitrogen balances in these children until the selenium levels are increased. Therefore, selenium may have a role in normal human liver metabolism and may be related to hepatic disease.

RESULTS AND DISCUSSION OF THE RESULTS:

The human subjects on an exercise study developed a calcium-magnesium deficiency within 30 days on liquid diets containing only 102 mg of calcium and 54 mg of magnesium per day. During the first two weeks on the liquid ration, fecal excretion of calcium and magnesium equalled or exceeded intakes and then decreased to less than 20 mg per day for each of the two minerals. Fecal excretion of these minerals remained low for 4-5 days after the ration was supplemented with the recommended daily allowances before excretion levels returned to normal. Urinary excretion of calcium and magnesium decreased rapidly with the deficient diet and returned to near normal within 2 days of supplementation. Balances for calcium and magnesium were near equilibrium, exclusive

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

of sweat losses, after 2 weeks on the deficient diet indicating a rapid adjustment to the low intakes. The serum levels were slightly decreased after 11 days on the deficient diets, no further analyses were performed until 2 days after the diets were supplemented at which time the serum levels had returned to normal. Serum potassium concentrations decreased concommitantly and remained low until 23 days after supplemention. With the exception of a negative potassium balance during the first week of the study, sodium and potassium were in balance throughout the study.

The influence of dietary selenium levels (0, 0.1, 0.25, 0.5 and 1.0 ppm) upon retention of an infused tracer dose of $^{75}SeO_3^=$ was studied. Retention was inversely related to the dietary level while urinary excretion was directly proportional to this level. The biological half-life of selenium in animals receiving unsupplemented diets was 60-70 days. The injection of 20 ug of non-radioactive selenium with the tracer increased excretion of the radioactivity during the first week. Examination of organ retention revealed that most tissues had a decreasing ability to retain the ^{75}Se as the dietary level was increased except for the blood and muscle which showed the opposite trend. A particularly significant observation was that the uptake of selenium by the testis was very large, especially in the deficient animals. Isolation of a serum protein which appears to bind ^{75}Se is progressing.

CONCLUSIONS:

Complete metabolic balance studies of Na, K, Ca and Mg are important during human studies to insure proper nutrition and to evaluate the effects of stresses upon changes in fluid compartments and total water balance. Under the conditions of these studies, males may have an increased requirement for selenium because the testis incorporate it. This may contribute to the more rapid development of nutritional liver necrosis in males as compared to females.

RECOMMENDATIONS:

Include mineral balances in all future human studies involving any stress which may affect water or electrolyte status of the man. Continuation of animal studies with the goal of elucidating the pathways of selenium metabolism and initiate human studies on selenium requirements and metabolism.

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

PUBLICATIONS:

1. Johnson, H.L., D.A. Brown, C.F. Consolazio, H.J. Krzywicki and R.F. Burk. A calcium-magnesium deficiency in normal man. Fed. Proc. 30:345 Abs., 1971. (Abstract).

2. Johnson, H.L., C.F. Consolazio, H.J. Krzywicki, G.I. Isaac and N.F. Witt. Metabolic aspects of caloric restriction: nutrient balances with 500 kilocalorie intakes. Am. J. Clin. Nutr. (To be published Aug 1971).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6338	71 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
70 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	6.102A	2A061102B71P	01	062
b. CONTRIBUTING	61145011	3A014501B71P	01	
c. CONTRIBUTING	CDOG 1412A	(2)		

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 71	0.1	19
FISCAL YEAR CURRENT 72	0.1	20

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital
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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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Metabolic Division

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

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TELEPHONE: 303 366 5311 X25193

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Hagler, L., LTC, MC

NAME:

21. GENERAL USE

Foreign Intelligence not Considered

DA

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.

24. (U) Riboflavin and ascorbic acid stimulate the metabolism of human red blood cells. The mechanism whereby these substances regulate the pentose phosphate pathway will be investigated by measuring the amount of $^{14}CO_2$ generated from 1-^{14}C-glucose in intact and hemolyzed preparations of red blood cells.

25. (U) 70 07 - 71 06 A method was developed for assaying riboflavin kinase activity in human red blood cells. During riboflavin deficiency in otherwise normal human volunteers red blood flavokinase activity increased. After riboflavin repletion flavokinase activity returned to normal.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498

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

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. 1. The effect of riboflavin deficiency on the flavokinase of human red blood cells.

We studied the effect of riboflavin deficiency in normal human volunteer subjects on red blood cell flavokinase activity. There was increased flavokinase activity which returned towards normal with the refeeding of riboflavin.

BODY OF REPORT

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

STUDY NO. 1. — The effect of riboflavin deficiency on the flavokinase of human red blood cells.

PROBLEM:

An assay for flavokinase utilizing 14^C-riboflavin has been developed in our laboratory. In conjunction with a vitamin B_2 deficiency study utilizing normal human subjects done in this laboratory the measurement of red cell flavokinase was performed to determine the effect of riboflavin deficiency on this enzyme.

RESULTS AND DISCUSSION OF THE RESULTS:

In the control state the activity of red blood cell flavokinase was 1.93×10^{-4} mmole FMN/mg protein/3 hrs incubation at 37°. Enzyme activity increased to 9.45×10^{-4} mmole FMN/mg protein/3 hr incubation at 37° at the end of 8 weeks on a vitamin B_2 deficient diet. Activity decreased to 3.65×10^{-4} mmole FMN/mg protein/3 hr at 37° after 2 weeks of repletion with vitamin B_2. These results are in contrast to animal studies which show a decrease in the activity of flavokinase and in contrast to human jejunum which showed no change in activity during the deficiency period.

CONCLUSIONS:

Red cell flavokinase shows an increase in activity during riboflavin deficiency. This may be a fruitful enzyme to study during riboflavin deficiency in man.

RECOMMENDATIONS:

These studies should be carried out in riboflavin-deficient animals. The kinetics of the red cell flavokinase should be studied. A method of precipitating hemoglobin prior to the preparation of a soluble fraction containing the enzyme might enhance measurement of enzyme activity.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6318	71 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. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 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	61102A	3A061102B71R	02	055
b. CONTRIBUTING	61145011	3A014501B71R	02	
c. CONTRIBUTING	CDOG 1412A (2)			

11. TITLE (Procede with Security Classification Code)*

(U) Experimental Surgery in Support of Medical Research (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

012900 Physiology; 005900 Environ. Biol.; 016200 Stress Phys.

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
54 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 70	0.9	12
FISCAL YEAR CURRENT 71	0.9	14

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

TELEPHONE: 303 366 5311 X26112

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) Surgical Techniques; (U) Cardiovascular Surgery; (U) Cardiac catheterization; (U) Anesthesia

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

23. (U) Surgical facilities are inherently necessary in, and are many times an integral part of biomedical research. The feasibility of many projects depends entirely upon the quality and versatility of the supporting surgical facility. It is the objective of this work unit to provide and maintain a facility for experimental surgery in support of the laboratory animal research that is conducted in other work units of the Physiology Division. Secondarily, this work unit provides surgical support to other investigators of the USAMRNL.

24. (U) The surgical facility is equipped to perform almost any type of surgical procedure. Knowledge to perform these experiments requires an explicit capability in surgical procedures and a facility to employ such instrumentation systems as gas anesthetic machines, positive pressure ventilator, electrocautery unit, heart-lung machine, physiological monitoring equipment, x-ray unit and developing laboratory, and fluoroscope.

25. (U) 70 07 - 71 06 Surgical procedures were developed to study the effects of cold on cardiac output, the effects of exercise on fatty acid metabolism, the hemodynamic effects of dextran, neurohumeral regulation and the effects of vasoactive drugs on coronary blood flow. Support was given to studies involving reconstruction of the superior vena cava, acute lead poisoning, and clot formation on intravascular catheters. Training of Clinical Specialists from FGH continued. This work unit is being terminated because the work performed is in support of research performed under other work units. Future work will be charged and reported under the appropriate work unit.

*Available to contractors upon originator's approval.

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

ABSTRACT

PROJECT NO.	3A061102B71R	Research in Biomedical Sciences
TASK NO.	02	Internal Medicine
WORK UNIT NO.	055	Experimental Surgery in Support of Medical Research

During the past year surgical support has been provided to several areas of investigation in USAMRNL projects: cardiovascular (CV) physiology, CV pharmacology and vascular surgery.

Surgical support for programs conducted by personnel at Fitzsimons General Hospital has consisted of 1) efforts to develop a surgical technique to replace the anterior vena cava and 2) the training of approximately 100 Clinical Specialists in operating room techniques.

BODY OF REPORT

WORK UNIT NO. 055 Experimental Surgery in Support of Medical Research

PROBLEM:

The primary objectives of this work unit are twofold: (1) to provide surgical support for research conducted by USAMRNL and; (2) to provide surgical support for research and teaching activities of Fitzsimons General Hospital.

RESULTS AND DISCUSSION OF THE RESULTS:

Surgical support for projects conducted by personnel at USAMRNL included an extensive study to determine the effects of low molecular weight dextran on the circulation in dogs. This study showed that peripheral blood flow increases significantly after dextran infusion. Experiments designed to elucidate the mechanism responsible for this increase are presently being conducted.

Several other studies have been initiated and are currently in progress: the effect of acute exposure to cold on cardiac output and blood pressure; the effects of exercise on fatty acid metabolism; the effects of various vasoactive drugs on cardiac output and coronary blood flow in the normal and denervated canine heart; prevention of clot formation in intravascular catheters treated with a new heparin compound; the effects of cardiac sympathectomy on coronary blood flow following the construction of an aorta to coronary shunt.

The surgical portion of a study to determine the effects of acute lead poisoning on various organs has been completed. The results of this study have not yet been compiled.

During the past year, personnel from Fitzsimons General Hospital have been studying various techniques for replacing the anterior vena cava in dogs. At present the external jugular vein is being used to construct the graft and a single arterial-venous shunt is being made. By use of this technique 100% of the last ten animals operated have survived. At this time, control studies indicate that the success of this procedure is dependent upon the arterial shunt. It is now believed that the shunt creates enough turbulence in the anterior vena cava to prevent clotting at the suture lines. Because of the more intense clotting mechanism in dogs as compared to humans the investigators feel this procedure will be successful in man.

The surgical facilities at USAMRNL are also being used to train clinical specialists in operating room procedures and in minor surgical techniques such as suturing and wound debridement. Approximately 100 students have utilized this facility in the

Experimental Surgery in Support of Medical Research (cont)

past year.

CONCLUSIONS:

Valuable surgical support has been provided to the research programs of USAMRNL and Fitzsimons General Hospital. This support will continue to be an important part in the research conducted by both organizations.

RECOMMENDATIONS:

Experimental surgery, as a separate work unit, will be terminated. Henceforth, funding for FGH projects will be allocated by the Clinical Research Office at FGH. Future work in conjunction with USAMRNL projects will be accomplished as an integral part of the studies described under Work Units 058, 059, 077 and 082.

PUBLICATIONS:

1. Sullivan, F. J. and J. J. Corneil. Low molecular weight dextran and reactive hyperemia. Federation Proc. 30:322, 1971. (abstract)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6320	71 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
70 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	61102A	3A061102B71R	02	057
b. CONTRIBUTING	61145011	3A014501B71R	02	
c. CONTRIBUTING	CDOG 1412A (2)			

11. TITLE (Procede with Security Classification Code): (U) Continuous Pathologic Studies of Diseases of Laboratory Animals and Maintenance of Animals for Research (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
002600 Biology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
58 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 70	6.5	135
FISCAL YEAR CURRENT 70	7.5	179

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: Ackerman, L. J., MAJ
NAME: Alden, C. L., CPT DA

21. GENERAL USE
Foreign Intelligence not Considered

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Diseases; (U) Histopathology; (U) Experimental Animals; (U) Histology; (U) Clinical Pathology; (U) Staining Technics

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 care for animals used by investigators of any division within the laboratory, (2) to provide pathology services, (3) to conduct studies of diseases affecting various species of laboratory animals, with emphasis on the etiology and lesions of specific disease entities, (4) to use materials derived from these studies in the Veterinary Pathology Preceptorship Program.
24. (U) Animals are housed, exercised and fed in accordance with experimenters' directions and humane principles. Routine and special pathologic and clinicopathologic technics are used to elucidate cause, pathogenesis and pathologic alteration of spontaneous and experimentally induced diseases in laboratory animals. Some technics used are light and electron microscopy, histochemistry, enzyme histochemistry, autoradiography, clinical examinations, clinical laboratory tests and photographic recording of lesions.
25. (U) 70 06 - 71 06 Laboratory animals, either procured commercially or supplied from intralaboratory breeding colonies were furnished as required to investigators of USAMRNL. Gross, clinical, and microscopic pathology services were provided in support of approved work units generated within the Laboratory. Study 7: Disease survey of laboratory dogs purchased from the city pound revealed that less than 30% of the dogs had intestinal parasites. Serological assays of 118 dogs revealed that 56% had titers of 1:100 or above against canine distemper virus; 13% had titers of 1:50, while the remainder had titers of less than 1:50. Only 9 dogs had positive titers for leptospirosis, an incidence of 7.6%. No positive titers for Brucella canis were found. Study 8: Chronic lead poisoning in dogs failed to produce erythrocytic fluorescence as reported in man. This work unit is to be terminated. Necessary ongoing studies will be transferred to the appropriate work units.

*Available to contractors upon originator's approval.

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

ABSTRACT

PROJECT NO.	3A061102B71R	Research in Biomedical Science
TASK NO.	02	Internal Medicine
WORK UNIT NO.	057	Continuous Pathologic Studies of Diseases of Laboratory Animals and Maintenance of Animals for Research

The following specific investigations have been conducted under this Work Unit:

STUDY NO. 7	A Disease Survey of Laboratory Dogs Purchased from the Denver Dog Pound
STUDY NO. 8	A Study of Lead Poisoning in Animals

1. Dogs for biomedical research are often obtained from city pounds in which pathogens abound. The opportunity for transmission of disease agents in such environments is probably optimal. It is important for the veterinarian charged with the care of laboratory dogs to know the incidence of spontaneous canine diseases in his specific geographic area in order that appropriate prophylactic and therapeutic measures may be taken. Dogs in the Animal Service Branch must be maintained free of disease not only at the time of issue to requesting investigators, but throughout the course of experiments.

2. Lead poisoning in animals occurs more commonly than generally believed. The development of a peripheral blood specimen for diagnosis of plumbism in animals by fluorescytosis would obviate the necessity for the complex and laborious laboratory analyses currently in use. Induced plumbism in dogs would permit evaluation of chemotherapeutic agents of potential value in lead poisoning of humans.

3. A significant portion of work assigned to this Work Unit has been performed over the last decade without specifically identifying it with a numbered study. Animals are reared or procured for biomedical research conducted within the Laboratory and pathology supportive services are rendered to requesting investigators of other divisions. In the conduct of these two broad functions during FY 71, 9781 animals were reared or procured from outside sources. These included 5067 rats, 3405 mice, 568 guinea pigs, 450 chickens, 122 dogs, 95 rabbits, 50 hamsters, 4 cats, and 22 monkeys. Nine hundred and fourteen cases were accessioned, an increase of 30% over FY 70. These resulted

in 2,772 paraffin blocks and 12,810 slides of which 3,494 required special stains and 9,316 routine stains. Additionally, 1,091 frozen sections were cut; of these, 930 received special histochemical stains and 161 routine hematoxylin-eosin. The following additional clinical laboratory determinations were made: 1072 complete blood cell counts; 1186 differential counts; 926 erythrocyte sedimentation tests; 82 Knott's tests; 217 fecal examinations; 75 urinalyses; 94 blood urea nitrogen; and 89 reticulocyte counts.

This Work Unit is being discontinued. Related studies will be placed under the appropriate Work Units.

BODY OF REPORT

WORK UNIT NO. 057 — Continuous Pathologic Studies of Diseases

STUDY NO. 7 — A Disease Survey of Laboratory Dogs Purchased from the Denver Dog Pound.

PROBLEM:

Dogs procured for biomedical research at this Laboratory are derived from the Denver pound. As in other similar holding facilities, the opportunity for spread of canine diseases is extremely favorable. The cornerstone for the formulation of an effective conditioning program against common canine infirmities is the knowledge of disease incidence.

RESULTS AND DISCUSSION OF RESULTS:

Fecal flotation and sedimentation tests indicated that intestinal parasitism was diagnosed in less than 30% of pound-procured dogs. Serologic assays in 118 dogs revealed that 56% had titers of 1:100 or above, against the virus of canine distemper; 13% had titers of 1:50, while the remainder had titers of less than 1:50. Thus, 69% of all dogs tested had titers in excess of 1:50 to the virus of distemper. Nine of 118 dogs had positive titers to leptospirosis, (both to L. canicola and L. icterohemorrhagiae) an incidence of 7.6%. No positive titers for Brucella canis were found.

CONCLUSIONS:

The data indicate a high prevalence of distemper in the dogs derived from the Denver pound. Distemper vaccinations will be continued in dogs newly arrived at the Laboratory. The 7.6% incidence of positive leptospiral titers was higher than expected.

RECOMMENDATIONS:

1. Similar surveys should be undertaken to determine the incidence of canine viral respiratory diseases other than distemper.

2. It would be of public health interest to determine what percentage of dogs with positive leptospiral titers are passing viable spirochetes in the urine.

PUBLICATIONS:

None.

Continuous Pathologic Studies of Diseases (Cont'd)

STUDY NO. 8 A Study of Lead Poisoning in Laboratory Animals

PROBLEM:

This study had primarily two objectives: (1) to evaluate erythrocytic fluorescence in plumbism including both the onset and the duration; (2) to correlate quantities of lead given with lesions in the blood, kidney, and liver.

RESULTS AND DISCUSSION:

This study has yielded information about both chronic and acute lead poisoning in dogs. We were able to demonstrate basophilic stippling of canine red blood cells by the end of 5 weeks of chronic lead toxicosis. A dog acutely poisoned by massive doses of lead finally died in convulsions after 23 days with basophilic stippling demonstrable during the final week. Dogs with chronic plumbism had typical lead inclusions in kidney and liver biopsy tissues, but inclusions were not found in the acute form. All dogs had characteristic attitudinal CNS disturbances exemplified by hypersensitivity and hyperirritability. Increased cerebrospinal fluid (CSF) pressure and papilledema were also observed. Much variability was seen in urinalyses, blood cell counts, and serum enzyme determinations. Of special importance is the fact that fluorescence of protoporphyrins within red blood cells due to the effect of lead was not demonstrable as in human subjects. Furthermore, responses of the endocrine system remain very poorly defined.

CONCLUSIONS:

Lead poisoning can be produced in dogs with highly variable clinical pictures resulting. Basophilic stippling, lead inclusions in hepatic and renal tissue, and central nervous system disturbances appear to be the most consistent diagnostic features. Erythrocytic fluorescence has not been demonstrated in animals.

RECOMMENDATIONS:

It would seem that continued study of chronic lead poisoning in dogs would be necessary to solve many of the unanswered questions regarding plumbism in animals and their use as models for the development of diagnostic procedures and for the evaluation of therapeutic agents of potential value in the treatment of humans.

PUBLICATIONS:

None.

Continuous Pathologic Studies of Diseases (Cont'd)

UNNUMBERED STUDIES

PROBLEM:

The detection, diagnosis, treatment, eradication, and prevention of disease among the animal colonies of the Laboratory is a function of this Work Unit. Within the Animal Service Branch, the animals are housed, fed, handled, and bred in accordance with experimenters' requirements. Another facet of this Work Unit is concerned with providing pathology services to requesting investigators within the Laboratory. Material of teaching value is used in augmenting didactic presentations during seminars and conferences.

RESULTS AND DISCUSSION:

Augmentation of primary research and supportive services by the Pathology Division was reflected in the increased number of animals issued and in the work produced during FY 71. A total of 9,781 animals were reared or purchased during the last fiscal year, representing an increase of about 28% over FY 70.

A total of 12,810 slides were produced (an increase of 80% over FY 70), including 3,494 specially stained slides (+ 74% over FY 70). Cases accessioned totaled 914, (versus 706 for FY 70) an increase of 30%.

The clinical pathology laboratory performed 3741 determinations including 1072 white blood cell counts, 1186 differential counts, 926 sedimentation tests, 82 Knott's tests (for microfilariae), 217 fecal examinations, 75 urinalyses, 94 blood urea nitrogen, and 89 reticulocyte counts. A total of 1091 frozen sections were cut in the cryostat; of these 930 were stained for special tissue enzyme activity and the rest received either routine hematoxylin-eosin or fat stains.

Facilities for housing dogs have been pitiably inadequate for years. Since only 32 dogs can be accommodated in our colony, it has been necessary to contract with a commercial boarding kennel for the housing of excess dogs. During FY 71 a total of $9,397 was spent for this purpose.

A function of this Division has been the training of veterinary pathologists; material for such training is derived largely from output of this Work Unit. During FY 71 one preceptee was certified by the American College of Veterinary Pathologists and two other preceptees were assigned to duty with this Division and are progressing commendably within the frame-work of the Veterinary Pathology Preceptorship Program. Additionally, there are four other officers who participate in the pathology seminars and slide conferences held daily for 1 hour.

Continuous Pathologic Studies of Diseases (Cont'd)

RECOMMENDATIONS:

This Work Unit will be terminated 30 June 1971. Necessary ongoing studies will be transferred to the appropriate work unit.

PUBLICATIONS:

1. Myers, Tony S. and C. H. Garvin. Multiple Neoplasia in a Dog. The Southwestern Veterinarian, Vol. XXIV, No. 1, Fall, 1970.

2. Kennedy, G. A. and G. S. Trevino. A Pituitary Adenoma in a Zebra. Pathologia Veterinaria, 7: 186-189, 1970.

3. Ackerman, Larry J., R. A. Kishimoto, and J. S. Emerson. Nonpigmented Serratia marcescens Arthritis in a Teju (Tupinambis teguixin). American Jour. of Veterinary Research, 32, No. 5, 823-826, 1971.

4. Painter, M. Ray, A. A. Borski, G. S. Trevino, and W. E. Clark. Urethral Reaction to Foreign Bodies. Am. J. of Urology. In press.

5. Demaree, R. S., Jr.: Isolation of Avian Trypanosomes by Selective Centrifugation and Subsequent Processing for Electron Microscopy. Jour. of Stain Technology, 45 (6), 304-306, 1970.

6. Demaree, R. S., Jr.: Morphogenesis of an Avian Trypanosome. Diss. Abstr. Internat. 30 (10), 4844, 1970.

7. Myers, T. S.: Renal Trematodiasis in a Boa Constrictor (Constrictor Constrictor). Laboratory Animal Science 21: 423, 1971.

8. Meng, H. C., L. D. Jones, L. J. Ackerman, D. G. Fairchild and T. J. Bucci: Effects of Antioxidants and Lipotrophic Agents in Rats Receiving Long-Term Administration of a Fat Emulsion, Intralipid. Advances in Parenteral Nutritions. Symposium of the International Society of Parenteral Nutrition, Prague, Sept. 3-4, 1969, pp. 222-232.

9. Jones, L. D., D. C. Fairchild and W. C. Morse: The Induction of Pulmonary Neoplasms in Mice by Isonicotinic Acid Hydrazide. Am. Rev. Respir. Dis. 103: 612, 1971.

10. Stedham, M. A. and T. J. Bucci: Spontaneous Tyzzer's Disease in a Rat. Laboratory Animal Care 20: 743, 1970.

11. Raica, H., Jr., M. A. Stedham, Y. F. Herman and H. E. Sauberlich: Vitamin A Deficiency in Germ Free Rats. In DeLuca, H. F. and Suttic, J. W.: The Fat Soluble Vitamins, Madison, Wisc. The University of Wisconsin Press, Dec. 1970, pp. 283-292.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6321	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUM'RY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 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	
c. CONTRIBUTING	CDOG 1412A (2)			

11. TITLE (Procede 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)
PRECEDING FISCAL YEAR 71	5.0	47
CURRENT FISCAL YEAR 72	5.0	88

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: 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 treadmill running will be used to study how dietary manipulation influences the ability of animals to perform. Simultaneous studies on enzyme patterns in carbohydrate and fat metabolic pathways will be conducted seeking nutritional means by which to delay fatigue. Further studies on the effects of strenuous exercise in trained and untrained animals will be performed to assess metabolic alterations in skeletal muscle, heart, and liver due to fatigue and exhaustion. Limited studies on the hormonal responses to training and exercise are planned. Mitochondrial function and structure will be investigated as studies begin to show differences in performance capabilities.

25. (U) 70 07 - 71 06 An environmental temperature of 68-70 degrees F appears beneficial during training phases compared to higher temperatures of 80 degrees F. Trained animals can be sufficiently exhausted to induce biochemical changes not evident in muscle mitochondria from untrained exhausted rats. Electron microscopic evidence from isolated mitochondria substantiates these findings. Supplementation of the diet of trained rats with carnitine is associated with enhancement of fatty acid oxidation. Studies are in progress to determine if carnitine also enhances performance capability.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

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. 5. Exhaustive exercise decreased palmitate oxidation in both heart mitochondria (HM) and skeletal muscle mitochondria (SMM) in untrained rats while trained rats demonstrated decreases in oxidation of palmitate, succinate and pyruvate as well as lower respiratory control index (RCI) and P/O ratios (moles phosphate esterified per gram-atom of oxygen taken up). Trained animals sacrificed in a resting state revealed that pyruvate and succinate oxidation in SMM was greater than that found for untrained rats. Electron micrographs of isolated SMM from trained exhausted rats revealed more swollen, disrupted mitochondria than those from untrained exhausted rats, a finding consistent with the earlier mentioned biochemical findings. Biochemical adaptations to exercise apparently are less evident in animals kept at higher environmental temperatures (78-80^{o}F) compared to lower temperatures (68-70^{o}F).

Study No. 6. Physically trained rats possessed greater oxidative capabilities as well as a greater capacity to mobilize energy sources. High fat, high carbohydrate, and carnitine diets all tended to alter energy generation. Carnitine appeared to be additive with the training effect while fat and carbohydrate were not.

BODY OF REPORT

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

STUDY NO. 5 — Biochemical Adaptation to Exercise

PROBLEM:

The initial problem concerning the choice of a training regimen to investigate metabolic adaptations due to exercise was discussed in the annual report for FY 70. Inconclusive data led to further investigations of the effects of moderate and exhaustive exercise upon trained and untrained rats. In addition to measuring various biochemical parameters of heart and skeletal muscle mitochondria (i.e., yield of mitochondrial protein/gm tissue, rate of O_2 uptake/mg protein/hr, P/O ratio, and RCI), a cooperative effort between this Division and the Pathology Division (work unit 91C-054) was designed to utilize electron microscopic techniques to study the morphology of mitochondria in both intact muscle and the isolated pellet. The effect of hypoxia upon heart and skeletal muscle mitochondrial metabolism was briefly investigated using trained and untrained animals. Rats run to exhaustion on the treadmill were compared against animals left at 25,000 feet in an altitude chamber for 24 hours.

RESULTS AND DISCUSSION OF THE RESULTS:

As previously noted in the FY 70 annual report, cytochrome c was a measure of physical conditioning. We have since found training to increase cytochrome c of quadriceps muscle by 30-40%.

One should expect to find an increase in mitochondrial protein/gm of muscle since cytochrome c is intramitochondrial. Since our data did not show such an increase it was postulated that we may have lost some of the larger mitochondria in our isolation procedures. Electron microscopic data has since shown no loss of large mitochondria at any step in our isolation procedures. A possible explanation for not observing a greater increase in cytochrome c as a result of training will be discussed later.

Detailed studies on the effects of moderate and exhaustive exercise have now been completed. Since trained rats can exercise longer at a given work load than untrained rats prior to exhaustion it was reasoned that energy metabolism adaptations in the former may be more evident following acute exhaustive exercise.

Nutritional and Metabolic Adaptations and Interrelationships (Cont'd)

Following 12 weeks of training on a rigorous schedule of treadmill running, trained and untrained rats were run at either 0.5, 1.0 or 1.25 mph to assess the effect of acute exercise on the activity of several mitochondrial enzyme systems. Animals were sacrificed immediately after exercise, and 600 x g supernatants and mitochondria were prepared from skeletal muscle and heart. Control animals were sacrificed at rest. Skeletal muscle mitochondria of untrained rats exhausted at either 0.5 mph or 1.0 mph showed a decrease in mitochondrial yield but no change in oxygen uptake, P/O ratio, or respiratory control with either pyruvate-malate or succinate as in vitro substrates. Heart mitochondria from untrained exhausted rats demonstrated no change in any of the above-mentioned parameters.

Mitochondria isolated from skeletal muscle of trained rats exhausted at 1.25 mph exhibited a decrease in oxygen uptake with pyruvate-malate and decreases in oxygen uptake, P/O ratio, and respiratory control with succinate as the substrate. There was no change in these parameters when heart mitochondria from trained exhausted rats were assayed.

The 600 x g supernatants of skeletal muscle and heart were assayed for their capacity to oxidize palmitate-1-^{14}C. Skeletal muscle from trained and untrained exhausted rats failed to oxidize plamitate as well as the rested controls. However, a statistically significant decrease could be demonstrated only for trained exhausted rats. Oxidation of palmitate by heart was higher in untrained rested than trained rested animals ($P < .05$). Palmitate oxidation by heart did not change when trained rats were exhausted, however, a significant decrease was observed when untrained exhausted rats were compared to untrained rested controls. Changes associated with an impaired capacity to synthesize adenosine triphosphate occurred in skeletal muscle of trained rats that were exhausted at 1.25 mph. Since these effects were not observed in untrained rats that were exhausted at a lower work load (0.5 mph) we were not able to ascertain if untrained rats respond differently to exhaustive exercise than trained rats or if the difference between the work loads and the duration of exercise caused the results observed.

In conjunction with the biochemical findings quantitative measurements of electron micrographs showed the cross-sectional area of SMM isolated from untrained exhausted rats to be 33-38 mm^2 compared to 55-60 mm^2 for those isolated from trained exhausted rats. Measurements on SMM from rested rats (trained and untrained) were approximately the same as the untrained exhausted rats. The larger variability observed in mitochondrial size of preparations from trained exhausted rats indicated to us a preferential adaptation by certain muscle fiber-types since untrained animals possess SMM more uniform in size. Studies are now in progress to determine if mitochondria from quadriceps tissues close to

the blood supply (i.e., around the bone) show different adaptations than those in the belly portion of the same muscle group.

Two identical experiments conducted at different times and different temperatures have led us to conclude that high environmental temperature (78-80°F) during the training phases may be important in determining whether metabolic adaptations due to exercise will develop as fully as if temperatures are kept around 68-70°F. This may help explain both the decreased magnitude and sometimes complete lack of exercise adaptations in resting trained rats reported by other workers. For example, we have recently noted 80% or more increases in cytochrome c along with 40-50% increases in mitochondria yield from skeletal muscle in rats trained at 68-70°F which agrees very well with Holloszy (J. Biol. Chem. 242: 2278, 1967) but which we did not observe in studies conducted at 78-80°F.

Since the lack of oxygen in the working muscle may be an important factor during strenuous exercise, an experiment was performed to determine the effect of hypoxia on the SMM and HM of trained and untrained rats. Trained and untrained rats were exposed for 24 hours to a simulated altitude of 25,000 ft. in an altitude chamber. Hypoxia caused all animals to decrease their food intake and, therefore, it was necessary to restrict the food intake of the normoxic control groups accordingly. This food restriction caused a decrease in palmitate and pyruvate oxidation by SMM and HM in both the trained and untrained normoxic groups. Therefore, to determine the effect of hypoxia the enzymatic activity of altitude-exposed animals was compared to normoxic animals that were food restricted.

Because of the experimental variability of the data obtained in this experiment no statistically significant conclusions could be drawn. However, there were several instances where trends were apparent that are under further investigation. Fatty acid oxidation by 600 x g homogenates of skeletal muscle and heart was decreased and increased respectively in both trained and untrained rats subjected to hypoxia. On the other hand, hypoxia caused pyruvate oxidation to be increased in SMM of untrained rats and decreased in SMM of trained animals, while pyruvate oxidation by HM was increased in untrained rats and unchanged in trained rats. These results show that hypoxia does change the energy metabolic capacity of SMM and HM and that in some instances trained animals respond differently than untrained animals.

CONCLUSIONS:

1. Exhaustive exercise at 1.25 mph for trained rats causes a greater reduction in the capacity for SMM to oxidize dicarboxylic and fatty acids than in preparations taken from untrained rats exhausted at 0.5 mph.

Nutritional and Metabolic Adaptations and Interrelationships (Cont'd)

2. SMM taken from trained exhausted rats demonstrate swelling, slight disorientations of the mitochondrial matrix and possess larger cross-sectional areas than SMM taken from either the resting controls or untrained rats rested and exhausted.

3. Exercise training will result in increased muscle cytochrome c levels, pyruvate succinate and palmitate oxidation rates in muscle and mg mitochondrial protein per gm muscle if the animals are trained at a sufficiently low environmental temperature (68-70°F).

4. Reduced food intake causes a reduction in mitochondrial enzyme activity within 24 hours.

PUBLICATIONS:

1. Huston, R. L., G. L. Dohm and E. W. Askew. Effects of exercise on mitochondria from trained and untrained rats. Fed. Proc. v30: 645, 1971 (Abstract).

2. Dohm, G. L., R. L. Huston and E. W. Askew. The effect of acute exercise on the activity of heart and skeletal muscle mitochondria from trained and untrained rats. Submitted to J. of Biol. Chem.

STUDY NO. 6 Dietary Control of Lipid Metabolism

PROBLEM:

Energy generation by fatty acid oxidation is controlled at several metabolic steps. Fatty acid mobilization, uptake, and oxidation were investigated under the influence of exercise and training to determine at which points metabolic adaptation occurs. The effect of diet was also studied to ascertain if nutritional measures could enhance biochemical adaptation to physical conditioning.

RESULTS AND DISCUSSION OF THE RESULTS:

Trained rats possessed greater capacity to oxidize fat and carbohydrate than did the non-trained controls. Trained rats also possessed greater adipose tissue hormone sensitive lipase activity than sedentary animals. These results indicate that physical training is associated with an enhanced ability to mobilize fatty acids from depot stores as well as an increased capability to oxidize both fatty acids and pyruvate to provide energy.

Rats fed 0.6% dietary D,L-carnitine possessed a slightly greater capacity to oxidize fatty acids than rats fed a control diet. The feeding of carnitine was also associated with decreased serum cholesterol

and small increases in serum triglyceride. Experiments are in progress to determine if trained rats fed carnitine possess a greater physical endurance capacity than similar rats fed a control diet.
The feeding of high fat and high carbohydrate diets to trained and untrained rats indicated that the effect of training was greater than the effect of diet in skeletal muscle mitochondria oxidizing pyruvate or palmityl carnitine. However in untrained animals, rats fed high fat diets tended to oxidize more pyruvate and palmityl carnitine than rats fed a high carbohydrate or control diet.

CONCLUSIONS:

Biochemical changes occurring in fat and carbohydrate metabolism in trained rats indicate that enhanced ability to generate energy accompanies increased physical endurance. Diet also affects these same adaptations.

RECOMMENDATIONS:

Subsequent experiments will be conducted to further elucidate biochemical adaptations occurring in response to exercise and training and to investigate the feasibility of enhancing physical performance by diet.

PUBLICATIONS:

1. Askew, E. W., G. L. Dohm, R. L. Huston and R. P. Dowdy. Effect of dietary D,L-carnitine on lipid metabolism in trained and untrained rats. Fed. Proc. v 30: 645, 1971 (Abstract).

2. Askew, E. W., G. L. Dohm, R. L. Huston and T. W. Sneed. Response of rat adipose epinephrine-sensitive lipolytic activity to physical training and exercise. Submitted to Biochim. Biophys. Acta

3. Dohm. G. L., R. L. Huston and E. W. Askew. The effect of acute exercise on the activity of heart and skeletal muscle mitochondria from trained and untrained rats. Submitted to J. of Biol. Chem.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6317	71 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. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 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	61102A	3A061102B71R	02	061
b. CONTRIBUTING	61145011	3A014501B71R	02	[redacted]
c. CONTRIBUTING	CDOG 1412A (2)			

11. TITLE (Precede with Security Classification Code)* (U) Work Performance and Body Composition as Related to Environment and Nutritional Status (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

002600 Biology; 012900 Physiology; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
56 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 71	2.0	53
FISCAL YEAR CURRENT 72	2.0	56

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

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Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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Bioenergetics Division

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

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TELEPHONE: 303 366 5311 X25222

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Consolazio, C. F.

NAME: Nelson, R. A. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Soldiers' Anthropometry; (U) Body Composition; (U) Work Performance of Soldiers; (U) Military Stress; (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) To determine what effect the soldier's age, duties, nutrition and environment has upon his body composition and physical fitness as related to his performance of military duties.

24. (U) Determine body fat by water displacement, protein by ^{40}K counting, body water with deuterium dilution, extracellular water with thiocynate dilution, and energy expenditure at various work loads on the treadmill utilizing continuous gas pattern analyzer for oxygen consumption, carbon dioxide production, heart and respiratory rates and pulmonary ventilation. These measurements will be made on soldiers of different ages, who perform various military duties and consume all types of rations while subjected to various environmental and nutritional stresses. The interrelationships between these parameters can then be calculated.

25. (U) 70 07 - 71 06 A variable speed stepping motor is being added to the operational ^{40}K whole-body counter which provides scanning speeds proportional to the live time of the multi-channel analyzer. Although elevated dietary protein and exercise for 8 weeks increased 8 men's dry protein mass, a 100 or 200 g contribution of daily protein intake to this body compartment was indistinguishable by body densitometry. Increased body water and decreased fat were noted, while pulmonary function was unchanged. Dentitometry or D_2O tracer studies indicated no edema in the Scurvy II subjects, since supplementation had been in progress for up to 10 days; after 5 months, increases were noted in body weight and fat. Only blood plasma ascorbate correlated with the dry protein body compartment. In a 12-week riboflavin deficiency study, body density measurement showed little change in body fat or dry protein, and 40_K did not show lean body mass changes. Lung compartments were unaffected from pulmonary function studies. This methodologically oriented work unit is being terminated, and the techniques developed will be utilized under work units with more specifically defined goals.

Available to contractors upon originator approval

ABSTRACT

PROJECT NO.	3A061102B71R	Research in Biomedical Science
TASK NO.	02	Internal Medicine
WORK UNIT NO.	061	Work Performance and Body Composition as Related to Environment and Nutritional Status.

Studies were conducted to observe the effects (a) of two protein levels; (b) two carbohydrate levels, (c) caloric restriction, and (d) riboflavin deprivation upon work performance. In all studies, short term physiological work performance was not impaired during the experimental period.

In another study, age and body weights proved to be poor factors in predicting submaximal oxygen uptakes. Pulmonary ventilation, with an r value of 0.77 showed the best relationship to $\dot{V}_{O_2}$.

The whole body shadow shield ^{40}K counter has been operational for laboratory and hospital studies. The calibration of the counter using ^{42}K in normal humans is progressing. Indications are that the calibration curve is a linear function of weight/height.

A data handling system which collects, stores, edits, calculates and displays data collected during the assessment of human energy expenditure has undergone extensive revision. These revisions have resulted in improved data editing, data display, and compatibility with other data storage and handling systems. The regular use of the system has proven the feasibility of off-line automation of the performance aspects of applied performance studies.

BODY OF REPORT

WORK UNIT NO. 061 Work Performance and Body Composition as Related to Environment and Nutritional Status.

PROBLEM:

The effects of various stresses upon the ability of military personnel to perform their duties are being evaluated. Studies have been done under conditions of abrupt exposure to high altitude, during short-term starvation and caloric restriction, on the effects of age in relation to man's ability to perform various military tasks, and with different levels of the various micro and macro nutrients. Evaluation of moderate and maximal work performance, body composition and pulmonary function were performed in collaboration with many studies performed studies under work unit numbers 073, 070 and 080 (Bioenergetics Division).

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.

RESULTS AND DISCUSSION OF RESULTS:

A study of the effects of dietary protein levels on work performance and body composition in humans was completed. This study of 8 young men (mean age 21) depicts the effects of two protein intake levels (200g/day for Gp I; 100g/day for Gp II), and heavy exercise for eight weeks upon changes in the fat, water and dry protein compartments of the body as derived from densitometry. Although dry protein mass was increased by 0.64 and 0.29 kg for Groups I and II, the values were not significantly different. Since no significant differences in body composition were found between groups, the data were combined. These data showed a significant body mass increase (1.70 kg) which was partitioned into an insignificant body fat loss (0.37 kg), a significant dry protein increase (0.47 kg), and body water (1.60 kg) increase. It appeared that exercise may be effective in building body protein mass, however the contribution of the protein intake level was not discernable. Performance data indicates no significant differences between groups during moderate or maximal work on the treadmill. Protein is an expensive source of calories and it would be uneconomical to increase the protein intake if it did not have any beneficial effects.

Work Performance and Body Composition as Related to Environment and Nutritional Status (Cont)

Six young men were studied during a 12-week riboflavin deficiency study (Chemistry Division work unit 072). The measurements made at weekly intervals included three submaximal work levels on the treadmill, body density and pulmonary function compartments. Preliminary inspection of the data indicates no significant changes in heart rates, pulmonary ventilation or oxygen uptakes, during the restriction period. Body density estimated by water displacement revealed no changes in body compartments, although the men lost 2.67 kg.

In a study, the oxygen uptake data indicated that 420 Cal/day of carbohydrate and 500 Cal/day of a carbohydrate-protein mixture with and without mineral supplementation were adequate in maintaining physical efficiency during short term submaximal and maximal treadmill work. The men in the 500 Cal/day study performed a stamina test each third day, which failed to show any significant differences in performance when comparing the 10 days of restriction to control data. Although the ability to perform aerobic and anaerobic work was not impaired in either group, other factors such as protein catabolism and body hypohydration indicated the detrimental effects of caloric restriction.

Body weight loss during a 500 Calorie dietary restriction study paralleled that noted on the 420 Calorie diet, however approximate dry protein losses were halved (0.4 kg) while body fat losses were doubled, up to 3.5 kg. The addition of minerals to the diet was effective in retaining body water.

In collaboration with the Computer Division, the evaluation of work performance data collected at military installations is in progress. The group under study consisted of 206 men between the ages of 17 and 53 years. Submaximal work performance at 60% of maximal proved to be a poor predictor of maximal work. Age was not significantly related to oxygen uptakes ($\dot{V}_{O_2}$) l/min with fairly low correlation coefficients of 0.11 and 0.05 for submaximal and maximal work, respectively, indicating that work was independent of age. The relationship of body weight to $\dot{V}_{O_2}$ was also poor and unexpected since better physical fitness with less body fat are usually associated with the better work performance. Heart rate/$\dot{V}_{O_2}$ relationships were only fair with an r value of 0.57.

The best correlations were observed between pulmonary ventilation, l/min and $\dot{V}_{O_2}$ l/min during both physical performance levels. The correlation coefficients were 0.77 for both work levels and regression equations showed very significant slopes.

Work Performance and Body Composition as Related to Environment and Nutritional Status (Cont)

Rats were maximally exercised weekly for a 12-week period, while consuming various dietary protein levels. The 5% protein group had a significantly lower growth rate compared to others. The 20% untrained protein group attained the higher weights. The 5% group were also able to run significantly longer than others, which may be related to the lesser body weights. Electrophoresis suggested that training and increased protein in the diet was reflected in an increased content of blood protein.

The whole body shadow shield ^{40}K counter has been relocated in a permanent facility in the basement of building 602. It is currently being used to measure total body potassium quantitatively, and also to determine the per cent retention of isotopes administered for clinical detection of residual thyroid tissue after a thyroidectomy. All data is placed on punched paper tape and is then analyzed by using the RCA 301 computer. The computer program corrects for instrumentation drift based on isotope standards which are counted daily.

The counter has been in fairly constant use with: (a) weekly measurements on 8 men for 8 weeks during the high protein/exercise study; (b) measurements of whole body ^{131}I retention on patients with thyroid carcinoma (the presence of the residual carcinoma is evidenced by a long biological half-life). determined by measuring whole body retention over a one week period, while other detection methods such as the body scan and gamma camera are being used to correlate with the whole body results; and (c) measurement of whole body retention of ^{59}Fe for a 19-day period in a Metabolic Ward patient.

Quantitative determinations of total body potassium were made possible by calibration using the isotope ^{42}K in 6 human volunteers. The calibration curve was found to be a linear function of weight/height, the independent variable.

A stepping motor that allows for 6 bed scanning speeds (in 10 to 60 minute increments) is currently being installed. It will also maintain any specific bed speed, and yet it will be controlled so that the speed remains proportional to the activity of the isotope being counted.

A data handling system is in use which automatically handles the data derived from a "continuous oxygen consumption analysis system". This system converts analog data to digital data; edits, formats and stores the digital data on magnetic tape files; retrieves and lists the raw data; retrieves, transforms and calculates gas analysis parameters; calculates, lists, and summarizes oxygen consumption data; and develops data messages of summary type information for permanent storage on magnetic tape files.

Work Performance and Body Composition as Related to Environment and Nutritional Status (Cont)

This system has been used extensively over the reporting period for oxygen uptake analysis in human subjects during rest and exercise. The data from 5 studies, representing 231 measurements, approximately one hour each, has been processed with a great deal of "user acceptability". This data processing has been in support of laboratory projects in the performance aspects of applied military nutrition and military environmental medicine.

An 18-unit small animal treadmill cage with a stimulation system has been designed. Numerous safety devices are present for protection of the animal while running. This apparatus is being satisfactorily used by this laboratory on work performance studies utilizing white rats.

CONCLUSIONS:

Submaximal and maximal work performance was measured (a) at two protein intake levels, (b) two carbohydrate intake levels, (c) during caloric restriction (420 Cal/day, 500 Cal/day), and (d) during a riboflavin deprivation period. In all studies, work performance was not significantly changed during the experimental periods. Although muscle mass was increased in the protein study, the contribution of the protein intake level was insignificant.

In a study concerning the relationship of age to physiological parameters, age and body weight were both poor factors in predicting oxygen uptakes. Pulmonary ventilations showed the best relationships to $\dot{V}_{O_2}$ with an r value of 0.77.

The whole-body shadow shield ^{40}K counter is operational. A computer program corrects for the instrumentation drifts based on isotope standards. The calibration of the ^{40}K counter, using ^{42}K in humans is in progress and preliminary information indicates the calibration curve to be a linear function of weight/height.

There appeared to be no significant differences in short term work performance ($\dot{V}_{O_2}$ ml/kg/min) on the treadmill when comparing a control to a calorie restriction period or between four groups consuming 603, 948, 1,362 or 3,382 Cal/day for a 10-day period. The body weight losses were the lowest observed in all of our calorie restriction studies ranging between 3.7 to 4.5% for the 3 restricted groups.

The automation of oxygen consumption measurements, even off-line to the measurement, can be an extremely useful tool for the performance physiologist. Data has been processed during this reporting period in approximately 30 hours which would have possibly required 2,300 manhours if it had been attempted by hand.

Work Performance and Body Composition as Related to Environment and Nutritional Status (Cont)

RECOMMENDATION:

None.

PUBLICATIONS:

1. Consolazio, C.F., H.L. Johnson and H.J. Krzywicki. Factors influencing physical performance. Proceedings of the Joint Malnutrition Conference sponsored by U.S.-Japan Cooperative Medical Science Program, Tokyo, Japan, 5-7 November 1970. (Abstract)

2. Consolazio, C.F. Energy metabolism during exposure to extreme environments. Proceedings of the 27th Aerospace Medical Panel Meeting, sponsored by AGARD-NATO, Garmisch-Partenkirchen, Germany, Conference Proceedings 82-71, p. 16: 1-9, 1971

3. Krzywicki, H.J., C.F. Consolazio and H.L. Johnson. Alterations in exercise and body composition with age. Excerpta Medica International Congress Series No. 213 - Proceedings 8th International Congress on Nutrition, Prague, Czechoslovakia, August 28-September 5, 1969, p. 522.

4. Consolazio, C.F. Energy metabolism and extreme environments (heat, cold and high altitude). Excerpta Medica International Congress Series No. 213, Proceedings 8th International Congress on Nutrition, Prague, Czechoslovakia, August 28-September 6, 1969, p. 324.

5. Johnson, H.L., C.F. Consolazio, H.J. Krzywicki and N.F. Witt. Metabolic Aspects of Calorie Restriction (500 Cal). Am. J. of Clin. Nutr. (In Press)

6. Consolazio, C.F., H.L. Johnson, H.J. Krzywicki, T.A. Daws, K. Calhoun. Relationship of restricted diets to work performance in a jungle environment. (Submitted for clearance)

7. Krzywicki, H.J., J.G. Dramise, S.O. Williams III, C.F. Consolazio and H.L. Johnson. Effects of exercise and dietary protein levels on body composition and respiratory function in humans. Federation Proceedings 30:646, 1971 (Abstract).

8. Consolazio, C.F. and H.L. Johnson. Measurement of energy cost in humans. Federation Proceedings (In press).

9. Consolazio, C.F., R.A. Nelson, T.A. Daws, H.J. Krzywicki, H.L. Johnson. Body weight, heart rate and ventilatory volume relationships to oxygen uptakes. Am. J. Clin. Nutr. (In press).

Work Performance and Body Composition as Related to Environment and Nutritional Status (Cont)

10. Consolazio, C.F. Studies of energy expenditure in military populations with the K-M respirometer. Am. J. Clin. Nutr. (In Press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6334	71 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. DOD'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	D Change	U	U	NA	NI	☐ 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 1412A (2)			

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)
PRECEDING FISCAL YEAR 71	2.5	37
CURRENT FISCAL YEAR 72	1.4	30

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
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RESPONSIBLE INDIVIDUAL
NAME: Canham, J. E., COL
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20. PERFORMING ORGANIZATION
NAME: US Army Med Rsch & Nutr Lab
Metabolic Division
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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]
ASSOCIATE INVESTIGATORS
NAME: Hagler, L., LTC, MC
NAME: Gozansky, D. M., MAJ, MC 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 rats and myotonic and normal goats. The troponin-tropomyosin complex will be studied. Should differences between normal and myotonic animals be found similar studies will be done in normal and myotonic human muscle. Muscle enzymes will be measured and an attempt will be made to correlate the enzyme levels with disease. The effect of various agents in various muscle diseases will be tested.

25. (U) 70 07 - 71 06 Study of the troponin-tropomyosin system in normal and myotonic goats has shown no differences. Studies in a patient with idiopathic myoglobinuria have shown that acidosis leads to a worsening of the condition and that improvement can be obtained by the use of alkalinizing solutions. Serum pyruvate kinase has proven to be a most sensitive enzyme for evaluating the severity of the patient's disease and for following the disease process serially.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO.	3A061102B71R	Research in Bio-Medical Sciences
TASK NO.	02	Internal Medicine
WORK UNIT NO.	062	Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet, and Steroids in Normal Man and Disease.

The following investigations have been conducted under this work unit:

STUDY NO. 1. Investigation into the control of muscle contraction and relaxation in myotonia and related muscle disorders.

STUDY NO. 2. Studies in a patient with idiopathic myoglobinuria.

Study No. 1. The abnormality in muscle metabolism in myotonia may be related to a defective interaction of troponin-tropomyosin with the contractile protein, actomyosin or to a defect in the troponin-tropomyosin complex itself. The dissociation between actin and myosin that occurs in the resting state is believed to be maintained by troponin and tropomyosin. When calcium is bound to this complex actin and myosin are then permitted to interact.

The effect of the troponin-tropomyosin complex on the ATPase activity of actomyosin is under study as a model for the contraction-relaxation cycle since the enzymatic activity decreases markedly on going from the contracted to the relaxed state. Rats were employed to prepare the system and then a normal and myotonic goat were studied. Inactivation of the actomyosin ATPase of the goats was encountered. We are endeavoring to overcome this complication in order that the method developed may be applied to the study of patients with myotonia.

Study No. 2. In studies of a patient with idiopathic myoglobinuria we have concluded the following. 1) The underlying defect in idiopathic myoglobinuria appears to reside in the muscle cell membrane. 2) The abnormality is characterized by abnormal membrane permeability. 3) Membrane permeability may be increased by acidosis and reduced by alkalosis. 4) Pyruvate kinase may be a more sensitive marker for membrane permeability than myoglobin, glutamic oxaloacetic transaminase (SGOT) or lactic dehydrogenase (LDH).

BODY OF REPORT

WORK UNIT NO.	062	Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet, and Steroids in Normal Man and Disease.
STUDY NO.	1	Investigation into the control of muscle contraction and relaxation in myotonia and related muscle disorders.

PROBLEM:

Muscle contraction and relaxation are believed to be controlled by a set of complex interactions between contractile proteins, control proteins and cations. In the resting state actin and myosin are dissociated so that there is little interaction between them. To maintain the dissociation two additional proteins, troponin and tropomyosin are required. It has been shown that calcium binding to the troponin of the troponin-tropomyosin complex releases the blocking of the interaction of actin and myosin. Thus, troponin prevents the activation of the myosin ATPase by actin when calcium is absent.

For muscle relaxation to occur, a calcium chelating system present in the tubules of the sarcoplasmic reticulum must remove calcium in order that actin and myosin again become dissociated. A number of workers have evaluated the calcium sequestration and transport of fragmented sarcoplasmic reticulum in normal individuals and patients with myotonia but have not been able to find any differences. In addition, we have not found consistent changes in sarcoplasmic reticulum ATPase activities. In a female patient with myotonia dystrophica, who experienced significant clinical improvement with procainamide, there were no changes in a wide range of cation values employed in assaying ATPase activity in the myofibrillar and sarcoplasmic reticulum fractions in muscle obtained by open biopsy.

Thus, it appears that myotonia and the control of relaxation in the normal state may well be more complex than hitherto appreciated. Widemann et al (Biochim. Biophys. Acta 205: 523, 1970) have demonstrated that troponin and tropomyosin are required not only for contraction, but moreover, are necessary for relaxation. This finding is in agreement with the concept that the defect in myotonia may lie in the interaction of the four proteins involved directly in the contraction-relaxation cycle of muscle.

RESULTS AND DISCUSSION OF THE RESULTS:

Efforts have been directed toward preparation of actomyosin, troponin and tropomyosin in order to study their interaction by assaying

actomyosin ATPase activity in the contracted and relaxed states. Actomyosin has been prepared by the method of Samaha et al (J. Biol. Chem. 245: 219, 1970) and involves fine comminution of muscle with a Sorvall homogenizer and recovery of the actin and myosin thus liberated.

The troponin-tropomyosin complex has been prepared by the method of Stewart and Levy (J. Biol. Chem. 245: 5764, 1970) with further purification achieved with ammonium sulfate as described by Watanabe and Staprans (Proc. Nat. Acad. Sci. 56: 572, 1966).

Rats were first studied to prepare the above protein components. The troponin-tropomyosin complex was removed from the rat actomyosin by trypsin treatment. After such treatment there was some loss in actomyosin ATPase activity indicating some inactivation of the actomyosin. However, addition of a certain fraction prepared by ammonium sulfate precipitation to the actomyosin ATPase assay revealed further decrease in activity suggesting that the troponin-tropomyosin complex was contained therein.

Recently muscle has been obtained from a myotonic and normal goat (in conjunction with Dr. Ward of C.S.U.) and has been studied as outlined above. Three separate experiments were performed and in each instance it appeared that trypsin treatment of the actomyosin resulted in such profound loss in ATPase activity that it was not possible to ascertain whether addition of fractions believed to contain the troponin-tropomyosin complex actually did so. Modification of the preparative procedure to study the myotonic goat is planned as a preface to studying this disorder in patients.

CONCLUSIONS:

1. There is evidence that the troponin-tropomyosin complex associated with the actin of the actomyosin complex can modify the activity of the actomyosin ATPase.

2. This control system may be involved in myotonia.

RECOMMENDATIONS:

The test system should be perfected in the goat for study in patients.

PUBLICATIONS: None.

STUDY NO. 2 Studies in a patient with idopathic myoglobinuria.

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

PROBLEM:

Idiopathic myoglobinuria (rhabdomyolysis with myoglobinuria) is a disease of unknown etiology characterized by repeated episodes of myoglobinuria associated with muscle stiffness, aching pain, and varying degrees of incapacitation. Episodes of myoglobinuria may be precipitated by fasting, infection, exercise and via other unrecognized mechanisms. There are many other causes of myoglobinuria including crushing wounds, electrical injuries, peripheral vascular accidents, Haff disease, specific infections such as clostridial disease and so-called march or stress myoglobinuria. In none of these, however, are there the repeated episodes of myoglobinuria and, of course, they are characterized by specific recognizable precipitating conditions. Long term risks of episodes of myoglobinuria are renal damage and perhaps cardiac arrythmia resulting from K^+ leakage associated with rhabdomyolysis.

The reason for myoglobin leakage from muscle in most conditions characterized by myoglobinuria, including idiopathic myoglobinuria is not clear. Faulty oxidative metabolism, abnormal energy generation, anoxia and other mechanisms have all been invoked as possible causes of the leakage of myoglobin. Myoglobin abnormality (akin to hemoglobin abnormality due to amino acid substitution) is a rare biochemical event and has not been found in association with myoglobinuria.

What is obvious, despite absence of clear understanding of the mechanisms involved, is that myoglobin leakage from muscle in idiopathic myoglobinuria is related to an abnormally permeable muscle membrane. Along with myoglobin leakage, numerous other constituents of muscle such as aldolase, SGOT, LDH and other enzymes, as well as potassium are known to "leak out" of muscle during clinically recognizable episodes. Thus, it would appear reasonable to focus attention on the muscle membrane as the avenue of as well as the mechanism for myoglobin loss, and to consider its relationship to the various conditions in which myoglobinuria is present. We postulate that in idiopathic myoglobinuria there is a primary defect in muscle membrane, analagous in concept to the abnormal erythrocyte (membrane) in paraxysmal nocturnal hemoglobinuria (PNH). The abnormal membrane is unusually permeable, or becomes so unusually easily, which allows for escape of myoglobin and other intracellular muscular constituents. In those conditions in which myoglobinuria is a secondary phenomenon, myoglobin leakage can also be reasonably explained on the basis of membrane damage, which may follow any of the conditions listed above.

The concept of membrane abnormality in idiopathic myoglobinuria fails to explain the intermittent nature of myoglobin leakage, since, if the membrane were defective, continuous leakage might be expected.

There must be additional factors which operate in concert with the presumed membrane abnormality to trigger the clinical episodes of myoglobinuria. Review of cases of idiopathic myoglobinuria reveals the presence of severe exercise, fasting, or infection immediately preceeding the onset of the clinical episode. One of the common denominators in these preceeding events is acidosis, which is known to occur with muscular activity, fasting, and in infections. We thus postulate that in idiopathic myoglobinuria there is a defect which renders the membrane abnormally permeable in the presence of acidosis. In concept, this is similar to our understanding of the defect in the PNH red cell, in which the RBC is abnormally sensitive to acid lysis. With the foregoing in mind, we studied a patient with idopathic myoglobinuria.

RESULTS AND DISCUSSION OF THE RESULTS:

This patient has undergone extensive study on the metabolic ward. His history is typical of patients with idiopathic myoglobinuria in that the disease remained unrecognized for a long while. By history the episodes of myoglobinuria have followed flu-like and/or viral upper respiratory symptoms or modest exercise. His exercise tolerance is extremely variable from day to day. The etiology of this variability is unclear. Preliminary studies indicate that systemic acidosis (provoked by NH_4Cl, Diamox and CO_2 breathing) tend to provoke symptoms and reduce his ability to exercise. Alkalosis seems to improve his symptoms and to increase his exercise tolerance. Other studies have shown that myoglobin, LDH, SGOT and other "traditional" serum markers of muscle membrane "leakiness" may be quite insensitive to early muscle membrane changes. In this patient pyruvate kinase (PK) has been markedly elevated all the time, and underwent further dramatic rises with exercise and/or acidosis. In some instances the PK rose spontaneously prior to the onset of clinical symptoms. This interesting finding supports the concept of an abnormal membrane, which becomes more abnormal when the appropriate triggering mechanism (acidosis is present.

CONCLUSIONS:

From the study of this one patient, and consideration of reported patients with idiopathic myoblobinuria the following conclusions may be drawn:

1. The underlying defect in idiopathic myoglobinuria appears to reside in the muscle cell membrane and is characterized by abnormal membrane permeability.

2. Membrane permeability may be increased by acidosis and reduced by alkalosis.

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

3. Pyruvate kinase may be a more sensitive marker for membrane permeability than myoglobin, SGOT or LDH.

RECOMMENDATIONS:

The study of this patient represents a unique opportunity to expand our understanding of muscle cell membrane function. The abnormality in this condition may be related to fundamental muscle physiology. These studies should be continued.

PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6328	71 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
70 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	063
b. CONTRIBUTING	61145011	3A014501B71R	02	
c. CONTRIBUTING	CDOG 1412A (2)			

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	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 71	2.0	55
FISCAL YEAR CURRENT 72	2.0	58

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

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Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

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TELEPHONE: 303 366 5311 X21108

20. PERFORMING ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
Microbiology Division

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

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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 are sought by examining the growth response to selected compounds, the enzyme content, and intermediary metabolism. Biochemical changes in host tissue are studied to establish the metabolic consequences of tuberculosis infection.
25. (U) 70 07 - 71 06 Despite the fact that in vivo studies have thus far failed to establish a role for α-amino-n-butyric acid (AANBA) as a chemotherapeutic agent in tuberculosis, work in this area has not been abandoned because of the extreme susceptibility of the unknown metabolic site in M. tuberculosis to inhibition by AANBA. New findings have shown that cells grown with AANBA excrete large amounts of aspartic acid and lysine, and greater than normal amounts of leucine, valine and isoleucine. A logical extension of this work would be to test the effect of derivatives of AANBA on the growth of M. tuberculosis. In related studies in vitro protein synthesis by ribosomal preparations from normal and tuberculous mice have been examined. Although all aspects of the analysis have not been completed, results to date indicate that protein synthesis in tuberculous liver tissue is depressed.

*Available to contractors upon originator's approval

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

ABSTRACT

PROJECT NO.	3A061102B71R	Research in Biomedical Sciences
TASK NO.	02	Internal Medicine
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. 1. New studies in this area were instituted with the objective of describing biochemical and metabolic events occurring in the animal host as the result of infection by M. tuberculosis. Efforts to date have been concerned with establishing techniques for the study of protein synthesis in normal and tuberculous mice. In in vivo experiments ^{14}C-labeled leucine was injected and after a suitable time interval, the amount of radioactivity present in liver microsomal protein was ascertained. Protein synthesis at the subcellular level has been evaluated by determining the ability of polyribosomal preparations from liver to incorporate radioactive amino acids into protein. Results from completed experiments have furnished evidence for both suppression and stimulation of protein synthesis during the course of infection with M. tuberculosis. The meaning of these findings in relation to the slow progressive pathogenesis of tuberculosis remains to be established.

Study No. 3. Investigations within this study have been concerned with examining the pathways of amino acid synthesis in variants of M. tuberculosis which are resistant to isoniazid and streptomycin. Results from these studies have failed to reveal readily discernable differences in biosynthetic pathways used by the mutants or parent strain to obtain their complement of essential amino acids. It is expected however that should quantitative differences exist between the amino acid pathways in these organisms, they will become apparent after the data has been analyzed statistically. In conjunction with this work, the ability of the non-essential amino acid, alpha-amino-n-butyric acid (AABA), to inhibit growth of M. tuberculosis has received additional attention. Careful in vitro testing has shown that between 1 and 2 µg/ml of AABA can be inhibitory for the growth of M. tuberculosis. Although the metabolic site at which AABA acts has not been established, cells grown with AABA were found to excrete large amounts of aspartic acid and lysine. Thus far attempts to assign a role to AABA as an antituberculous agent have given only marginal success.

BODY OF REPORT

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

STUDY NO. 1 — Enzymes in Mycobacterium tuberculosis

PROBLEM:

To study in vivo and in vitro protein synthesis in animals infected with Mycobacterium tuberculosis.

RESULTS AND DISCUSSION OF THE RESULTS:

Initial efforts in this study have been aimed at comparing protein synthesis in normal and tuberculous mice. In one phase, intact animals were injected with 2 μc of ^{14}C-leucine and the incorporation of radioactivity into liver microsomal protein determined after 60 minutes. Maximum incorporation of radioactivity was found to occur within this interval. In conjunction with the in vivo measurements, protein synthesis at the subcellular level has been estimated by determining the ability of polyribosomal preparations from liver to incorporate carbon-14 labeled amino acids into protein. Utilizing methods developed in the USAMRNL laboratory for rat liver, polyribosomal and cell-sap enzymes of high activity have been obtained from mouse liver. Protein synthesis at this level might be expected to agree with findings obtained from in vivo experiments involving the whole animal, and in addition, point to whether differences in protein synthesis between control and infected animals resides within the cell-sap enzymes or polyribosomes. Profiles of the polyribosomes have been obtained by sucrose density centrifugation. Examination of these patterns indicate that little degradation occurred during isolation as judged by the absence of low molecular weight species. Because of the necessity of moving the spectrophotometric equipment to a safe room, polyribosomal profiles have been determined only for control animals. Eventually these patterns from control animals will be compared with similar preparations from infected animals. Despite success in developing experimental procedures, comparison of subcellular protein synthesis by control and infected animals has thus far been inconclusive. Marked stimulation of protein synthesis in infected animals was observed in one case and a slight depression with another group. Because the animals are infected by aerosolization, which corresponds closely to the natural pathway of infection, it is difficult to control the precise state of the disease. Other modes of infection, such as the injection of virulent M. tuberculosis into the animal, might result in a clinical picture which is incorrect in terms of the pathogenesis of respiratory system tuberculosis.

CONCLUSIONS:

Experimental procedures have been developed for the study of *in vivo* and *in vitro* protein synthesis in normal and tuberculous mice. Results thus far have shown that such studies are feasible and that differences in protein metabolism exist between normal and tuberculous mice.

RECOMMENDATIONS:

It is recommended that these studies be extended to an examination of changes in ribonucleic acid (RNA) metabolism in normal and tuberculous animals. Based on current knowledge regarding the mechanism of protein synthesis, it is likely that disruption of protein metabolism will be reflected by change in RNA metabolism.

PUBLICATIONS:

None.

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

PROBLEM:

To examine the pathways of amino acid synthesis in drug-susceptible and drug-resistant *Mycobacterium tuberculosis*.

RESULTS AND DISCUSSION OF THE RESULTS:

In keeping with the established objective of investigations concerned with describing pathways of amino acid synthesis in drug-susceptible *Mycobacterium tuberculosis*, similar studies have been carried out with strains of *M. tuberculosis* which are resistant to streptomycin and isoniazid. Through use of the "isotopic competition" technique it has been possible to demonstrate that the pathways utilized by the drug-resistant mutants for the biosynthesis of amino acids do not differ significantly from pathways found in drug-susceptible strains. In both classes of organisms, the amino acids lysine, threonine, methionine, and isoleucine are apparently derived from aspartic acid, while glutamic acid functions as a precursor of arginine as well as proline. Detailed examination of these biosynthetic sequences showed homoserine to be a key intermediate in the synthesis of methionine and threonine, with isoleucine being derived

from threonine. Exogenous threonine is converted to glycine, which furnishes carbons 1 and 2 of serine. Carbon from ornithine may flow into proline as well as arginine. Comparisons in a quantitative sense between results obtained from drug-resistant and drug-susceptible strains are rendered difficult because of the propensity of M. tuberculosis to recycle carbon into various pathways. The above studies have resulted in the finding that alpha-amino-n-butyric acid (AABA) is a very effective growth-inhibitor for M. tuberculosis. Depending on the size of the inoculum it is possible to show an inhibitory effect for as little as 2 µg/ml of alpha-amino-n-butyric acid. Thus far, the metabolic site which is susceptible to the action of AABA has not been established. None of the amino acids which are structurally related, such as leucine, valine, and isoleucine have been found effective in reversing the effect of AABA. AABA, however, may have a disruptive effect on normal amino acid metabolism since we have observed that M. tuberculosis grown with AABA excretes large amounts of aspartic acid and lysine into the growth medium. Levels of leucine, valine, and isoleucine were elevated over normal values. Despite the ease of demonstrating the toxicity of AABA for M. tuberculosis in test tube assay, as well as attaining by dietary supplementation serum levels of AABA which in some cases have run as high as 300 µg/ml, only marginal success in demonstrating the efficacy of AABA as an antituberculosis agent has been observed. Much of the ability of AABA to inhibit growth of M. tuberculosis is lost in serum. It is believed that the reversing effect of serum is related to the amino acid content since simulated mixtures of serum amino acids also increase the level of AABA required for growth inhibition.

CONCLUSIONS:

The acquisition of resistance to isoniazid and streptomycin apparently does not result in significant modification of amino acid pathways. Despite these negative results it is expected this work will serve to clarify the amino acid metabolism of M. tuberculosis.

RECOMMENDATIONS:

It is recommended that structural analogs of AABA be tested against M. tuberculosis.

PUBLICATIONS:

O'Barr, T. P. and K. A. Everett. Effect of L-Homoserine on the growth of Mycobacterium tuberculosis. Infection & Immunity 3: 328, 1971.

Whitten, B. K., G. Beecher, C. G. Liddle and T. P. O'Barr. A comparison of $^{14}CO_2$ trapping agents used in *in vitro* metabolic studies. USAMRNL Laboratory Report No. 324, September 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6355	71 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
70 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	61102A	3A061102B71R	02	064
b. CONTRIBUTING	61145011	3A014501B71R	02	
c. CONTRIBUTING	CDOG 1412A	(2)		

11. TITLE (Precede with Security Classification Code)*

(U) Bio-Medical Information Systems Design (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

004200 Computers

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 70	2.0	38
FISCAL YEAR CURRENT 71	3.0	50

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Boog, M. L.

NAME: Ferguson, M. J. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Digital Computer; (U) Medical Information; (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 and develop a biomedical information system programmed for a digital computer.

24. (U) Biomedical information is provided by the laboratory divisions of USAMRNL and by cooperating elements of FGH. Generalized computer programs are developed by Computer Division, USAMRNL, to support the information system.

25. (U) 70 06 - 71 06 Study No. 1, "Long Term Effects of Selected Drug Regimens" - An additional 1500 records have been entered into the data file in cooperation with the Dept. of OB/Gyn, FGH. Analysis of the data will be forthcoming. Study No. 2, "The Development of a Natural Language Retrieval System for the Tumor Registry, FGH" - This study has not progressed and is to be terminated at the request of the Dept. of Surgery, FGH, due to the lack of trained personnel to fulfill the FGH commitments to the study. The study with the Dept. of OB/Gyn, FGH, will be transferred to Work Unit No. 166, "Design of Military Biomedical Research Information Systems." The remainder of this work unit will be terminated.

*Available to contractors upon originator's approval

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

ABSTRACT

PROJECT NO.	3A061102B71R	Research in Biomedical Sciences
TASK NO.	02	Internal Medicine
WORK UNIT NO.	064	Biomedical Information Systems Design

The following investigations have been conducted under this work unit:

STUDY NO. 1 Long Term Effects of Selected Drug Regimens

STUDY NO. 2 The Development of a Natural (English) Language Information System for the Tumor Research Registry, Fitzsimons General Hospital

STUDY NO. 3 General Programming and Support Development

This work unit serves to develop methods for automating medical data in cooperation with Fitzsimons General Hospital. Coordination with the hospital is through the services concerned and the Clinical Research Service.

Study No. 1: This study, in collaboration with the OB-GYN Service, FGH, is a system to collect, store, retrieve and analyze patient data relevant to the evaluation of oral contraceptives. Analysis of data on 3500 women collected between June 1969 and June 1970 was completed during FY 71. A complete report of this data will be forthcoming.

Study No. 2: This is a joint study with the Tumor Registry, FGH, for the development of a computer based system which will accept the English prose generated by the physician and maintain this information in a logical file system. The Dept. of Surgery, Fitzsimons General Hospital, has not been able to identify personnel to provide the input required as part of their commitment to this study. Hence there has been no input during FY 71 and the Dept. of Surgery has requested cancellation of the study.

Study No. 3: This study serves to develop the necessary supporting programs for the effective use of the computer. This work includes control system programming, language usage programming, statistical programming and support. Data files have been developed to support nearly every division of USAMRNL. Statistical analysis of data in these files is currently in progress. Several macro routines have been written and have proven invaluable in speeding development of application programs.

BODY OF REPORT

WORK UNIT NO. 064 — Biomedical Information Systems Design

STUDY NO. 1 — Long Term Effects of Selected Drug Regimens

PROBLEM:

There is a need to develop computer based information systems which have the capability to process fully automatically, the requirements for evaluating long term medical regimens. Such programs present unique problems as regards information processes of data collection, storage, analysis and retrieval. The long-term administration of oral contraceptives provides an excellent model for such an information system. Specifically, since oral contraceptives are administered to large numbers of women (8.5 million in the U. S. alone) in the absence of any disease, it is necessary to continuously monitor and evaluate the effects of long-term use of this particular drug through statistical methods and systematic classification techniques. Moreover questions have been raised by a variety of studies concerning possible long-term serious side effects of these drugs.

RESULTS AND DISCUSSION OF THE RESULTS:

The study was begun on 1 March 1967. A questionnaire was administered to each patient upon entry into the OB-GYN family planning section. A physician summary was completed for each of the patients. A number of subjects were selected from other sections of the service to provide additional information. This data was continuously updated to form a chronological patient record. Analyses of these records showed that the data which was collected was not comprehensive enough to make a reasonable evaluation of the effects of oral contraceptives. The data collection procedure was revised in the form of a second questionnaire to include more physician generated information and data concerning other drugs which the subjects were taking. Provisions were also made to include a physician's comment. The second questionnaire has been in use since June 1969.

The data file currently consists of 14,000 questionnaires on 9,600 patients collected over a period of four years. Data collected for the period June 1969 to June 1970 was statistically analyzed during FY 71. The study group consisted of 3548 women of whom 2990 had taken or were currently taking oral contraceptives. The mean age of the study group was 29±6 years. More than 65% of the

group had taken oral contraceptives for more than 2 years; 21% has taken oral contraceptives for more than 5 years. Table 1 highlights some of the 120 comparisons made during the analysis. Only medical problems for which there was a statistically significant difference (99.5% level) between groups are listed. The most common reasons for stopping the use of the pill were non-medical, such as, "wanted to become pregnant," or "husband was away." A complete analysis is being prepared and should be published in the near future.

CONCLUSIONS:

Use of an automated data file is extremely valuable in the study of large research populations. This is especially true when many variables (more than 120 items surveyed) are associated with each individual (9600 women) in the population.

RECOMMENDATIONS:

1. Revise the questionnaire to include more clinical data.

2. Transfer this study to Work Unit 166, "Design of Military Biomedical Research Information Systems."

STUDY NO. 2

The Development of a Natural (English) Language Retrieval System for the Tumor Research Registry, Fitzsimons General Hospital

PROBLEM:

Methods tailored to the structural and functional characteristics of the electronic digital computer must be developed to process, under automatic controls, medical information documented in natural language. Past experience has demonstrated that the time-honored technique of assigning codes to information documented in natural language prose gives rise to formidable problems. This study aims to achieve a means for processing the computer input and retrieval of information in the English language familiar to medical personnel. These functions must be achieved with no encoding of information prior to computer input or preliminary to communication with the computer and no decoding of information output from the computer.

RESULTS AND DISCUSSION OF THE RESULTS:

A programming system to take unformated and uncoded Tumor Registry data has been completed to facilitate the information handling of large bodies of unstructured data. The program converts the data into a structured file format for update in the general patient master file. This data is stored in a retrievable form under conditions of logic expressed by the participating service. The data may be retrieved by location, disease, process, modifier processes, person, time or any combination of the preceding. Only one case has been inputted into the files since the start of the project, and no information has been updated to this file in FY 70 due to lack of support from FGH. The Dept. of Surgery, FGH, has requested that they be relieved of their commitment to provide input into this study.

CONCLUSIONS:

FGH is no longer interested in this project due to personnel turnover and shortages. FGH requests that the study be terminated.

RECOMMENDATIONS:

Terminate the study.

STUDY NO. 3 — General Programming and Support Development

PROBLEM:

To facilitate the application of the computer, certain basic programs must be developed. This type of programming is of a general nature and applicable to many different projects.

Biomedical Information Systems Design (Cont'd)

RESULTS AND DISCUSSION OF THE RESULTS:

Data files have been developed for the following MRNL projects:

Division	Project	[1]No. of Messages	Special Application Programs
Administrative	Property Book	-	-
Bioenergetics	Energy Expenditure	1300	general statistical
Chemistry	Vitamin A Study	7500	interpolation, general statistical
Metabolic	Human Thermoregulation	45000	heat generation, auto-correlation, cross correlation, statistical analysis
Microbiology	TB Culture Data	35000	culture result by specimen type, contamination by clinics, contamination by patients, general statistical
Pathology	Nutritional Aspects of Military Dog Performance	1000	work factors, general statistical analysis
Inter-divisional	Military Nutrition Surveys	19000	nutrient intake, clinical data, biochemical data, general statistical analysis

[1]A message is a group of related items: i.e., the "21" message in the Microbiology Division file contains 36 different items related to the processing of TB specimens. Generally a message is limited to 80 alphabetic or numeric characters.

Biomedical Information Systems Design (Cont'd)

In depth statistical analysis is being performed on the data stored in research files. In addition, several specific programs have been written for each of these files. For example, a summary printout of all clinical and biochemical data (more than 50 items) for each individual subject studied in the Ft. McClellan nutrition survey was prepared and sent to the individuals for their personal use. Detailed results of the analyses of the files are discussed in the work units for each project.

A binary search macro routine has been written and added to the division library. The routine automatically decodes information using a standard dictionary. The routine is especially useful in developing file display programs where the information to be displayed is stored in code form and the display is to be in English language.

A macro routine to block and write output records has been written. This routine removes the burden of output blocking from the programmer. The routine can be used with all file programs.

The Fortran compiler is being revised to permit programmers to access the data files using simple Fortran commands. Program development time associated with data file analyses will be significantly decreased when this is completed. In addition, much more powerful analyses of the data will be readily available.

The data file structure for the MRNL biomedical research information system has been greatly improved. Four edit routines have been replaced by a single program, thus allowing complete file corrections to be made on a single pass rather than on two or more passes. In addition, several types of file corrections can be made on the same pass. This system also includes a conversion to batched type files which will result in shorter magnetic tape files and consequently faster file maintenance and data processing. For example, the prime master file consisted of greater than 19000 blocks on two reels of magnetic tape prior to batching. After batching the number of blocks was reduced by a factor of ten and the batched file required less than one tape reel for storage. A typical update of 5000 records to a one-reel data file now requires 30 min. The old update system required a minimum of 8 hours to do the same job.

CONCLUSIONS:

1. The use of automated data files is an extremely valuable tool in research.

Biomedical Information Systems Design (Cont'd)

2. Most major projects and divisions at USAMRNL maintain an active data file.

3. Comprehensive data analysis can be performed on data files with a minimum of human intervention and results can be returned while the study is still relevant.

RECOMMENDATIONS:

1. Transfer the study to Work Unit 166, "Design of Military Biomedical Research Information Systems." The design of the new work unit will permit better organization and management of data banking, data retrieval and data analysis.

2. Develop generalized programs to make data analysis and queries easily accessible to non-computer oriented researchers.

PUBLICATIONS:

None.

TABLE 1

COMPARISON OF GROUPS OF WOMEN WHO HAVE NEVER TAKEN ORAL CONTRACEPTIVES WITH THOSE WHO HAVE

Item		Group 1 Currently on the Pill	Group 2 Have Taken Pill But Not at Present	Group 3 Never Taken the Pill	Total
1. Number of women studied		2013	977	558	3548
2. Religious Preference					
Protestants	(% of total)	58.5%	28.8%	12.7%	2410
Catholics	"	52.1	25.2	22.7	952
Jewish	"	50.0	32.1	21.7	28
None	"	50.4	24.5	15.1	106
Others	"	52.2	17.4	30.4	23
No preference indicated	"	58.6	17.2	24.1	29
3. Ethnic Group					
Caucasian	(% of total)	56.7%	28.3%	15.0%	3192
Negro	"	58.8	19.6	21.6	245
Oriental	"	50.0	19.2	28.8	52
American Indians	"	50.0	33.0	17.0	6
No Ethnic Group indicated	"	52.8	26.5	20.8	53
4. Marital Status					
Have been married	(% of total)	57.6%	28.0%	14.4%	3442
Never married	"	29.2	11.3	59.4	106
5. Child Bearing Factors					
Successful pregnancies			2.6 ± 1.9		2985
No. of miscarriages	(% of group)	400 (19.9%)	183 (18.7%)	75 (13.4%)	658

TABLE 1 - Comparison of Groups of Women Who Have Never Taken Oral Contraceptives with Those Who Have (Cont'd)

Item	Group I Currently on the Pill	Group 2 Have Taken Pill But Not at Present	Group 3 Never Taken the Pill	Total
5. Child Bearing Factors (cont'd)				
Normal pregnancies (% of ea.group)	87.1%	91.6%	-	903
Normal babies "	91.1	93.2	-	928
Pregnant while on pill "	1.2 (24)	2.1 (21)	-	45
Pregnancy and baby normal "	66.7	71.4	-	31
Pregnant on other forms of contraceptives "	15.0	14.2	9.1	492
Pregnancy and baby normal "	88.1	88.5	96.0	483
6. Medical Problems (Statistically significant difference at 99.5% level)				
Excessive weight gains (% of group)	18.4%	18.4%	9.3%	716
Migraine headaches "	4.3	6.9	3.2	260
7. Surgery (Statistically significant at 99.5% level)				
Appendectomy (% of group)	13.5%	12.5	6.6	422
D&C "	3.9	3.7	6.8	430

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6312	71 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
70 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 1412A	(2)		

11. TITLE (Precede 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 Env. 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 71	1.8	39
	CURRENT 72	2.2	30

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

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20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
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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 (Precede 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. Precede text of each with Security Classification Code.)

23. (U) To elucidate the biochemical and physiological changes in animals and man associated with abrupt altitude exposure and to measure acclimatization, dietary effects and the influence of training on these changes in order to improve the health, well-being and physical and mental capacities of soldiers having to function under conditions requiring abrupt exposure to high terrestrial altitude.

24. (U) Determine serum levels of metabolites and measure activities of enzymes involved in intermediary metabolism during altitude exposure using the rat. The initial series of animal studies are designed to elucidate the interrelationships of diet, physical activity and altitude exposure upon muscle and liver glycogen contents, serum glucose levels and several key or rate-limiting enzymes involved with carbohydrate metabolism. Measure glucose turnover rates in man at altitude with ^{14}C-glucose to estimate pool sizes, release of glucose from liver stores, and glucose uptake by tissues.

25. (U) 70 07 - 71 06 A study comparing glucose metabolism at 300 and 4300 meters was conducted using ^{14}C-glucose. The labeled glucose (30 uci) was infused to 4 men after 18 hrs, and to 4 men after 42 hrs of 4300 m exposure; to 8 pair-fed men at 300 m. Fasting glucose levels were lower in the altitude exposed men. However, no differences in glucose specific activity between altitude and control subjects were found. A similar, detailed study will be conducted this summer using different techniques. In another study, pulmonary function and blood acid-base levels were compared at 1600 and 4300 m. Inspiratory and total lung capacities were increased at 4300 m. Blood pH values remained unchanged with a decreased pCO_2 compensated thru a decreased HCO_3. The data are indicative of an increased ventilatory response to reduced atmospheric oxygen with compensatory changes in acid-base values.

*Available to contractors upon originator's approval.

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

ABSTRACT

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

The following investigations were conducted under this work unit.

STUDY NO. 12	Physiological and metabolic aspects of high altitude. Effects of dietary carbohydrate-fat levels upon metabolic responses to exercise.
STUDY NO. 13	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

STUDY NO. 13

The blood glucose and specific activity of glucose were measured after infusion, in pair fed subjects at altitude and at sea level. Fasting glucose levels were decreased after 18 and 40 hours at altitude, but specific activities of carbon 14 labelled blood glucose did not appear to be affected.

STUDY NO. 14

Both high temperatures and altitude increase hypoxia at the cellular level. Studies with mice showed, at sublethal and lethal temperatures, that blood lactate levels and lactate to pyruvate ratios are increased. However, the blood pH's were decreased. Further studies are in progress to attempt to counteract these effects by injection of metabolic intermediates.

Two experiments have been conducted with rats fed 30-80% carbohydrate calories with and without treadmill exercise. Several of the carbohydrate metabolizing enzymes were affected by both diet and exercise at Denver.

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 high altitude. Effects of dietary carbohydrate-fat levels upon the metabolic responses to exercise.
	STUDY NO. 13	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:

STUDY NO. 12

Several studies have been designed to test the hypothesis that carbohydrate metabolism may be altered after abrupt exposure to altitude. The affects of altitude, dietary carbohydrate-fat levels, and treadmill exercise upon the activity of certain key inducible carbohydrate metabolizing enzymes are being evaluated using the laboratory rat.

RESULTS AND DISCUSSION OF THE RESULTS:

Two of the five experiments on the effects of diets, activity and altitude upon enzymatic activities have been completed. Half of the rats were fed ad libitum and half were fed for 8 of 48 hours in an attempt to reduce variation in the activities of creatinine phosphokinase, hexokinase pyruvic kinase, glucose-6-phosphate dehydrogenase, transketolase, succinate dehydrogenase and malic dehydrogenase. The time after feeding before sacrifice in the rats fed intermittently, and the amount of carbohydrate or fat in the diets altered the enzyme activities, liver size and blood glucose; however, intermittent feeding did not reduce variability. The second experiment evaluated the effects of treadmill exercise and carbohydrate to fat ratios of the diets, on these same parameters. The data is being prepared for computer analyses. The third study will be conducted this summer to compare diet-induced changes at 4,300 vs 1,600 meters.

CONCLUSION:

Increases in the carbohydrate content of the diet and treadmill exercise, increases most of carbohydrate metabolizing enzyme activities. Intermittent feeding schedule did not appear to significantly reduce the variability between animals.

PROBLEM:

STUDY NO. 13

To further study the influence of acute exposure to altitude on carbohydrate metabolism, changes in glucose spaces and metabolism are measured in human subjects through the infusion of glucose-U-^{14}C, with subsequent determination of blood glucose specific activity and the specific activity of expired CO_2. Pulmonary diffusion capacities and blood acid-base alterations will be concommitantly evaluated.

RESULTS AND DISCUSSION OF THE RESULTS:

The infusion of 30 uci of glucose-U-^{14}C in men after 18 and 42 hours at altitude, and in pair-fed men at sea level indicated that the specific activity of blood glucose was not affected by altitude exposure. Fasting blood glucose levels appeared to be decreased after 18 and 40 hours of altitude exposure 4,300 m. A similar study will be conducted this summer to determine the sizes of various glucose spaces, the specific activity of expired carbon dioxide, changes in blood acid-base parameters and changes in pulmonary diffusion capacities.

CONCLUSIONS:

Data from the ^{14}C-glucose study with humans indicated that glucose metabolism was not affected by altitude although fasting glucose levels were decreased after 18 and 40 hours of altitude exposure. Another study is required to confirm these results and to gather further data. This study has been approved and will be conducted in August-September 1971.

PROBLEM:

STUDY NO. 14

It is postulated that hypoxia may be the primary factor involved in heat death. This study was designed to investigate various tissue parameters in association with high sublethal and lethal temperature in order to elucidate the role of hypoxia in thermal death in rats.

RESULTS AND DISCUSSION OF THE RESULTS:

Hypohydration was evident in mice at thermal death with hematocrits of 58% compared to 52% in sublethal temperature treated animals and 47% in the controls. Decreases in other blood parameters of animals at terminal respiratory movement compared to controls were as follows: pH, 6.91 vs 7.25; pO_2, 5.7 vs 32.9 mm Hg; standard bicarbonate, 8.1 vs 15.8 mEq/l; and actual bicarbonate, 9.5 vs 15.4 mEq/l; with increases of pCO_2, 49.0 vs 37.2 mm Hg; and base excess, -25.3 vs 11.1 mEq/l. Determinations of lactate and pyruvate in the brain, muscle and liver indicated that lactate levels and lactate to pyruvate ratios were increased while pyruvate concentrations were decreased in thermal stress, but these changes were less than in another group subjected to fatal hypoxia.

CONCLUSION:

Hypoxia may be involved in heat death, but it does not appear to be a primary factor.

RECOMMENDATIONS:

1. Conduct Part B of the mouse study of lethal heat effects; summarize the data and prepare a draft of the results.

2. Conduct Experiment 3 of the rat study on the effects of diet, altitude and exercise upon enzymatic adaptations during the summer of 1971; summarize all of the data on the first three experiments, and prepare a draft of the results. The results of these experiments should provide considerable information about the effects of carbohydrate and exercise during abrupt altitude exposure.

3. Upon completeion of the ^{14}C-glucose study with humans, the calculations of various glucose spaces, changes in acid-base balances and pulmonary diffusion capacities and the expiration rates of labelled carbon dioxide should be correlated and compared in the altitude-exposed vs non-exposed men.

PUBLICATIONS:

1. Bodenhausen, G.E. and H.L. Johnson. Design of an 18-unit rodent activity treadmill cage and stimulation system. J. Appl. Physiol. (In Press)

2. Burk, R.F.Jr., H.L. Johnson, R.D. Johnson and C.C. Scaief III. Glucose metabolism at high altitude. Fed. Proc. 30:405 Abs., 1971 (Abstract)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6339	71 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
70 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 1412A (2)			

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 71	1.1	32
FISCAL YEAR CURRENT 72	4.3	103

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
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RESPONSIBLE INDIVIDUAL
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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, E. 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) 70 07 - 71 06 Anorexia and body weight loss regularly occur in animals and humans acutely exposed to high altitude. Initial stages of high altitude exposure are associated with a reduction in the efficiency of food utilization and a more rapid gastric emptying time. Under these environmental conditions an increase of intracellular sodium takes place. As a result there is a transfer of water from the extracellular to intracellular compartment. An increase in tyrosine oxidation also has been observed.

*Available to contractors upon originator's approval.

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

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. 24	Effects of altitude on body fluids
Study No. 25	Effects of translocation to high altitude on mechanisms regulating food intake.
Study No. 26	Synthesis and degradation of catecholamines at high altitude

Significant differences were observed between the spontaneous and locomotor activity of rats exposed to high altitude. In addition, inhibition of locomotor activity at high altitude does not substantially alter free-locomotor activity of rats following return to Denver altitude. The body fluid shifts observed during an acute high altitude exposure appears to be due to a transcellular exchange of hydrogen and sodium ions. Such a shift could seriously affect the normal cellular function of some tissues. Efficiency of protein utilization is markedly reduced during the initial stages of high altitude exposure. This may be the result of an increased rate of ingesta flow. Oxidation of tyrosine is markedly increased during the first three days of high altitude exposure.

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:

Few experiments have examined the possible interactions among length of exposure to high altitude, food consumption and spontaneous activity. This study, involving four experiments, is an attempt to determine the differences between various measures of animal activity following exposure to hypoxic environments.

RESULTS AND DISCUSSION OF THE RESULTS:

Results of the first experiment were reported previously. The second experiment confirmed earlier reports that exposure for six days to 4,300 meters produced temporary reductions in food and water consumption, and body weight of albino rats. This exposure was associated with temporary reductions in feeding activity but produced only minor decreases in spontaneous activity.

Experiments 3 and 4 were designed to assess differences in spontaneous and locomotor activity during exposure to 4,300 meters and the potential inhibitory effect such exposure might exert upon locomotor activity after return to 1,600 meters. Results of these experiments can be summarized as follows: (a) strong diurnal activity patterns were observed throughout the procedure, with night-time activity being approximately 8% greater than day-time activity; (b) spontaneous activity decreased less and recovered faster than locomotor activity both during and following altitude exposure; (c) rats prohibited from locomotor activity during altitude exposure showed no difference in activity following exposure as compared to a similarly inactive group housed at 1,600 meters; however, both groups showed reduced levels of activity when compared to animals allowed to exercise at 4,300 meters.

CONCLUSIONS:

These results indicate that the activity of animals exposed to hypoxic environments is greatly dependent upon the properties of the measuring instrument, i.e., running wheels or home-cage. Such evidence suggests changes in activity are triggered by environmental stimuli, not strictly a reflection of internal drives. Furthermore, data from Experiment 4 indicates that forced inactivity is prepotent to hypoxia in altering

subsequent free-locomotor activity of animals. Such a result is counter to the hypothesis that the potential increased fatigue produced by locomotor activity in a decreased oxygen environment serves to inhibit recovery of such activity upon return to a normoxic environment.

RECOMMENDATIONS:

1. Complete analysis of the data.

STUDY NO. 24 Effects of altitude on body fluids

PROBLEM:

Acute altitude exposure causes a rapid and marked reduction in plasma volume. The decrease is associated temporally, with a proportionately similar reduction in interstitial volume. In soldiers, for example, total extracellular fluid volume was reduced by 3.5 liters after one week on Pikes Peak (14,110 feet). This reduction is almost exactly compensated by an increase in intracellular water. Questions arising from these observations include the following: what are the responsible mechanisms, which tissues and organs are involved, and what are the effects on various tissue and organ functions? These questions have been studied in a series of animal experiments conducted in the Denver and Pikes Peak Laboratories.

RESULTS AND DISCUSSION OF RESULTS:

Earlier human studies (Work Unit 073) have shown, indirectly, that the extra- to intracellular fluid transfer is accompanied by a massive transfer of electrolyte, principally sodium. On the basis of this observation it would appear that the fluid shift is due to an increase in intracellular osmotic activity. To further evaluate this phenomenon twenty rabbits were exposed for either 2, 4, 8 or 21 days to an elevation of 14,110 feet on Pikes Peak. Total body hydration and extracellular water were estimated by the dilution of intravenously injected tritiated water and $^{35}SO_4$, respectively. Tissues were removed to ascertain the sites as well as the magnitudes of the extra- to intracellular fluid shifts and the associated electrolyte changes. Partially completed analyses have revealed that a) altitude exposure as in early human studies, had little effect on total body water, but did cause a shift from the extra- to the intracellular compartment; b) this shift was particularly prominent in the liver; and c) sizeable increases in total tissue and intracellular sodium occurred in liver and muscle after four days' exposure.

In a study of rats the effects of high altitude anorexia on mineral intake were evaluated with a pair feeding experiment lasting fourteen days. This study showed that most of the changes in mineral balance

observed at high altitude are directly attributable to anorexia.

Pure altitude effects included chloride, phosphate and potassium retention, calcium and magnesium losses, and a sustained reduction in ammonia excretion.

The role played by respiratory alkalosis in the fluid and electrolyte shifts observed at high altitude is currently being evaluated in dogs. During a two-hour hyperventilation period a) little change in extracellular space was observed, b) alkalosis appeared to be associated with an exchange of intracellular hydrogen ion for extracellular sodium and potassium. Hyperventilation caused a prompt and marked increase in bicarbonate excretion.

CONCLUSIONS:

The body fluid shifts observed during acute altitude exposure appear to be attributable in large measure to a transcellular exchange of hydrogen and sodium ions, the latter accumulation causing an increase in intracellular osmolarity and concomitant water transfer. The increase in intracellular water, and perhaps more importantly sodium, should seriously affect the normal cellular functions of some tissues.

RECOMMENDATIONS:

1. Complete the tissue electrolyte analyses from the rabbit study.

2. Continue the hyperventilation studies in dogs to delineate the differences, if any, between normoxic and hypoxic alkalosis.

3. Initiate studies to measure in vivo transmembrane potentials in various tissues during normoxic and hypoxic hyperventilation, and hypoxic normal ventilation.

4. Initiate in vivo and in vitro studies to assess the degree of functional decrement in organs, such as the heart, which is attributable to the foregoing fluid and electrolyte shifts.

STUDY NO. 25 — Effects of translocation to high altitude on mechanisms regulating food intake

PROBLEM:

Reduced food intake and depressed growth rates have been observed in rats exposed to altitudes greater than 10,000 feet. Accordingly, two experiments were conducted to determine whether hypoxia decreases food intake by affecting the satiety center of the ventromedial

hypothalamus and/or by reducing gastric motility to an extent that added gastric fullness inhibits food consumption.

RESULTS AND DISCUSSION OF RESULTS:

Mice, injected with gold thioglucose to produce lesions in the ventromedial hypothalamus, were exposed to a simulated altitude of 14,000 feet. This procedure eliminated the anorexic effects of altitude. In the second experiment, the flow of ingesta through the gastrointestinal tract of rats was compared after continuous exposures of 1, 3 and 8 days to 14,000 feet altitude, and after similar intervals of ad libitum and pair-feeding at low altitude in Denver (5,280 feet). During the acute stages of altitude exposure gastric emptying time was shortened, and intestinal peristalic activity and the rate of ingesta flow were markedly increased. There was an associated reduction in the efficiency of protein digestion and absorption. These responses were attributable in part to hypoxia and in part to altitude anorexia.

CONCLUSIONS:

It was concluded that the anorexic effects of altitude exposure, which is seen in all species including man, are not due to stomach distension. Rather, it appears that suppression of appetite is locally mediated in the hypothalamus itself.

RECOMMENDATIONS:

Future studies in this particular aspect of the work unit should be directed to the effects of altitude on the intestinal digestion and absorption of food, particularly protein.

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 RESULTS:

Metabolic, Physiological and Psychological Effects of Altitude (cont)

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 the injection, the rats were killed, tissue samples were removed and frozen. Urine was collected for 24 hours. The control animals were kept in Denver (5,200 feet).

Compared to the control, 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 for 1, 3, 6 or 13 days, respectively. Total radioactivity in the urine followed a pattern similar to that observed in $^{14}CO_2$. Further biochemical evaluations of samples are in progress.

CONCLUSIONS:

An acute exposure to high altitude apparently alters metabolic pathways of catecholamines precursors.

RECOMMENDATIONS:

1. Complete the tissue and urine catecholamine analyses.

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

PUBLICATIONS:

1. Klain, G. J. and J. P. Hannon. High altitude and protein metabolism in the rat. Proc. Soc. Exp. Biol. Med. 134:1000-1004, 1970.

2. Hannon, J. P., D. D. Schnakenberg and G. J. Klain. Effects of high altitude and caloric restriction on electrolyte metabolism. Federation Proc. 30:428, 1971 (abstract).

3. Schnakenberg, D. D. and R. F. Burlington. Effect of high carbohydrate, protein, and fat diets and high altitude on growth and caloric intake of rats. Proc. Soc. Exp. Biol. Med. 134:904-908, 1970.

4. Krabill, L. F. and J. P. Hannon. Effects of high altitude on rate of ingesta passage. Am. J. Physiology 1971 (submitted).

5. Schnakenberg, D. D., L. F. Krabill and P. C. Weiser. Anorexic effect of high altitude on weight gain, nitrogen retention and body composition of rats. J. Nutrition 101:787, 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6356	71 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
70 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 1412A (2)			

11. TITLE (Precede 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 71	4.0	30
FISCAL YEAR CURRENT 72	3.0	9

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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ASSOCIATE INVESTIGATORS

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

21. GENERAL USE

Foreign Intelligence not Cc

22. KEYWORDS (Precede 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. Precede 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 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) 70 06 - 71 06 Subhuman primates rapidly transported to 14,110 feet showed significant increases in the right ventricular/total heart weight ratios after 5 days. After 3 months this ratio increased approximately 15% over sea level control values. This change was greater than that found in dogs but less than that in rats or rabbits after prolonged exposure. A mild, perivascular cerebral edema occurred in some monkeys at 14,110 feet for 1 to 5 days. During that time electron dense tracers passed through endothelial cytoplasm and basement membranes of cerebral tissues, which was not seen at sea level. Monkey cardiac muscles showed edematous capillary endothelial cells after acute exposure to altitude, while prolonged exposure caused swollen mitochondria and sarcoplasmic reticula with separation of myofibrils. Additional monkeys are being studied to determine the role of diuretics in alleviating high altitude sickness.

*Available to contractors upon originator's approval

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

ABSTRACT

PROJECT NO.	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. 4	Effects of Altitude on the Cebus Monkey (Cebus apella) with Emphasis on Cerebrospinal Fluid Pressure and Brain Water Content
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 Cerebral Edema Cardiac Hypertrophy, Food Intake, and Growth Rate at Altitude

The general objectives of Studies 4, 6, and 7 are to characterize the effects of altitude on the myocardium and the central nervous systems of animals and to correlate these where possible with physiological and subcellular functional changes.

In Study 4, monkey cardiac muscles showed edematous capillary endothelial cells after acute exposure to altitude, while prolonged exposure also caused swollen mitochondria and sarcoplasmic reticula with separation of myofibrils. After 5 days and 3 months exposure to altitude, the right ventricular/total heart weight ratios increased significantly when compared to sea level control values.

In Study 6, an electron dense tracer (Thorotrast) passed through cerebral capillaries of Cebus apella monkeys rapidly transported to 14,110 feet. No such capillary leakage was observed in sea level controls.

Data collected from Study 7 on the preconditioning of rats by exercise prior to exposure to altitude is being statistically evaluated.

BODY OF REPORT

WORK UNIT NO. 085 — Effects of Altitude on Myocardium of Animals

STUDY NO. 4 — Effects of Altitude on the Cebus Monkey (Cebus apella) with Emphasis on Cerebrospinal Fluid Pressure and Brain Water Content.

PROBLEM:

It has been suggested that cerebral edema may play an important role in initiating and perpetuating symptoms of "acute altitude disease." To study the role of increased intracranial pressure in the causation of the symptoms (ultimately, hypoxia is the basic change) monkeys were used to quantitate some of the alterations.

Mean cerebrospinal fluid pressures, arterial blood pressures, and right heart pressures were all elevated at high altitude when compared to sea level controls. Also a mild perivascular edema was seen in some altitude-exposed monkeys.

In addition to the above, samples of right ventricles from sea level and altitude-exposed monkeys were processed for electron microscopy. These tissues were collected to determine if cardiac hypertrophy, similar to that in non-primate species, does occur in Cebus monkeys with chronic or acute exposure to high altitude.

RESULTS AND DISCUSSION OF RESULTS:

All physiological measurements and electron microscopic observations of brain tissues were reported in the U.S. Army Medical Research and Development Technical Report 30 June 1970.

Cebus apella right ventricle from sea level controls resembled typical primate cardiac muscle. Cardiac ultrastructural morphology of monkeys exposed to high altitude for 1 - 5 days demonstrated prominent edema of many capillary endothelial cells. Myofibrillar and mitochondrial morphology were normal when compared to sea level controls.

After 3 months' residence at high altitude, ultrastructural alterations indicative of right ventricle hypertrophy were observed. Numerous swollen mitochondria, with reduced matrix density, and disorganized and sometimes degenerate cristae were prominent. Sarcoplasmic reticula were often distended. Edematous capillaries identical to those seen with acute altitude exposure were observed. Myofibrils were separated by swollen mitochondria and occasional large edematous vesicles. Myofibrillar morphology was normal and intercalated discs were unaltered.

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

The ultrastructural alterations in primate cardiac muscle following acute and prolonged exposures to high altitude suggest a sequential pathogenesis. In acute exposure, only edematous capillary endothelial cells were seen, while prolonged exposure produced alterations characteristic of right ventricular hypertrophy, which has been reported for many species of animals with chronic exposure to high altitude. Apparently the edematous capillaries are instrumental in producing the cardiac hypertrophy.

CONCLUSIONS:

The results of this study were enlightening and contributory in elucidating the sequential pathogenesis of cardiac hypertrophy associated with "acute mountain sickness."

RECOMMENDATIONS:

The prevention of cardiac hypertrophy should be investigated. Possibly diuretics could prove useful to prevent capillary edema and thus eliminate the apparent initial step in the pathogenetic sequence.

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

PROBLEM:

Cebus apella monkeys were used in Study No. 4 to determine the acute effects of altitude, particularly on the brain, cerebral blood flow and cerebrospinal fluid (CSF). This study revealed increased CSF pressure after 1, 3, and 5 days' exposure to high altitude. During this 5-day interval, there was also an increased cerebral blood flow which began to subside on the fifth day. During this period there was also noted a significant, although subtle, perivascular cerebral edema. The basic alterations consisted of swelling of astrocytes and astrocytic processes with some ruptured cell membranes and separation of the neural elements. These changes suggest an alteration in the blood-brain barrier. To properly evaluate alterations in blood-brain barrier sophisticated experimental techniques are required. Such techniques include perfusion of the brain in vivo to minimize artifacts, and the use of an electron dense tracer substance to localize alterations in the blood-brain barrier.

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

RESULTS AND DISCUSSION OF RESULTS:

A constantly-monitored sustained pressure perfusion apparatus was devised to perfuse monkey brains for electron microscopic examination. Sea level monkey brains preserved using this apparatus demonstrated normal ultrastructural morphology. An electron dense tracer (Thorotrast) injected intravenously 30 minutes prior to termination of the monkeys did not pass through capillary endothelial cells.

In monkeys exposed to high altitude for 1 day, Thorotrast particles were seen mainly in endothelial cells and basement membranes, with a few particles on the periphery of the neuropil nearest capillaries. At three days' exposure to high altitude similar Thorotrast distribution was observed. At 5 days' exposure to high altitude, extravasation of Thorotrast particles into cerebral cortex neuropil was observed in every monkey studied. The Thorotrast particles were not seen in endothelial cell junctions. Instead, particles apparently passed through endothelium and basement membranes in small pinocytotic vesicles.

On the basis of this study, it appears that there is no breakdown of endothelial cell junctional complexes. Small particles leaving capillaries had to travel directly through endothelial cell cytoplasm and basement membranes to reach neuropil. Fluids such as those responsible for cerebral edema may also follow a similar route.

CONCLUSIONS:

Cerebral cortex capillaries of monkeys exposed to high altitude were shown to be permeable to the electron dense tracer (Thorotrast).

RECOMMENDATIONS:

It seems desirable to study the effects of various drugs, especially diuretics, in alleviating this mild perivascular cerebral edema associated with "acute mountain sickness."

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 cerebral edema in some of the monkeys which were transported to 14,110 feet. The extent of this edema was not determined during that study.

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

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

It is the purpose of this study to try to determine if cerebral edema occurs in rats at high altitude and the extent of the edema. In addition to this, it is of interest to determine the effect of preconditioning by exercise on cerebral edema as well as its effect on cardiac hypertrophy, daily food intake and growth rate.

RESULTS:

Histologic and morphometric examinations have been completed. Data are currently being statistically evaluated.

PUBLICATIONS:

1. Brown, G. L., M. V. Rothlauf, R. H. Heinzerling and T. S. Myers. Antibody Formations in Rabbits Adapted and Nonadapted to High Altitude. Infection and Immunity, 3: 358-360, 1971.

2. Bucci, T. J., Dean, W. D., Bishop, G. W., Frics, L. A., and M. B. Bischoff: Species Comparisons of Cardiac Hypertrophy in Animals Chronically Exposed to High Altitude, USAMRNL Report # 326, Jan 1971.

3. Stedham, M. A., T. J. Bucci and W. D. Dean: Effects of High Altitude on Erythropoiesis in Several Animal Species. In Myeloproliferative Disorders of Animals and Man, Clark, Howard and Hackett Eds, Oak Ridge, Tennessee, USAEC, June 1970, pp. 371-378.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6300	71 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
70 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	065
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 1412 A(2)			

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	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 71	3.5	86
FISCAL YEAR CURRENT 72	2.5	83

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS
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NAME:

21. GENERAL USE

Foreign Intelligence not Considered

DA

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 provides controls on laboratory methodology, procedure changes and on investigative procedures. Continuous improvement and updating of laboratory methodology and quantitation of results are imperative. Equivocal results from ethambutol agar susceptibility tests of M. tuberculosis indicate that loss of drug activity probably occurred during storage of drug plates (5°C) and/or during testing (36°C) resulting in false resistance readings; the stability of antituberculosis drugs used under these conditions are being tested.

25. (U) 70 07 - 71 06 (1) A computer data bank of mycobacteriologic data on 1479 tuberculosis ward patients containing 35,000 messages was made operational. (2) Four to six modifications of the 7H10 and 7H11 agar media are being evaluated for optimal recovery of mycobacteria from patients; specimens. (3) Incubation at 36°C of 7H10 agar media containing rifampin or ethambutol results in rapid loss of drug activity. (4) Additional improvements have been made in the blue-light microscopy system for demonstrating auramine O-stained mycobacteria. (5) Heat fixing of sputum smears for 2 hours at 65°C was not sufficient to kill all M. tuberculosis; exposure to 10% formaldehyde fumes sterilized the slides without affecting the staining reaction.

*Available from contractors upon originator's approval

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

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 mycobacteriologic laboratory methods

(a) A computer data bank containing 3 1/2 years of mycobacteriologic data on Fitzsimons General Hospital tuberculosis patients has been established and is in routine operation. Some analyses of these data are presented.

(b) Comparisons of three modifications with the Middlebrook 7H10 OA agar medium, pH 6.6, (7H10-pH 6.8, 7H11-pH 6.6, 7H11-pH 6.8) reveal the 7H11-pH 6.8 medium to be superior for recovery of M. tuberculosis from clinical specimens. Other studies have shown that the incorporation of catalase (or contaminating substances in the catalase solution) in the 7H10 medium to be detrimental to growth of drug susceptible M. tuberculosis.

(c) Incubation at 36°C of 7H10 OA agar medium containing ethambutol or rifampin results in rapid loss of drug activity, which can result in erroneous reports of resistance, especially in the case of ethambutol susceptible mycobacteria.

(d) Additional refinements to the blue-light excitation system for microscopic examination of auramine O-stained mycobacteria include an additional usable barrier filter and another microscope which fulfills requirements for this procedure.

(e) "Routine" methods of heat-fixing sputum smears preparatory to acid-fast staining are not sufficient to kill the M. tuberculosis in these preparations; an adequate method is described.

BODY OF REPORT

WORK UNIT NO. 065 — Microbiological Research in Tuberculosis

STUDY NO. 1 — To improve mycobacteriologic laboratory methods

PROBLEM:

Large numbers of U. S. military personnel are stationed in foreign countries and continually exposed to populations whose primary medical problem is tuberculosis. In these countries the problem is increased by the high incidence of drug-resistant tuberculosis strains in the population. Disease caused by mycobacteria other than Mycobacterium 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 greatest accuracy. 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) All mycobacteriologic data on Fitzsimons General Hospital tuberculosis patients are presently being input to three computer files. A data bank of 35,000 messages on 1479 patients was made operational during this report period. Two files containing 53,000 messages were merged into one file containing fewer messages but more information. A program was completed which prints out the patient's essential mycobacteriologic data, i.e., all cultures, drug susceptibility tests, organism identification, etc., on sheets which could be placed in his medical records for future reference. Another program provides monthly printouts of quantitative smear and culture data and contamination rates which furnishes laboratory quality control information. Computer analyses of the data files revealed that in this laboratory, 71% of culture-positive patients infected with drug susceptible M. tuberculosis were found with the first sputum specimen, 80% with 2, 84% with 3, 89% with 4, 91% with 5, 94% with 6 and 97.8% with 10 specimens; 2.2% of patients required 11-16 sputa before a positive culture was found. The number of sputa required to demonstrate culture positivity from patients infected with drug resistant strains was slightly greater in each category. Analyses of culture data also revealed that specimen cultures, especially those containing drug

resistant organisms, must be held for at least eight weeks to insure recovery of slow growing mycobacteria; 23.3% of 640 cultures on 62 patients required either six weeks (19.5%), eight weeks (2.7%) or 10 weeks' incubation before growth appeared. Another analysis of culture data indicates that certain patients disproportionately contribute to specimen contamination rates, predominately caused by a gram-positive spore former or a gram negative rod. Of 558 patients with 15 or more sputum specimens, 35.1% had no contaminated cultures while the culture contamination rate of 10.8% of these patients exceeded the 95% confidence limits of the mean rate (7.4%). The mean contamination rate has been subsequently lowered by increasing the malachite green concentration of the 7H10 or 7H11 media to 0.05 µg/ml. Studies are in progress to elucidate the interesting contamination problem which appears to be related to asymptomatic carriage of these contaminants.

(b) Comparisons of the growth promoting qualities of three modified 7H10 or 7H11 media with the Middlebrook-Dubos 7H10 Oleic Acid Albumin Agar (7H10-OA) medium, pH 6.6, are underway. It was found that incubation under 10% CO_2 lowered the reaction of the 7H10-OA medium from pH 6.6 to 6.4, so a 7H10-OA medium, pH 6.8, was included (pH after CO_2:6.6). The 7H11-OA medium (modified 7H10-OA with added enzymatic casein hydrolysate) has been reported to enhance growth of certain drug-resistant strains, but it also allows more growth of undesirable non-acid fast organisms. Therefore, the malachite green concentration in this medium was increased from 0.25 µg/ml to 0.5 µg/ml. Two media, 7H11-OA, 0.5 µg malachite green, pH 6.6 and the same medium at pH 6.8 were included in the comparison studies.

Sets of the four media were equally inoculated using concentrates from a single specimen. Each medium's growth promoting qualities, based on number and size of mycobacterial colonies, was compared and ranked with the other three. These data were computerized as part of the regular patient data file.

Comparisons using 100 patient specimens showed that mycobacterial strains differed considerably in their growth response to the four media. Overall, growth of M. tuberculosis was best on medium 7H11, pH 6.8, although in many instances growth response was equal on the other media; 7H10, pH 6.8, ranked second. Growth response on the other two media at pH 6.6 was about equal but inferior to 7H11-6.8 and 7H10-6.8. Although only a few specimens were tested, Runyon groups I, III and IV appeared to prefer the 7H10, pH 6.6 medium over the other three.

Additional observations on a small number of specimens revealed that the presence of catalase in the 7H10 or 7H11 medium often retarded growth of both isoniazid-sensitive and resistant M. tuberculosis. Growth response on the media without catalase was usually better and never less than on catalase-containing media. Whether this effect is due to catalase per se or a contaminating substance in the catalase solution has not been determined.

(c) Experiences with false results of resistance of M. tuberculosis to ethambutol (EMB) in this and other laboratories has caused us to investigate the problem. In direct drug susceptibility tests, M. tuberculosis colonies often appear on the EMB medium, indicating resistance, if the culture plates are held for the "standard" 4-week period. Usually there was no growth on these media after three weeks' incubation. Indirect susceptibility tests showed the same phenomenon, except that it occurred one week earlier. Subsequent testing of "resistant" colonies arising on the drug media resulted in a recurrence of the above phenomenon, indicating that the apparent resistance of these organisms resulted from deterioration of drug in the medium.

Experiments were designed to determine the rate of rifampin (RMP), EMB, and kanamycin (KM) activity loss in the 7H10 medium after storage at 5°, 24°, or 36°C for varying periods. An indirect system of measuring drug activity loss after storage consisted of taking cylindrical plugs from stored drug agar plates and using them in the same fashion as for disk antibiotic tests, employing a suitable test organism sensitive to the particular drug. A linear relationsip exists between the diameter of the zone of growth inhibition and the log of the drug concentrations employed, therefore loss in activity could be equated with reduction in zone diameter for a given concentration of drug.

RMP and EMB rapidly lost activity at 36°C, the optimal growth temperature for M. tuberculosis. The level of RMP in media incubated at 36°C for three days dropped from 1 µg/ml to about 0.6 µg/ml; after six days the level dropped to about 0.2 µg/ml. Incomplete results from EMB studies indicate an equal or greater activity loss at this temperature. Loss of KM activity was considerably less under these conditions, correlating with actual laboratory observations.

In the case of EMB, RMP, and probably ethionamide (ETA) and capreomycin (CM), the rapid loss of drug activity at 36°C coupled with the extremely slow growth rate of M. tuberculosis (generation time - 20 hours) allow the slower growing members of the test population to be less inhibited by the drug that remains when cell division begins. In practice, providing adequate growth occurs on the control (no drug) medium, drug

susceptibilities are reported as follows: isoniazid, KM, p-aminosalicylic acid, streptomycin: direct - 4 weeks, indirect - 3 weeks, and RMP, EMB, ETA, CM: direct - 3 weeks, indirect - 2 weeks.

(d) A system using blue-light excitation, instead of ultraviolet light, for microscopic examination of fluorochrome (auramine O)-stained specimen smears for mycobacteria was developed and has been in routine use in this laboratory for over three years (USAMRNL Report No. 317, May 1968). Refinements to the original procedure include a simpler stain, more efficient barrier filters, and a light source built into the microscope (USAMRNL Report No. 323, November 1969). Until recently, only the equipment manufactured by the Carl Zeiss Co. performed adequately. However, the American Optical Co. Micro-Star, Series 20, Microscope with the quartz-halogen illuminator, equipped with the proper exciter and barrier filters, was tested and found to be suitable for viewing fluorochrome-stained mycobacteria. In addition to those previously described, the 2" x 2" Kodak No. 16 gelatin Wratten filter was found to be an excellent and economical barrier filter.

(e) Slides, containing smears of specimen concentrates are normally heat fixed for two hours at 65°C. To determine if this heat fixing rendered the M. tuberculosis non-viable, smears of sputum concentrates containing numerous bacilli (> 2 cells/high power field) were made and processed in the usual manner. The material from the slide was then taken up on a sterile moistened swab, placed in 7H10 broth, and incubated for growth. Viable M. tuberculosis was found in material from five of ten slides tested, indicating that some slides were still potentially infectious. Exposure of fresh smears to 10% formalin fumes in closed Coplin jars for 10 minutes also proved inadequate for killing the mycobacteria. However, exposure to 10% formaldehyde fumes for 10 minutes was sufficient to kill all M. tuberculosis, although formaldehyde-fixed material did not resist washing as well as heat-fixed. The latter difficulty was overcome by heat fixing for 30 minutes at 65°C prior to exposure to formaldehyde fumes. Comparison of the staining reactions of heat-fixed smears with those treated with formaldehyde showed no differences. This simple method should render unstained microscopic slides containing M. tuberculosis safe for storage or shipment.

CONCLUSIONS:

(a) A readily accessible computer bank of mycobacteriologic data is operational for combinatorial analyses with patient clinical data and experimental studies.

Microbiological Research in Tuberculosis (Cont'd)

(b) The growth promoting qualities of 7H11 medium, pH 6.8, were superior to three others tested.

(c) Heat lability of the antituberculosis drugs rifampin and ethambutol in drug test agar and the slow growth characteristics of M. tuberculosis probably accounts for occasional false-resistance results in these drug susceptibility tests. Indirect and direct EMB, RMP, ETA and CM susceptibility tests should be examined at two and three weeks, respectively.

(d) The blue light system for microscopy of fluorochrome-stained mycobacteria has been further improved.

(e) Heat fixing does not kill all M. tuberculosis in microscopic smears. Unstained smears can be rendered safe by exposure to 10% formaldehyde fumes for 10 minutes.

RECOMMENDATIONS:

(a) Improvement of methods for computer storage and analyses of patient laboratory data should be continued.

(b) Initiate studies to determine (1) the pH of the 7H11 medium for optimal initial isolation of mycobacteria from clinical specimens, and (2) elucidate the observed effect of catalase on initial growth of M. tuberculosis.

(c) Complete the studies in progress on rifampin, ethambutol and kanamycin and determine the rates of drug activity loss at 36°C for streptomycin. The antituberculosis drugs isoniazid, p-aminosalicylic acid and ethionamide should be examined if a suitable test system can be developed.

(d) Continue the search for improvements for this excellent microscopic method for detecting acid fast bacteria.

(e) This study has been completed and will be submitted for publication.

PUBLICATIONS:

Ackerman, L. J., R. A. Kishimoto and J. S. Emerson. Nonpigmented Serratia marscesens arthritis in a Tegu (Tupinambis teguixin). Am. J. Vet. Res. 32: 823, 1971.

Microbiological Research in Tuberculosis (Cont'd)

Blair, E. B. "Media, Test Procedures and Clinical Reagents." In: Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections, by H. L. Bodily, E. L. Updyke and J. O. Mason. New York: American Public Health Association, Inc., 1970. Chapter XXII, p. 791.

Blair, E. B., A. H. Tull, C. J. Messa and D. H. Andres. Computer storage and analysis of routine mycobacteriologic data. Presented to the 30th Annual VA-Armed Forces Pulmonary Disease Research Conference. Cincinnati, Ohio, 25-26 January 1971.

Blair, E. B., O. L. Weiser and A. H. Tull. Mycobacteriology Laboratory Methods. 23rd Annual Symposium on Pulmonary Diseases, Fitzsimons General Hospital, Denver, Colorado, pp 352-391, September 1970.

Jones, L. D., D. C. Fairchild and W. C. Morse, The induction of pulmonary neoplasms in mice by Isonicotinic Acid Hydrazide. Am. Rev. Resp. Diseases 103: 612, 1971.

Tull, A. H. and E. B. Blair. Staphylococcal carriage in a hospital population: Throat vs. nasal carriage and persistence of carriage. USAMRNL Laboratory Report No. 325, August 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6301	71 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
70 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	066
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 1412 A	(2)		

11. TITLE (Precede 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	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 71	2.9	59
FISCAL YEAR CURRENT 72	4.0	61

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
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Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab
Microbiology Division
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Denver, Colorado 80240

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: 303 366 5311 X24234
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME: Weiser, O. L.
NAME: DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Melioidosis; (U) Pesudomonas; (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. Precede text of each with Security Classification Code.)

23. (U) A substantial number of fatal and other severe melioidosis infections (due to Pseudomonas pseudomallei) have occurred in U. S. troops in Vietnam, as were experienced by the French and the British in Southeast Asia. This disease can erupt months to years after leaving an endemic area with no previous sign or symptoms evidenced. A large 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) 70 07 - 71 06 A P. pseudomallei strain has been partially characterized. L phase variants of this strain have been induced in vivo and in vitro. Animals inoculated with this variant tolerated it for several months without disease, but when cultured yield the classical organism. An attempt to induce wall defective variants of BCG with cycloserine is underway.

*Available to contractors upon originator's approval

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

Many U. S. military personnel have contracted acute melioidosis. There is also the long term problem of latent disease developing months or years after exposure. Progress has been made on better characterization of the organism, *Pseudomonas pseudomallei* and its wall defective variant.

Strain CA was lyophilized and various facets of it quantitated. Antigen analysis and lethal dose standarization are in progress. Wall defective variants of *P. pseudomallei* were induced *in vivo* and *in vitro* and tolerated by mice when given via aerosol. Attempts to induce wall defective variants of *Mycobacterium tuberculosis* or BCG have, thus far, been unsuccessful.

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 a reference strain of *Pseudomonas pseudomallei* and its L-phase variant

PROBLEM:

Melioidosis is a severe disease due to a tropical soil bacterium, *Pseudomonas pseudomallei*, which is widely distributed in Southeast Asia. U. S. troops in this area of the world have been incurring approximately 100 cases per year of the disease in its many forms: hyperacute, acute, subacute, chronic, subclinical and latent. The incidence of the acute forms will undoubtedly rapidly diminish with troop withdrawals but a legacy of chronic and latent cases may persist for 10 to 15 years. It is not known how *P. pseudomallei* can remain dormant in an apparently healthy individual for months or years but one possible mechanism would be as cell wall defective variants (L forms) of the organism. It has been found that wounded and burned Vietnam casualties have a much higher frequency of both significant ($\geq$1:40) and lower indirect hemagglutination antibody (IHA) titers than control groups. Speculation has arisen as to the incidence of the latent disease in veterans of the Southeast Asian conflict and whether those with an elevated antibody titer are any more at risk of being a latent carrier than those with a negative titer.

Another disease with prolonged latency is tuberculosis. Wall defective variants of *M. tuberculosis* have been induced *in vitro* and it is suggested that such naturally occurring variants might play a role in the latency of tuberculosis. Vaccination against tuberculosis with BCG is not generally recommended in this country, because although partial protection is conferred, there is conversion of the tuberculin skin test to positive. It is theorized that by inducing a wall defective variant of BCG and using this for immunization, protection might be conferred without conversion of the tuberculin skin test.

RESULTS AND DISCUSSION OF THE RESULTS:

Typically, P. pseudomallei isolated from patients grow on solid media as smooth and rough colonies. A highly wrinkled, rough colonial type isolated from the sputum of patient CA at Fitzsimons General Hospital was selected and lyophilized in large quantity as a reference strain for future studies.

It has been noted that P. pseudomallei when first seen in patient specimens, have a characteristic cell morphology which has been correlated with strain virulence. These cells are faintly staining, gram negative, strongly bipolar, are ovoid to round in shape and some resemble closed safety pins. However, studies in this laboratory have shown that when the same agar culture was repeatedly sampled over an 11-day period, the initial weakly bipolar rod appearance gradually evolved to the above described picture. The relative virulence of cells from these samplings at early and later stages has not yet been assessed. Better microscopic visualization of individual cells in the bipolar state is derived from a crystal violet stain rather than the complete gram stain.

The growth curve of P. pseudomallei CA in trypticase soy broth at 35°C using roller tube aeration showed that log phase growth began 6 1/2 hours after inoculation, and continued approximately 3 hours. The generation time was approximately one hour. The end of log phase growth, using optical density measurements, was not clearcut because of clumping of growth and pellicle formation. The latter was not dispersible by tube agitation or by growth in 0.05% Tween-80 broth. The biochemical characteristics and antibiotic susceptibilities of P. pseudomallei CA generally agreed with published findings.

Wall defective variants of P. pseudomallei were induced in vitro on penicillin agar plates and in vivo in animals suboptimally treated with cycloserine and erythromycin. These wall defective variants were maintained in various "transitional phases" by using urine agar and frequent agar block transfers. Clinical signs were absent in aerosol-infected mice using a wall defective variant, however the parent P. pseudomallei strain was recovered at two weeks and again at 3 1/2 months after aerosolization from apparently well mice from the aerosol-infected group. It was not possible to determine whether the organisms existed as wall defective variants in vivo and reverted immediately on plating out, or whether they had existed most of the time in vivo as avirulent mutants.

Standardization of the Tri-R, Model A3 airborne infection apparatus is underway. There are various hypotheses regarding the exact number

of organisms reaching the lungs of the individual exposed animals. In preliminary tests mice were exposed to a 15-minute aerosol of 3 to 4 ml of a suspension which had been filtered through a 5 micron filter and contained 10^6 organisms per ml. Plate counts from lungs of these mice, taken within 1-2 hours post-aerosol revealed very small numbers of organisms in spite of a 65% mortality within 14 days of mice exposed at the same time.

In preliminary studies on the induction of wall defective variants of BCG with cycloserine, BCG Glaxo was found to be sensitive to cycloserine at 30 to 50 micrograms per cc. It has been reported in the literature that BCG is resistant to cycloserine and, indeed, this level might not be achieved *in vivo* without toxicity.

CONCLUSIONS:

A selected passage of *Pseudomonas pseudomallei* CA has been preserved in large quantity by lyophilization as a reference strain from which experiments are initiated using a standardized method. Biochemical, antibiotic susceptibility, growth curve, cellular and colonial morphology characteristics have been elucidated concerning this reference strain. Aerosols of the strain resulted in high mouse mortality in spite of a very small number of organisms originally cultured from the lungs of simultaneously exposed mice. Wall defective variants of *P. pseudomallei* have been induced *in vivo* in mice and *in vitro*. Mice receiving transitional phase, wall defective variants of *P. pseudomallei* tolerated the organisms for up to 3 1/2 months in apparent good health but yielded the parent *P. pseudomallei* on culture when sacrificed. BCG was found to be sensitive to cycloserine in the 30 to 50 microgram per ml range.

RECOMMENDATIONS:

Biological studies to include biochemical characteristics, antigenic analysis, and lethal dose standardization of *P. pseudomallei*, strain CA, should be continued. IHA antibody titers should be determined on a sample group of 500-1,000 wounded and nonwounded U. S. military personnel who have served in Southeast Asia; those who have significant and near significant titers would then be cultured for *P. pseudomallei* and/or its wall defective variants. Attempts will be made to induce wall defective variants on *M. tuberculosis* and BCG.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6303	71 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. DISB'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
70 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	068
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 1412A	(2)		

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)
FISCAL YEAR PRECEDING 71	2.0	13
FISCAL YEAR CURRENT 72	2.5	21

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

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20. PERFORMING ORGANIZATION

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Computer Division

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Denver, Colorado 80240

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Werner, J. E., PFC 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) 70 07 - 71 06 An automated system for analyzing continuous oxygen consumption data has undergone refinement to better edit raw data from a digital magnetic tape file and to render the system compatible with some automated statistical systems. The system has been in continuous use on studies in applied military nutrition evaluation, work performance and on the influence of high terrestrial altitude.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine

WORK UNIT NO. 068 Computer Instrument Linkage

The following investigation has continued under this work unit:

STUDY NO. 1 Continuous Oxygen Consumption Measurement

A data handling system which collects, stores, edits, calculates and displays data collected during the assessment of human energy expenditure has undergone extensive revision. These revisions have resulted in improved data editing, data display, and compatibility with other data storage and handling systems. The regular use of the system has proven the feasibility of off-line automation of the performance aspects of nutrition or environmental studies conducted inhouse or during applied field studies.

BODY OF REPORT

WORK UNIT NO. 068 — Computer Instrument Linkage

STUDY NO. 1 — Continuous Oxygen Consumption Measurement

PROBLEM:

A need exists 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 is 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:

A data handling system is in use which automatically handles the data derived from a "continuous oxygen consumption analysis system" (USAMRNL Report No. 318). This data handling system converts analog data to digital data; edits, formats and stores the digital data on magnetic tape files; retrieves and lists the raw data; retrieves, transforms and calculates gas analysis parameters; calculates, lists, and summarizes oxygen consumption data; and develops data messages of summary type information for permanen storage on magnetic tape files.

The system has undergone extensive updating during this report period to increase its versatility and render the output easier to use by investigators. A source of consternation in the use of the system has been evident when a component instrument in the gas analysis system failed, leading to erroneous data. Editing has been expanded in the data analysis system to alleviate many of these problems.

The PLOT portion of the output display was rewritten and incorporated into the system. This resulted in a clearer display of oxygen consumption variables and also resulted in a ten fold increase in data processing speed during plotting. The detailed numerical listing of data was also modified for greater clarity.

A summary of data collected during a performance measurement is developed for inclusion on data files for future retrieval

and statistical analysis (Work Unit 166 - Design of Military Biomedical Research Information Systems). This is a general purpose record keeping system for research data which has proven invaluable for maintaining data in a concise manner on a data file common with other information derived from the same subjects.

A provision has been added to the data handling system which allows the output on cards of the same data which is displayed in detail on the output listing. The period of time which each card represents is flexible and controlled by the user in the same manner in which he is able to control the time interval covered on each line of printout. This data output provision has served to tie this system into the FORTRAN mathematical system (Military Internal Medicine, Work Unit 082 - Mathematical and Computer Support of Military Bio-Medical Research) for further and more complete analysis of the dat .

This system has been used extensively over the reporting period for the analysis of oxygen uptake in human subjects during both rest and exercise. The data from 5 studies, representing 231 measurements, each for approximately one hour, has been processed with a great deal of user acceptability. This data processing has been in support of laboratory projects in the performance aspects of applied military nutrition and military environmental medicine.

CONCLUSIONS:

The automation of oxygen consumption measurements, even off-line to the measurement, can be an extremely useful tool for the performance physiologist. Data has been processed during this reporting period in approximately 30 hours which would have required approximately 2300 manhours if it had been attempted by hand; with detail and accuracy which would not have been possible by manual manipulation. In addition, the oxygen uptake data is normally available within one day for use by the investigator or for subsequent data analysis.

RECOMMENDATIONS:

1. Refinements will continue to be made as the need arises. Specifically, a provision should be made by the Bioenergetics Division for recording on a free channel of the gas analysis instrument the elevation of the treadmill and an indication of when the treadmill starts and stops. Monitoring of this information can then be included in the data handling system which

Computer Instrument Linkage (Cont'd)

would permit more precise determinations of the total elapsed time of exercise and a more accurate determination of the physical work which was accomplished by the subject during the exercise.

2. As magnetic tape units are added to the digital computer system, this "energy expenditure analysis system" will be modified to allow the output of oxygen uptake variables on magnetic tape for subsequent data analysis in the FORTRAN system.

3. Long range plans may beneficially include the investigation of the incorporation of an on-line, small computer to the gas analysis system for the real time analysis of data during an experiment.

PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6307	71 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
70 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	072
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 1412A (2)			

11. TITLE (Precede with Security Classification Code)

(U) Studies in Human Nutrition (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

000800 Argi. Economics; 002300 Biochemistry; 003500 Clin. Chemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
56 03	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 70	6.8	78
FISCAL YEAR CURRENT 71	9.7	116

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

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RESPONSIBLE INDIVIDUAL

NAME: Canham, J. E., COL

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20. PERFORMING ORGANIZATION

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ADDRESS: Chemistry Division
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Raica, N., Jr.

NAME: Tillotson, J. A. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Military rations and feeding; (U) Nutr. Disorders; (U) Nutr. Surveys; (U) Nutritional requirements; (U) Food Wholesomeness

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 operational military rations in terms of wholesomeness and nutritional adequacy and storage stability; obtain information concerning human requirements for specific nutrients in order to provide guidance for changes in rations and military feeding that will permit optimum nutrition and health of the soldier in any given environment or military situation; investigate the nutritional status of military populations for the purpose of appraising and improving their nutritional status; develop new and practical biochemical techniques for the evaluation of the nutritional status of military personnel.

24. (U) Part of the desired information will be obtained through the use of volunteer human subjects under controlled conditions receiving test military rations or known foods or menus. The evaluations will be supported by appropriate clinical and biochemical measurements. The volunteer studies, along with blood or urine samples, will be employed in the development of biochemical techniques useful in evaluating the nutritional status of individuals.

25. (U) 70 07 - 71 06 The human vitamin A study in cooperation with the Univ. of Iowa Med. School will be completed 30 Jun 71. Data obtained from this study is now being evaluated. Vitamin A liver stores were low in 22% (range 12-33%) of the 372 human liver samples collected from 5 states. A recent study with 6 human male volunteers has shown that the activity coefficient of the human erythrocyte glutathione reductase is an excellent measure of the riboflavin nutritional status in man. Collaborative studies with USAIIDR on the influence of infection on vitamin requirements of man reveal an increased requirement of vitamin B_2 and B_6 in the acute phase of infection. This work unit is being terminated. The microbiological phase of the wholesomeness studies will be performed by the Food Hygiene Divison while the other phases will be performed under other work units.

A reprint or preprint may be furnished upon originator's approval.

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

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine

WORK UNIT ? . 072 Studies in Human Nutrition

The following investigations have been conducted under this work unit:

STUDY NO. 3 Vitamin C Metabolism and Requirement in Man

STUDY NO. 4 Thiamin Metabolism

STUDY NO. 10 Experimental Vitamin A Deficiency in Humans

STUDY NO. 12 A Study of Erythrocyte Glutathione Reductase as a Measurement of Riboflavin Status in Man

Study No. 3. Ascorbate-3-sulfate has been demonstrated as an essential metabolite of ascorbic acid in humans. The compound was characterized by use of dual label experiments coupled with co-chromatography with synthetic ascorbate-3-sulfate in a variety of solvent systems and by direct spectrophotometric measurements of the isolated material.

Study No. 4. A method to determine the metabolites of thiamin in urine is based on the incubation of urine with yeast. During the incubation, yeast converts some of the metabolites to thiamin so that the vitamin may be measured by a fluorometric procedure. The validity of the method depends on yeast using only those molecules derived from thiamin and converting them quantitatively to thiamin. Because the metabolites are excreted in such small quantities we cannot determine the extent to which yeast may convert each of these to thiamin. However, we have found only one major fluorescent material when yeast is incubated with urine. This has been identified as thiochrome, the oxidation product of thiamin. The yeast appears to require molecules that are structurally related to thiamin for the synthesis of thiamin.

Study No. 10. (A) The clinical phase of the cooperative study with the University of Iowa Medical School on the male human adult vitamin A requirement was terminated 30 June 1971. Eight subjects were depleted of their vitamin A stores and six were repleted with graded doses of either vitamin or β-carotene. The available completed data has been committed to the computer for analysis.

(B) Details concerning this study were reported for FY 1970 (30 June 1970, Report Control Symbol MEDDH 228R1). Livers from 372 subjects (from 5 states) were assayed for vitamin A. Average

concentration and median value for all subjects was 146 ± 151 µg/g and 106 µg/g, respectively. The overall incidence of low (<40 µg/g) concentration was 22%; however, more than 35% of the subjects in the 11-40 year age group had low liver vitamin A concentrations. No correlation was found between adipose pesticide and liver vitamin A concentrations.

(C) Rat growth assay procedure for estimating the level of vitamin A active substances in a modified Sheffield diet (natural foods selected for their low carotene and vitamin A content) was completed. This diet was used during the latter phase of vitamin A depletion of human subjects. Based on rat growth, this diet supplied about 0.25 µg of vitamin A equivalents per rat per day. Food consumption was 10.6 g/day/rat (dry weight), or 52.26 kcal/day. Assuming 2700 kcal per day/man, this would supply about 13 µg vitamin A equivalent per day. A similar study is in progress to estimate the biologically active vitamin A substances in a meat-flavored soy protein diet that was also fed to the human subjects on the vitamin A study.

Study No. 12. A recent study with six human male volunteers has shown that the activity coefficient of the human erythrocyte glutathione reductase is an excellent measure of the riboflavin nutritional status in man.

BODY OF REPORT

WORK UNIT NO. 072 Studies in Human Nutrition

STUDY NO. 3 Vitamin C Metabolism and Requirement in Man

PROBLEM:

Ascorbic acid is not only an essential nutrient to prevent scurvy but, in larger amounts, may have functions to improve stress resistance. In this report we describe studies to identify ascorbic acid metabolites in man, to study their physiological role, and to show their relationship to the nutritional needs of ascorbic acid.

RESULTS AND DISCUSSION OF THE RESULTS:

Recent studies by this group show that about 3% per day of the ascorbate pool in human volunteers is excreted as urinary metabolites. Analysis of urine from these volunteers who were given ascorbic acid doubly labeled with carbon-14 and tritium show a number of metabolites not previously described. One group of compounds that rapidly decreased in amount with the onset of symptoms of scurvy has been examined in detail and appears to be a mixture of sulfate derivatives of ascorbic acid. Resolution of this mixture of derivatives on ion exchange columns and by thin-layer chromatography (TLC) indicates that the principle component is a compound tentatively identified as ascorbate-3-sulfate. The identity of this metabolite has been established by comparison with synthetic ascorbate-3-sulfate and by a variety of labeling procedures using C-14, H-3 or S-35. A moot question remains as to whether it is really the 3-sulfate or possibly a 1- or 2- sulfate, but this must be established by further chemical studies.

Ascorbate-3-sulfate had previously been identified in brine shrimp cysts. We have now shown its existence not only in man but in rainbow trout, rats and guinea pigs. It is probably ubiquitous in vertebrates. It is probable that ascorbate sulfate is an important metabolite of ascorbate and accounts for part of the nutritional role of vitamin C. Several workers have shown an *in vitro* and *in vivo* sulfation role for this compound including the sulfation of cholesterol leading to elimination of cholesterol in the bile. This could give a rational explanation to the Russian scientists claim (not substantiated by Western studies) that ascorbate in large doses gives some protection against atherosclerosis. Studies at USAMRNL in 1963 disclosed that the ingestion of three grams of ascorbic acid/day for 45 days did not influence serum lipids in young adult males.

Incomplete studies show evidence for an ascorbate-2-sulfate and possibly a 2,3-cyclo-ascorbate sulfate in human urine. These compounds could also have an essential role in ascorbate nutrition. Because of the probable importance of ascorbate sulfate in human nutrition analytical procedures for assay of this compound in urine and blood samples are being developed. At present a fairly specific dinitrophenylhydrozine procedure has been demonstrated and is being quantified. It will be used in answering the important question of whether low levels of ascorbate sulfate in human are related to nutritional deficiencies.

CONCLUSIONS:

Ascorbate-3-sulfate has been demonstrated as a significant metabolite of ascorbic acid excreted in human urine. Other ascorbate sulfates also appear to be present. A variety of results suggests that this compound has an important physiological role, including that of a sulfation agent. Further studies are in progress and are urgently needed to clarify the biological role of ascorbate sulfate, whether it has importance as a nutrient in man and animals, and whether body levels of this compound can be used to evaluate nutritional status.

RECOMMENDATIONS:

It is recommended that patent for synthesis and use of ascorbate sulfate be applied for through the U. S. Army Medical Research and Development Command.

PUBLICATIONS:

1. Baker, E. M., R. E. Hodges, J. Hood, H. E. Sauberlich, S. C. March and J. E. Canham. Metabolism of ^{14}C- and ^{3}H-labeled L-ascorbic acid in human scurvy. Am. J. Clin. Nutr. 24: 444, 1971.

2. Hodges, R. E., J. Hood, J. E. Canham, H. E. Sauberlich and E. M. Baker. Clinical manifestations of ascorbic acid deficiency in man. Am. J. Clin. Nutr. 24: 432, 1971.

3. Kinsman, R. A. and J. Hood. Some behavioral effects of ascorbic acid deficiency. Am. J. Clin. Nutr. 24: 455, 1971.

4. 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 In press, 1971.

5. Tolbert, B. M., D. J. Isherwood, R. W. Atchley and E. M. Baker. Ascorbic acid sulfate: Synthesis and properties. Fed. Proc. 30: 1819, 1971 (Abstract).

Studies in Human Nutrition (Cont'd)

6. Baker, E. M., D. C. Hammer, J. E. Canham and B. M. Tolbert. Identification of ascorbate sulfate in human urine. Fed. Proc. 30: 1820, 1971 (Abstract).

7. Johnson, C. L., D. C. Hammer, J. E. Halver and E. M. Baker. Urinary metabolites of ascorbate in the rainbow trout. Fed. Proc. 30: 1822, 1971 (Abstract).

STUDY NO. 4 Thiamin Metabolism

PROBLEM:

Experiment 2: Identification of thiamin and thiamin-like compounds extracted from yeast following incubation with urine.
In the assessment of nutritional status, it has been found practical to measure the amount of the vitamin excreted in the urine and to correlate this with sufficient or deficient intakes of the vitamin. This procedure fails to consider the amount of the vitamin which has been metabolized and may appear in the urine in an altered form. In fact, it may be said that the excreted vitamin is spill-over or excess vitamin. A measure of the utilized vitamin might be more meaningful in understanding the body's metabolic processes. The yeast resynthesis method has been shown to be capable of combining the two moieties of thiamin to form thiamin. Because of the complex nature of the substances found in the urine, it is necessary to establish the specificity of the yeast method so that the thiamin measured in the assay may be related directly to thiamin metabolites in the urine.

RESULTS AND DISCUSSION OF THE RESULTS:

The fluorescent spots derived from oxidized extracts of yeast incubated with urine have been characterized in terms of their contribution to the measurement of thiamin. The only spot with activating and emitting wavelengths which would be measured in our fluorometer was thiochrome. The isolation procedures used for the assay of thiamin appear to remove the non-thiochrome fluorescent spots in untreated urine.

CONCLUSIONS AND RECOMMENDATIONS:

Having established the specificity of the yeast method insofar as the product we measure as thiamin, the question of the source of this thiamin must be investigated. Possible precursors of thiamin may be found in urine.

PUBLICATIONS:

1. Ziporin, Z. Z. and P. P. Waring. Thin-layer chromatography for the separation of thiamine, N'-methylnicotinamide, and related compounds.

In: Methods in Enzymology, McCormick and Wright ed., Academic Press, New York, 1970, vol 18A, pp 86-90.

2. Ziporin, Z. Z. and P. P. Waring. The use of yeast to assay the separate moieties of thiamine. In: Methods in Enzymology, McCormick and Wright, ed., Academic Press, New York, 1970, vol 18A, pp 195-199.

STUDY NO. 10 Experimental Vitamin A Deficiency in Humans

PROBLEM:

A. The objectives of this study with male human volunteers were: 1) to measure, by labelling 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.

B. This study also involves continuing assay of human liver samples for vitamin A content to determine the vitamin A nutritional status of the American population.

C. The Sheffield type diet fed to the human subjects was found to contain low levels of β-carotene when assayed by chemical methods. To estimate the biological vitamin A activity, a rat growth assay was required.

RESULTS AND DISCUSSION OF THE RESULTS:

A. Six of eight adult human male volunteers achieved vitamin A deficiency as defined by significant changes in electroretinograms and dark adaptation tests. The remaining two subjects were released after 617 and 679 days of the study after developing cutaneous and buccal mucosal changes characteristic of vitamin A deficiency. One of these subjects developed significant impairment of dark adaptation but showed no change in electroretinograms while the other subject showed no significant change in either parameter.

The repletion phase of the study was carried out with graded doses of either labeled vitamin A or β-carotene. Excretion of the ^{14}C isotope in urine, feces and breath was monitored throughout the study. Preliminary results indicated that significant amounts of label were excreted by all three routes. A greater amount of label was excreted as $^{14}CO_2$ when the subjects were normal than was excreted when relabelled at

maximum depletion. The total label excreted in the first 21 days after repletion labeling was higher for the subjects receiving the vitamin A supplement than those receiving the β-carotene supplement. The plasma vitamin A values did generally, over a long period of time, show the declining body stores of vitamin A and the approach of deficiency but individual plasma values did not correlate well with the state of vitamin A nutrition.

Considerable progress has been made on urinary metabolite fractionation and purification methodology. Column and thin-layer chromatrography have afforded the opportunity to fractionate large amounts of urine in an effort to obtain large enough quantities of metabolites to identify.

B. A total of 372 liver samples from human subjects were assayed for vitamin A (five geographic areas were represented). Average concentration was 146 ± 155 µg/g, median concentration was 106 µg/g. For subjects that died of accidental causes, the results were 107 ± 81 µg/g and 96 µg/g for average and median values, respectively. Low (less than 40 µg/g) concentrations were found in 22% of the subjects, with the highest incidence (35-40%) in the 11-40 year age group. Low concentrations were found in 23% of the accidental deaths. There was a 4.6% incidence of critical (less than 10 µg/g) concentration. No correlation was found between adipose pesticide and liver vitamin A concentrations.

C. A modified Sheffield diet, which consisted of natural foods selected for their low carotene and vitamin A content, was fed to human subjects during the latter phase of vitamin A depletion. Chemical analysis showed the diet to contain small amounts of β-carotene (provitamin A) but no detectable vitamin A. Because of the difficulty in estimating the biological vitamin A equivalents of the diet from chemical analysis for humans and because a reliable estimate of dietary "vitamin A" intake was required, a rat growth assay was performed. By the bioassay test the diet supplied about 0.25 µg of vitamin A equivalents per rat per day. Average daily food consumption (dry weight) was 10.6 g per rat per day or 52.26 kcal per day. Assuming that the human subjects consumed the diet at a rate of 2700 kcal per day per man, this would be equivalent to an intake of about 13 µg of vitamin A per day.

A similar study is in progress to estimate the vitamin A equivalence of a meat-flavored soy protein diet.

CONCLUSIONS:

A. The clinical phase of this study has been terminated but laboratory analysis of samples is continuing. The completed available data has been committed to the computer for analysis. Interpretation of the data is

pending completion of the laboratory studies and the inputting of the final results into the computer.

B. The highest incidences (35-40%) of low liver vitamin A were found in the 11-40 year age group.

C. The Sheffield diet contained 13 µg of vitamin A per 2700 kcal.

PUBLICATIONS:

A. (1) Wallace, D. L., E. M. Baker, J. E. Canham, N. Raica, H. E. Sauberlich and R. E. Hodges. Metabolism of vitamin A in the human. Fed. Proc. 30: 2176, 1971 (Abstract).

(2) Canham, J. E., E. M. Baker, D. L. Wallace, N. Raica, H. E. Sauberlich and R. E. Hodges. Aspects of vitamin A nutrition in man. Abstracts of the 161st National Meeting of the Am. Chem. Soc. - 29 Mar - 2 Apr 1971 (Abstract).

STUDY NO. 12 — A Study of Erythrocyte Glutathione Reductase as a Measurement of Riboflavin Status in Man

PROBLEM:

Present analytical methods limit the evaluation of the metabolic utilization of riboflavin in man. Urinary and red blood cell riboflavin reflect the previous day's dietary riboflavin. Further, a 24-hour urine collection is preferred for measurement. This is impractical if not impossible to obtain during nutrition surveys requiring sampling of large numbers of the population. A preferred assay is a measure of tissue-bound riboflavin which is not mobilized until the supply of riboflavin circulating in the blood has been depleted. The erythrocyte glutathione reductase (EGR) has been proposed as a measure of the riboflavin utilization in man. The activity of the EGR is altered *in vivo* by dietary riboflavin and *in vitro* by flavin adenine dinucleotide (FAD), a phosphorylated form of riboflavin. Thus, the degree of FAD saturation of EGR may be a measure of the riboflavin status in the intact organism. The activity of EGR is measured *in vitro* with and without FAD and the results expressed as the degree of stimulation or activity coefficient (AC).

It has been demonstrated that the AC of EGR is a measure of the nutritional status of the rat, Work Unit 074, Study No. 4. EGR in the rat and man respond similarly to added FAD *in vitro* differing in degree of stimulation. The purpose of this study was to evaluate the AC of EGR as a measure of the riboflavin status in man during partial riboflavin depletion and subsequent repletion. Further, the relationship of riboflavin status of an individual to his psychological behavior was studied.

Studies in Human Nutrition (Cont'd)

RESULTS AND DISCUSSION OF THE RESULTS:

A 12-week study evaluating the activity coefficient (AC) of the human erythrocyte glutathione reductase (EGR) during partial riboflavin depletion and the subsequent repletion of six human male volunteers has been completed. The subjects were confined to the Metabolic Ward and fed liquid formulated diets throughout the study period. No variation of the AC was seen during the control period. The AC increased slightly by day-7 of depletion in all subjects. A continued increase of the AC was seen during the first five weeks of the depletion period after which the AC plateaued. The urinary riboflavin decreased as expected during the depletion period but daily measurements showed an erratic riboflavin excretion pattern in all subjects. A significant decrease of the AC was seen after the subjects received 6.0 mg riboflavin within 4 days. The AC of all subjects were in the normal range following a massive dose of riboflavin giving each man a total of 24.5 mg riboflavin within six days. An increase in urinary riboflavin wasn't seen until the day following the initial decrease of the AC. Psychological testing was done throughout the study and the results are reported under Work Unit 059, Study No. 5.

CONCLUSIONS:

It has been shown that the AC of the human EGR is a measurement of the metabolic utilization of riboflavin in the human. The AC of EGR will be advantageous for the evaluation of the nutritional status of individuals during nutrition surveys. Further, measurement of this parameter will identify not only individuals suffering from severe aribоflavinosis but will identify individuals receiving marginal levels of riboflavin over a prolonged duration which heretofore was not possible.

OTHER ANCILLARY STUDIES

A. Effect of Sandfly Fever Virus Infection on Vitamin Metabolism in the Human

B. Nutrition Survey of Civilian and Military Populations

PROBLEM:

A. To study systematically aspects of vitamin metabolism during an experimentally-induced Sandfly Fever Virus infection in the human.

B. As a part of MRNL's continuing interest in the nutritional status of civilian and military populations as it reflects on the overall defense preparation planning, assistance through cooperative efforts on the analysis of biological samples has been continued.

Studies in Human Nutrition (Cont'd)

RESULTS AND DISCUSSION OF THE RESULTS:

A. Subjects excreted significantly more urinary riboflavin and pyridoxine but not thiamin during acute infection with Sandfly Fever Virus than during control periods.

B. Over 3300 samples of urine, sera, whole blood and diet composites were analyzed for vitamins. A portion of the total sera, analyzed for vitamins C and A, were obtained from the Florida Study and the Pittsburg Summer Recreation Program as part of the National Nutrition Survey. Diet Composites analyzed for vitamins A, B_1, B_2, B_6 and niacin were daily menus from the Malaysia British Troop Ration Study and the Fort McClellan Survey. Urinary B_1, B_2 and B_6 and serum vitamins A, B_{12} and folacin and whole blood folacin were measured in samples from the Fort McClellan Nutrition Survey. Serum samples from Army and Air Force hospitals were also analyzed for vitamins B_{12} and folacin. Results were submitted to proper individuals for compilation.

CONCLUSIONS:

A. These data suggested an increased requirement for riboflavin and pyridoxine during the acute phase of Sandfly Fever virus infection.

RECOMMENDATIONS:

A. Collaborative studies to assess the nutritional needs of man in disease states under controlled conditions should be continued.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6308	71 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
70 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	
c. CONTRIBUTING	CDOG 1412A (2)			

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 71	2.8	95
FISCAL YEAR CURRENT 72	2.8	115

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

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RESPONSIBLE INDIVIDUAL

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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) Envir.

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 develope predictive measures for improving performance.

25. (U) 70 07 - 71 06 Data processing and computation from the Ft. McClellan WAC survey is progressing. Clinical and biochemical data reports are being prepared for mailing to the individuals studied. Dietary intake data is being evaluated using the computer. Nitrogen and mineral balances data is being analyzed from the British study in the Malaysian jungle where sweat rates of 1.5 and 3.0 liters per 2 hours were observed. Based on studies of nutrient/calorie restriction performed in a laboratory setting and on troops in the jungles of Panama, a report indicating that nitrogen balances approach equilibrium with 1,500 Cal/day intakes is being prepared.

*Available to contractors upon originator's approval.

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

ABSTRACT

PROJECT NO.	3A062110A822	Military Internal Medicine
WORK UNIT NO.	073	Applied Nutrition Studies of Military Populations

The sixth in a series of nutrition surveys was conducted at Ft. McClellan, Alabama to evaluate the nutritional status of Women's Army Corps personnel. The "nutrition survey system" instituted by the Computer Division was utilized in this study to determine the nutrient intake of the WAC personnel. Studies will be continued in FY 1972 at Lowry Air Force Base, Colorado and Ft. Lewis, Washington to determine the food consumption of troops using the new feeding systems.

In the calorie restriction studies, it appears that negative nitrogen balances can be expected at caloric intakes below 1,000 Cal/day. Short term caloric restriction less than this level is not recommended in military personnel required to be physically active.

Preliminary information indicates great mineral and nitrogen losses (including sweat losses) during a 12-day period of caloric restriction in a Malaysian jungle when physical activity was maintained at a high level.

BODY OF REPORT

WORK UNIT NO. 073	Applied Nutrition Studies of Military Populations
STUDY NO. 1	Nutrition Surveys of Military Populations and Installations
STUDY NO. 2 (a-d)	Metabolic Aspects of Calorie Restriction (10 days) -
2 e	Malaysia

STUDY NO. 1

PROBLEM:

Annual 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 under 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 the support of the military nutrition surveys.

STUDY NO. 1

RESULTS AND DISCUSSION OF RESULTS:

Nutrition surveys at military installations were continued. The sixth study was conducted at Ft. McClellan, Alabama on Women's Army Corps personnel. This study was designed to further evaluate the nutrient intakes and nutritional status of the military female.

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 by WAC personnel at Ft. McClellan.

The survey analysis indicated some minor to marked discrepancies between the calculations based on the handbook data and the actual values determined by chemical analysis of collected samples of the food consumed. Investigation indicated some discrepancies in the mathematics for handling the cooking losses of some items, particularly meat items. This problem is being studied by recalculating the Ft. McClellan study utilizing handbook values for prepared food items, using detailed recipe evaluation to the minimum extent possible. A preliminary analysis of this approach seems to indicate a more reliable estimation of nutrient intake utilizing handbooks for food nutrient content. Food intakes calculated by this system are presented in Table 1.

The statistical portions of the system are being used to analyze the data and to prepare a report for distribution to the personnel surveyed. The forementioned report which includes the findings on the physical examination and the results of various biochemical tests for a specific individual have been sent to each individual who participated in the McClellan survey. Work performance, anthropometric, body composition and respiratory function data collected during the nutrition surveys have been computer programmed.

TABLE 1

Food Intake - Ft. McClellan, Alabama

Computer Programming*

	Basic Trainees	Students	Cadre	All Subjects
Calories	2707	2402	2382	2551
Protein, gms	98.4	89.1	100.0	95.6
Fat, gms	126.8	125.8	118.1	125.0
Carbohydrate, gms	301.8	234.4	234.6	268.2
Calcium, mg	1443	1215	1321	1347
Phosphorus, gms	1720	1545	1662	1652
Iron, mg	13.9	13.0	12.8	13.4
Sodium, mg	2994	3135	2382	2939
Potassium, gms	3256	2972	2868	3097
Vit A, IU	6162	5543	4954	5754
Thiamine, mg	1.51	1.47	1.47	1.49
Riboflavin, mg	2.56	2.34	2.54	2.49
Niacin, mg	15.4	13.8	16.8	15.1
Ascorbic acid, mg	84	98	72	86

*Abbreviated program using standard food tables.

Applied Nutrition Studies of Military Populations (Cont)

RESULTS AND DISCUSSION OF RESULTS:

The energy metabolism of the troops on maneuvers in a jungle environment (Panama) was studied. In summary, there appeared to be no significant differences in short-term physiological work performance ($\dot{V}_{O_2}$ in ml/kg/min) on the treadmill when comparing a control period to caloric restriction. In addition, no significant differences in performance were observed between the four groups who consumed either 603, 948, 1,362 or 3,382 Cal/day for a 10-day period. In comparison with our previous studies, body weight losses were the lowest observed, averaging 4.5% to 3.7% of the initial total body weight for three calorie-restricted groups. Body composition studies showed body weight changes to be due primarily to the caloric deficits and the loss of body fat. The data indicates that caloric intakes between 600 to 1,362 Cal/day, with mineral supplementation, are adequate to maintain physical efficiency during short term maximal work on the treadmill for a period up to 10 days.

The evaluation of B-vitamin excretions during starvation and calorie restriction has been completed. It appears that under conditions of acute starvation and calorie restriction, the daily minimal allowances of the B-vitamins are influenced by the caloric and protein intake, the degree of negative nitrogen balances and the subsequent protein catabolism. The suggested tryptophan-to-niacin conversion ratio of 60 to 1 may not apply during conditions of caloric restriction.

CONCLUSIONS:

1. The data suggests that under conditions of caloric restriction, the daily minimal allowances of vitamin B_1, B_2 and B_6 are influenced by the calorie, protein and vitamin intakes, the degree of negative nitrogen balance and the subsequent protein catabolism.

2. In a 500 Cal/day study, negative nitrogen balances were still observed (44 and 41 grams of protein/day) indicating that this was the best balance attainable under these conditions. Short term calorie restriction at this level of intake is not recommended.

RECOMMENDATIONS:

1. Initiate a study of the thiamine, riboflavin and niacin requirements as related to heavy energy expenditure.

2. Consolidate all of the calorie restriction results for a summary report.

CONCLUSIONS:

1. The computation of the dietary intake and the biochemical data from the Ft. McClellan WAC survey is completed. Data is being statistically analyzed. Individual data has been sent to each WAC participating in the survey.

2. There was considerable variation in nutrient intake data based on USDA Handbook 8 data and the actual intake based on chemical analysis. Part of the discrepancy appeared to be a technical problem.

RECOMMENDATIONS:

1. Continuation of the military nutrition surveys, to include concentration upon new feeding systems being adopted by the Armed Forces.

2. Conduct one nutrition survey in a cold environment (Alaska).

3. The weight loss in meat items during cooking due to fat and water loss should be studied, and an improved algorithm developed for the "Nutrient Survey System".

4. The investigation of the use of handbook values for prepared recipes versus the use of detailed recipe observations from the military kitchen should be continued. The results of this should be used to develop an improved data handling system.

STUDY NO. 2a-d

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.

3. Continue studies on the relationship of tryptophan and niacin during rapid growth. Is conversion more efficient under these conditions?

4. Study the degree that alteration of protein, fat and carbohydrate proportions in the diet affect the human vitamin requirements.

STUDY NO. 2e

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:

A member of this division participated in the British study conducted in West Malaysia which was designed to evaluate performance in relation to caloric restriction (1,800 Cal/day for 12 days). In this study, the daily energy expenditure averaged 3,700-4,000 Calories, with sweat rates ranging between 6 to 10 liters/day. All blood, urine, sweat and food samples have been analyzed and are being evaluated. Preliminary inspection of the data indicates that the nitrogen and mineral concentrations in sweat, during caloric restriction, are fairly high, and by the end of 12 days, practically unchanged from control values. The daily excretions of nutrients in sweat in a jungle environment are dependent on the sweat rates.

CONCLUSION:

A manuscript is being prepared evaluating nitrogen and mineral balances (including sweat losses) during a calorie restriction period. In this Malaysian jungle study, sweat losses ranged between 6-10 liters/day for a 12-day period.

RECOMMENDATION:

1. Completion of data analysis and manuscript preparation.

2. Initiating of protein metabolism studies to determine the effect of physical activity and extremely hot environments on protein requirements.

Applied Nutrition Studies of Military Populations (Cont)

PUBLICATIONS:

1. Consolazio, C.F. Institutional feeding, military type. Excerpta Medica International Congress Series No. 213, Proc. 8th Intl. Cong. on Nutr., Prague, Czechoslovakia, August 28 - September 5, 1969, p. 642.

2. Consolazio, C.F., H.L. Johnson, H.J. Krzywicki, T.A. Daws, and R.A. Barnhart. Excretion of the B-vitamins during acute starvation and calorie restriction. (Accepted for publication, Am. J. of Clin. Nutr.)

3. Johnson, H.L., D.A. Brown, C.F. Consolazio, H.J. Krzywicki and R.F. Burk, Jr.. A calcium-magnesium deficiency in normal man. Fed. Proc. 30:345, 1971 (Abstract).

4. Consolazio, C.F. Nutrition and Work Performance Proceedings of a Symposium on "Progress in Human Nutrition". AVI Publishing Co. (In Press)

5. Consolazio, C.F. B-vitamin excretions during starvation and caloric restriction (10 days). Proceedings Western Hemisphere Nutrition Congress II. Chicago: American Medical Association, 1969, p. 249. (Abstract)

6. Consolazio, C.F., H.L. Johnson and H.J. Krzywicki. Metabolic imbalances and body hypohydration during food deprivation (10 days). Proceedings of the 27th Aerospace Medical Panel Meeting, sponsored by AGARD-NATO, Garmisch-Partenkirchen, Germany, CP 18-71, p. 17, 1-6, 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6345	71 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
70 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	
c. CONTRIBUTING	CDOG 1412A (2)			

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 71	6.0	77
	CURRENT 72	8.0	106

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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Chemistry Division

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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) 70 07 - 71 06 A recent study involving the erythrocyte glutathione reductase activity as a measure of riboflavin status in the rat has been completed. A secondary iron deficiency (measured by iron absorption and tissue distribution) appears to result from folacin deficiency in the rat. The folate deficient animals are found to have diminished liver vitamin A stores in weanling rats. Modified Sheffield diet was found to contain 15 micrograms of vitamin A activity per 2700 calories (the normal daily calorie intake). Fats extracted from irradiation or thermally sterilized beef were equally inhibitory to lipase (in vitro assay). Serum GOT and GTP methods have beer optimized and will be used on the next nutrition survey. Progress is being made on optimizing the method for measuring O-phosphoryl ethanolamine in urine. This method will provide another means of evaluating vitamin B_6 nutritional status in humans and animals.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

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. 1. Experiment 3. Comparative metabolism of ascorbic acid in rainbow trout. The rainbow trout was found to have an ascorbic acid metabolism similar to that of man. Further, ascorbic-3-sulfate was found to be the major metabolite of ascorbic acid in the trout.

Studies No. 2 and 3. Nutritional and wholesomeness aspects of irradiated foods. Assistance and consultation in the final preparation of an irradiated beef feeding and testing protocol was given to the U. S. Army Medical Research and Development Command. The contract for this study has been awarded and the study is in progress. Emulsions of irradiated pure triglycerides and fats extracted from beef sterilized by thermal or ionizing radiation processing were tested against lipase by an *in vitro* procedure. Irradiated saturated triglycerides did not inhibit lipase activity but fats extracted from sterilized beef were inhibitory. The magnitude of inhibition was essentially equal for the thermal and irradiation processed beef.

Study No. 4. A recent study has shown that the activity coefficient of the rat erythrocyte glutathione reductase is a measurement of the riboflavin nutritional status of the animal.

A method has been developed for the determination of O-PE (O-phosphorylethanolamine) in urine. Also, a modification of optimal GPT (glutamic pyruvic transaminase) and GOT (glutamic oxalacetic

transaminase) assays has been developed for measurement of the enzymes in red blood cells. The methods will be tested for their usefulness in determining B_6 nutrient status.

Study No. 8. Folic acid deficiency in rats appears to result in a secondary iron deficiency as evidenced by increased uptake of radioiron into the duodenum, increased uptake and turnover of ^{59}Fe in plasma, and increased isotope uptake in the spleen following an oral dose of iron-59 administered to deficient rats.

Study No. 9. Because of personnel losses very little progress was made on this study. New experiments are planned during FY 71-72 to continue these studies.

BODY OF REPORT

WORK UNIT NO. 074 — Nutritional and Metabolic Aspects of Nutrients

STUDY NO. 1 — Ascorbic Acid: Chemistry and Biological Functions

PROBLEM:

Experiment 3: Comparative metabolism of ascorbic acid in rainbow trout. A joint study with Dr. J. E. Halver of the Western Fish Nutrition Lab on the metabolism of ascorbic acid in the trout has been completed. The reason for this study was that the rainbow trout requires vitamin C and further metabolizes his ascorbate in a manner similar to man.

RESULTS AND DISCUSSION OF THE RESULTS:

Recent nutrition studies have demonstrated that ascorbic acid is an essential nutrient for coho salmon and rainbow trout. Little is known about metabolism of ascorbate in fish. Ascorbic acid ^{14}C-1, ^{3}H-4 was intubated in a single dose into 250 gm rainbow trout catheterized and confined in metabolism chambers. Total urinary, branchial and fecal wastes were collected for 72-hour post insult. Urinary excretion of both ^{14}C and ^{3}H increased over a 3-day period and corresponded to 3% of ingested dose. Direct chromatography of urine demonstrated no free ascorbic acid. Urinary metabolites were separated into fractions with lead acetate at pH4 and pH8 plus the soluble (S) filtrate. Labeled metabolites were observed in all fractions but most occurred in the pH4 and (S) fractions.

The following were found:

1. That 3% of the ingested dose was eliminated via the urine within the first three days post intubation, and one-third of this appeared as ascorbate-3-sulfate.

2. Liver, head kidney, posterior kidney and skin showed high specific activity for ascorbate or its metabolites. In the organs studied the major portion of the labeled metabolites appeared as ascorbate-3-sulfate.

CONCLUSIONS:

1. Four percent of ingested ^{14}C and ^{3}H-labeled ascorbic acid was eliminated by the rainbow trout within the first 72 hours.

Nutritional and Metabolic Aspects of Nutrients (Cont'd)

2. About 30% of that material excreted via the urine co-chromatographed with referenced pure ascorbate-3-sulfate.

3. Studies of liver, head kidney, posterior kidney and skin showed that the major labeled ^{14}C-^{3}H metabolite was ascorbate-3-sulfate.

STUDY NO. 2 Irradiation Studies on Dietary Nutrients

STUDY NO. 3 Assistance to the Office of the Surgeon General on Irradiated Food Activities

Nutritional and wholesomeness aspects of irradiated foods.

PROBLEM:

Data on irradiated fats and oils, in most instances, have demonstrated an inhibition of in vitro lipase activity. This was not supported by long-term feeding studies in which no impairment of digestion or utilization of irradiated fats was found. As a result of conflicting data irradiated fats are considered by some individuals to be detrimental. Past studies with irradiated foods were considered inadequate by Food and Drug Administration (FDA) to prove the safety of irradiated foods. Since irradiation processing can economically make more "fresh" foods available to the military, even under the most adverse conditions, new in-depth studies to prove the safety of irradiated foods were considered necessary. Accordingly, a new protocol and testing study was considered essential to answer questions raised by FDA.

RESULTS AND DISCUSSION OF RESULTS:

The protocol for investigating irradiated fats has been reactivated (USAMRNL, Ann. Research Prog. Rpt. 30 June 1967, Study No. 3, pp 204-5, 209-12) and has been modified to include fats extracted from the beef being used in the current feeding studies. The beef (enzyme inactivated) was processed as follows: (a) frozen control (no further processing), (b) thermally treated (Fo = 6), (c) electron irradiated and, (d) gamma irradiated (4.6 - 7.1 Mrad). Pancreatic lipase activity was tested in vitro against the extracted fat. In every case the lypolysis rate was less, by as much as 25%, for the thermal or irradiation sterilized fats as compared to the control. The magnitude of inhibition was dependent upon the extraction procedure, however, the inhibition of the thermal was always equal to the irradiated fat. Irradiation of the fat extracted from the control beef did not significantly affect lipase activity. Peroxide values and TBA numbers of the extracted fats were not significantly different. The observed inhibition of lipolysis cannot be explained; however, it may be significant that both the thermal and irradiation treated beef was stored at room temperature for several

months while the control beef was stored frozen. Studies will be continued to determine the factors that contribute to the observed decreased lipase activity.

CONCLUSIONS:

Fats extracted from thermally or irradiation sterilized beef were equally inhibitory to *in vitro* lipase activity when compared to fat from control beef. Irradiation of fat extracted from the control beef did not cause inhibition of lipase activity. Storage temperature may be of greater significance than irradiation (or thermal) processing.

PUBLICATIONS:

1. Raica, N., Jr. The nutritional quality of irradiated foods. ACS National Meeting, Los Angeles, 1971. Abstract No. AGFD 069.

2. Raica, N., Jr., J. Scott and W. Nielsen. The nutritional quality of irradiated foods. Accepted for publication in Radiation Reviews.

STUDY NO. 4 Tissue and Blood Enzymes

PROBLEM:

Experiment 2: A study of erythrocyte glutathione reductase as a measurement of riboflavin status in the rat.
Present analytical methods limit the evaluation of the metabolic utilization of riboflavin in man. Urinary and red blood cell riboflavin reflect the previous day's dietary riboflavin. Further, a 24-hour urine collection is preferred for measurement which is impractical if not impossible to obtain during nutrition surveys requiring sampling of large numbers of the population. A preferred assay is a measure of tissue-bound riboflavin which is not mobilized until the supply of riboflavin circulating in the blood has been depleted. The erythrocyte glutathione reductase (EGR) has been proposed as a measure of riboflavin status in man. Eq. 1

$$NADPH + H^+ + GSSG \xrightarrow{ENZ \cdot FAD} NADP^+ + 2GSH \qquad \text{Eq. 1}$$

The activity of the glutathione reductase (EGR) is altered *in vivo* by dietary riboflavin and *in vitro* by flavin adenine dinucleotide (FAD), a phosphorylated form of riboflavin. The degree of FAD saturation of the EGR may be a measure of the riboflavin status in the intact organism. The activity of EGR is measured *in vitro* with and without FAD and the results expressed as the degree of stimulation or activity coefficient (AC). The EGR in man and the rat respond similarly to FAD *in vitro* differing in the degree of stimulation.

It was the purpose of this study to evaluate the EGR during riboflavin depletion of the rat and the subsequent repletion. The rat was chosen to enable a large sampling for experimental conditions and to establish parameters for a human study if the results warranted it.

RESULTS AND DISCUSSION OF THE RESULTS:

EX-2. The activity coefficient (AC) of the rat erythrocyte glutathione reductase (EGR) in 65 animals was studied during riboflavin depletion and the subsequent repletion by daily oral supplements followed by incorporation of the vitamin into the diet. Measurement of the AC was made periodically throughout the study. The AC of the control animals remained constant with values of 1.15 to 1.30. A significant increase of the AC was seen by day-9 of the depletion period and continued to increase reaching a plateau during the third to fourth week. A decrease of the AC was seen in all animals soon after the daily supplements were given regardless of the level of the supplement. However, the AC did not return to the normal levels after 28 days. Once riboflavin, at three levels, was incorporated into the diet the AC of all the animals returned to the normal range. The time required for the saturation of the tissue protein, which appeared to be equivalent to a normal AC, varied according to the level of riboflavin in the diet.

PROBLEM:

Experiment 4: Experiment to determine an individual B_6 nutrient status from blood and/or urine assays.

There is a need for an accurate, fast and reproducible method to determine the B_6 status in humans regardless of age, sex or dietary intake. Three assay methods warrant detailed study because they show possible usefulness (used either separately or in conjunction with each other) in providing the desired assay. O-phosphorylethanolamine (O-PE) has been shown to reach very high levels in the urine of weanling and adult rats on a high protein diet void of vitamin B_6. O-PE appears to be ubiquitous to nearly all vertebrate tissues. The enzyme (O-PE phospho-lyase, requiring pyridoxal-P for activity) involved in the metabolism of the compound has been found in the liver of all species studied and is present at low levels in the kidneys of some of these. Pyridoxal phosphate (PLP) is one of the forms of vitamin B_6 most frequently used by enzymes. It has been suggested that O-PE may play a significant role as a metabolite in the pathway of ethanolamine's carbon atoms into fatty acids.

The other two assays also involve the use of PLP as a cofactor necessary for the activity of the two enzymes GOT and GPT. Evidence for and against stimulation of blood GOT and GPT when PLP is added to an in vitro

assay system is inconclusive. Part of the reason for discrepancies in this area is due to the use of poor assay methods. Ideally one would like to sample only blood and/or urine from an individual in order to evaluate his B_6 status.

RESULTS AND DISCUSSION OF RESULTS:

EX-4. Using a given amount of O-PE phospho-lyase purified from rats fed a 60% or 80% protein diet and measuring the change of O.D. for 30 minutes, one can obtain a standard curve for O-PE between 10 and 70 nmoles of O-PE/ml of water or urine. Measured values approach theoretical values when 60 to 80 mU (milliunits) of enzyme are added to the reaction mixture. One can also obtain a linear curve for O-PE concentration with 15 mU of enzyme; however, the sensitivity of the assay is decreased 33%. This method which has been developed (using purified O-PE phospho-lyase to assay O-PE) is superior to previous methods using the amino acid analyzer because of the increased number of samples wwhich can be assayed per unit of time.

Until April 1971 the best kits commercially available for measuring GPT and GOT spectrophotometrically gave only 32% of the GPT and 76% of the GOT activity which could be measured under optimal conditions. Since April 1971 kits which provide optimum conditions for these two enzyme assays have become commercially available. Modification of the commercial procedure has been made which allows one to measure GPT and GOT levels in plasma or red blood cells (RBC's) using 0.2 to 0.5 ml of whole blood.

CONCLUSIONS:

EX-2. It has been demonstrated that the AC of the rat EGR is a measure of the riboflavin status in the rat.

EX-4. Two methodological tools (the assay of O-PE in urine and the optimized assay of GOT and GPT in red blood cells) have been set up which show possible usefulness in evaluating B_6 nutrient status from the two most convenient sources of human sample collection, blood and urine.

RECOMMENDATIONS:

EX-4. Continue the studies with animals such as the experiment being performed at the present time which utilizes high (60%) and low (20%) protein diets plus or minus B_6 (pyridoxine). Parameters being measured are urine O-PE, Ca and Mg; blood GPT, GOT, serum alkaline phosphatase, Ca and Mg; Ca and Mg of kidney and brain. If an effect of $-B_6$ (correlation of deficiency with one or more measurements) is observed, further study would be warranted. Similar experiments would be performed

to ascertain whether or not the effect is specific for B_6 or can be observed under other vitamin deficiencies. The ultimate goal of the experiment is to devise a test(s) to determine the early stages of a B_6 deficiency in humans.

PUBLICATIONS:

1. Tillotson, J. A. and H. E. Sauberlich. Effect of riboflavin depletion and repletion on the erythrocyte glutathione reductase in the rat. Accepted for publication in the J. of Nutr., Nov 1971.

2. Tillotson, J. A. and E. M. Baker. Effect of riboflavin deficiency on glutathione reductase in the rat. Fed. Proc. 30: 2190, 1971 (Abstract).

3. Fleshood, H. L. and H. C. Pitot. The metabolism of O-phosphorylethanolamine in animal tissues. I. O-phosphorylethanolamine phospho-lyase: partial purification and characterization. J. Biol. Chem. 245: 4414, 1970.

4. Fleshood, H. L. and H. C. Pitot. The metabolism of O-phosphorylethanolamine in animal tissues. II. Metabolic regulation of O-phosphorylethanolamine phospho-lyase in vivo. Arch. Biochem. & Biophys. 141: 423, 1970.

STUDY NO. 8 Malabsorption, Diarrhea, Steatorrhea and Nutritional Deficiency Syndromes

PROBLEM:

Experiment 3: Absorption and metabolism of essential minerals in the germ-free animal.

Anemia is a perplexing disease in that many factors may be responsible for its development. Iron and folic acid deficiencies can result in anemia. However, there are very little data available to ascertain how these factors may interact in the development of the anemic syndrome. This study was designed to determine the effect of folic acid deficiency upon normal iron metabolism.

RESULTS AND DISCUSSION OF THE RESULTS:

Weanling rats were fed either a control or folic acid (FA) deficient diet for 4 weeks. At that time they were orally dosed with 5 µCi of ^{59}Fe. At varying time intervals following isotope dosage, at least 5 rats from each group were sacrificed. Blood, liver, kidney, heart, spleen, duodenum, and femur were removed for isotopic assay. The duodenum of the FA-deficient rats contained significantly greater amounts of ^{59}Fe one-half hour following dosage than controls. The deficient rats' plasma also showed greater initial radioactivity and a more rapid ^{59}Fe turnover rate

than controls. The relative turnover rate of ^{59}Fe in bone (marrow) of the FA-deficient group was more rapid than in control rats. The spleen of the deficient rats showed markedly greater incorporation of the isotope 12 hours following dosage (approximately 50% greater than controls). These data would seem to suggest that Fe absorption was enhanced by FA deficiency and that the spleen of the deficient rat may have assumed a greater role in hematopoietic function.

CONCLUSIONS:

The data from the study reported here suggest that a secondary iron deficiency may result from folic acid deficiency in the rat. Further, the patterns of ^{59}Fe incorporation and turnover in the bone (marrow) and spleen suggest that there may be reduced hematopoietic function in the marrow with a concomitant increase in splenic erythropoiesis in the FA-deficient rat.

RECOMMENDATIONS:

Continue to study the metabolic and nutritional interactions between iron and folic acid in order to ascertain optimum dietary levels of both nutrients so that anemia and the related symptomatology will not be a limiting factor in physical performance and endurance.

PUBLICATIONS:

1. Dowdy, R. P. and Y. F. Herman. Effect of folic acid deficiency upon absorption and tissue distribution of iron-59. Fed. Proc. 30: 239, 1971 (Abstract).

2. Nielsen, Forrest E. and H. E. Sauberlich. Evidence of a possible requirement for nickel by the chick. Proc. Soc. Exptl. Biol. and Med. 134: 845, 1970.

STUDY NO. 9 Vitamin A and Carotene

PROBLEM:

Experiment 2: The metabolism of radioactively labeled vitamin A and carotene in the rat.

Previous data obtained and reported (1970 Annual Report) suggested that the utilization of vitamin A was more efficient in the vitamin A deficient than in the vitamin A sufficient rat and that urinary metabolites of vitamin A are unique entities. Excess dietary vitamin A is generally stored; however, it has not been established whether newly ingested vitamin A is mixed with existing pools or whether it is preferentially

utilized. Neither has it been established whether storage/utilization is the same during repletion as during vitamin A sufficiency,

RESULTS AND DISCUSSION OF THE RESULTS:

It is proposed to study the problems presented above with radioactive vitamin A. The rate of excretion of urinary metabolites has been inadequately studied during both vitamin A insufficient and sufficient nutritional states. If qualitative or quantitative differences exist then it should be possible to develop a reliable procedure to evaluate vitamin A nutriture. These studies had been initiated and progress reported but because of the loss of personnel very little progress had been made during FY 71.

PUBLICATIONS:

Raica, N., Jr., M. A. Stedham, Y. F. Herman and H. E. Sauberlich. Vitamin A deficiency in germ-free rats. In: The Fat-Soluble Vitamins, H. F. DeLuca and J. W. Suttie, eds. The University of Wisconsin Press, Madison, 1970, pp 283.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6337	71 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
70 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 1412A	(2)		

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 71	6.2	176
FISCAL YEAR CURRENT 72	7.2	207

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: 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) 70 07 - 71 06 Analytical support was provided to several laboratory research projects relevant to military medicine, basic and applied nutrition of military personnel, and nutrition of military working animals. Sample requirement for serum iron methodologies was halved as a result of chemical and physical modifications of the automated system. Methodology has been developed which enables satisfactory automated determination of total nitrogen in stool specimens. An improved triglyceride procedure has been evaluated and incorporated into the automated serum lipid assay series. An automated sucrose assay was developed for application to urine and stool supernatants.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

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

Several additional manual and automated methodologies were developed and/or incorporated into services available to research projects. Preliminary investigation has begun in an effort to broaden the capacity of the data processing systems for flexibility and final summary preparation.

BODY OF REPORT

WORK UNIT NO. 076 Analytical Biochemistry

STUDY NO. 1 Analytical Support and Services

PROBLEM:

In order to satisfactorily meet the requirements for branch services, it is necessary to be continually conscious of the need for functional efficiency in all phases of the laboratory operation. Services are in a dynamic state of expansion through incorporation of additional methodologies as required by the investigators. Large quantity sample inputs and growing lists of determinations dictate the necessity for continual attempts to automate analytical procedures and improve data handling systems.

RESULTS AND DISCUSSION OF THE RESULTS:

Service was provided to approximately 83 project and subproject requests representing studies in the areas of military medicine and nutrition of military personnel and working animals. While many of the determinations requested were already in the repertoire of the Branch several new methodologies were added including automated serum glutamic-oxalacetic transaminase, automated alkaline phosphatase, and manual methods for aldolase, lactic dehydrogenase, serum and urinary myoglobin, erythrocyte proto- and coproporphyrin, urinary porphyrin and porphobilinogen, erythrocyte delta-aminolevulinic acid dehydratase, and urinary delta-aminolevulinic acid. A method for blood NH_3 has been evaluated and implemented for servicing studies in muscle metabolism.

An automatic glucose analyzer (which provides "true" glucose values employing glucose oxidase) has proved very effective in handling small sample lot inputs for both blood and urine, replacing a cumbersome manual method in the latter case.

Study has been initiated of improvements in data handling systems in cooperation with the Computer Division. A program is being developed which will provide off-line preparation of final summary statements of data from automatic analyzers. A sample input form and coding system, and a sample and quality control loading scheme, have been developed to be used in conjunction with this program. A program is also being developed to permit time-shared use of the gas chromatography-amino acid analyzer data processing equipment.

An electronic integrating unit has been modified to enable direct simultaneous print-out of peak areas as electrophoresis scans are being recorded by the densitometer.

CONCLUSIONS:

Expansion of analytical services through implementation of additional methodologies has broadened the demands for support on the Branch. Wherever feasible the implementation of automated methods has been desirable as it improves the performance capabilities of the staff. However, combined with expansion of manual services provided, this results in an impressive data handling problem for both data reduction and summarization. The expansion of services increases the value of the Branch to the laboratory, and successful accomplishment of the programming studies will improve the overall operational efficiency and relieve senior staff for developmental work.

RECOMMENDATIONS:

Work should be continued in the area of the most critical problem of the Branch, the production of edited machine-generated summary statements. The Branch will continue to respond to requests for new and existent services.

STUDY NO. 2 Development of Analytical Biochemistry Procedures

PROBLEM:

Manual methods must be automated whenever possible as demand for each determination necessitates in order to achieve maximum efficiency with a fixed level of staff. In response to specific requests, the Branch must attempt to innovate methods for desired analyses, as well as modifying existent methodology for more satisfactory performance.

RESULTS AND DISCUSSION OF THE RESULTS:

Complete automation of the serum iron and iron-binding capacity method was not achieved, however physical modification of the system and use of a more sensitive chromogen resulted in a requirement for approximately half the volume of serum normally used. A report on this current methodology is in preparation.

Total nitrogen in stool specimens has been successfully performed on the automated system normally used for urines. The procedure requires the preparation of a stable suspension prior to loading on the sampler.

The processing of diet samples is also being evaluated. A report on these techniques is being prepared.

Analytical Biochemistry (Cont'd)

It is felt that a rapid screening technique can be developed for a limited number of amino acids in physiological fluids using an automatic chemical derivatizer and gas chromatography. At present the derivatizer needs some modification to provide maximum and reproducible recoveries. The serum lipid assay series has been updated with the evaluation and incorporation of an improved automated triglyceride procedure.

An automated sucrose assay employing invertase was developed which gave satisfactory performance in urine and stool supernatants.

An automated procedure for xanthurenic acid has been implemented and awaits evaluation in a test series.

CONCLUSIONS:

While attempts to fully automate serum iron and iron-binding capacity methods were not successful, the decreased sample requirement is a worthy achievement at a time when increasing numbers of determinations are requested on a single blood collection.

The successful application of the automated nitrogen analysis system to urines and stools meets a critical need resulting from the balance studies involved in many laboratory projects. In addition to increasing the operating capacity, this method eliminates much of the tedium and subsequent potential error of the manual Kjeldahl method.

The xanthurenic acid method should be useful in determining tryptophan metabolism in relation to nutritional studies of vitamin B_6.

RECOMMENDATIONS:

The achievement of peak operational efficiency requires a continuing examination of currently used methods with the object of eliminating manual steps (such as extraction, deproteinization or pH adjustments) in semi-automated methods, and automating of completely manual methods. Efforts should be made to obtain more information from smaller volumes of blood specimens by means of miniaturization of methods or performing multiple analyses on single aliquots of each specimen. Development of methods and adaptation of new procedures to automation must be attempted whenever required by projects being serviced.

PUBLICATIONS:

Baker, E. M., R. E. Hodges, James Hood, H. E. Sauberlich, S. C. March and J. E. Canham. Metabolism of ^{14}C- and ^{3}H-labeled L-ascorbic acid in human scurvy. Am. J. Clin. Nutr. 24: 444-453, 1971. Appendix, E. M. Baker and J. H. Skala, Am. J. Clin. Nutr. 24: 453, 454, 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6324	71 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
70 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 1412A (2)			

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 71	6.0	85
	CURRENT 72	9.0	90

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

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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) 70 07 - 71 06 Experimental starvation followed by refeeding produces alterations in metabolic pathways of leucine. Fatty acid synthesis in adipose tissue is markedly enhanced during refeeding. Fatty acid synthesis is also increased by excessive intake of adenine. A high intake of dietary protein enhances activities of specific enzymes involved in amino acid degradation. Apparently, this is an adaptive response to an increased nitrogen load.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 67 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine

WORK UNIT NO. 077 Nutritional Physiology

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

Study No. 6 Metabolic effects of starvation-refeeding

Study No. 7 Kidney response to a high protein intake and to cold in the rat

Study No. 10 Metabolic effects of adenine toxicity

Study No. 11 The effects of diet and cold-exposure on lipogenesis

Experimental starvation and refeeding in rats causes alterations in metabolic pathways of leucine. In the liver, oxidation of leucine to CO_2 is increased during fasting. During subsequent refeeding this effect is reversed. Leucine incorporation into lipids of adipose tissue on the other hand, is markedly increased during refeeding and depressed during starvation. These data indicate that fasting enhances the catabolic utilization of leucine at the expense of fatty acid synthesis.

Rats exposed to cold or fed a high-protein diet develop enlarged kidneys. These gross observations are associated with increased activities of specific renal enzymes mediating a degradation of amino acids. High protein intake also increases the levels of renal DNA and RNA with no difference between groups exposed to environmental temperatures of 7° C or 25° C.

Excessive intake of dietary adenine increases the activities of several enzymes associated with fatty acids synthesis. Incorporation of glucose or acetate into fatty acids is also enhanced. These biochemical changes are associated with a profuse deposition of crystalline material in the renal cortex. The latter has been identified as a very insoluble metabolite of adenine, 2,8-dihydroxyadenine.

BODY OF REPORT

WORK UNIT NO. 077 Nutritional Physiology

STUDY NO. 6 Metabolic effects of starvation-refeeding

PROBLEM:

The metabolism of branched-chain amino acids (leucine, isoleucine and valine) appears to be directly associated with lipid metabolism. In man and swine branched-chain amino acids in serum have been reported to increase during fasting and decrease after refeeding. Diverted utilization of these amino acids to provide energy is the most likely explanation for the elevated levels observed during fasting. Increased utilization probably explains the decrease of plasma levels during refeeding. Since leucine is normally metabolized via acetyl-CoA, experiments were designed to study *in vitro* and *in vivo* lipogenesis from leucine-^{14}C in fasted and fasted-refed rats.

RESULTS AND DISCUSSION OF RESULTS:

Male, Holtzman rats, weighing 180-200 grams, were fed a casein-sucrose diet for seven days. The rats were then divided into the following experimental groups: I. controls, fed ad lib; II. fasted for five days; and III. fasted for five days and refed for four days. In the control animals 19.16 $\pm$ 0.8% (SEM) of injected leucine-^{14}C was converted to $^{14}CO_2$ in three hours. In the fasted animals the conversion was significantly increased to 35.10 $\pm$ 6.8%, while in the fasted-refed animals it was significantly reduced, to 12.9 $\pm$ 1.4%. *In vitro* experiments showed the amount of leucine oxidized by liver, kidney and diaphragm tissue was similar in the three groups. In adipose tissue, however, leucine oxidation was higher in the fasted-refed group.

In vivo and *in vitro* incorporation of leucine into fatty acids of liver and adipose tissue were decreased in the fasted group and increased in the fasted-refed group. In the fasted-refed group *in vitro* lipogenesis from leucine was also increased in diaphragm. In *in vitro* experiments glucose and glucose plus insulin increased leucine incorporation into fatty acids in liver, diaphragm and adipose tissue in all three groups. The effect of glucose and insulin was significantly greater in the fasted-refed group than in the controls.

CONCLUSIONS:

The foregoing study suggests that during fasting ketone bodies produced from leucine are mainly oxidized to CO_2. In contrast, during refeeding a greater quantity of ketone bodies produced from leucine are utilized for lipogenesis.

RECOMMENDATIONS:

The quantity of ketone bodies produced in the liver from amino acids during fasting should be determined by isolated liver perfusion. The dietary factors (quantity of protein, fat and carbohydrate) contributing to ketone body formation from amino acids should also be investigated.

STUDY NO. 7 — Kidney response to a high protein intake and to cold in the rat

PROBLEM:

Marked increases in kidney size are observed in animals exposed to cold or fed a high-protein diet. In fact, this effect of cold and diet appears to be additive, as evidenced by the markedly greater kidney enlargement seen in cold-exposed rats receiving a high-protein diet as compared to those receiving a high-carbohydrate diet. Presumably, the cold-induced or diet-induced enlargement of the kidneys represents a compensatory response to an added functional load. In the case of high-protein diets, and probably in the case of cold exposure during which caloric intake may be increased two-fold, this added functional load could be caused by an increased metabolic turnover of body nitrogen. Accordingly, experiments have been designed to study various aspects of intermediary nitrogen metabolism as influenced by both cold exposure and high-protein diets.

RESULTS AND DISCUSSION OF RESULTS:

Male rats, weighing approximately 300 gm, were used in all experiments. The control diet contained 20% casein and 73% sucrose, while the high-protein diet contained 85% casein and 8% sucrose. Adequate levels of vitamins and minerals were included in both diets. These regima were fed for two weeks to rats exposed either to 25° C or 7° C. At the end of the feeding period the rats were decapitated and kidney homogenates were prepared and assayed for the following enzymes: glutamic-oxalacetic (GOT), glutamic-pyruvic (GPT) and tyrosine-alpha ketoglutaric (TKT) trans-aminases, arginase (ARG), arginine synthetase (ARG-S) and glutamic dehydrogenase (GDH). In addition, concentrations of nitrogen, lipids, DNA and RNA in the kidneys were determined. Rats exposed to 7° C and fed the control diet developed slightly enlarged kidneys: 0.89% body weight vs. 0.69% for the controls kept at 25° C. When the level of dietary casein was increased to 85%, kidney weights of cold-exposed animals were further increased to 1.21% body weights vs. 0.88% for the controls. Cold exposure had no effect on the activity of GDH in rats fed the control diet; however, high protein intake increased this enzyme's activity by 80% at 25° C and 160% at 7° C. Responses of similar

magnitude were observed in the activities of GOT, GPT, ARG and ARG-S.

Cold exposure also increased the concentrations of kidney DNA and RNA in animals fed the control diet. Essentially the same results were obtained with high protein diets. There were no differences between the groups at 7° C and 25° C. This effect of cold and diet appears to be additive, representing an adaptation to handle the increased nitrogen load.

CONCLUSIONS:

Excessive intake of dietary protein causes a marked enlargement of the kidneys. Apparently, the kidney adapts to handle the increased nitrogen load; thus the enlarged kidneys would represent hypertrophy secondary to an increase in the metabolic pathways necessary to handle the increased load of dietary protein.

RECOMMENDATIONS:

To further study basic mechanisms underlying the foregoing metabolic alterations. These would include renal function studies, creatinine and urea clearance, glomerular filtration and renal plasma flow.

STUDY NO. 10 Metabolic effects of adenine toxicity

PROBLEM:

Adenine supplementation of blood stored for future transfusion is effective in maintaining a high adenine nucleotide level of erythrocytes, thus prolonging their post-transfusion survival. Adenine, however, has been reported to be relatively toxic to mammalian cells grown in vitro in dogs, rats and humans.

Rats consuming a purified diet containing 0.5% supplemental adenine develop enlarged kidneys, associated with uremia. The renal failure is apparently the consequence of deposition of crystalline masses of a very insoluble metabolite of adenine in renal tubules. Ingestion of excessive adenine also causes a derangement of renal and hepatic glucose metabolism. In particular, gluconeogenic processes are depressed while glycolysis is stimulated. In addition, acetate incorporation into fatty acids is increased both in the liver and in the kidney. Consequently, the purpose of the experiments described below was to study the effect of excessive dietary adenine on the activities of several enzymes associated with fatty acid synthesis in liver, kidney and adipose tissue.

RESULTS AND DISCUSSION OF RESULTS:

Male rats, weighing 200-220 gm, were fed a complete casein-sucrose

diet, without or with 0.5% of supplemental adenine, for ten days. At the end of the feeding period the activities of the following enzymes were studied: malic dehydrogenase, citrate cleavage enzyme, glucose-6-phosphate dehydrogenase and isocitric dehydrogenase. These results indicate that the activities of all three dehydrogenases were stimulated by excessive adenine. In contrast, the activity of the citrate cleavage enzyme was not altered. The crystalline masses in the kidneys have been identified as 2,8-dihydroxyadenine.

CONCLUSIONS:

Excessive intake of dietary adenine stimulates activities of several enzymes associated with fatty acid synthesis in liver, kidney and adipose tissue. This effect does not appear to be related to an increased flow of substrates over specific metabolic pathways.

RECOMMENDATIONS:

Further studies are required to delineate the causes of adenine toxicity and their implications with respect to long-term kidney function.

STUDY NO. 11 The effects of diet and cold-exposure on lipogenesis

PROBLEM:

Previously reported data on the effect of cold on lipogenesis is very conflicting. Further, knowledge about the effects of various diets and their composition on lipogenesis is limited, not only at 25° C, and even more so at low environmental temperatures are limited. Since most reports on animals exposed acutely or chronically to cold disregarded increased food intake of the animals it is very difficult to distinguish between the effect of cold per se and the effect of increased food intake.

RESULTS AND DISCUSSION OF RESULTS:

The oxidation and metabolism of ethyl-^{14}C acetoacetate will be measured in rats maintained at 25° C and 5° C and fed a high fat (80% of total calories from beef tallow or corn oil, carbohydrate free) or a high carbohydrate diet (70% of total calories from sucrose, 3% corn oil). As part of this study, incorporation of acetoacetate into fatty acids and cholesterol will be determined in various organs, tissues and blood plasma.

PUBLICATIONS:

1. Klain, G. J., F. J. Sullivan and A. W. Meikle. Dietary orotic

acid and lipogenesis in the rat. J. Nutrition 100:1431-1436, 1970.

2. Klain, G. J. Kidney response to cold and high protein intake. Federation Proc. 30:259, 1971 (abstract).

3. Meikle, A. W. and G. J. Klain. The effect of fasting and fasting-refeeding on the metabolism of leucine in rats. Federation Proc. 30:519, 1971 (abstract).

4. Hannon, J. P., R. E. Beyer, R. F. Burlington, R. P. Somero and J. C. Bland. A guide for future studies of low temperature metabolic function. Chapter in book. Am. Elsevier Co. Publ. (Submitted for publication) 1971.

5. Whitten, B. K., Beecher, G., Liddle, C. G. and O'Barr, T.P. USAMRNL Laboratory Report No. 324. A comparison of $14CO_2$ trapping agents used in in vitro metabolic studies. September 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6343	71 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
70 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 1412A (2)			

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 71	5.1	107
FISCAL YEAR — CURRENT 72	5.5	106

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

21. GENERAL USE

Foreign Intelligence not Considered

ASSOCIATE INVESTIGATORS

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NAME: Stifel, Fred B. DA

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 diseases 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 a substantial proportion of disabling gastrointestinal disease is related to a failure of gastrointestinal enzyme adaptation to diet. Impaired gut absorption and impaired utilization of dietary carbohydrate and lipid by muscle will lead to suboptimal performance. It is essential that the optimum amounts of dietary lipid and carbohydrate necessary for maximum muscle function and normal gastrointestinal tract enzyme adaptation be determined.

24. (U) The response of gastrointestinal enzymes of normal human volunteer subjects and selected patients with specific gastrointestinal diseases to specific dietary substances and certain oral drugs (e.g., anti-malarial agents) is under study. The response of blood lipids and their utilization by muscle to similar diets will be studied in normal subjects. The basic laboratory studies are covered in work unit 059.

25. (U) 70 07 - 71 06 Anti-malarial drugs in normal human subjects cause variable responses of jejunal glycolytic enzymes. Ethyl alcohol stimulated jejunal adenyl cyclase while clofibrate decreases human adipose tissue adenyl cyclase. Fructose increased the activity of an abnormal sucrase in a patient with sucrase-isomaltase deficiency. The adaptation of jejunal glycolytic enzymes was modified by adrenalectomy and the effect repaired by corticosterone replacement. Normal subjects fed no carbohydrate diets developed gastrointestinal symptoms and abnormal glucose tolerance tests with an actue challenge of sucrose, glucose or fructose as compared to the response after being given as isocaloric diet containing 50% of calories as glucose and fructose.

*Available to contractors upon originator's approval

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

ABSTRACT

PROJECT NO.	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. 3. Study of patients with endogenous hypertriglyceridemia.

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. 8. Study of a patient with severe anxiety, tachycardia, syncope and a family history of sudden and/or unexplained death.

STUDY NO. 9. The effect of dietary fructose on jejunal sucrase in a patient with sucrase-isomaltase deficiency.

STUDY NO. 10. The effect of isocaloric carbohydrate-free diets and diets containing either sucrose, fructose or glucose on oral carbohydrate tolerance (physiologic carbohydrate intolerance).

STUDY NO. 11. Study of the adaptive responses of jejunal glycolytic enzymes in patients with dermatitis herpetiformis.

STUDY NO. 12. Lack of jejunal glycolytic enzyme adaptation in patients with chronic gastrointestinal disease: the gastrointestinal maladaptation syndrome.

STUDY NO. 13. Clotting defects in fasting patients.

STUDY NO. 14. Effect of riboflavin deficiency.

STUDY NO. 15. Flavokinase activity in Acrodermatitis Enteropathica.

Metabolic Response of Man to Nutrition or Disease (Cont'd)

STUDY NO. 16. The *in vitro* effect of alcohol on adenyl cyclase activity.

STUDY NO. 17. Study of a patient with possible hyperaldosteronism.

Studies have been directed to various areas exploring the relationship between adaptation of jejunal glycolytic enzymes to dietary substances and certain gastrointestinal disorders that appear to result as a consequence of a failure of the enzyme adaptation mechanism. Oral and intramuscular (I.M.) sex steroids (natural or synthetic congeners, free or conjugated) increase jejunal glycolytic enzymes in the rat. The response is blocked or diminished by inhibitors of protein synthesis, folate deficiency, and adrenalectomy. Antimalarial drugs produce variable effects. Histidine activates jejunal and other tissue pyruvate kinase *in vitro*. Jejunal adenyl cyclase activity is stimulated *in vitro* by various alcohols. Hypogonadal men have decreased jejunal glycolytic enzyme activities which increase with I.M. or oral testosterone. Dietary fructose increased the defective sucrase activity in a patient with sucrase-isomaltase deficiency and thus increased the patient's tolerance to dietary sucrose. Normal individuals fed a no-carbohydrate diet had gastrointestinal symptoms with acute challenges of dietary sugars and abnormal glucose tolerance which did not occur with the same acute challenge after an isocaloric 50% carbohydrate diet. In 2 out of 3 patients with dermatitis herpetiformis but with no gastrointestinal symptoms abnormal jejunal morphology and enzyme adaptation was found. A gluten-free diet did not affect the skin lesions of these 2 patients. In some of the patients with gastrointestinal symptoms associated with a failure of enzyme adaptation a kinin-like substance has been found using an *in vitro* guinea-pig ileum assay system.

Other studies have been the effect of clofibrate on the adenyl cyclase of fat and jejunum in patients with type IV hypertriglyceridemia, study of a patient with hyperaldosteronism due to hyperplasia of the zona glomerulosa of the adrenal, studies of the effect of adrenergic blockade on pituitary hormone release, the effect of clomiphene in oligospermic conditions, study of a patient with symptoms and signs apparently due to hyperepinephrinemia who recovered after sub-total adrenalectomy, study of the clotting mechanism during starvation, and the measurement of flavokinase activities of the jejunum in normal subjects on a riboflavin deficient diet and in a patient with acrodermatitis enteropathica.

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:

Gastrointestinal enzymes are adaptively regulated by diet, drugs and sex steroids. Both the nature and mode of administration of the adaptive factors are important in the regulatory process. The knowledge of these responses in normal human subjects and animals have enabled us to evaluate these same responses in patients with various gastrointestinal diseases, particularly the maladaptation syndrome. Thus, studies have been conducted to further elucidate the nature of the adaptive responses of glycolytic enzymes in the jejunum to various physiologic factors such as diet, folic acid, sex steroids, and several non-physiologic factors such as chloroquine, primaquine and dapsone.

RESULTS AND DISCUSSION OF THE RESULTS:

In normal rat jejunum, dietary substances and oral sex hormones produce adaptive changes in glycolytic and folate-metabolizing enzyme activities. Oral testosterone and estradiol produce adaptive changes in the activities of jejunal phosphofructokinase and pyruvate kinase in castrate and non-castrate male and female rats. Intramuscular estradiol produced significant increases in jejunal pyruvate kinase activity in castrate female rats, but had no effect in non-castrate female rats. One possible explanation for the failure of intramuscular sex hormones to alter jejunal enzyme activities in non-castrate rats may be that the glucosiduronide forms are metabolically inactive in the jejunum. Our data demonstrate that the glucosiduronide forms of estradiol and testosterone when given orally do produce significant increases in the activities of jejunal pyruvate kinase in female and male rats, respectively. Thus, the conversion of intramuscular sex steroids to the glucosiduronide forms would not account for the failure of intramuscular sex steroids to affect non-castrate rat jejunum. We suggest that the endogenously produced circulating sex hormones may participate in the formation of a "blood-gut barrier" only on the serosal side of the jejunum. In castrate rats, with the absence of endogenous sex hormones, the "blood-gut barrier" would be minimal and one would expect both oral and intramuscular sex hormones to be effective which, indeed, was found.

Oral sex hormones (testosterone in male rats and estradiol in female rats), produce adaptive changes in the activities of several jejunal

folate-metabolizing enzymes in normal and castrated male and female rats. The same sex hormones administered intramuscularly had no effect on these enzymes in non-castrate rats. Specifically, in the jejunum of normal and castrated male and female rats, oral testosterone and estradiol, respectively, adaptively increased the activities of serine hydroxymethyltransferase and methylenetetrahydrofolate dehydrogenase. In rat jejunum from normal male and female rats, oral sex hormones decreased the activity of formyltetrahydrofolate synthetase. Oral testosterone caused a similar decrease in the activity of the same enzyme in castrated male rats. Conjugated sex hormones also produced adaptive increases in the activities of serine hydroxymethyltransferase and methylenetetrahydrofolate dehydrogenase in normal rat jejunum.

Actinomycin, an inhibitor of DNA-dependent-RNA synthesis, and ethionine, an inhibitor of protein synthesis, both effectively decreased the adaptive responses of jejunal glycolytic and folate-metabolizing enzymes to diet and oral sex hormones. These findings lend support to the hypothesis that stimulation of synthesis of certain RNA species is probably one of the primary mechanisms by which sex hormones (17-beta-estradiol and testosterone) and diet produce adaptive increases in the activities of certain jejunal glycolytic and folate-metabolizing enzymes.

Oral 17-beta estradiol and various estrogen-like oral contraceptives produced significant adaptive increases in the activities of a cytoplasmic glycolytic enzyme, pyruvate kinase, and a membrane-bound enzyme, adenyl cyclase. Progesterone and progesterone-like oral contraceptives have no effect on the same two enzymes. These observations suggest that the responses seen in the cytoplasmic glycolytic enzymes might be due to a primary effect of the membrane bound enzyme, adenyl cyclase, which catalyzes the formation of an "intracellular messenger" cyclic AMP. When pharmacologic amounts of nucleotides were administered orally to rats changes were seen in the activities of two cytoplasmic glycolytic enzymes and the membrane-bound enzyme adenyl cyclase. Oral cyclic AMP and dibutyryl cyclic AMP had stimulatory effects on the two glycolytic enzymes, while ATP had an inhibitory effect. Cyclic GMP had a significant effect only on phosphofructokinase activity.

Studies in folate-deficient rats demonstrated that folic acid is necessary for the adaptive response of jejunal pyruvate kinase activity to oral and intramuscular sex hormones. In folate-deficient rats, sex steroids had no stimulatory effect upon jejunal pyruvate kinase activity. In all cases, the activity of each glycolytic enzyme measured was higher on the folate-supplemented diet than on the folate-deficient diet. No adaptive responses were seen in the rats (non-castrate) when the sex steroids were administered intramuscularly.

Adrenalectomy in rats resulted in a marked decrease in activity of three jejunal glycolytic enzymes and one jejunal gluconeogenic enzyme, fructosediphosphatase. However, the responses to different diets (high fructose, glucose and casein) were qualitatively the same in adrenalectomized rats as in normal rats. When corticosterone was administered to the adrenalectomized rats, the responses to different diets were also quantitatively similar to those observed in the normal, non-adrenalectomized rats. In contrast to the jejunum, the adrenal gland appeared to be necessary for normal qualitative adaptive responses of hepatic glycolytic enzymes to diet. The fluoride-stimulated adenyl cyclase activity was higher on the high-carbohydrate diets than on the high-casein diet in both the jejunum and liver.

The anti-malarial drugs (chloroquine, primaquine and dapsone) gave highly variable results upon two jejunal glycolytic enzymes in normal human subjects. In one subject, each of the anti-malarial agents caused a marked decrease in the activity of the two enzymes measured, while in another subject the same agents increased the activities of the same enzymes. Since the prevalence of gastrointestinal symptoms with combined chloroquine-primaquine tablets is variable, it is not surprising that the responses of individual normal human subjects is also variable. However, it should be noted, that these data are preliminary and many assays remain to be done.

Histidine stimulates pyruvate kinase activity of most rat tissues and especially intestine in vitro and in relation to dietary histidine. Extensive studies suggest that this may be a regulatory mechanism for pyruvate kinase activity.

CONCLUSIONS:

Oral and intramuscular sex steroids increased jejunal pyruvate kinase and phosphofructokinase activities in castrate rat jejunum while only oral sex steroids were effective in normal rat jejunum. The oral glucosiduronide forms were also effective as were the estrogen-like oral contraceptive steroid agents. Protein synthesis inhibitors blocked most of the adaptive response of jejunal pyruvate kinase activity to oral sex steroids. Folic acid is necessary for the jejunal response to oral sex steroids, while the adrenal gland is necessary for the normal quantitative adaptive responses of jejunal glycolytic enzymes to diet. Our preliminary data shows that anti-malarial drugs (chloroquine, primaquine and dapsone) cause variable responses of two jejunal glycolytic enzyme activities in normal human subjects.

RECOMMENDATIONS:

Studies concerning regulation of gastrointestinal enzymes have been particularly helpful in elucidating the nature of normal intestinal

metabolism and in uncovering various gastrointestinal disorders, particularly the maladaptation syndrome. Progress should be continued toward elucidating the mechanism of regulation of jejunal enzymes and toward uncovering the biochemical basis of the maladaptation syndrome.

PUBLICATIONS:

1. Stifel, F. B., R. H. Herman and N. S. Rosensweig. Dietary regulation of glycolytic enzymes. IX. The effect of oral, intramuscular and conjugated sex steroids on jejunal glycolytic enzyme activities in normal and castrated male and female rats. Biochim. Biophys. Acta 222: 65, 1970.

2. Stifel, F. B., R. H. Herman and N. S. Rosensweig. Dietary regulation of glycolytic enzymes. X. The effect of oral, intramuscular and conjugated sex steroids on jejunal folate-metabolizing enzyme activities in normal and castrated male and female rats. Biochim. Biophys. Acta 222: 71, 1970.

3. Stifel, F. B., R. H. Herman and N. S. Rosensweig. Dietary regulation of glycolytic enzymes. XI. Effect 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.

4. Stifel, F. B., H. L. Greene and R. H. Herman. Effect of oral contraceptive steroids on jejunal pyruvate kinase and adenyl cyclase activities. Endocrinology. (accepted for publication).

5. Rosensweig, N. S., R. H. Herman and F. B. Stifel. Dietary regulation of small intestinal enzyme activity in man. Amer. J. Clin. Nutr. 24: 65, 1971.

6. Herman, R. H. The effect of diet on jejunal enzymes. Dimensions of Nutrition. J. Dupont, Editor., Colo. Assoc. Univ. Press, 1970, p. 77.

7. Rosensweig, N. S. and R. H. Herman. Dose response of jejunal sucrase and maltase activities to isocaloric high and low carbohydrate diets in man. Am. J. Clin. Nutr. 23: 1373, 1970.

8. Stifel, F. B. and R. H. Herman. Effect of L-histidine on human and rat jejunal pyruvate kinase activity. Fed. Proc. 30: 234, 1971 (abstract).

9. Stifel, F. B. and R. H. Herman. Effect of L-histidine on human jejunal pyruvate kinase activity. Canadian J. Biochem. (accepted for publication).

10. Stifel, F. B. and R. H. Herman. Histidine metabolism. Amer. J. Clin. Nutr. 24: 207, 1971.

11. Anderson, J. W. and R. H. Herman. Treatment of reactive hypoglycemia with sulfonylureas. Amer. J. Med. Sci. 261: 16, 1971.

STUDY NO. 3 Study of patients with endogenous hypertriglyceridemia.

PROBLEM:

Type IV hyperlipemia is a disorder characterized by excessive plasma triglycerides and possibly also excessive plasma cholesterol. The etiology and pathogenesis are unknown, but these patients typically respond to carbohydrate feeding with an elevation of plasma triglycerides (TGL) and respond to carbohydrate restriction and clofibrate therapy with a decrease in TGL. Previous studies with normal subjects have shown that plasma triglycerides increase with sucrose feeding. An initial elevation of TGL occurs, followed by a decrease in TGL almost to normal levels. We have continued our studies in hypertriglyceridemic patients. The mechanisms by which alcohol ingestion causes hyperlipemia in some, but not all, patients with alcoholism are unknown. Data obtained in our laboratory have shown that there is stimulation of jejunal adenyl cyclase activity in rats by alcohol. Thus, we measured blood lipids and jejunal and adipose tissue adenyl cyclase activity in a patient who exhibited both alcoholism and hyperlipemia (Type V).

RESULTS AND DISCUSSION OF THE RESULTS:

A 62-year old retired medical technician who has been treated for hypertension, obesity, and diabetes mellitus has been studied. Hyperlipemia (Type IV) was discovered in December 1970 and studies were carried out in the metabolic ward in February 1971. Sucrose feeding produced a lowering of plasma triglycerides. Clofibrate lowered plasma TGL, jejunal and adipose tissue adenyl cyclase, and also adipose tissue cyclic AMP. Jejunal cyclic AMP varied during clofibrate treatment.

A 43 year old Army sergeant who has had a heavy alcohol intake since age 18 was studied. Turbid serum had been present for at least 5 years, which had recently been shown to result from Type V hyperlipemia. The patient responded to a low-carbohydrate diet with lowering of plasma triglyceride (from 305 to 142 mg/100 ml) and then responded to ethanol ingestion, 30 ml q.i.d. for 7 days, with a rise in plasma triglycerides to 360 mg/100 ml. There was no significant change in jejunal or adipose tissue adenyl cyclase activity following the alcohol feeding.

CONCLUSIONS:

The results confirm our previous suggestion that clofibrate therapy lowers adenyl cyclase activity in jejunum and adipose tissue of patients with Type IV hyperlipidemia. Alcohol feeding elevated plasma TGL, but caused no change in jejunal or adipose tissue adenyl cyclase activity. The mechanisms by which alcohol raises TGL in the patient remain unknown.

RECOMMENDATIONS:

The results to date are sufficienty promising so that more patients should be studied to confirm and extend our observations. Future studies of hyperlipemia should perhaps be restricted to Type IV disorders.

PUBLICATIONS: None.

STUDY NO. 5 The effect of alpha and beta blockade upon the release of growth hormone, insulin and ACTH.

PROBLEM:

The release of certain pituitary hormones in humans is subject to adrenergic influences. In the USAMRNL Annual Report dated 30 June 1970, we described the study of pituitary hormones and insulin release with and without adrenergic blockade by propanolol (a beta blocking agent) and phentolamine (an alpha blocking agent) in 8 normal subjects. Since that report, assay of blood samples for human growth hormone (HGH) and insulin has been completed in all subjects. Thyroid stimulating hormone (TSH) and ACTH assay have been completed in one subject each.

RESULTS AND DISCUSSION OF THE RESULTS:

As noted by others, insulin-induced hypoglycemia results in the release of HGH, which is significantly increased by propanolol and moderately reduced by phentolamine. We have shown for the first time that a brisk release of ACTH also occurs. This response is increased by propanolol and blunted by phentolamine. Plasma cortisol levels reflect the changes in plasma ACTH but are of much smaller magnitude. The recovery from insulin-induced hypoglycemia is delayed by propanolol and hastened by phentolamine. Free fatty acid (FFA) levels are decreased by insulin-induced hypoglycemia. This response is increased by propanolol and abolished by phentolamine.

An arginine infusion causes the release of HGH and insulin, a rise

in blood glucose and a fall in FFA. Adrenergic blockade does not alter these responses in glucose, FFA or HGH. A moderate increase in insulin release was noted with both propanolol and phentolamine, but this was not statistically significant.

Vasopressin injection causes the release of HGH, a rise in blood glucose and a fall in FFA, unchanged by adrenergic blockade. Vasopressin causes no release of insulin. Piromen injection causes the release of HGH, a fall in FFA and no change in blood glucose or insulin. These changes were unaffected by adrenergic blockade. Isoproterenol inhalation causes a brisk rise in FFA, a small rise in glucose, a small rise in insulin and a small decrease in HGH.

The results add further support to the concept that adrenergic activity modulated the release of certain pituitary hormones. In particular, beta blockade increases the release of HGH and ACTH, and alpha blockade does the reverse.

CONCLUSIONS:

The release of HGH and ACTH in humans after insulin-induced hypoglycemia, but not after other stimuli, is modulated by adrenergic blockade with propanolol or phentolamine infusions.

RECOMMENDATIONS:

Assay of more samples from this study for ACTH and TSH should be performed, if this can be arranged. The recent demonstration that L-DOPA releases HGH in patients with Parkinson's disease suggests further studies in animals or humans, in which L-DOPA could be given with adrenergic blocking drugs and the release of pituitary hormones tested.

PUBLICATIONS: The results are being prepared for publication.

STUDY NO. 6 The effect of clomiphene on serum FSH and LH levels and sperm production in male hypogonadism.

PROBLEM:

We have been studying men who have idiopathic oligospermia. These men have persistently subnormal sperm counts (less than 20 million/ml), no abnormality in thyroid function, normal appearing testicular biopsy, and are otherwise in good health. They have been unsuccessful in impregnating their wives who have no abnormalities of the reproductive tract. This study was initiated in 1970 to learn whether these men

have any abnormality of plasma FSH and LH levels, and to learn whether they would respond to long-term therapy with clomiphene citrate (50 mg daily by mouth for 90 days).

RESULTS AND DISCUSSION OF THE RESULTS:

Five men have entered the study to date. One dropped from the study after one month when he left the army. One man has not yet completed the study. Three men have completed the study. Of the three men who completed the study, two impregnated their wives. One of these wives delivered a healthy baby in December 1970. The other expects delivery in December 1971. This wife became pregnant approximately 4 months after her husband's clomiphene was stopped, raising the question whether rebound increase in spermatogenesis occurred after discontinuance.

CONCLUSIONS:

It is of great interest that pregnancy occurred in three couples related to the use of clomiphene citrate by the husbands. Since no hormonal studies will be performed until more patients have finished the study, conclusions cannot be made now.

RECOMMENDATIONS:

We recommend that the study be continued until at least 10 men have been treated.

PUBLICATIONS: None.

STUDY NO. 7 The effect of testosterone on jejunal glycolytic enzyme activities in male hypogonadism.

PROBLEM:

We have shown previously that oral sex hormones (estradiol in females, and testosterone in males) produce increased activity of jejunal pyruvate kinase (PK) in both castrate and non-castrate rats. Intramuscular (IM) sex steroids were effective only in castrate animals. Because of these findings in animals the effect of testosterone on jejunal enzyme activities was studied in humans with testosterone deficiency due to hypogonadism.

RESULTS AND DISCUSSION OF THE RESULTS:

Four men with Klinefelter's syndrome and 3 men with other forms of

hypogonadism were studied. Jejunal tissue was obtained by peroral biopsy capsule. The activities of glycolytic enzymes after isocaloric diets: control (25% glucose, 25% fructose, 20% protein, 30% fat); high glucose (50% glucose); high fructose (50% fructose); and control diet plus oral testosterone (10 mg/day for 2 days) or IM testosterone (100 mg/day for 2 days) were measured.

In comparison with the normal men studied previously, the hypogonadal patients showed significantly decreased activities of jejunal PK, fructose-1-phosphate aldolase (F1PA) and fructose-diphosphate aldolase (FDPA) and no change of hexokinase (HK). Both normal and hypogonadal men showed markedly increased activity of PK after oral testosterone; hypogonadal men showed moderately increased activity of PK after IM testosterone. The hypogonadal men showed less adaptive enzyme changes to dietary manipulation than did the normals.

CONCLUSIONS:

Testosterone-deficient adult males have decreased activities of certain jejunal glycolytic enzymes. Activity of PK is increased moderately by IM testosterone and markedly by oral testosterone, as previously shown in the rat. Jejunal biopsy provides a useful means of studying the effect of hormones on human enzyme activities.

RECOMMENDATIONS:

Since hypogonadal patients have reduced adaptive response of jejunal enzymes to dietary changes, we recommend further studies to determine whether prolonged testosterone administration to such patients will improve their adaptive response.

PUBLICATIONS:

1. Lufkin, E. G., F. B. Stifel and R. H. Herman. Effect of testosterone on jejunal pyruvate kinase (PK) activity in normal and hypogonadal males. The Endocrine Society (Program of the Fifty-Third meeting) June 1971 (Abstract #362).

STUDY NO. 8 — Study of a patient with severe anxiety, tachycardia, syncope and a family history of sudden and/or unexplained death.

PROBLEM:

A 26-year old white female was studied who had a long and puzzling history of progressively more frequent and severe anxiety, fear,

tachycardia, faintness, weakness, diarrhea, syncope, mild hypertension, and episodes of hypotension. Extensive studies failed to reveal the nature of her disease. Her family history was most significant in that her father had similar symptoms, used alcohol to excess, had mild diabetes and hypertension and died suddenly at age 56. A meat impaction was found but there is reason to believe that he collapsed first and then aspirated a bolus of food. Autopsy was quite unrevealing. Iron deposition in several organs and a small adrenocortical adenoma were found. A paternal uncle died suddenly at age 51 after an 11 year history of episodic tachycardia, anxiety, pain and hallucinations. Autopsy was unrevealing. Two other paternal uncles died at ages 49 and 52 respectively. Another paternal uncle has tachycardia and unexplained syncope. A paternal aunt has tachycardia and multiple unexplained symptoms. A cousin has tachycardia as do 2 brothers.

RESULTS AND DISCUSSION OF THE RESULTS:

Careful studies revealed the following pertinent findings. A glucose tolerance test (GTT) was abnormal. Her ECG became quite abnormal during the test showing unusual ST segment-wave changes and the degree of change being roughly proportional to the blood glucose level. On a few occasions, 24 hour urinary total catecholamines were slightly elevated. Epinephrine levels were greater than norepinephrine levels which is a reversal of the normal situation. Throughout hospitalization she had persistent tachycardia of 120 beats/min. and mild hypertension. Severe anxiety and fear of death occurred frequently. A tyramine test produced significant hypertension but a glucagon test was negative. Studies of control patients and subjects showed little if any ECG change during a GTT and no response to tyramine by an identical procedure. Surgery was done in the summer of 1970. No adrenal tumor could be found. The left adrenal and 1/2 of the right adrenal were removed. The post-operative course was uneventful. The patient has been maintained on cortisone and extra dietary NaCl without difficulty. Just prior to surgery propranolol and dibenzyline relieved all symptoms. During the past year the tachycardia, syncope, diarrhea, faintness and severe anxiety have disappeared. Anxiety of a milder degree is present. The patient has gained increased strength and self-confidence and has done increasingly well. Retesting at 6 months showed an abnormal GTT with the same ECG changes but to a lesser degree than prior to surgery. A tyramine test was negative. A second post-operative retesting is in progress. This would appear to be a new entity of either increased secretion of epinephrine or an epinephrine-like substance and/or increased sensitivity to epinephrine. Since epinephrine is made exclusively in the adrenal medulla surgery should be expected to effect a cure. This patient has been transformed from a completely disabled patient to an almost normal functioning person.

CONCLUSIONS:

This patient probably represents a new disease entity which may be due to over-secretion of epinephrine or an epinephrine-like substance. The continued secretion leads to a progressively worsening state which then causes otherwise unexplained death.

RECOMMENDATIONS:

This patient's family should be studied. The long-term physiological effects of epinephrine should be investigated.

PUBLICATIONS: None.

STUDY NO. 9 The effect of dietary fructose on jejunal sucrase in a patient with sucrase-isomaltase deficiency.

PROBLEM:

Previous studies in this laboratory have shown that jejunal disaccharidases, excepting lactase, may be increased with certain dietary sugars. Sucrase activity can be significantly increased with dietary fructose. Patients with jejunal sucrase deficiency are usually infants or children who develop diarrhea and abdominal pain when only small amounts of dietary sucrose are ingested. Since many foods have added sucrose, it is difficult to treat patients with sucrose-free diets. For this reason a child with intestinal sucrase-isomaltase deficiency was given a diet high in fructose to test the response of jejunal sucrase. As a measure of the therapeutic effectiveness of fructose jejunal disaccharidases were measured and oral sucrose tolerance tests were performed. During the tolerance tests, urinary and fecal sucrose measurements were done as a measure of the ability of the gut to hydrolyze dietary sucrose.

RESULTS AND DISCUSSION OF THE RESULTS:

Synthetic diets containing 0%,40%,50% or 60% of the calories as fructose were fed to total 1200 Calories a day. On the fructose diets jejunal sucrase activity increased to 3 times that seen during the no fructose period. Concommitantly there was an improvement in two sucrose tolerance tests. There were fewer symptoms, an increase in blood glucose and a decrease in the amount of sucrose excreted in the urine and feces. When jejunal tissue from the patient was mixed with normal tissue there was no evidence of a sucrase inhibitor being present in the patient's tissue. Following the study, the patient was returned to an ad libitum sucrose-deficient diet in which fructose was the

source of all sugar. Despite the fact that she takes sucrose occasionally the patient has remained symptom-free for 6 months on the high fructose diet. These studies suggest that an abnormal enzyme is made which is only partially active. Thus, fructose can cause a larger amount of the protein to be synthesized and therefore the active portion is also increased in quantity. The biochemical defect in sucrase deficiency is probably at the level of enzyme protein synthesis.

CONCLUSIONS:

1. A patient with sucrose deficiency fed a high fructose diet had a qualitatively normal increase in jejunal sucrase activity.

2. The increase in jejunal sucrase activity was coincident with a clinical and biochemical improvement in sucrose tolerance tests.

3. It is suggested that patients with sucrase deficiency produce an abnormal enzyme in normal amounts which is only partially active. By stimulating more protein production with fructose, there is a concommitant increase in the amount of enzymatically active protein.

RECOMMENDATIONS:

This study has suggested that an abnormal protein is produced in sucrase deficiency, perhaps an amino acid substitution. Studies should be extended to identify the nature of enzyme. This might be done by immunologic techniques since many enzymatically inactive enzymes can still react with antibody. Conceivably a similar approach can be tried in lactase deficiency which is a more common disease.

PUBLICATIONS:

1. Greene, H. L., F. B. Stifel, R. H. Herman. Dietary stimulation of sucrase in a patient with sucrase-isomaltase deficiency. Lancet I: 651, 1971 (Letters to the Editor).

STUDY NO. 10 The effect of isocaloric carbohydrate-free diets and diets containing either sucrose, fructose or glucose on oral carbohydrate tolerance (physiologic carbohydrate intolerance).

PROBLEM:

It has been observed repeatedly that some people have a transient period of food intolerance immediately following acute diarrheal episodes. Similar observations have been made in patients who have been fasted for more than a week and in starving individuals upon refeeding. During periods of a low carbohydrate intake or fasting jejunal disaccharidase activities decrease over a period of 3 days. Jejunal glycolytic enzyme activities decrease within 24 hours on a low carbohydrate intake. Conversly, the same disaccharidases and glycolytic enzymes may be increased significantly by giving diets high in fructose or by treatment with oral folic acid. We have found a group of patients with chronic diarrhea who show poor carbohydrate tolerance and an inability to increase their jejunal glycolytic enzymes. The relationship between the poor glycolytic enzyme response and carbohydrate intolerance is not known. If there is a direct relationship between the glycolytic enzyme levels and carbohydrate intolerance, then fasting or a carbohydrate-free diet should decrease jejunal glycolytic enzyme activities to the levels seen in patients with carbohydrate intolerance. There should also be a transient period of poor carbohydrate tolerance similar to that seen in patients whose glycolytic enzymes cannot adapt normally. To test this hypothesis, 4 normal male subjects were fed an isocaloric carbohydrate-free diet during one period and a diet consisting of 50% of calories as equal amounts of fructose and glucose. Jejunal disaccharidase activities and glycolytic enzyme activities were measured during both periods. Tolerance to acute loads of sucrose, glucose and fructose was tested at the end of each dietary period.

RESULTS AND DISCUSSION OF THE RESULTS:

There was a significant decrease in disaccharidase activities while on the carbohydrate free diet. Although two of the subjects complained of mild abdominal pain and several loose stools following the sucrose tolerance test, there was no statistical difference between the blood sugar and urine sucrose concentrations between the two treatment periods.

There was a significant decrease in glycolytic enzyme activities during the carbohydrate-free period. There was a significant increase in blood sugar and plasma insulin levels during the carbohydrate-free diet after challenge with both glucose and fructose. The most striking finding was the development of symptoms with both glucose and fructose during the carbohydrate free period. The symptoms consisted of abdominal pain, flushing, dizziness, sweating, nausea, blurred vision and one or two watery stools within 30 to 45 minutes following the sugar load. These symptoms are identical to those described by patients with an inability to increase their glycolytic enzyme

activities in response to diet (the G.I. maladaptation syndrome).

With reinstitution of the diet with 50% of the calories as equal amounts of glucose and fructose, the glycolytic enzymes returned to control levels. There were no untoward symptoms with glucose and fructose loads and the blood sugar and plasma insulin levels were normal. These studies suggest a cause and effect relationship between human jejunal glycolytic enzymes and gastrointestinal symptoms.

CONCLUSIONS:

1. Ingestion of a carbohydrate-free diet causes a significant decrease in jejunal disaccharidase activities (except lactase), as compared to an isocaloric diet in which 50% of the calories consist of equal amounts of glucose and fructose.

2. There was significant decrease in jejunal glycolytic enzymes during the carbohydrate free diet.

3. When the subjects were challenged with sucrose there was some increase in symptoms during the carbohydrate free period, however the blood glucose and total urinary sucrose excretion did not change significantly with the two diets.

4. When the subjects were challenged with glucose or fructose, there was a significant increase in symptoms during the carbohydrate-free period. There was evidence also of decreased glucose and fructose tolerance as reflected by blood sugar and insulin concentrations.

5. The symptoms the subjects described with glucose and fructose while on the carbohydrate-free diet were identical to those described by the patients with an inability to adapt their jejunal glycolytic enzymes to diet.

6. It is suggested that there is a cause and effect relationship between the level of jejunal glycolytic enzymes and gastrointestinal disease.

RECOMMENDATIONS:

Since the jejunal glycolytic enzymes can also be increased by folic acid, sex hormones and cyclic nucleotides we plan to use these agents during a carbohydrate-free period to see if the clinical symptoms and the biochemical evidence of poor carbohydrate tolerance can be reversed.

PUBLICATIONS: None.

Metabolic Response of Man to Nutrition or Disease (Cont'd)

STUDY NO. 11 Study of the adaptive responses of jejunal glycolytic enzymes in patients with dermatitis herpetiformis.

PROBLEM:

It has been found that patients with dermatitis herpetiformis may or may not have small intestinal lesions that resemble those seen in celiac disease (gluten enteropathy). Treatment of such patients with a gluten-free diet has given variable responses: no change in the skin lesions, clearing of skin lesions on lower dosages of medication, and clearing of skin lesions without additional therapy. Some of the patients with jejunal lesions have steatorrhea but not all. Some of the symptomatic patients respond to a gluten-free diet but not all of the patients with steatorrhea. We investigated 3 patients with dermatitis herpetiformis to determine if gastrointestinal lesions were present and if jejunal glycolytic enzymes responded to diet or folic acid.

RESULTS AND DISCUSSION OF THE RESULTS:

All 3 patients had no gastrointestinal symptoms whatever. One patient had no histological lesion and had normal glycolytic enzyme adaptive responses. A gluten-free diet was tried empirically but it seemed to have little effect on her skin lesions. The other 2 patients had abnormal histology of the jejunum and abnormal adaptive responses of the glycolytic enzymes. A trial of a gluten-free diet in each patient did not affect either the skin lesions, the jejunal histology or the glycolytic enzyme adaptive responses. The nature of the jejunal lesions in dermatitis herpetiformis and the relationship to the skin lesions remains unknown.

CONCLUSIONS:

Abnormal jejunal histology and glycolytic enzyme adaptive responses were found in 2 of 3 patients with dermatitis herpetiformis. A gluten-free diet did not have any therapeutic effect. The relationship of the skin lesions to the jejunal lesions remains unknown.

RECOMMENDATIONS:

Further investigations need to be performed in dermatitis herpetiformis to determine the relationship of the jejunal and skin lesions.

PUBLICATIONS: None.

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, oral folic acid, and a variety of drugs. An understanding of these responses in normal individuals has provided a basis against which the responses of patients with various gastrointestinal symptoms may be compared. We have continued to evaluate the effects of various diets, folic acid, and different drugs on jejunal glycolytic enzyme activity in patients with gastrointestinal disease and have attempted to relate their chronic symptomatology to differences in jejunal enzyme responses.

RESULTS AND DISCUSSION OF THE RESULTS:

Nine patients with "functional" gastrointestinal disease were studied. Several of these were studied in FY 70 and are discussed in the annual report of that year. All had a long history of diarrhea with other gastrointestinal complaints variably present or absent. Most had undergone repeated evaluations including multiple radiographic studies which were normal. All had normal "routine" laboratory studies, and normal jejunal mucosal morphology. Most had been treated with a variety of symptomatic regimens with moderate to no control of symptoms. These patients were evaluated in the same manner on the metabolic ward. All underwent multiple small bowel biopsies to evaluate glycolytic enzyme activity on a variety of diets and therapeutic regimens. All showed varying degrees of failure of glycolytic enzyme adaptation to diet and/or oral folic acid. In most, a high carbohydrate diet provoked obvious worsening of the diarrhea. In all there seemed to be improvement with oral folic acid and carbohydrate restriction. In some of these patients prolonged carbohydrate restriction has provided continuing freedom from diarrhea, and diminution in other gastrointestinal complaints.

Special adjunctive studies were conducted in some of these patients. An *in vitro* guinea pig ileum preparation was set up in a standard fashion. The guinea pig ileum is an extremely sensitive tissue and responds vigorously to low concentrations of a variety of vasoactive and myoactive substances. The response of the system was standardized with histamine (@ 1 μg). The response of the guinea pig ileum was then measured following addition of a small amount of the homogenate of patient's jejunum obtained at biopsy. It was found that the jejunal biopsy from 2 patients contained a material which markedly stimulated the guinea pig ileum. Tissue from other patients and from normal

subjects had no effect on the guinea pig ileum preparation. Studies are in progress to characterize the stimulatory material from patient's jejunal tissue.

CONCLUSIONS:

1. Chronic diarrhea may be the result of jejunal mucosal enzyme abnormalities which render the patient sensitive to dietary carbohydrate.

2. Elimination of dietary carbohydrate in certain patients may result in improvement of the diarrhea.

3. The intestine of some patients with diarrhea contains a substance which stimulates guinea-pig ileum in vitro and which may be related to the hypermotility seen in these patients.

RECOMMENDATIONS:

The relationships between dietary intake and acute and chronic diarrhea are aspects which require further study and understanding. The influence of diet and drugs on gastrointestinal enzyme function and the nature of controlling and regulating mechanism all warrant further investigation. Finally, the apparent link between gastrointestinal enzyme activity and chronic gastrointestinal symptoms provides clues that may help to establish the etiology of other so called "functional" gastro-intestinal disease.

PUBLICATIONS: None.

STUDY NO. 13 Clotting defects in fasting patients.

PROBLEM:

Coagulation problems in chronically ill patients have been noted frequently, usually involving the vitamin K-dependent clotting factors. Four of our patients who bled after jejunal biopsy were fasting. One of these was found to have prolonged bleeding and partial thromboplastin times and a factor IX deficiency. This study was done to determine if fasting leads to deficiencies of various clotting factors.

RESULTS AND DISCUSSION OF THE RESULTS:

One patient was fasted for two 3-week periods separated by a 3 week period using an 800 Calorie diet. This patient developed prolonged partial thromboplastin, prothrombin and bleeding times at the end of the first and second fast. The defects rapidly were corrected upon refeeding. This data is very suggestive that fasting leads to coagulation defects in man.

Metabolic Response of Man to Nutrition or Disease (Cont'd)

CONCLUSIONS:

Fasting is apparently related to the development of coagulation defects.

RECOMMENDATIONS:

This area should receive further study.

PUBLICATIONS: None.

STUDY NO. 14 Effect of riboflavin deficiency.

PROBLEM:

An assay for flavokinase utilizing ^{14}C-riboflavin has been developed in our laboratory. In conjunction with a vitamin B_2 deficiency study utilizing normal subjects measurements of jejunal flavokinase were performed to determine the effect of riboflavin deficiency on this enzyme.

RESULTS AND DISCUSSION OF THE RESULTS:

The flavokinase activity of jejunal tissue of 6 normal subjects on two levels of dietary protein were measured during a control period, after 8 weeks of deficiency and after 2 weeks of refeeding with riboflavin. The activity of the enzyme did not change through these periods. These results are different from those found in the rat liver where a decrease in the enzyme activity occurs. In human red cells the activity is increased during the deficient period. These results suggest that flavokinase responds differently to riboflavin deficiency in different species.

CONCLUSIONS:

In the jejunum of man there is no change in flavokinase activity during periods of riboflavin deficiency.

RECOMMENDATIONS: None.

PUBLICATIONS: None.

STUDY NO. 15 Flavokinase activity in Acrodermatitis Enteropathica.

Metabolic Response of Man to Nutrition or Disease (Cont'd)

PROBLEM:

In the rare disease entity, Acrodermatitis enteropathica, a possible relationship to riboflavin deficiency was suggested by the similarity in the clinical appearance of the lesions to those in monkeys made riboflavin deficient. To test this hypothesis a ^{14}C-riboflavin assay for flavokinase was developed and tested in this condition and compared to normal control subjects.

RESULTS AND DISCUSSION OF THE RESULTS:

The flavokinase activities of jejunum and red blood cells of a patient with acrodermatitis enteropathica during and after diodoquin therapy and in normal human subjects were determined. No decrease in activity of the patients flavokinase was found compared to normal controls. These results negate the hypothesis that a flavokinase deficiency is responsible for the clinical findings in acrodermatitis enteropathica.

CONCLUSIONS:

Flavokinase deficiency is not present in acrodermatitis enteropathica.

RECOMMENDATIONS: None.

PUBLICATIONS: None.

STUDY NO. 16 The *in vitro* effect of alcohol on adenyl cyclase activity.

PROBLEM:

Ethanol has been used for a number of years, both for its nutrient value (7 Kcal/gm) and as an intoxicating agent. Its mechanism of action largely remains unknown. Recent observations have suggested that certain changes in liver triglycerides and glycogen, the urinary excretion of adrenal steroids, blood glucose and insulin concentrations that occur after chronic or acute ethanol ingestion may also be seen with agents that are known to cause increased tissue cyclic AMP (cAMP) concentrations. This suggested that some of the effects of *in vivo* ethanol might result from the activation of adenyl cyclase. To test this hypothesis varying amounts of ethanol were added to a tissue preparation containing adenyl cyclase. Assays were performed on human intestine and rat kidney, intestine, liver, fat and brain.

RESULTS AND DISCUSSION OF THE RESULTS:

Rats weighing between 200 and 250 gm were sacrificed, tissues immediately

removed and prepared for the assay of adenyl cyclase. Several short chain alcohols were found to be capable of stimulating rat intestinal adenyl cyclase activity with methanol being the most potent. The stimulation of cAMP varied inversely as the number of carbons in the alcohol increased. Other rat tissue adenyl cyclases were stimulated by ethanol but not to the degree seen in intestinal tissue. Human intestinal adenyl cyclase activity was stimulated by ethanol. However, maximal activity was obtained only after homogenization of the tissue with the detergent Lubrol WX. These studies suggest that some of the physiologic effects of ethanol may be mediated through its activation of adenyl cyclase and the resultant increase in tissue cyclic AMP concentrations.

CONCLUSIONS:

1. Several short chain alcohols are capable of stimulating rat intestinal adenyl cyclase.

2. Ethyl alcohol gives maximal stimulation in rat intestine but shows some stimulation in liver, kidney, brain and fat.

3. Ethyl alcohol gives some stimulation of human intestinal adenyl cyclase but not to the same degree as seen in rat intestine.

4. Some of the mechanisms of the physiologic effects of ethyl alcohol are not known at present. These data suggest that at least one mechanism of action of ethanol may be via the activation of adenyl cyclase.

RECOMMENDATIONS:

Similar studies should be extended to in vivo analysis of cyclic AMP concentrations in response to ethanol in both animals and humans. This would help further characterize the mechanism of action of ethanol.

PUBLICATIONS:

1. Greene, H. L., Herman, R. H., Kraemer, S. The effect of short chain alcohols on rat tissue adenyl cyclase. Central Society for Clinical Research 43: 94, 1970 (abstract).

2. Greene, H. L., Herman, R. H., Kraemer, S. Stimulation of jejunal adenyl cyclase by ethanol. J. Lab. Clin. Med. (In press).

STUDY NO. 17 Study of a patient with possible hyperaldosteronism.

Metabolic Response of Man to Nutrition or Disease (Cont'd)

PROBLEM:

A 41-year old married Negro mess sergeant was referred to the endocrinology service because of hypertension and hypokalemia. He had not used thiazide diuretics for four years, and thus it was necessary to consider hyperaldosteronism as a cause of his hypertension. Because of the need to control his sodium intake it was necessary to complete the studies under careful supervision in the metabolic ward.

RESULTS AND DISCUSSION OF THE RESULTS:

The studies on the metabolic ward revealed persistent hypertension despite rather large doses of Aldomet (500 mg q.i.d.) with pressures averaging about 150/105, accompanied by gr. ii retinopathy and diffuse ST-T changes on EKG. However, he remained asymptomatic. Mild hypokalemia and hypernatremia persisted. On a high sodium intake the aldosterone secretion rate and plasma aldosterone were considerably elevated and plasma renin was absent. On low sodium intake (8 mEq/day) the plasma aldosterone and renin was unchanged and unaffected by quiet standing for two hours. His urine sodium at this time was inappropriately high. There was no significant suppression of aldosterone secretion by DOCA, 10 mg b.i.d. IM. Samples of adrenal vein blood revealed slightly elevated plasma aldosterone from the left adrenal vein. No tumor was seen on IVP. Lasix, 40 mg, orally, caused no change in plasma renin or aldosterone. These results suggested aldosteronism, and because of failure of suppression of aldosterone excretion by DOCA, an adrenal cortical adenoma was suspected. During surgery the adrenals were found to be finely nodular but normal in size and consistency with no sizable nodules or adenoma formation. The entire left adrenal and one-half of the right were removed. Histology revealed diffuse hyperplasia of the zona glomerulosa as the major abnormality.

Postoperatively, his blood pressure, EKG, serum sodium and potassium returned to normal. Repeat studies in the metabolic ward in March 1971, revealed very low plasma aldosterone and normal plasma renin, both of which increased with quiet standing and Lasix. His blood pressure has slowly risen and now requires low doses of medication for control. His glucose tolerance was abnormal both pre- and postoperatively, as were plasma insulin levels. (The plasma and urine aldosterone studies were kindly performed by Dr. Fred Katz, University of Colorado Medical Center).

CONCLUSIONS:

Isolated hyperplasia of the zona glomerulosa is a rare cause of hyperaldosteronism, and has not previously been shown to respond to DOCA by non-suppression of aldosterone secretion.

Metabolic Response of Man to Nutrition or Disease (Cont'd)

RECOMMENDATIONS:

The patient should be followed carefully and watched for recurrence of hyperaldosteronism.

PUBLICATIONS: In preparation.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6357	71 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
70 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 1412A (2)			

11. TITLE (Precede 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)
FISCAL YEAR PRECEDING 71	1.0	60
FISCAL YEAR CURRENT 72	.5	54

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: DA

NAME:

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military Research Projects; (U) Radioisotopes; (U) Instrumentation; (U) Data Acquisition

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

23. (U) To develop and adapt new concepts in radioisotope techniques to research programs, and provide radioisotope support to all projects requiring the use of radioisotopes within the laboratory. 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 MRNL mission.

24. (U) Radioisotope procedures will be developed or adapted by direct innovation or revision of existing techniques. Eight automatic sample changing radiation detection instruments operate continuously to detect beta or gamma radiation. Total capacity is approximately 1,000 samples. All aspects of the radiological protection program as required by AEC licensure are conducted.

25. (U) 70 07 - 71 06 In collaboration with the Computer Division, the existing computer programs for quench correction of samples counted in the liquid scintillation counters have been reviewed, updated and standardized. Current efforts are directed toward development of programs to allow investigator specified computations beyond "disintegrations/minute" conversions, directly from the counting tape output.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 64 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO.	3A062110A822	Military Internal Medicine
WORK UNIT NO.	079	Radioisotope Support for Military Medical Research

Radioisotope support is currently being provided for twenty-six (26) investigators who are performing military medical research involving animals and human subjects. The studies are being performed within USAMRNL, at the Pikes Peak Laboratory Facility and in coordination with universities. Radioisotope Branch instrumentation includes 6 liquid scintillation counters, one gamma counter, a sample oxidizer, and several monitoring instruments.

BODY OF REPORT

WORK UNIT NO. 079 Radioisotope Support for Military Medical Research

PROBLEM:

The precision and sensitivity of metabolic and biochemical studies can be greatly enhanced by the use of radioisotope procedures. The Radioisotope Branch is responsible for the support of such procedures by orderly procurement and disposal of radioisotopes and related supplies, maintaining health protection procedures, providing for the efficient utilization and maintenance of radiation detection instruments, and providing advice and counsel to investigators regarding the use of radioisotopes.

RESULTS AND DISCUSSION OF THE RESULTS:

Twenty-six investigators within USAMRNL are currently listed as authorized radioisotope users. Numerous 14-carbon and 3-hydrogen labeled organic compounds are procured and counted in one of the 6 liquid scintillation counters which are maintained in the Branch. A Packard Model 305 sample oxidizer is employed in the preparation of some of the samples for counting. One crystal scintillation counter is also maintained for the use of investigators employing gamma emitting radioisotopes in their research.

Five of the liquid scintillation counters are equipped with teletype and punch tape printout, and computer programs are currently available for counting efficiency corrections. The original computer programs were developed with relatively specific aims for use of each machine. In FY 71 we have collaborated with the Computer Division to standardize to a single program capable of receiving data from the various instruments and making the computations appropriate for that instrument and counting solution. Also, Radioisotope Branch personnel have taken on the added responsibility of converting the tapes, delivering them to the Computer Division with appropriate instructions, picking up the results and having them available to the radioisotope user with his counted samples. As a result, the computer program is being more widely utilized by more personnel than prior to the revisions.

CONCLUSIONS AND RECOMMENDATIONS:

The use of radioisotopes is considered to be essential to the mission of the Laboratory. The centralized facility being

employed would appear to be the most efficient approach to providing radioisotope related services to the Laboratory as a whole. The only disadvantage to the system that is apparent is the inconvenience of investigators carrying their samples to the central area for counting. The advantages include the following:

a. Radioisotopes can be made available to investigators with approved protocols on 3-4 days notice instead of 3 to 4 weeks or more through usual supply channels. Such procedures would not be practical without the centralized control being employed.

b. Since all counters are centrally located, investigators seldom have to wait for the use of a counter in spite of the near capacity operation of the counters. The same degree of availability in a dispersed system would require several more counters at a cost of approximately $16,000 to $22,000 each.

c. Computer use to save personnel time is more practicable with the centralized system since only the Radioisotope Branch personnel need to be familiar with the procedures and programs, whereas each of the 26 users, and their technicians, or at least a member of each division, would need such knowledge if the facilities were dispersed.

d. Operation of the centralized counting facility helps justify the maintenance of a more highly trained staff and thus provides better monitoring and health protection procedures than might be possible otherwise.

It is recommended that the current policy of centralized radioisotope service facilities be maintained.

PUBLICATIONS:

Whitten, B. K., Beecher, G., Liddle, C. G., and O'Barr, T. P.: A Comparison of $^{14}CO_2$ Trapping Agents used in *in vitro* metabolic studies. USAMRNL Report #324, September 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6360	70 12 31	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. ORD'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 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	080
b. CONTRIBUTING	62156011	3A025601A822	00	
c. CONTRIBUTING	CDOG 1412A (2)			

11. TITLE (Provide with Security Classification Code)

(U) Virologic Clinical Research - Respiratory Viruses and Vaccines (06)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

010100 Microbiology; 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 09	70 12 31	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: 70	1.0	20
FISCAL YEAR CURRENT: 71	.3	4

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 (Provide name, title, and business address, telephone)
NAME: Eller, J. J.
TELEPHONE: 303 366 5311 X24234
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME: Seiber, O., LTC, MC
NAME: Moran, K. J. DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Provide each with Security Classification Code) (U) Myxovirus; (U) Vaccines; (U) Fluorescent Antibody Technic; (U) Hemagglutination; (U) Microscopy; (U) Electron; (U) Kinetics; (U) Immunity

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

23. (U) Vaccines are often the only available approach in combating viral diseases in military populations. The nature of the protective immune response to vaccines is not understood, especially the failure of certain virus vaccines intended to protect against respiratory disease--the principal disease category causing loss of men from active duty. Some inactivated vaccines, i.e., respiratory syncytial virus, measles, contain agents that may injure human beings. Purification of the vaccines, development of protective measures and basic mechanistic studies should be pursued.

24. (U) The nature of the immune response evoked by respiratory virus vaccines is studied to determine if there is a difference in the serum or secretory immune response evoked by vaccine as compared to natural infection and if cell-bound immunity is different.

25. (U) 70 07 - 70 12 Due to loss of the principal investigator on 30 Jun 70 and non-replacement of virology capability, this study is terminated. Progress appears in USAMRNL annual reports (1968-70) and the publications: Eller, J. J. Inactivated myxovirus vaccines, atypical illness and delayed hypersensitivity. J. Pediatrics 74: 664, 1969. Fulginiti, V. A., Eller, J. J., et al. Respiratory virus immunization. 1. A field trial of two inactivated respiratory vaccines; an aqueous trivalent para-influenza vaccine and an alum precipitated respiratory syncytial virus vaccine. Am. J. Epidemiol. 89: 435, 1969. Nagahama, H., Eller, J. J., et al. Direct immuno-fluorescent studies of infection with respiratory syncytial virus. J. Infect. Dis. 122: 260, 1970. Additional published reports, on the basis of six abstracts submitted to national scientific meetings, are expected to appear in the future. Also, see progress reports (1968-70), Univ. of Colorado Med. Ctr. Project: "Respiratory Virus Vaccine Evaluation and Surveillance," contract No: PH-43-62-477, Vaccine Development Branch, NIAID, NIH, Bethesda, Md., as MAJ Eller was project coordinator.

* Applicable to Contractors only (Optional)

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

ABSTRACT

PROJECT NO. 3A062110A822 Military Internal Medicine

WORK UNIT NO. 080 Virologic Clinical Research - Respiratory Viruses and Vaccines

The following investigations have been conducted under this work unit. (This is a final report.)

STUDY NO. 7 Hong Kong Influenza virus IgE antibody

Repetition of studies to substantiate the protective effect of IgE against infection of monkey kidney tissue culture cells with Hong Kong A_2 influenza virus confirmed results of prior experiments. However, IgE overlay followed by additional overlay of myeloma IgE did not maintain the protective effect as when Ige overlay of tissue cells immediately preceded virus challenge.

BODY OF REPORT

WORK UNIT NO. 080 — Virologic Clinical Research - Respiratory Viruses and Vaccines

STUDY NO. 7 — Hong Kong influenza virus IgE antibody

PROBLEM:

Is Ige, in secretions and sera of atopic allergy patients as a consequence of either natural influenza infection or intranasal immunization with influenza vaccine, capable of fixing in vitro to primary rhesus monkey kidney (MK) cells and preventing infection by subsequent homologous challenge?

RESULTS AND DISCUSSION OF THE RESULTS:

Eller (Annual Report 1970) demonstrated that specific Ige influenza antibody in respiratory sections and sera was capable of fixing to MK cells and inhibiting growth of a subsequent virus challenge. Repeat studies have confirmed this observation. However, IgE overlay followed by addition overlay of myeloma IgE did not maintain the protective effect as when IgE overlay of tissue cells immediately preceded virus challenge.

CONCLUSIONS:

The exact mechanism of virus growth inhibition at the cell surface by specific influenza IgE is yet unclear.

RECOMMENDATIONS:

This study and work unit have been terminated because of loss of the senior investigators.

PUBLICATIONS:

Marks, M. I. and J. J. Eller. Intradermal influenza immunization. Experience with Hong Kong vaccine. Am. Rev. Resp. Dis. 103: 579, 1971.

Nagahama, H., J. J. Eller, V. A. Fulginiti and M. I. Marks. Direct immunofluorescent studies of infection with respiratory syncytial virus. J. Infect. Dis. 122: 260, 1970.

Newcomb, R. W. and J. J. Eller. Nasal immunization of asthmatic children with killed influenza virus. J. Allergy 47: 229, 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6364	71 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
70 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 1412A (2)			

11. TITLE (Procede 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)
FISCAL YEAR PRECEDING 71	4.0	54
FISCAL YEAR CURRENT 72	6.0	51

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
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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: Messa, C. J., CPT, MSC
TELEPHONE: 303 366 5311 X25130
SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS
NAME: Bougie, M. L., PFC
NAME: Goforth, H. H., PFC DA

21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Procede 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. Procede 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 GE 600 Remote teletype terminal and a Mathatron 4280A 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. Study No. 2 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. Study No. 3 will make mathematical and statistical services and consultations available to laboratory investigators.

25. (U) 70 07 - 71 06 Study No. 1 - A library of mathematical, statistical and utility routines has been written and added to the Fortran library. An easy-to-use statistical system has been develped for use by research investigators. The Fortran compiler has been modified to accept Fortran "DATA" statements. Study No. 2 - The nutrient intake survey system is operational. Modifications to the nutrient factor dictionary are made periodically to insure that the most current values of nutrient factors are used in the computations. Study No. 3 - Statistical support has been given to many laboratory divisions. A series of routines to support liquid scintillation counting of radioisotopes have been written and are currently being used by several research investigators.

*Available to contractors upon originator's approval

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

ABSTRACT

PROJECT NO.	3A062110A822	Military Internal Medicine
WORK UNIT NO.	082	Mathematical and Computer Support of Military Bio-Medical Research

The following investigations have been conducted under this work unit:

STUDY NO. 1 Computer Software Library Development

STUDY NO. 2 Nutrition Survey Computation System

STUDY NO. 3 Mathematical and Computer Programming Support

This work unit provides for mathematical and computer support of bio-medical research at USAMRNL.

Study No. 1: This study is concerned with organizing, documenting, maintaining and utilizing a library of computer programs related to the areas of mathematics, statistics, file manipulations and data reduction. More than 100 mathematical, statistical and utility routines have been added to the Fortran library during FY 71. In addition, significant modifications have been made to both the Fortran compiler and the RCA 301 assembler.

Study No. 2: This study resulted in the development of a computerized system to calculate the nutrient content of meals audited through the USAMRNL Military Nutrition Survey Program and with the statistical correlation of this data with the health status of the personnel eating in surveyed facilities. Analysis of the data from the Ft. McClellan Nutrition Survey has uncovered discrepancies between food handbook data and chemical analyses of food samples. The algorithms used to compensate for food losses are being investigated and updated in view of this new information.

Study No. 3: Statistical, computational and computer programming support has been given to nearly every division at USAMRNL. In addition, special purpose programs have been written to support the liquid scintillation system, to plot and analyze human diurnal temperature variations and to extend the usage of the PDP-8S computer. A portable FM tape recording system is being developed to interface laboratory instruments with the RCA 301 computer.

BODY OF REPORT

WORK UNIT NO. 082 — Mathematical and Computer Support of Military Bio-Medical Research

STUDY NO. 1 — Computer Software Library Development

PROBLEM:

The utilization of a digital computer to support biomedical research requires the use of many recurring utility and computational routines (i.e., the calculations of means, standard deviations, etc.) It is highly inefficient to write programming to perform these functions for each new application. Consequently, many such general purpose functions are being programmed for use by all laboratory investigators. These routines will be stored on library tapes so that they can be easily employed for many different applications.

The programming library tapes and the system compilers require maintenance to implement new techniques which will increase system efficiency and ease of use. Software development for this maintenance is performed under this study.

RESULTS AND DISCUSSION OF THE RESULTS:

The RCA 301 Assembler Program has been revised to run as a library program on the division library tape. This significantly reduces the problem of tape changing and concurrently increases the number of programs which can be run per unit time. The new system is loaded via a single initialization card. This minimizes the operator intervention required to bring the system up at the start of the work day. The RCA 301 Assembly System has been modified to automatically execute assembly programs provided that the programs do not contain compilation error flags. Error flags are special codes set by the assembly system to indicate that syntactical or logical errors have been found in a source computer program. If error flags are found, the next program is automatically processed. These modifications relieve the system operator from the tasks of checking undebugged assembly programs for error flags and from compiling and executing assembly programs as separate operations. Program throughput has increased as a result of these modifications.

The following modifications have been made to the Fortran compiler:

1. BLANK data fields are read in as 0.1E-98 instead of zero (0.0 E-99) therefore missing data can be distinguished from

values which are equal to zero. This will be especially useful in statistical analysis.

2. Over 100 statistical, mathematical and utility subroutines have been added to the Fortran subroutine library. These range from simple data plotting routines to complex mathematical algorithms such as autocorrelation and spectral analysis. These routines are in subprogram form and can readily be called by any Fortran program. The programmer does not have to understand the mathematical or internal operation of these subroutines to be able to use them.

3. The Fortran "DATA" statement has been added to the MRNL Fortran compiler. This permits many Fortran IV programs to be run in the RCA 301 computer without change.

4. A "users library" has been established to store and retrieve Fortran object programs. The system provides for storage of segmented Fortran programs and permits the programmer to call segments in random order. This is especially useful for complicated mathematical analyses programs which are too large to run as a single segment on the RCA 301 system.

A generalized research analysis statistical system (GRASS) has been written which will satisfy most of the statistical computational requirements of laboratory investigators. The system was designed to be readily used by research personnel who are relatively unfamiliar with computer programming. In addition, it will relieve the Computer Division from the requirement of setting up routine jobs so that more effort can be devoted to complex projects.

A program which simulates the operation of an analog computer has been procured. The system permits rapid and straightforward solutions of ordinary differential equations. It will be used for the mathematical study of complex-interrelated biomedical phenomena.

CONCLUSIONS:

A firm foundation of generalized mathematical, statistical and utility routines has been implemented. This has significantly increased the capability of the Computer Division to support laboratory research projects.

Mathematical and Computer Support of Military Bio-Medical Research (Cont'd)

RECOMMENDATIONS :

An operating system monitor should be developed to further reduce the necessity for system operator interaction. This would speed program turnaround time.

Work should be continued on both functional and utility general purpose programs.

STUDY NO. 2 Support of Military Nutrition Surveys

PROBLEM:

To use the digital computer to handle the filing and calculation of data involved in the support of Military Nutrition Surveys.

RESULTS AND DISCUSSION OF THE RESULTS:

The "Nutrient Survey System" uses data derived from handbooks and laboratory analytical analysis together with observations 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 intake of Women's Army Corps personnel at Ft. McClellan, Alabama, (Military Internal Medicine, Work Unit 073 - Applied Nutrition Studies of Military Populations, Study No. 1 - Nutrition Surveys of Military Populations and Installations).

The analysis of this survey indicated some discrepancy between the use of handbook data and the chemical analysis of collected samples of the food actually consumed. Investigation indicated some problems in the algorithm for handling the loss of weight of food items during cooking, particularly meat items. This problem is being studied by recalculating the study at Ft. McClellan utilizing handbook values for prepared food items and using detailed recipe evaluation to the minimum extent possible. A preliminary analysis of this approach seems to indicate a more reliable estimation of nutrient intake utilizing handbooks for food analysis.

CONCLUSIONS:

The use of the "Nutrient Survey System' has allowed some study of the use of handbook food analysis data to estimate nutrient

intake of military populations eating in dining halls. The effect of using detailed analysis of recipe observations versus the use of handbook values of prepared recipes can be studied and the ability to predict nutrient intake improved.

RECOMMENDATIONS :

The algorithm for handling the loss of weight in meat items during cooking due to fat and water loss should be studied and an improved algorithm developed for the "Nutrient Survey System."

The comparative investigation of the use of handbook values for prepared recipes, the use of detailed recipe observations from the kitchen, and chemical analysis of food composites should be continued. The results of this investigation should be used to develop an improved data handling system.

Modifications, revisions and expansions should be made to the system as investigators develop the need through the use of the system.

STUDY NO. 3 Mathematical and Computer Programming Support

PROBLEM:

To provide mathematical, statistical and computer programming service and consultations to laboratory investigators.

RESULTS AND DISCUSSION OF THE RESULTS :

The liquid scintillation programs which convert counts/min to disintegrations/min. have been revised to (1) allow periodic update of system calibration curves and (2) provide for simple linkage to applications programs. Applications programs have been written for the Metabolic and Chemistry Divisions. These programs convert the output of the liquid scintillation programs (DPM) to concentration parameters readily usable by the investigator. In addition, they perform simple statistical computations.

A new project has been initiated in cooperation with the Metabolic Division. The objective of the project is to automatically record and statistically analyze data on the peristaltic movement of guinea pig ilium which has been subjected to various chemical stimuli.

Mathematical and Computer Support of Military Bio-Medical Research (Cont'd)

A clinical plot program has been written for the Metabolic Division. The program accepts a patient's temperature record for a 24-hour period and plots the temperatures against time. Extenuating circumstances (e.g., medication, illness, etc.) are listed for edification of the temperature pattern. Parameters indicating the degree of fever over a 24-hour period are calculated and printed out with the plots. A metabolic patient file has been initiated to store the temperature profiles for all patients and healthy volunteers of the Metabolic Ward. The data will provide a base for studying the diurnal and annual temperature variations of individuals over long periods of time.

Work has been initiated in the Chemistry Division to implement a Priority Interrupt System, through hardware and programming, in the Digital Equipment Corporation, PDP-8S digital computer to allow additional use of computer during free periods. Approximately 95% of the time is currently utilized for collection of data from amino acid determinations with only 5% of the time actually used for computing. With interrupt capabilities, the idle time (95%) could be utilized by other analytical instruments. A magnetic tape input/output routine has been written to facilitate storage of programs. The amino acid program can now read into the PDP-8 in 8 min. whereas 68 min. are required to read it in from paper tape.

The hardware portion of the FM tape recording system is nearly complete. A subroutine has been written to accept, edit and produce a Fortran compatible tape from data collected via this system. When complete, this system will be able to record, on-site, analog signals from laboratory instruments and punch paper tape for computer input.

Statistical support has been given to the following projects:

1. Physiology Division - Work and Heart Rate Correlation
   Rat Activity at Altitude
   Psychological Parameters in Vitamin A Deficiency

2. Microbiology Division - Antibody Production at Altitude
   TB Culture Data

3. Metabolic Division - Thermoregulation in Normals

4. Bioenergetics Division - O_2 Uptake Patterns
   Rat, Environment and Nutrition

Mathematical and Computer Support of Military Bio-Medical Research (Cont'd)

5. Pathology - Nutritional Aspects of Military Dog Performance

6. Interdivisional Projects - Correlation of Biochemical Factors with Nutritional Intake

A systems analysis of each laboratory division has been performed by the Computer Division. Many research projects could significantly benefit from the use of ADP techniques. Programs to assist these projects will be developed in the near future.

CONCLUSIONS:

Mathematical and computer support has proven extremely useful to many divisions in the laboratory. This is especially true in the area of data reduction and analysis. The computational ability of the digital computer has been shown (1) to increase the timeliness of results (2) to minimize the incident of computational errors and (3) to free investigator personnel for more important tasks.

RECOMMENDATIONS:

Cooperative projects with other research divisions should be developed to a greater extent. The use of mathematical simulations could assist in correlating complex biomedical parameters and would be an area where cooperative projects might be beneficial.

PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6370	71 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
70 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 1412A (2)			

11. TITLE (Procode 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 71	1.2	50
	CURRENT 72	2.4	88

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: Henderson, J. E., COL, VC

TELEPHONE: 303 366 5311 X22223

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) 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. Procode 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; apply findings to specifications of new or existing military subsistence items.

24. (U) Maintain liaison with military preventive medicine, Public Health Service, Food & Drug Administation and food science and technology departments of Universities to keep abreast with problem areas of food hygiene and to prevent duplication of on-goirg research. Conduct research in-house or collaborate on research contracts with other food research organizations. Maintain current reference information on individual specialists in the fields of microbiology, mycology, virology, toxicology, chemistry and radiobiology as related to food research and processing.

25. (U) 70 07 - 71 06 On 1 February 1971, the laboratory facilities were completed and the Food Hygiene Division was established as an integral function of the USAMRNL. The first study under investigation is a microbiological analysis of the Food Packet, Long Range Patrol when subjected to potential field conditions.

*Available to contractors upon originator's approval.

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

ABSTRACT

PROJECT NO.	3A062110A822	Military Internal Medicine
WORK UNIT NO.	083	Military Food Hygiene

A nucleus for a continuing research program in military food hygiene was established in consonance with the expanded mission of the USAMRNL. Rapport has been established with some universities, USPHS, Food and Drug Administration, military and industrial laboratories as well as the veterinary food inspection service working in areas of food science and technology.

The Food Hygiene Laboratory became operational in February 1971, equipped and partially staffed to initiate research in potential problem areas of military subsistence. The first study under investigation is an in depth microbiological analysis of the Food Packet, Long Range Patrol after exposure to varying environmental conditions and subjecting the rehydrated entree items to simulated field conditions. Primary emphasis is being placed on the detection and isolation of human pathogenic organisms.

BODY OF REPORT

WORK UNIT NO. 083 Military Food Hygiene

PROBLEM:

As directed by AR 30-11, The Surgeon General has the responsibility for the wholesomeness, safety and hygiene of military rations. There had been no activity under The Surgeon General which was investigating the wholesomeness of newly developed military rations or ration items. Specifications for procurement of freeze-dehydrated food products and other "convenience" type foods being procured in large quantities permit total microbiological counts up to 3 million per gram on the finished product. This figure seems unusually high under present day techniques of processing, but the potential hazard of this microbiological level has not been evaluated.

An investigation into the possible role of exported rations on the high incidence of diarrheal disease in theaters of operations appears indicated. Army area medical laboratories and Army and Air Force Veterinary Inspection Service conduct routine examinations and oversee contractor laboratory testing of procured products but with suspected specification laxity, absence of testing under simulated field conditions, and lack of examination of the product at time of consumption, the actual wholesomeness is unknown. The goal of this project is to establish a research program to develop standards of hygiene and sanitation for military subsistence.

RESULTS AND DISCUSSION OF RESULTS:

The Food Hygiene Division was activated on 1 February 1971. Following approval of a Manpower Utilization Survey Report, the staffing of this Division was accomplished by an intra-activity transfer of two civilian spaces and HQ, USAMR&D Command changed the personnel authorization at USAMRNL to add an additional Veterinary Corps officer and an enlisted man. The civilian spaces have been filled and the new military personnel should be available by October 1971.

The laboratory facilities were completed in late January 1971, previously procured supplies and equipment were installed and the first project was initiated.

Military Food Hygiene (Cont'd)

This project involves a microbiological analysis of the Food Packet, Long Range Patrol, a highly acceptable food packet for troops in the field. Specifications permit a maximum of 200,000 per gram standard plate count and 40 per gram coliform count which are relatively high and are primarily indicators of microbiological quality. The freeze dehydration process produces only minor bacteriocidal action therefore a pathogenic bacterial evaluation seems indicated. The testing includes standard plate count, total coliform, fecal coliform, fecal streptococcus, coagulase positive staphylococcus and salmonella on the following menus: chicken and rice, beef hash, chili con carne, spaghetti w/meat sauce, beef w/ rice, chicken stew, pork w/escalloped potatoes, and beef stew. Samples of these packets were stored for at least nine months at room temperature, refrigerated temperature (38-42°F) and at ambient temperature atop Pikes Peak at the Pikes Peak Laboratory Facility. The products are rehydrated and examined at 0, 2, 4, 6, 8, 12 and 24 hours after being held at 72°F and 100°F in an attempt to simulate potential field conditions.

Preliminary results on the first two menus which were stored at room temperature indicate that the pathogenic bacterial load is not significant but the levels of other organisms which have not been typed may be of sufficient numbers to result in competitive inhibition of pathogenic organisms. Growth patterns indicate that these products harbor massive bacterial loads when incubated at 100°F for six (6) hours after rehydration. At six (6) hours after rehydration a slight off odor can be detected which could act as a deterrent for its consumption.

Plans for the detection of toxins are being developed, especially for the clostridium species as an extension of the original study. This will include animal inoculation and an evaluation of the actual hazard in the food product as opposed to selective anaerobic growth and toxin development.

This Division reviews proposed new and revised specifications to be published by Army Materiel Command (Natick Laboratories). Comments are forwarded to the Office of the Assistant for Veterinary Services, OTSG, for inclusion in their official reply.

Liaison activities are being maintained through attendance at Research and Development Associates and Institute of Food Technologists meetings.

SUMMARY AND RECOMMENDATIONS:

The establishment of this research laboratory for the conduct of research on the wholesomeness and safety of military subsistence is needed to evaluate potential hazards of specially processed

military subsistence and to assist in the planning stages of the DoD Food Program. It is anticipated that sufficient expertise will be available to resolve problems of food safety and wholesomeness as they are encountered by personnel in the field. Liaison and updating of current information in the field of food science and technology should be maintained.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6371	71 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
70 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	084
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procode 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	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 71	2.1	41
FISCAL YEAR CURRENT 72	2.5	34

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

ADDRESS:* Fitzsimons General Hospital
Denver, Colorado 80240

RESPONSIBLE INDIVIDUAL

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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 (Procode 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. Procode 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 factor of high altitude (14,400 ft.) are evaluated.
25. (U) 70 07 - 71 06 1. Mice vaccinated with viable BCG, formalin-killed M. tuberculosis, wax C components, or washings from these cells developed high degree of acquired resistance; currently, isolation and purification of M. tuberculosis components are in progress for evaluation as immunogens. 2. Latex particles have been successfully sensitized with various tuberculo-proteins; sensitivity and reproducibility of this test as a serodiagnostic procedure for tuberculosis is being studied. 3. Evaluation of primary and secondary immune response and the amount of humoral antibody was increased; however, limited protection was afforded the same animals on challenge infection; additional studies are in progress.

*Available to contractors upon originator's approval.

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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. 1 To characterize the immunologic response evoked by Mycobacterium tuberculosis

STUDY NO. 2 *Melioidosis: Serologic studies on U. S. Army personnel returning from Southeast Asia

STUDY NO. 3 *Possible granulomatous changes in lung similar to sarcoidosis secondary to long-term use of aerosol-propellent underarm deodorants

Study No. 1. Investigative studies revealed: (a) mice vaccinated with formalin-killed M. tuberculosis, wax C component, or washing from the killed cells developed a high degree of acquired resistance to tuberculosis; (b) amount of antibody recorded as PFU (plaque forming units) during primary and secondary immune responses in mice sensitized during stress of high altitude (14,100 ft) was enhanced; however, limited protection was afforded the same animals on challenge infection; (c) latex and bentonite particles were sensitized successfully with various tuberculo-proteins; these particles have demonstrated high sero-specificity and sensitivity for detecting tuberculous antibodies.

Study No. 2. Military personnel (500) returning from Vietnam were evaluated serologically for melioidosis using a hemagglutination procedure (HA); test erythrocytes were sensitized with polysaccharide antigen extracted from Pseudomonas pseudomallei. The recorded diagnostic titers (i.e., $\geq$ 1:80) were: (a) wounded patient group, six (3%); medically-ill and U. S. control groups, one (1%) each group, respectively. Antibody activity in sera with diagnostic titers were mercaptoethanol resistant, and had no complement fixing properties. This study demonstrated that the HA test is a valuable serologic tool for the evaluation of clinical or subclinical melioidosis.

Study No. 3. Observations at Fitzsimons General Hospital have shown numerous cases which appeared to be clinically and histologically sarcoidosis. In each case an exposure history to one of several commercial brands of aerosolized underarm deodorants was evident. Preliminary immuno-diffusion studies using various types of propelled

*Collaborative studies with Fitzsimons General Hospital: Project Number 3A062110A826

underarm deodorants and samples from these sarcoidosis patients showed development of questionable precipitin bands. Guinea pigs were then aerosolized daily for three (3) months with these deodorants and were subsequently evaluated histologically for evidence of sarcoidosis and serologically for humoral antibodies. The only significant animal findings were histologic changes in the lung tissue which showed definite lesions with no granulomas.

BODY OF REPORT

WORK UNIT No. 084 — Immunologic Research in Tuberculosis

STUDY NO. 1 — To characterize the immunologic response evoked by M. tuberculosis

PROBLEM:

M. tuberculosis and its components initiate production of different antibodies whose functional properties remain unsolved. With approximately 500,000 U. S. troops exposed in world areas where incidence of active tuberculosis is between 15-20 million cases annually, the importance of a diagnostic test for case finding and for epidemiologic index of tuberculosis has increased. To meet the objectives for an adequate T.B. control program there are requirements for: a single serologic test to supplement other criteria for detecting active tuberculosis, development of a vaccine which protects without eliciting a positive skin test, and evaluation of immune responses as affected by physiological extrinsic stress factors.

RESULTS AND DISCUSSION OF THE RESULTS:

Mice vaccinated with viable BCG, formalin killed M. tuberculosis, or wax C component developed a high degree of acquired resistance to tuberculosis. Delayed hypersensitivity was noted only in animals vaccinated with viable BCG. These results support findings for the efficacy of non-living vaccines; it appears that immunogen responsible for evoking acquired resistance is a residue-type of antigen.

Evaluation of immunologic responses in vaccinated mice maintained at 14,100 ft. showed that the amount of antibody recorded as plaque forming units (PFU) or as circulating titer was enhanced. On the other hand, limited protection was afforded to these animals on challenge infection to tuberculosis. During the period of high altitude exposure, (14,100 ft) the following changes were noted in the test animals: transient decrease of body weight, maximum recorded on the 10th day; a rapid early increase of spleen weight, peak recorded on the 6th day; a rapid increase of PFU units followed by a rapid decrease, 4th and 5th days respectively; peak circulating antibody was observed on the 5th day.

Serum samples from animals immunized with M. tuberculosis were tested with a modified form of the latex flocculation test; polystyrene latex, 0.81 micron, was sensitized with purified protein derivative (PPD), H37Ra filtrate antigen (Ra filtrate), or old tuberculin (OT). Using criteria favoring sensitivity and specificity it was found that this

test detected antibodies against tuberculosis; immune sera gave positive flocculation reactions and no false positive reactions were noted with negative sera. A marked variability for sensitivity of reaction was noted; latex particles tr ..ed with PPD detected antibody with serum dilution range of 1:10,240; latex treated with Ra filtrate or OT detected the same antibody at dilution ranges of 1:1280 and 1:10 respectively.

CONCLUSIONS:

Acquired resistance in mice to tuberculosis does not require vaccination with living M. tuberculosis cells.

The increased level of antibody brought about by the stress of high altitude does not represent a condition of plasma concentration. Increase of PFUs, amount of circulating antibody, and increase of spleen weight support a finding that stress of high altitude stimulates lymphoid tissue proliferation with corresponding release of antibody. The high level of available circulating antibody does not increase the acquired resistance mechanisms of the stressed animal.

Flocculating antibodies, as demonstrated with a modified form of the latex test, were found only in the sera of animals previously immunized with various strains of M. tuberculosis. The antigen(s) required for optimum sensitization of the latex particle are confined to the protein component of the filtrate from the bacterial culture.

RECOMMENDATIONS:

Studies concentrating on the isolation and purification of the M. tuberculosis immunogen which appears responsible for evoking resistance against tuberculosis should be continued.

Further evaluate the effects of high altitude stress on immunoglobulin synthesis and functional properties of these immune globulins to infection.

Human sera representing samplings from tuberculous patients or individuals converted to positive skin test should be evaluated serologically with the modified procedure of the latex flocculation test.

PUBLICATIONS:

Brown, G. L., M. V. Rothlauf, R. H. Heinzerling, and T. S. Myers.

Antibody formation in rabbits adapted and nonadapted to high altitude. Infection and Immunity 3: 358, 1971.

Brown, G. L. and P. L. Carpenter. Characteristics of cytophilic antibody in the mouse. Infection and Immunity 3: 637, 1971.

STUDY NO. 2 Melioidosis: Serologic studies on U. S. Army personnel returning from Southeast Asia

PROBLEM:

Melioidosis is interesting from an ecologic viewpoint in that primary infections have been found only in areas between 20° north and south parallels of latitude. The clinical manifestations of melioidosis range from acute fulminating bacteremia to subclinical infections which occasionally exacerbate to acute disease after months or years of dormancy. The purpose of this study was to evaluate serologically 500 U. S. Army personnel returning from Vietnam for melioidosis antibodies.

RESULTS AND DISCUSSION OF THE RESULTS:

Results of the serologic evaluation (HA test) showed that in the wounded patient group 6 (3%) of 200 sera gave diagnostic titers ($\geq$ 1:80). Only one specimen each from the medically ill and the control patient groups had titers $\geq$ 1:80. There were 100 serum samples from each group respectively. Serum samples showing positive HA titers were negative for complement fixing (CF) antibodies to pseudomallei antigens. This finding established that anti-pseudomallei globulin does not have CF properties.

The recorded HA titers from all serum samples tested were: 91.2% $\leq$ 1:2, 5.8% $\leq$ 1:80, and 3.0% $\geq$ 1:80. The incidence of 3% of apparent subclinical unrecognized melioidosis found in the wounded soldier group was considerably higher than in the other groups tested ($p=0.1$). Furthermore, titers of <1:80 found in 5.8% samples pose an interesting epidemiologic question as to what constitutes serologic evidence of infection by this organism. The finding of a "diagnostic" titer ($\geq$ 1:80) suggests that the individual had a positive contact with the organism during his tour in southeast Asia. A question now arises as to whether this individual experienced subclinical melioidosis and may, at a later day, again experience exacerbations of acute disease.

CONCLUSIONS:

The polysaccharide antigen from P. pseudomallei, used in the HA test,

was shown to be serologically specific for the detection of pseudomallei antibodies in U. S. soldiers returning from Vietnam. Significant anti-P. pseudomallei titers were recorded in 3% of wounded personnel tested.

RECOMMENDATIONS:

The present study is completed. It is recommended that additional studies be undertaken to evaluate Vietnam returnees for prevalance of subclinical melioidosis.

PUBLICATIONS:

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 Medicine, August 1971 (in press).

STUDY NO. 3 — Possible granulomatous changes in lung similar to sarcoidosis secondary to long-term use of aerosol-propellent underarm deodorants

PROBLEM:

The etiologic agent(s) eliciting cutaneous and systemic lesions of sarcoid have remained an enigma. Atypical mycobacteria, biological antigens, as well as chemical agents (aluminum, beryllium, zinc) have been postulated to be causes of sarcoidosis. In this study, selected sera from clinically diagnosed sarcoidosis patients were subjected to immuno-diffusion studies using propelled underarm deodorant and sera as source of antigen and antibody. In addition, immunological and histological studies were undertaken to determine if long-term exposure of aerosol propellent underarm deodorant on guinea pigs results in lung toxicity changes similar to sarcoidosis.

RESULTS AND DISCUSSION OF THE RESULTS:

Immuno-diffusion studies using propelled underarm deodorant and samples of sera from sarcoidosis patients showed development of questionable precipitin bands. Further studies suggested that the band was denatured protein formed due to the extreme acidity, pH 2.8 - 3.2 of the aerosol. The guinea pigs which were aerosolized daily for three months to various types of underarm deodorants developed lesions in the lung tissue with no evidence of granulomas; histological changes included bronchial mucosa hypertrophy, lymphatic interstitial infiltration, and some

histiocytes or mast cells with suggestion of early giant cell formation. The question now arises as to whether the extreme acidity of the deodorant can cause protein denaturation in situ resulting in an altered protein which then acts as a foreign body antigen, the latter possibly initiating an antigen-antibody reaction with altered cellular immunity and eventually leading to the development of an epithelioid tissue reaction.

CONCLUSIONS:

The possibility exists that long exposure of underarm deodorant preparation may cause lung lesions in susceptible humans and other animal species.

RECOMMENDATIONS:

Continuation of fluorescent antibody studies with aerosol deodorant as the antigen, patient and animal sera, and immune sera from animals sensitized with deodorant-treated normal sera will provide data for evaluating probable cellular immunity involved in this disease process.

PUBLICATIONS:

None except that preliminary report of findings was presented at 1971 Annual Meeting:

Ward, G. W., Jr., D. R. Hazlett, G. L. Brown and A. S. Woo, Jr., Lung changes secondary to inhalation of underarm aerosol deodorants. American Thoracic Society, Los Angeles, 16-19 May 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6347	71 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. DOD'N INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
70 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	
c. CONTRIBUTING	CDOG 1412A (2)			

11. TITLE (Procede 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:
b. NUMBER: Not Applicable
c. TYPE:
d. KIND OF AWARD:
EXPIRATION:
e. AMOUNT:
f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PRECEDING FISCAL YEAR 71	1.8	22
CURRENT FISCAL YEAR 72	2.0	45

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 (Provide YEAR if DOD 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 (Procede 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 (Provide brief individual paragraphs identified by number. Procede 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) 70 07 - 71 06 Energy requirements appear to be increased at altitude; however, the specific dynamic activity of nutrients was unaffected. Acid-base changes of Denver natives (1,600 m) at 4,300 m showed a decreased pCO_2 compensated for by a decreased bicarbonate valueresulting in a relatively constant pH while pulmonary changes revealed increased total lung and inspiratory capacities. Results from other studies indicated that altitude adaptation was facilitated by (a) increased physical activity and conditioning before exposure, (b) consumption of at least 320g of carbohydrate; (c) food intake maintenance, and (d) a natural diuresis with subsequent total body and intracellular water decreases.

*Available to contractors upon originator's approval

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

ABSTRACT

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

The following conditions appear to be highly beneficial in high altitude adaptation: (a) heavy physical activity and physical conditioning before altitude exposure; (b) consumption of at least 320 grams of carbohydrate per day; (c) maintenance of normal food intake, and (d) a natural diuresis with a subsequent total body water and intracellular water decrease.

Data in three studies at 4,300 meters indicates that the energy requirements appear to be increased at high altitude. These increases may be due to the increased metabolic cost of cardiac and respiratory work or to a decreased work efficiency.

Studies of respiratory function including pulmonary diffusion capacity, with concurrent arterialized blood acid-base parameters are in progress. Preliminary results suggest an increased ventilatory response to low atmospheric oxygen with compensating acid-base dynamics.

Specific dynamic activity after ingestion of various nutrients, indicate no significant differences between 1,600 or 4,300 m.

BODY OF REPORT

WORK UNIT NO. 070 — High Altitude Bioenergetics - The Physiological Consequences of Altitude Exposure Upon the Soldier

The following investigation has been conducted under this work unit:

STUDY NO. 12 — Effect 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 decrease in 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 diffusion capacities), cardiovascular, and biochemical parameters (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, in human subjects exposed to high altitude, the influence of dietary manipulations and heavy physical conditioning on the symptoms of acute mountain sickness.

RESULTS AND DISCUSSION OF THE RESULTS:

The possibility of an increase in energy requirements at high altitude led to completion of a study on the specific dynamic action (SDA) of food after meals of various nutrients. This information should provide additional data on energy requirements at altitude. Arterialized blood and acid-base parameters were evaluated under conditions in which men were eating normally at altitude. Under normal conditions,

one observes an anorexia at altitude which possibly could account for some of the acid-base abnormalities. Preliminary data indicates no significant difference in SDA between 1,600 and 4,300 m.

In the same study completed in 1970, alterations in respiratory function and acid-base balance at high altitude were evaluated. Alterations in respiratory function at 1,600 m suggest greater responses in acid-base dynamics and pulmonary function with the lower atmospheric oxygen pressures at 4,300 m. Changes in pulmonary volumes were observed in 12 males (mean age 30 years) residing at 1,600 m (Denver) after translocation to 4,300 m for a 48-hour period. No significant change occurred in functional residual capacity (FRC: 1,600 m (a) = 3.47 l, 4,300 m (b) = 3.65 l, 1,600 m (c) = 3.36 l), and vital capacity (VC: (a) = 5.57 l, (b) =5.48 l, (c) = 5.55 l). The inspiratory capacity (IC) increased + 0.20 l at 4,300 m with a corresponding decrease of -0.28 l in the expiratory reserve volume (ERV). The elevated residual volume (RV: -0.45 l) revealed no change in FRC at 4,300 m; however, the IC increase is noted in the enlarged total lung capacity (TLC: +0.37 l). All volumes reverted to pre-4,300 m (a) values upon return to 1,600 m. A relatively constant pH (a) = 7.395, (b) = 7.405, (c) = 7.375) was maintained at 4,300 m with pCO_2 decreased (-3.2 mmHg) values compensated by a proportional decrease in HCO_3. Results suggest an increased ventilatory response to low atmospheric O_2 with compensating acid-base dynamics.

Preliminary information indicates that the energy expenditure remained unchanged at altitudes up to 3,475 m. At 4,300 m significant increases of oxygen uptake, pulmonary ventilation and heart rates were observed during moderate and fairly heavy steady state work on the treadmill. Oxygen uptakes in comparison to sea level values were increased by 3-15% after 5 days, and 17-35% after 9 days of exposure to 4,300 m. The significant increases in these parameters are all suggestive of an increase in energy requirements during acute high altitude exposure of 4,300 m, and may be due to the increased metabolic cost of cardiac and respiratory work, or to decreased efficiency of work performance.

A summary of the results of our altitude studies prior to 1970 is given below. The daily food intake of human subjects can be maintained after abrupt exposure to high altitude. Under conditions of normal dietary intake, it has been observed that: (a) positive nitrogen balances can be achieved; (b) body weight losses are greatly reduced; (c) mineral balances are positive; (d) blood electrolyte levels are normal, and (e) fasting glucose levels and glucose tolerance curves are normal. It appears that many of the biochemical changes that occur during high altitude exposure which were previously attributed to hypoxia

may be partially due to anorexia and the subsequent caloric deficit. However, negative water balances with decreased total body water are still observed and appear to be essential for high altitude adaptation.

The following conditions appear to be highly beneficial in adapting to high altitude: (a) heavy physical conditioning prior to altitude exposure; (b) the consumption of a minimal quantity of carbohydrate (at least 320g/day); (c) the maintenance of a normal food intake at altitude, and (d) a natural diuresis with a subsequent decrease in total body water and intracellular water.

Very limited information is available on pulmonary diffusion capacity (D_LCO), functional residual capacity and other lung compartments during high altitude exposure. Complete pulmonary function measurements were made and evaluated at 1,600 m (Denver), during exposure to 4,300 m (Pike's Peak), and after return to 1,600 m. The study was designed to measure D_LCO by three separate techniques to evaluate diffusion capacity of the lungs. Although these techniques are based upon different measured parameters, the values for the diffusion capacity should be comparable (for individuals free of lung pathology). The $PaCO_2$ and P_ACO_2 and P_A CO will be incorporated to derive diffusion capacity values. Differences in final D_LCO alveolar or blood values are indicative of possible lung pathology. This system of cross checking will greatly aid in defining and for evaluating changes in respiratory function when translocating from sea level to high altitudes. Measurements were made twice a day on 4 healthy individuals during acute altitude exposure, for 5 days. Data has been computer programmed and statistical analyses are in progress.

CONCLUSIONS:

Energy requirements appear to be increased at high altitude and are related to the significant increases in oxygen uptakes, pulmonary ventilation, and heart rates during altitude exposure. These increases are due to the increased metabolic cost of respiratory and cardiac work or to the decreased efficiency of work performance.

Pulmonary function and blood acid-base parameters have been evaluated in two studies. The results suggest an increased ventilatory response to low atmospheric oxygen with compensating acid-base dynamics.

RECOMMENDATIONS:

1. Further studies on the effects of normal consumption 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 work will be studied.

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

2. Physiological, biochemical and nutritional studies at altitudes above 4,300 m in sea level natives during abrupt altitude exposure.

3. Studies will be conducted to measure the effects of both high altitude and cold stress during acute exposure.

PUBLICATIONS:

1. 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., 1971. (In Press)

2. Nielsen, W.C., Jr., H.L. Johnson, H.J. Krzywicki and C.F. Consolazio. The evaluation and application of gas chromatography to determine deuterium oxide concentrations in body fluids. (Accepted for publication in J. Appl. Physiol., 1971)

3. Consolazio, C.F., H.L. Johnson and H.J. Krzywicki. Adaptation to High Altitude (In press - J. Physiologie France, 1971)

4. Consolazio, C.F., H.L. Johnson and H.J. Krzywicki. Body fluids, body composition and metabolic aspects of high altitude adaptation. Proceedings of a symposium on Physiology Adaptations: Desert and Mountain, in honor of Dr. D.B. Dill. (In press - Academic Press).

5. Johnson, H.L., C.F. Consolazio, T.A. Daws and H.J. Krzywicki. Energy requirements of man abruptly exposed to high altitude. (Submitted for publication, 1971)

6. Consolazio, C.F., H.L. Johnson, H.J. Krzywicki, T.A. Daws and W.C. Nielsen, Jr. The reduction in the severity of the mountain sickness syndrome during abrupt altitude exposure. (Submitted for publication, 1971)

7. Dramise, J.G., C.F. Consolazio, H.J. Krzywicki and S.O. Williams III. Alteration in respiratory function and acid-base balance in humans at high altitude. Fed. Proc. 30:428, 1971 (Abstract).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6350	71 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
70 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	62110A	3A062110A827	00	073
b. CONTRIBUTING	62156011	3A025601A827	00	
c. CONTRIBUTING	CDOG 1412A	(2)		

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 71	5.9	111
FISCAL YEAR CURRENT 72	3.7	95

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Med Rsch & Nutr Lab

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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Physiology Division

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

21. GENERAL USE

Foreign Intelligence not Considered

ASSOCIATE INVESTIGATORS

NAME: Sullivan, F. J.

NAME: Sterner, R. T., CPT, MSC DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Environmental Stress; (U) Physiological Adaptation; (U) High Altitude; (U) Human Factors; (U) Biochemistry; (U) Military Stress

23. TECHNICAL OBJECTIVE,* 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) 70 07 - 71 06 Reevaluation of General High Altitude Questionnaires from previous studies has verified the delineation of the subjective assessment of acute mountain sickness into four symptomatalogy subscales: Arousal Level, Somatic Discomfort, Tired and Mood. These subscales have provided improved estimates of acute mountain sickness and can be of use in the assessment of various treatments directed at the amelioration of the sickness. A study evaluating cardiopulmonary function and performance during prolonged exposure to altitude will be conducted this year.

*Available to contractors upon originator's approval

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

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. 7	Treatment of acute mountain sickness with diuretic and motion sickness drugs
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 days of exposure, the phenomenon of acute mountain sickness becomes manifest with most of the symptoms disappearing within a week. Psychomotor, mental and sensory functions decrement during acute exposure; however with prolonged exposure, performance improves. Further analysis of the General High Altitude Questionnaire has supported the separation of AMS symptoms into subscales of Arousal Level, Somatic Discomfort, Tired and Mood. Hypoxia does not affect vestibular functioning. A new study (No. 9) will evaluate cardiopulmonary function and performance during prolonged exposure to high altitude.

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:

Much information has been gathered concerning the biochemical and physiological alterations that occur when man is rapidly exposed to high altitude. Less information is known about the phenomenon of acute mountain sickness (AMS) which is manifest in the majority of low altitude residents when brought to 14,110 feet. The General High Altitude Questionnaire (GHAQ) has been developed to quantify the symptoms of AMS. However, reevaluation of the GHAQ is continually required to improve this technique in the assessment of the subjective aspects of AMS. In this way the GHAQ will provide improved estimates of AMS that will be useful in assessing the relative effectiveness of selected treatments to ameliorate the condition. It will also allow better correlation of AMS symptomatology to the alteration in physical, psychomotor and mental performance as well as the physiological and biochemical changes which are observed during acute altitude exposure.

RESULTS AND DISCUSSION OF THE RESULTS:

As in previous years, considerable effort was devoted to analyzing data obtained in past high altitude experiments. Specifically, such analysis dealt with the development of a statistically-improved instrument for quantifying subjective aspects of mountain sickness symptomatology. Through the use of preset key-cluster analysis techniques, sets of items contained in a revision of the General High Altitude Questionnaire were combined to form four symptomatology subscales; i.e., Arousal Level, Somatic Discomfort, Tired and Mood. Rapid transition from low to high altitude resulted in significant increases of these subscale scores.

CONCLUSIONS:

A selected 16-item, four-dimension breakdown of the GHAQ yielded subscale measures which provide improved estimates of AMS symptomatology. Such measures should prove useful in the assessment of any treatment procedure intended to ameliorate the sickness.

RECOMMENDATIONS:

Since the described subscales offer a means for assessing AMS symptomatology

less prone to high standard error of measurement than single discrete-item procedures, it is recommended that future studies utilizing symptomatology measurements of AMS use subscale estimates.

STUDY NO. 7 Treatment of Acute Mountain Sickness with Diuretic and Motion Sickness Drugs

PROBLEM:

Certain AMS symptoms are quite similar to the symptoms of sea or motion sickness. They are also similar to those observed clinically in patients with high intracranial pressure. With respect to the latter, hypoxia is known to cause an increase in cerebrospinal fluid pressure and consequently intracranial pressure. In view of these facts, a study is being initiated to test the effectiveness of a common motion sickness drug and several diuretic drugs in reducing AMS symptomatology.

EXPERIMENT NO. 1: Relationship of Hypoxia, Anxiety and Nystagmus

Due to the problems consequent to the retention of military enlisted personnel in excess of 30 days, as volunteer research subjects, Study No. 7 could not be completed as a unit. It is, therefore, being broken into smaller, more workable experiments. Experiment 1 was designed to evaluate the effect of hypoxia and anxiety vestibular functioning.

RESULTS AND DISCUSSION OF RESULTS:

In this experiment the subjects were segregated into groups subjected to high and low anxiety stress situations. During each measurement period each subject was first placed on a breathing mixture of 10 or 20% oxygen in nitrogen for 10 minutes. At the end of this period, with the particular breathing mixture being continued, vestibular excitation was produced by irrigating the external ear with 0° C water. Vestibular activity was recorded by means of an electronystagmogram. Measurements were made of the slow phase, rapid phase, latency, and duration of nystagmic movements. No differences were found in any of the measures between high and low oxygen groups. The only significant difference found was that the fast phase amplitude was significantly greater in the low anxiety group as compared with the high anxiety group. These results are not congruous with past findings and theoretical arguments and thus require further analyses before a complete interpretation can be made.

CONCLUSIONS:

It appears that brief, hypoxic, normobaric conditions do not directly

influence vestibular functioning. Therefore, if vestibular disturbances contribute to the symptomatology of acute mountain sickness, longer periods of hypoxia or hypobaria are required for manifestations.

RECOMMENDATIONS:

Study No. 7 should be completed at the earliest possible date so that a number of theoretical and practical problems associated with AMS can be solved.

STUDY NO. 9 — The interrelationships of cardiopulmonary function and performance during prolonged altitude exposure of 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, mental 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, and the rate at which they occur, 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 study of the long-term changes in human physical, psychomotor and mental performance will be studied on Pikes Peak. Simultaneously, the cardiovascular, pulmonary, biochemical and psychological factors which limit or control performance ability will also be studied.

RESULTS AND DISCUSSION OF RESULTS:

Eight female volunteer subjects for this study have been recruited. The various experimental procedures have been selected and tested. A low altitude testing laboratory was established at the University of Oregon Institute for Human Performance in Eugene (elevation 400 feet). The low altitude measurements commenced on 3 June 1970 and were completed on 13 June. The high altitude measurements on Pikes Peak commenced on 16 June 1970 and will be completed on 3 September 1970.

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

RECOMMENDATIONS:

1. Proceed as planned.

PUBLICATIONS:

1. Stamper, D. A., R. T. Sterner and R. A. Kinsman. Symptomatology Subscales for the Measurement of Acute Mountain Sickness. Percept. and Motor Skills (In Press - 1971).

2. Chinn, K.S.K., and J. P. Hannon. Effects of diet and altitude on the body composition of rats. J. Nutr. 100:732, 1970.

3. Stamper, D. A., R. A. Kinsman and W. O. Evans. Subjective symptomatology and cognitive performance at high altitude. Perceptual and Motor Skills 31:247, 1970.

4. Whitten, B. K., L. E. Schrader, R. L. Huston, and G. R. Honold. Hepatic polyribosomes and protein synthesis: seasonal changes in the hibernator. Internat. J. Biochem. 1:406-408, 1970.

5. Hannon, J. P., C. W. Harris and J. L. Shields. Alterations in the serum electrolyte levels of women during high altitude (4,300 m) acclimatization. Int. J. Biometeor. 14:201-209, 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6358	71 07 01	DD-DRAE(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 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	62110A	3A062110A830	00	061
b. CONTRIBUTING	6215601A	3A025601A830	00	
c. CONTRIBUTING	CDOG 1412A (2)			

11. TITLE (Procede 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	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	5.0	35
FISCAL YEAR CURRENT 72	4.0	35

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

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21. GENERAL USE

Foreign Intelligence not Considered

22. KEYWORDS (Procede 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. Procede 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 evaluate means of increasing the acceptability of diets after strenuous forced exercise.

24. (U) Fifteen 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 and recover from fatigue, maintenance of body weight, and recording of other physiologic parameters. Food consumption will be recorded and correlated with ambient temperature, relative humidity and forced exercise. Attempts to increase food intake to maintain a positive nitrogen balance will be done by incorporating certain additives to render the ration more palatable.

25. (U) 70 07 - 71 06 Daily food consumption of 6 dogs fed Purina Dog Chow (PDC) averaged 1.43 lbs/dog; 6 dogs fed Pooch Tasty Nuggets (PTN) averaged 1.63 lbs/dog; and 5 dogs given Military Stress Diet (MSD) consumed a daily average of 1.12 lbs/dog. Daily average cost of these diets/dog was: PTN, 13.61¢; PDC, 16.00¢; and MSD 51.52¢. Three groups of 5 dogs from each diet regimen were exercised on a treadmill at an average speed of 5.5 mph and 11% elevation. A Work Factor (WF) utilizing the dog's weight, time to fatigue, speed and elevation of treadmill, and number of runs was computed. Mean WF for the diets fed were: MSD, 60.94; PTN, 57.5; and PDC, 52.4. Biopsies of triceps muscle obtained at rest and immediately after swimming or walking the treadmill to fatigue were compared by means of histochemical strains and by electron microscopy. There were morphologic differences between resting and fatigued muscles at the EM level.

*Available to contractors upon originator's approval

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

ABSTRACT

PROJECT NO.	3A062110A830	Biosensor Systems
WORK UNIT NO.	061	Nutritional Aspects of Military Dog Performance
	STUDY NO. 1	To evaluate three test rations and the performance of military dogs undergoing field training at Ft. Benning, Georgia
	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

Since April 1970, studies have been in progress to compare the adequacy of 3 test rations in a group of 15 German shepherds both at rest and under induced physical stress. Daily individual food consumption has been recorded since the beginning of the experiment. Body weights have been taken at regular intervals.

Between June and September 1970, the animals were forced to swim to fatigue once weekly in a galvanized metal tank 8 feet in diameter and 3.5 feet deep. From September to December, they were exercised to fatigue on a treadmill. Blood samples were collected immediately before and after each swim and treadmill run. During exercise, deep body temperature and heart rate were continuously monitored and recorded. A triceps biopsy from each dog was collected immediately after each swim or treadmill run and respective portions processed for light and electron microscopy. Samples of fatigued and resting muscle were compared.

A large volume of data has been collected and some is still being analyzed.

BODY OF REPORT

WORK UNIT NO. 061	Nutritional Aspects of Military Dog Performance
STUDY NO. 1	To evaluate three test rations and the performance of military dogs undergoing field training at Ft. Benning, Georgia

This study has been completed.

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 caloricallly 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 caloricallly 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 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. Group A received Maximum

Stress Diet 198 (MSD, Hill's Division of Riviana Foods, Topeka, KA); Group B was fed Purina Dog Chow (Ralston Purina Co., Checkerboard Square, St. Louis, Mo.); and Group C Pooch Tasty Nuggets (Safeway Stores, Inc., Head Office: Oakland, Calif.) At first, the dogs were offered, once daily, a weighed quantity of the respective food estimated to be near the upper limits of their daily consumption; on the next day the amount of food that had remained uneaten was weighed, subtracted from the quantity that had been offered, and the net daily intake of each ration was calculated for each animal. In each instance, the ration offered each animal was adjusted so that only a minimal portion would be left uneaten each day; thus, in effect, the animals were fed ad lib with each dog receiving that amount of food that previous experience had shown to be its maximum intake. Water was also offered ad lib. For 7 weeks the animals were maintained at rest while they adjusted to their environment and their specific diets. Weekly weights have been obtained.

Approximately 15cc of blood was drawn from each animal weekly and two 24-hour specimens of urine were collected from each animal during the initial 7-week interval. A muscle biopsy from the triceps group was obtained from each animal prior to exercise; a portion of each biopsy was processed for electron microscopy and another portion frozen on a cryostat chuck using isopentane cooled by liquid nitrogen.

Chemical profiles of the blood specimens were performed weekly using the Technicon SMA-12 analyzer for the following: Total protein, Na^+, K^+, Cl^-, CO_2, Ca^{++}, alkaline phosphatase, total bilirubin, BUN, glucose, and SGOT; assays of free fatty acids and creatine phosphokinase were also determined. Complete hemograms were done for each animal. For light microscopic studies the muscle biopsies were cut at 10μ in the cryostat at -20 degrees Centigrade, and the following histochemical procedures and stains were performed: diphosphopyridine nucleotide - tetrazolium reductase ((DPNH-TR), phosphorylase, adenosine triphosphatase (ATPase), periodic acid Schiff, modified trichrome and hematoxylin-eosin. The 24-hour urine specimens were analyzed for vanilmandelic acid.

Between June and September, 1970, the animals were placed in a tank and forced to swim. To accelerate the onset of fatigue additional stress was placed on the animals by attaching a spring device on the tail and by elevating the hind quarters slightly. This procedure forced the animals to swim so that they were unable to reach the sides of the tank. The temperature of the water was 40° C. During exercise the heart rate and EKG were monitored by means of silver/silver chloride leads taped to the skin of the chest; body temperature was recorded by a thermistor placed in the rectum. The animals were forced to swim until their heart rate reached 300/min., and their body temperature rose to 107.6 degrees Farenheit (42° C). If they became cyanotic or unable to swim any more, they were immediately pulled out of the tank regardless of the status of the heart rate and body temperature.

Immediately before and after exercise blood was drawn, and a muscle biopsy was made from the triceps group as soon as the animals were removed from the tank. Following the biopsy, the animals were placed in individual metabolism cages and the urine collected for 24 hours for assaying vanilmandelic acid.

From September to December, 1970, the animals were forced to run to exhaustion once a week on a treadmill at an average speed of 5.5 mph and 11% elevation. The same parameters were monitored during the treadmill exercises as were recorded for the swims, and blood samples and biopsies of triceps muscle were again taken from each animal as described above.

RESULTS:

a. Food Consumption. Table 1 presents the average food consumed by each dog in pounds per day, for each test ration during the interval between June 1970 and May 1971.

TABLE 1
Daily Average Food Consumption for One Year
In Adult German Shepherds on Three Test Rations

Ration	Dog No.	Average Body Weight Pounds	Average Daily Food Consumed Pounds	Remarks
	1	58	1.417	Manufacturer of this Diet:
	4	56	1.611	Perk Foods, Co., Chicago,
Pooch	5	64	1.357	IL, Distributed by Safeway
Tasty	7	65	1.374	Stores, Inc., Head Office:
Nuggets	16	63	1.444	Oakland, California
	17*	65	1.811	
Average		61.83	1.502	
	6	68	1.573	Manufactured by Ralston
	9	57	1.336	Purina Co., Checkerboard
Purina	10*	45	1.076	Square, St. Louis, MO
Dog	13	46	1.126	
Chow	14	61	1.384	
	15	56	1.333	
Average		55.50	1.304	
	2	53	.914	Manufactured by Special
	3	63	1.196	Products Dept., Division
MSD	8	71	1.125	of Riviana Foods, Topeka,
	11	44	.771	KA 66603
	12	49	.878	
Average		56.0	.976	

*Supernumerary; not exercised.

Nutritional Aspects of Military Dog Performance (Cont'd)

b. Pre- and post-exercise blood and urine findings. Pre- and post-exercise values for total protein, calcium, chloride, potassium, CO_2, sodium, glucose, inorganic phosphates, cholesterol, uric acid, creatinine, bilirubin, alkaline phosphatase, creatine phosphokinase, lactic dehydrogenase, SGOT, and blood urea nitrogen have all been recorded and are being statistically analyzed. Total white blood cell and differential counts are being studied. The effects of exercise on these physiologic parameters will be the subject of a future laboratory report. The procedure for determining urinary vanilmandelic acid in the dog has not yet been perfected.

c. Muscle biopsies. Excellent differentiation of fiber types was obtained with histochemical stains for light microscopic demonstration of ATPase, DPNH, and phosphorylase activity. Routine stains, such as hematoxylin-eosin, periodic acid-Schiff, and trichrome complemented the demonstration of enzymatic localization. Differences in pre- and post-exercise muscle specimens were observed but these represented changes due to fatigue and were not related to diet. Type III fibers were more numerous in fatigued muscle than in resting muscle. Glycogen was more abundant in resting Type II fibers than in the post-exercise specimens. However, the use of these histochemical stains as absolute quantitative indices is not recommended, as the techniques are not designed to measure amounts of a specific ingredient, but rather to assess the site and quality of activity, in addition to fiber size and shape, size and location of nuclei, and condition of sarcoplasm. Electron microscopy of fatigued muscle biopsies revealed swollen mitochondria. This change was observed mainly in untrained animals. There were no significant changes due to exercise in the transverse tubular systems, bands, nuclei, or organelles.

d. Additional results. Between June of 1970 and May 1971 daily food consumption of 6 dogs fed Purina Dog Chow (PDC) averaged 1.304 lbs/dog; 6 dogs fed Pooch Tasty Nuggets (PTN) averaged 1.502 lbs/dog; and 5 dogs given Military Stress Diet (MSD) ate an average of .976 lbs/dog. Daily average cost of these diets/dog was: PDC, 14.34¢; PTN, 12.61¢; and MSD, 44.8¢. No difference can be detected in the appearance of these 3 groups of dogs. It has been noted that dogs on MSD seem to have only one bowel movement a day, while those on PDC and PTN have between 3 and 5 stools a day. Under actual field operations, it would appear undesirable for dogs on patrol to have frequent and copious stools. An advantage of possible logistical import appears to favor MSD in the reduced shipping space required to transport this high caloric dog food to overseas areas. For example, the dogs fed MSD had a mean body weight that was equal to that of the group fed PDC and was about 90% of the weight of the PTN-fed dogs; yet the MSD dogs ate, in pounds, only 75% as much as The PDC group and only 65% as much as the PTN group. Table 2 represents the weight gains and losses experienced by the 3 groups between May and December, 1970.

TABLE 2
Weight Gains and Losses in German Shepherds
On Three Different Dietary Regimens

Dietary Group	Average Weight at Start	Average Weight at End of 6 Months
Purina	59.2 lbs.	60.0 lbs.
MSD	57.2 lbs.	55.8 lbs.
Pooch Tasty Nuggets	60.2 lbs.	61.0 lbs.

CONCLUSIONS:

Many conclusions have been made as noted under RESULTS. Final evaluation of findings will be done when all physiologic parameters are analyzed.

RECOMMENDATIONS:

Swimming the dogs should not be resumed, as treadmill runs appear to fatigue the animals in a more sustained and natural way.

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1. Trevino, G. S.: Histochemistry of Canine Skeletal Muscle. (Abstract of presentation) 7th Histopathology Conference, Wash., D. C., 1970.

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APPENDIX A

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APPENDIX B

DIRECTORY OF 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. (U. of Maryland)
	Robert L. Morrissey, CPT, VC D.V.M. (Univ. of Ill.) Ph.D. (Cornell Univ.)
Computer Division	
Chief	Charles J. Messa, CPT, MSC Ph.D. (Lehigh Univ.)
	Richard A. Nelson, GS-12 A.B. (Univ. of Calif.)
Metabolic Division	
Chief	Robert H. Herman, COL, MC M.D. (U. of Illinois) Biochemistry (Univ. of Penn.)
Ass't Chief	Louis Hagler, LTC, MC M.D. (U. of Colo. Med. Sch.)
	Robert M. Cohn, MAJ, VC M.D. (Yale Med. Sch.)
	Harry L. Greene, MAJ, MC M.D. (Emory Med. Sch.)
	Irene T. Haupert, MAJ, ANC B.S. (Iowa State Univ.)
	Clara L. Miller, MAJ, AMSC B.S. (Florida A&M)
	Oscar D. Taunton, MAJ, MC M.D. (Univ. of Alabama)
	Janis C. Schiedeman, CPT, ANC B.S. (Ft. Hays Kansas State College)

Directory of Professional Staff (Cont'd)

	Deborah E. Wall, 2LT, ANC (Hurley Hosp. Sch. of Nursing)
	Frederick B. Stifel, GS-12 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., CPT, 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.)
	Neil J. Corneil, CPT, VC D.V.M. (Okla. State Univ.)
	Wayne R. Price, CPT, MSC Ph.D. (Univ. of Arkansas)
	Ray T. Sterner, CPT, MSC Ph.D. (Univ. of Wisc.)
	George J. Klain, GS-14 Ph.D. (Univ. of Ill.)

Directory of Professional Staff (Cont'd)

Chemistry Division	
Chief	Eugene M. Baker, III, COL, MSC Ph.D. (Georgetown Univ.)
Chief **(Fellowship Vanderbilt Univ.)**	Howerde E. Sauberlich, PL-313 Ph.D. (Univ. of Wisc.)
	Eldon W. Askew, CPT, MSC Ph.D. (Michigan State)
	Gerald L. Dohm, CPT, MSC Ph.D. (Kansas 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-11 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)

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Ralph M. Coan, MAJ, MC
M.D. (Georgetown Univ.)

Thomas P. O'Barr, GS-13
Ph.D. (Univ. of Texas)

Orman L. Weiser, GS-12
M.S. (Univ. of Denver)

Pathology Division
Chief — Gilberto S. Trevino, LTC, VC
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