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AD819586

ANNUAL RESEARCH PROGRESS REPORT

30 JUNE 1967

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USARMY MEDICAL RESEARCH AND NUTRITION LABORATORY

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ANNUAL RESEARCH PROGRESS REPORT

30 June 1967

US ARMY MEDICAL RESEARCH AND NUTRITION LABORATORY

Fitzsimons General Hospital

Denver, Colorado 80240

U. S. ARMY MEDICAL RESEARCH AND NUTRITION LABORATORY
Fitzsimons General Hospital
Denver, Colorado 80240

ANNUAL RESEARCH PROGRESS REPORT
1 July 1966 - 30 June 1967

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

5011 - 6.11.45.01.1 - Defense Research Sciences, Army

3A014501B71P - Basic Research in Support of Military Medicine

01 - Biochemistry
Work Units 058 - 062

07 - Pharmacology
Work Unit 030

3A014501B71R - Research in Biomedical Sciences

02 - Internal Medicine
Work Unit 055
Work Units 057 and 058
Work Units 061 - 064

05 - Environmental Medicine
Work Units 080 - 085

5016 - 6.11.30.01.1 - In-House Laboratory Independent Research

3A013001A91C - In-House Laboratory Independent Research

Work Unit 041
Work Unit 046
Work Units 049 - 052

5028 - 6.21.56.01.1 - Biomedical Investigations

3A025601A822 - Military Internal Medicine

Work Units 065 - 068
Work Units 070 - 074
Work Units 076 - 078

3A025601A827 - Military Environmental Medicine

Work Units 070 - 074

SUMMARY

Basic Research in Support of Military Medicine:

Studies of Performance Physiology, Molecular Biochemistry, Studies in Lipids, Nutrition and Metabolism, Mineral Metabolism, and Haemopoietic Metabolism.

Research in Biomedical Sciences:

Experimental Surgery, Maintenance of Animals, Nutritional and Metabolic Adaptations and Interrelationships, Work Performance and Body Composition, Muscle Metabolism, Microbial Metabolism, High Altitude Bioenergetics, Cardiovascular and Pulmonary Responses, Microbial Flora and Myocardium of animals have been studied utilizing humans, dogs, and small laboratory animals using various techniques.

In-House Laboratory Independent Research:

Research has been conducted under the following work units during the past year: Symbiosis and Intestinal Flora, Means for Measurement of Work Decrement in the Rat, Body Temperature Control by Adrenal Steroids, Examination of Mature German Shepherd Dogs Fed Rice Base Diets, Coronary Blood Flow Studies and Natriuretic Property of 16-Alpha-Hydroxyprogesterone.

Biomedical Investigation:

These studies include: Tuberculosis Research, Computer Classification of Pulmonary Disability, Computer Instrument Linkages, Effects of INH on Animal Histology, Intravenous Fat Emulsion, Human Nutrition, Nutritional Studies of Military Populations, Nutritional Physiology, Analytical Biochemistry, Nutrients and Response of Man to Nutrition or Disease.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

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RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
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10a. CURRENT NUMBER/CODE: 61145011 3A014501B71P 01 058

10b. PRIOR NUMBER CODE: None

11. TITLE: (U) Molecular Biochemistry (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry; 014000 Radiation Chemistry; 003500 Clin. Med.	02 64	NA	OTHER / DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST	a. PROFESSIONAL MAN YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 67	2	73
		CURRENT FY 68	2	65

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21. TECHNOLOGY UTILIZATION: Radiation protection; cancer therapy; clinical chemistry

22. COORDINATION: None

23. KEYWORDS: Radiation, enzymes, lysozyme, enzyme inactivation, protein, enzyme assays, peptides

24. (U) Technical Objective: To determine the mode of radiation inactivation of lysozyme and to develop ultramicro analytical procedures to facilitate this work. Such information will be useful in better understanding radiation damage resulting from cancer therapy or military situations. To increase the efficiency and precision of enzyme assays, such as transketolase assays for use in assessing thiamine deficiencies.

25. (U) Approach: Paper electrophoresis and ion exchange chromatography are being used to separate and quantitate peptides from irradiated lysozyme products. Characterization of the isolated peptides is being attempted with sensitive chemical procedures (dansylation reaction) and spectrophotometric measurements. Transketolase assays are being automated.

26. (U) Progress: About half the peptides from several lysozyme radiation products can be isolated in sufficiently pure form for characterization. The dansylation and other reactions have been automated to facilitate peptide characterization. A Gamma-cell cobalt-60 source has been installed to permit expanded enzyme radiation studies. Transketolase assays have been automated.

Publication: Radiation produced aggregation and inactivation in egg-white lysozyme. J. Biol. Chem. 242: 1821, 1967.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
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31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

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35. EST. FUNDS (In thousands) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biochemistry
WORK UNIT NO.	058	Molecular Biochemistry

The following investigations have been conducted under this work unit:

STUDY NO. 1 Modes of ionizing radiation inactivation in lysozyme

STUDY NO. 2 Radiation effects in ribonuclease

STUDY NO. 3 Increase efficiency and precision of conducting enzyme assays via automation

1. Work continued on determining the mode of ionizing radiation inactivation of egg-white lysozyme. Procedures were refined for large-scale isolation of chromatographically homogeneous lysozyme and components from irradiated lysozyme. Purity checks have been performed on isolated components chromatographically, and an analytical ultracentrifuge is being developed for more precise and efficient purity checks subsequently. A partial automatic method has been devised for measuring disulfide bonds in radiation products from lysozyme. A fluorometric marker has been synthesized for use in studying radiation disruption of three-dimensional structure in lysozyme. Both methods are being applied and will help answer the question as to whether radiation damage in lysozyme is localized and/or the result of disruption of 3-dimensional structure. About half the peptides from tryptic hydrolysates of radiation products can now be isolated in sufficiently pure form for characterization. Dansylation and other ultrasensitive analytical reactions, in conjunction with diagonal peptide mapping, are now being automated to facilitate this characterization.

2. Studies have been initiated on radiation effects in ribonuclease, utilizing the same approach as with lysozyme. To facilitate this work and expansion of these studies to other enzymes and enzyme systems (e.g., in vivo systems), a Gamma-cell Cobalt-60 source has been installed. Definitive results are not yet available to support any valid conclusions.

3. Work has been initiated to increase the efficiency and precision of conducting enzyme assays by automation, particularly those of use in assessing animal and human nutritional status and those of use in protein or peptide degradation procedures. Transketolase assays (thiamine deficiency assessment) have been automated and applied to about 1200 hemolysate samples from a nutrition survey in Panama and other collaborative studies.

BODY OF REPORT

WORK UNIT NO. 058	Molecular Biochemistry
STUDY NO. 1 and STUDY NO. 2	To determine the modes of ionizing radiation inactivation in lysozyme and ribonuclease

PROBLEM:

Separation and purification of active and inactive components from dry irradiated lysozyme samples has been accomplished. Previous studies established reversible aggregation as one mode of inactivation. Another mode which must be considered is general disruption of 3-dimensional structure in the irradiated enzyme. Such disruption can be studied by using fluorometric markers whose fluorescence is decreased in aqueous solutions by disrupted protein structure to a greater degree than by the native structure of the enzyme.

Chemical characterization of active and inactive components from irradiated lysozyme and ribonuclease will also be necessary to ascertain whether radiation damage is a localized effect and/or a general structural rupture effect in the enzyme. Characterization of these components involves splitting these component large molecules via limited proteolysis into smaller fragments more amenable to available analytical techniques. Purification of these fragments is essential prior to characterization. Evolvement of more sensitive, precise and efficient analytical procedures, particularly for disulfide groups and end groups, is dictated by the larger number of materials (fragments) becoming available in micro amounts for characterization.

RESULTS AND DISCUSSION OF THE RESULTS:

Separation, and to some extent purification, of active and inactive components from dry irradiated lysozyme samples had been accomplished in previous studies. Purity of the isolated components has been checked using rechromatography on the same resin used for isolation. In most cases, single symmetrical peaks were obtained. This degree of purity provided sufficient basis for successful attempts to break a few of the components down, via limited proteolysis, into smaller fragments that could be separated on columns and characterized by available analytical methods or more sensitive methods in the process of being developed. The smaller fragments are available in micro quantities at this stage so that more sensitive methods are being developed. Existing ultra-micro methods are being automated to accommodate the large number of fragments that are becoming and will become available for

characterization. These methods include fluorescent or colorimetric disulfide analyses and fluorescent dansylation procedures for end groups analyses (Sieler and Wiechmann, Experentia, 20: 559-60, 1964).

It has already been established that localized radiation effects at disulfide bonds are responsible for a significant (20-30%) portion of enzymic inactivation in disulfide bonds. Mechanisms accounting for the remainder of the damage can lie in localized effects in other portions of the molecule and/or general disruption of the molecule resulting from primary random damage events in the molecule. Column chromatography and the diagonal peptide-mapping procedure (Lamm et al., Fed. Proc., 26: 480, 1967) followed by characterization will answer part of this question.

Bearing more specifically on general disruption of structure can be used the technique of McClure and Edelman (Biochemistry, 5: 1908, 1966). 2-p-Toluidinylnaphthalene-6-sulfonate is used as a hydrophobic probe here for determining the conformation state of proteins. This compound is not commercially available so that synthesis of the material was necessary; this has been accomplished in good yield and purity and is now being used in preliminary studies of the conformational states of soluble components from irradiated lysozyme.

Recent installation of a gamma-cell Cobalt-60 radiation source has been accomplished. This will permit expanded enzyme radiation investigations not previously possible. These can include larger scale radiation studies on lysozyme, expansion to other dry enzymes (particularly ribonuclease) and studies of a simulated in vivo system (ribonuclease in solution and/or as it exists in bovine pancreas).

Studies of radiation effects have been initiated in dry, solid ribonuclease. A rapid manual method for chromatography of the enzyme and its derivatives or radiation products has been adapted to use in this laboratory and is in the process of being automated. A sensitive, rapid, precise procedure for assay of the enzyme has been adapted for use in this laboratory and it, too, is in the process of being automated.

An analytical ultracentrifuge (Beckman Model E) has been installed and is in the process of being equipped with the necessary accessories for its utilization in these research efforts as well as those of other investigators in this laboratory.

WORK UNIT NO. 058 - BODY OF REPORT

CONCLUSIONS:

Purity of isolated components from irradiated lysozyme has been sufficiently established to permit characterization via two approaches:

a. Direct study of conformation structure on the component via fluorometric probes to evaluate possible changes in hydrophobic structure.

b. Reduction of purified components to fragments amenable to available manual ultra-micro analytical procedures and automatic procedures in the development stage, based on the manual procedures.

Preliminary work with ribonuclease has progressed to the stage where studies similar to those performed on lysozyme can be applied to this enzyme with expectation of success.

Installation of the gamma-cell Cobalt-60 source is permitting more extensive studies with these two enzymes and expansion to other enzymes and enzyme systems approaching in vivo conditions. Its presence has also provided the opportunity for more collaborative efforts with other investigators in the university and medical school areas.

The analytical ultracentrifuge, due to technical difficulties in its installation and procurement of essential parts or accessories for its operation, has not been utilized as effectively as desired. This situation has been largely corrected, and it is anticipated that more extensive use of this equipment will facilitate not only these research efforts but assist other investigators in this laboratory in the conduct of their research efforts.

RECOMMENDATIONS:

Radiation studies should be continued along the same lines as followed previously with these points in mind. Increased emphasis should be placed on studies of solid dry ribonuclease and in vivo or simulated in vivo systems containing ribonuclease. Acceleration of improved analytical techniques for characterization work should be accomplished by use of the automatic equipment on hand, supplemented with the addition of newer, more efficient equipment which will facilitate evolvement of automatic techniques. In particular, additional moderate cost, low temperature devices and light sources are required, as well as a new piece of equipment which enables one to simultaneously develop and scan thin-layer chromatographic plates. This item will facilitate rapid development of procedures for separating and purifying fragments of radiation products from enzymes and be amenable to automation in a

fashion compatible with existing equipment. It will also be of use generally within the laboratory for application by other investigators in their research efforts.

The gamma-cell Cobalt-60 source should be made available to other investigators in the Denver area on a controlled but liberal access policy since it has the capacity to handle either large samples or many small samples and can easily accommodate our laboratory's need as well as those of other investigators.

The analytical ultracentrifuge should have its capability increased by utilizing either Polaroid photographic techniques or, ultimately, addition of the ultraviolet monitoring system provided by Beckman Instruments. This provides a recorder readout which should be adaptable to computer treatment of data on a direct or indirect basis, depending upon the amount of use to which this instrument will be placed.

PUBLICATIONS:

1. Stevens, C. O. and G. R. Bergstrom (Introduced by H. E. Sauberlich). The multiple nature of crystalline egg-white lysozyme. Proc. Soc. Exp. Biol. Med., 124: 187-191, 1967.

2. Stevens, C. O., H. E. Sauberlich and G. R. Bergstrom. Radiation produced aggregation and inactivation in egg-white lysozyme. J. Biol. Chem., 242: 1821, 1967.

STUDY NO. 3

To increase the efficiency and precision of conducting enzyme assays via automation, particularly those of nutritional interest and of use in protein characterization work

PROBLEM:

Considerable success has been obtained in automating transketolase assays. The principal aim is to automate other enzyme assays that would be useful in protein characterization work and assessing nutritional deficiencies, disease states, or stress conditions. Little work has been done in this area insofar as protein characterization work is concerned. Manual procedures are still primarily being used. In the medical area, automated methods are available,

but they are not as precise as the manual methods. There is also considerable room for improvement insofar as the speed with which such automated assays can be conducted. Eventual linkage of recorder output with the digital computer for data processing is anticipated.

RESULTS AND DISCUSSION OF THE RESULTS:

A partially automatic method for transketolase assays has been developed and is being refined and evaluated. The present method for transketolase assay consists of manual incubation of the sample with ribose-5-phosphate, precipitation of the sample protein and analysis of the residual ribose-5-phosphate by an automatic colorimetric method utilizing an orcinol-ferric ammonium sulfate reagent. This method has been applied to approximately 1200 hemolysate samples derived from a nutrition survey in Panama and collaborating investigators. Reproducibility of the method is satisfactory. Reagent stability is excellent for prolonged runs or storage. Accuracy is satisfactory, as judged from the use of standards and internal standards. Accuracy can be improved by reducing the speed at which the assays are performed. In addition, the method will be completely automated by the use of a temperature controlled time delay coil for the enzymic portion of the reaction followed by use of a dialyzer to remove sample protein from the substrate or product before colorimetric measurement. Statistical analysis of the available data is in progress and will provide a basis for future direction on refinement of the method.

CONCLUSIONS:

Satisfactory progress has been made in automating a method for transketolase determinations in a short period of time. Refinement of the method is required and can be accomplished quickly. It is a valuable method for the rapid assessment of thiamine deficiency states and, in conjunction with thiamine excretion data, should provide an accurate assessment of suspected thiamine deficiency states.

RECOMMENDATIONS:

Refinement of the automatic transketolase method should continue. Efforts here should, in the immediate future, be concerned with the study of automation of other enzymes close to transketolase in the pentose phosphate pathsay since, in severe thiamine deficiencies, general enzyme synthesis may be

depressed and this would not be reflected in the present transketolase assays since ribose-5-phosphate is not the immediate substrate of the enzyme. An intermediate enzyme converts ribose-5-phosphate to xylulose-5-phosphate which then is converted to fructose-6-phosphate and sedoheptulose-7-phosphate by transketolase. Therefore, two things must be done. The present transketolase assay should be modified so as to use xylulose-5-phosphate as substrate and either the substrate or product measured colorimetrically. Secondly, moderate and severe thiamine deficiency in rats should be studied as it relates to transketolase and other closely associated enzyme and coenzyme levels. Red cell transaminases are of immediate importance in evaluation of vitamin B_6 nutritional status, using much the same approach as with transketolase (M. C. Cheney et al., Can. J. Physiol. Pharmacol., 45: 343, 1967).

Automated enzyme assay procedures should be investigated for other enzymes that are related to or altered in nutritional deficiencies, disease states, or stress conditions. Eventually, automated procedures for characterization of proteins, in particular the abnormal hemoglobins, will be of tremendous medical and military importance.

PUBLICATIONS:

1. Stevens, C. O. and J. L. Long. An automatic method for transketolase assays. To be presented at the Technicon Symposium, "Automation in Analytical Chemistry," 2-4 October 1967, New York, N. Y.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
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10a. CURRENT NUMBER/CODE: 61145011 3A014501B71P 01 059

10b. PRIOR NUMBER CODE: None

11. TITLE: (U) Basic Studies in Lipids (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT COMPL DATE	15. FUNDING AGENCY
002300-Biochemistry 012900-Physiology; 002600-Biology	04 66	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	a. DATE	a. RESOURCES EST	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA		PRIOR FY 67	1	42
	c. TYPE	d. AMOUNT	CURRENT FY 68	1	49

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21. TECHNOLOGY UTILIZATION
Understanding the pathogenesis of disease

22. COORDINATION
None

23. KEYWORDS: Lipids, phospholipids, steroids, fatty acids, bile acids, diet, nutrition, free fatty acids, obesity, hyperlipemia

24. (U) Technical Objective: (a) The effect of various carbohydrate diets on plasma triglycerides will be studied in normal man and in patients with hypertriglyceridemia. (b) The bile steroid pathway in animals and in patients with hepatic disease will be investigated and the effect of various controlling factors will be studied. (c) Hypoglycemic states will be investigated. Plasma insulin in growth hormone will be studied. (d) The regulation of gastrointestinal enzymes by dietary constituents will
25. be studied.

25. (U) Approach: (a) Normal subjects will be placed on varying saccharide diets and the response of plasma triglycerides will be studied. Lipoproteins will be studied. In patients with hypertriglyceridemia the response to various dietary regimens will be investigated and the level of acetyl CoA carboxylase in the liver will be assayed. (b) Various bile steroids will be synthesized especially radioactive bile steroids. (c) Patients with reactive hypoglycemia will be studied on a variety of dietary regimens and the response of insulin and growth hormone to a variety of test substances will be studied. (d) The response of gastrointestinal disaccharidases to various
26. dietary sacharides will be studied in normal man.

26. (U) (a) The synthesis of lipids by the liver is independent of levels of a-glycerophosphate. The regulation of lipid synthesis from glucose fructose and sucrose depends upon the amount of acetyl-CoA presented to the enzyme acetyl-CoA-carboxylase which regulates lipid synthesis in the liver. Fructose produces large amounts of Acetyl-CoA whereas glucose produces smaller amounts because of the limitations imposed by hexokinase and phosphofructokinase. Studies indicate that reactive hypoglycemia is indicative of early latent diabetes. It was observed that a gastrointestinal disaccharidase can be induced by dietary sucrose.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71P	Basic Research in Support of Military Medicine
TASK NO.	01	Biochemistry
WORK UNIT NO.	059	Basic Studies in Lipids

1. The relationship of varying dietary carbohydrates to plasma lipid concentrations was studied in normal human volunteers, patients with hypertriglyceridemia, and in rats. A dose-response between dietary sucrose and increase in serum triglyceride levels has been demonstrated. No such change occurred with serum cholesterol and phospholipid levels. Dietary glucose in varying amounts was less effective in elevating serum triglyceride levels. The diagnosis of carbohydrate-induced hypertriglyceridemia was proven in two patients. Atromid was effective in lowering serum triglycerides in one patient and in bringing serum insulin levels to normal. The serum insulin levels remained normal even after cessation of atromid therapy even though serum triglycerides rose to their previous high levels. Serum insulin however was normal in the second patient. The enzymes mediating fatty acid synthesis are presently being measured in liver tissue obtained from the first patient by closed liver biopsy while atromid therapy was discontinued. Studies of rats on varying carbohydrate diets have disclosed that acetyl CoA is produced at a more rapid rate from fructose whether free or in the form of sucrose as compared to glucose and chow. These studies indicate that dietary fructose as such or in the form of sucrose can increase plasma triglyceride levels to a greater degree than can glucose and is not directly related to cholesterol or phospholipid levels.

2. The transformation of cholesterol into bile steroids is under investigation. To study the enzymatic transformations involved in this pathway in the liver it is necessary to obtain suitable bile steroid intermediates. A number of chemical approaches are being considered to effect the chemical synthesis of various of the bile steroid intermediates.

3. Using an immunoreactive double antibody assay system for plasma insulin, studies on insulin responsiveness have been carried out in a number of differnet situations. Patients with reactive hypoglycemia frequently have a delayed response of insulin to a glucose load. The symptoms and hypoglycemia in these individuals is aggravated by a high protein diet. Sulfonylurea therapy has alleviated this reactive hypoglycemia. These patients also had an alteration of glucose metabolism during fasting which was much more striking than that demonstrated by normal subjects. Studies are being carried out on normal and obese subjects to determine the influence of potassium supplementation on glucose metabolism during fasting. Investigations of maximal inductable insulin stimulation have indicated that multiple insulinogenic stimuli produce a much greater insulin response than does a standard glucose tolerance test.

4. Dietary sucrose is able to increase the activity of jejunal sucrase but has no effect on maltase and lactase activities. The increase in sucrase activity is possibly due to an induction of enzyme activity by the specific sucrase substrate. Dietary glucose and maltose had no effect on any of the disaccharidases.

BODY OF REPORT

WORK UNIT NO. 059 Basic Studies in Lipids

STUDY A:

DESCRIPTION:

The problem of the relationship of diet to plasma lipid concentration is under investigation. This has been approached by studying the effect of varying carbohydrate diets on plasma lipids in normal man, by studying the problem of carbohydrate-induced hypertriglyceridemia, and by studying the effect of varying carbohydrate diets in rats on the concentration of glycolytic intermediates in the liver, the level of activity of glycolytic and fatty acid synthesizing enzymes, and the incorporation of radioisotopically labeled sugars into lipids in vitro in rat liver slices.

Normal human volunteers have been placed on a 3000 calorie diet divided into four equal meals consisting of 15% of calories as casein, 20% as sucrose, and 65% as corn oil. After 3 weeks the sucrose content was increased to 40% of calories and the corn oil decreased accordingly. Thus, at 3 week intervals the sucrose content was 20, 40, 60, and 80% of dietary calories. A similar study was carried out with glucose instead of sucrose. One individual was studied with fructose at 20 and 40% of calories.

Two patients with hypertriglyceridemia were studied and the diagnosis confirmed demonstrating a change in serum triglyceride levels on a high-fat low-carbohydrate diet. The effect of Atromid was studied. The level of certain hepatic enzymes was measured in specimens obtained by closed liver biopsy.

Rats were fed diets varying as to chow, sucrose, glucose, and fructose. At the end of the dietary periods the animals were killed and serum and hepatic content of cholesterol, triglycerides, and phospholipids were measured. The level of glycolytic intermediates in the liver was also measured as was the activity of various glycolytic and fatty acid synthesizing enzymes. Liver slices were incubated with radio-isotopically labeled sugars (glucose and fructose) and the incorporation into hepatic lipids was determined.

PROGRESS:

In normal human volunteers we have found that plasma triglycerides increase as the content of sucrose in the diet increases. At the higher level of sucrose hypertriglyceridemia occurred in some of the subjects. That is, the serum triglyceride level was higher than at the start of the study. At the lower levels of sucrose the serum triglyceride level was less than the initial triglyceride level. Serum cholesterol and phospholipid levels fell and rose only to a slight degree compared to the changes in plasma triglycerides. There were only slight changes in body weight during the study. The glucose diet produced lesser increases in serum triglyceride while the serum cholesterol and phospholipid dropped from initial levels and remained constant throughout the study.

Thus we have demonstrated a dose-response relationship between the amount of dietary carbohydrate and the plasma triglyceride level. In addition, and most important, the type of dietary carbohydrate is related to the degree of triglyceridemia with sucrose being more potent than glucose. We interpret the data, particularly in conjunction with the animal data, to mean that the fructose content of the sucrose is responsible for greater elevations of serum triglycerides.

The patients with hypertriglyceridemia were shown to indeed have the carbohydrate-induced variety of hypertriglyceridemia. One patient responded to atromid with a marked decrease in his serum triglyceride levels. On discontinuation of atromid his serum triglycerides promptly rose to their previous high levels. Before therapy serum insulin levels after oral glucose ingestion were abnormally high. On atromid therapy the insulin levels after glucose ingestion became normal and remained so even after the cessation of atromid therapy. During the hyperlipemic phase a closed liver biopsy was performed and enzyme levels of certain fatty acid synthesizing enzymes were measured. Liver obtained from patients undergoing elective cholecystectomy was used as control tissue, since these represent as nearly normal liver tissue as it is possible to obtain. Our second patient with hypertriglyceridemia has normal serum insulin before atromid therapy and thus differs in this respect as compared to the first patient.

The animals fed varying amounts of dietary carbohydrates were found to have higher values of hepatic and serum triglycerides when on the high sucrose and fructose diets as compared to the glucose and chow diets. The intermediates and enzymes in the liver gave data which can be interpreted to mean that glucose is metabolized via enzymes that necessarily limit the amount of glucose that can be transformed into acetyl CoA. Fructose on the other hand is metabolized at a much more rapid rate thereby giving rise to larger amounts of acetyl CoA. Rats and man have a fructokinase which is able to handle large amounts of fructose as such in rats and in the form of sucrose in man and rats. The glucokinase of rats and man however cannot handle comparable amounts of glucose and thus the rate of glucose metabolism is not as rapid as that of fructose. Further, the enzyme phosphofructokinase, can be inhibited by citrate and this may further limit the metabolism of glucose. No such limit is imposed upon fructose since its metabolic pathway bypasses this enzyme. The data obtained by measuring the incorporation of radioactive sugars into the lipids in liver slices confirmed these findings.

SUMMARY:

The relationship of dietary carbohydrate to plasma lipid concentration has been investigated. It has been found that dietary sucrose gives rise to increased levels of serum triglyceride in a dose response fashion with a lesser response with dietary glucose. The fructose content of sucrose is believed to be responsible for these findings.

Basic Studies in Lipids (cont'd)

Serum cholesterol and phospholipid levels changed only to slight degree with dietary sucrose and not at all with dietary glucose. We have proven the diagnosis of carbohydrate-induced hypertriglyceridemia in two patients and have controlled the condition in one patient with atromid. Serum insulin levels after oral glucose was abnormally high in one patient and normal in the other. On Atromid therapy the insulin level returned to normal levels and remained normal after cessation of the atromid therapy. Studies in rats indicate that fructose is metabolized at a more rapid rate than glucose and thus gives rise to greater substrate levels necessary for triglyceride synthesis. Other studies have shown that glycerol levels in the liver decrease as increased triglyceride synthesis occurs and thus cannot account for the increase in triglyceride synthesis due to high fructose and sucrose diets in the rat.

PUBLICATIONS:

1. David Zakim and Robert H. Herman. Relationship of Varying Amounts of Dietary Sucrose to Serum Lipid Concentration in Normal Males. Am. J. Clin. Nutrition 20:364 (1967) (Abstract No. 8). Presented at the Am. Soc. Clin. Nutrition, 7th Ann. Mtg., Atlantic City, New Jersey, April 29, 1967.

2. David Zakim, Ronald Pardini, Robert H. Herman and Howerde Sauberlich. The Relation of Hepatic alpha-Glycerophosphate Concentration to Lipogenesis in Rat Liver. Biochim. Biophys. Acta 137:179, 1967.

3. David Zakim, Robert H. Herman, and James W. Anderson. Effect of Atromid on the Relation Between Plasma Insulin and Triglyceride Concentration in Carbohydrate Induced Hypertriglyceridemia. Clin. Res. 15:333, 1967.

4. David Zakim and Robert H. Herman. Glucokinase Activity in Human Adipose Tissue. Nature, in press.

5. David Zakim, Ronald Pardini, Robert H. Herman, and Howerde Sauberlich. Relation of High Carbohydrate Diets to Fatty Acid Synthesis in Rat Liver. Seventh International Congress of Biochemistry, Tokyo, Japan, Aug. 24, 1967, p. 69, Abstract No. 9.

6. David Zakim, Ronald S. Pardini, Robert H. Herman, and Howerde E. Sauberlich. Mechanism for the Differential Effects of High Carbohydrate Diets on Lipogenesis in Rat Liver. Biochim. Biophys. Acta, in press.

Basic Study in Lipids (cont'd)

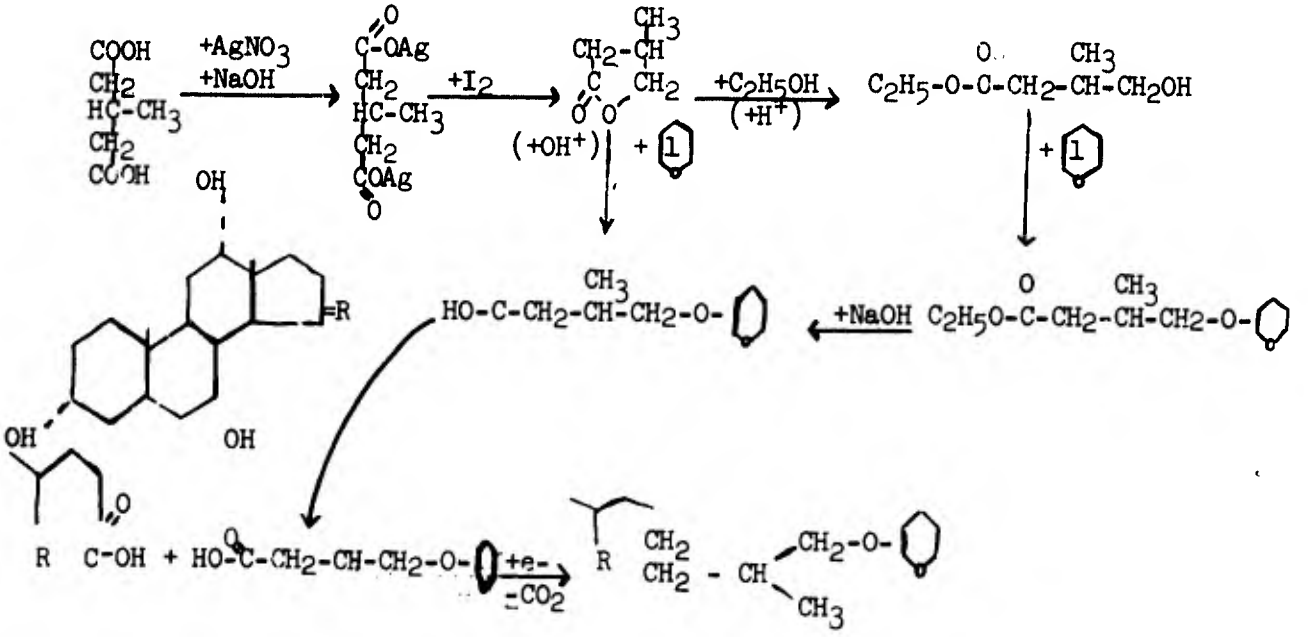
STUDY B:

DESCRIPTION:

Cholesterol is synthesized by the liver in large quantities daily. The quantitatively most important derivatives of cholesterol in the liver and in the body are the bile steroids. The exact pathway by which cholesterol is converted into bile steroids, the controlling factors of such a pathway, the importance of bile steroids in liver disease, and the exact function of the bile steroids is unknown. We have undertaken to determine certain of the details of the bile steroid pathway as an initial step in answering these important questions. The main difficulty in studying the problems is obtaining intermediate bile steroids. These must be synthesized from cholic acid or chenodeoxycholic acid chemically or synthesized from trihydroxycoprostanic acid which is an end-product of bile acid metabolism in certain reptilia. Our efforts have been focused on methods for obtaining tetrahydroxycoprostane.

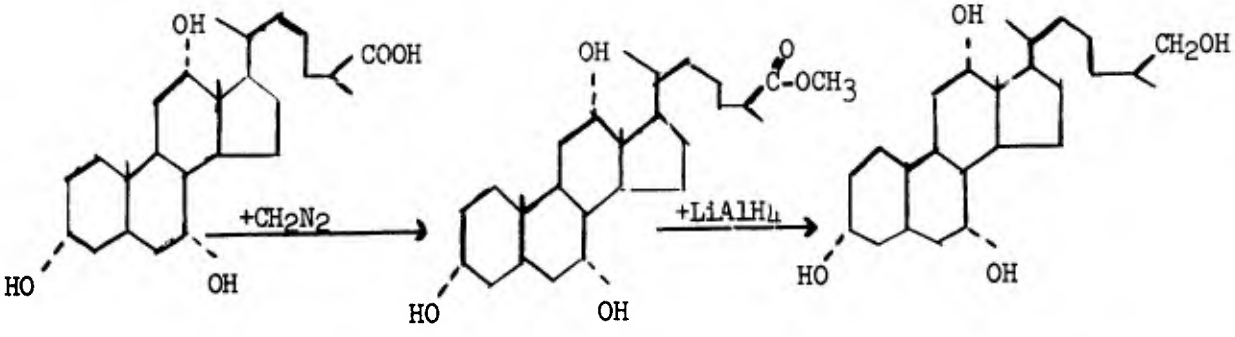
PROGRESS:

3-methyl butyrolactone has been synthesized from 3-methylglutaric acid. It is possible to open the lactone ring with alkalai and protect the free hydroxyl group with dihydropyran. If the dihydropyran also reacts with the carboxyl group then the lactone can be converted to the corresponding ethyl ester by refluxing in acid ethanol. With the carboxyl group protected the hydroxyl group can be protected with dihydropyran, the ester group removed by saponification and the free acid coupled electrolytically with cholic acid to form the tetrahydropyran derivative of tetrahydroxycoprostane.



Basic Study in Lipids (cont'd)

The tetrahydropyran group can then be removed by refluxing in phosphoric acid. Several other approaches are being investigated to synthesize radio-isotopically labeled compounds utilizing Grignardreagents of 2-bromopropanol with the hydroxyl group protected by dihydropyran. An alternative is to convert the silver salt of triformylcholic acid into the corresponding nitrile by treatment with bromine and react this with the ^{14}C-ethyl ester of 2-bromopropionic acid in the presence of activated zinc, the so-called Reformatsky reaction. This would result in an imide derivative which could be converted to the keto derivative by refluxing with pyruvic acid. The keto group can be removed by heating with hydrxazine hydride and removal of formyl and ethyl groups can be effected by saponification. We have recently obtained bile from crocodilians and will prepare the trihydroxycoprostanic acid. This can be easily converted into the tetrahydroxycoproste by esterification with diazomethane and reduction with lithium aluminum hydride:



With the preparation of these compounds we will be ready to carry out the definitive studies on the transformations of these intermediates by hepatic enzymes.

SUMMARY:

The transformation of cholesterol into bile steroids by liver enzymes is being investigated. In order to carry out this work it is necessary to obtain appropriate bile steroid intermediates to test with various enzyme preparations. Various methods are outlined to obtain the necessary intermediates from bile steroids that are readily accessible.

PUBLICATIONS:

None.

Basic Study in Lipids (cont'd)

STUDY C:

DESCRIPTION:

The influence of various diets on glucose tolerance has been recognized for years. Recently it has been noted that amino acids as well as glucose can stimulate insulin secretion. The mechanisms by which these stimuli influence the release of insulin remains unclear. Studies of patients with reactive hypoglycemia have revealed that certain individuals are made symptomatically better on high protein diets while other have an aggravation of their symptoms. These patients frequently have had a delay or asynchrony in their insulin release in response to glucose. We have investigated the serum insulin levels after oral glucose ingestion in patients with so-called reactive hypoglycemia after preparation with high carbohydrate and high protein diets, and in a number of other experimental situations.

PROGRESS:

In patients with severe reactive hypoglycemia (glucose less than 30mg%) plasma glucose and serum immunoreactive insulin levels were measured during a standard glucose tolerance test (GTT). On a control diet (carbohydrate 64% of calories, protein 13% of calories) two patients with protein-aggravated and one patient with carbohydrate-aggravated hypoglycemia had an average maximal glucose rise to 137mg%. The insulin response was above normal in two and delayed in the third patient. After a high protein (51% of calories), low carbohydrate (26% of calories) diet each had a diabetic GTT with an average maximal glucose value of 222mg%. The average GTT of five normal subjects remained normal on this diet. The patients were not sensitive to leucine or arginine. Sulfonylurea therapy relieved the hypoglycemia of these patients.

Fasting for 48 hours causes marked abnormalities of the GTT in these patients with the maximal glucose values ranging from 236 to 292 mg%. Under similar conditions four normal subjects averaged maximal glucose values of 180 mg%. Potassium supplementation had no effect on the GTT in normal subjects fasted for 48 hours.

With intravenous and oral glucose together with intramuscularly administered glucagon, non-obese subjects showed maximal insulin values above that seen only with a standard GTT. Under these conditions, obese patients showed an even greater insulin response.

SUMMARY:

Studies of patients with reactive hypoglycemia have indicated that in certain cases the hypoglycemia is worsened by protein ingestion. Sulfonylurea therapy alleviated the reactive hypoglycemia. An alteration in glucose metabolism occurs in these patients with fasting as indicated by an abnormal GTT. Under similar conditions of fasting

normal individuals show much less abnormal change in GTT. Multiple insulinogenic stimuli are capable of producing a much greater insulin response than seen during a standard GTT.

PUBLICATIONS:

1. Anderson, James W. and Herman, Robert H. Protein aggravated reactive hypoglycemia: Response to sulfonylureas. Diabetes, in press (abstract).

2. James W. Anderson and Robert H. Herman. Reversal of Hypoglycemia and Asynchronous Insulin Release by Tolbutamide Therapy. Presented at the Colorado Chapter, American College of Physicians, Colorado Springs, Colorado, Jan. 27, 1967.

STUDY D:

DESCRIPTION:

The effect of various carbohydrate diets on the disaccaridase activity of jejunal mucosa has been investigated. Normal human subjects were placed on control diets of 40% of calories as carbohydrate, 45% as fat and 15% as protein. The carbohydrate varied as to glucose, lactose, and sucrose. At varying intervals jejunal mucosa was obtained by means of an intestinal biopsy capsule. The mucosa was frozen until the end of the study when all specimens were assayed for lactase, maltase, and sucrase activity.

PROGRESS:

On the glucose and lactose diets no change occurred in the activity of the lactase, maltase or sucrase. On the sucrose diet a significance increase in sucrase activity occurred, while no change occurred in the lactase and maltase activities. The data can best be interpreted as indicated that enzyme induction took place under the influence of the specific substrate. However, other explanations can be invoked to explain the changes but these seem less likely.

SUMMARY:

Dietary sucrose is able to increase the activity of jejunal sucrase but has no effect on maltase and lactase activities. Dietary glucose and lactose have no effect on any of the disaccharidases.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6344	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 07 66)	U NAT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71P 01 060	61145011 3A014501B71P 01 055 056 057

11. TITLE:
(U) Basic Studies of Nutrition and Metabolism (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry	07 66	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	1	115
		CURRENT FY 68	3	130

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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TEL. 202 OXford 6 5472	TEL 303 366-5311 X24214 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Nutrition; Clinical Medicine Nutritional Diseases	None

23. KEYWORDS Lipid Metab.; Mineral Metab.; Proteins; Protein Metab.; Proteins, Lipids, Carbohydrates & Related Compounds; Trace Elements; Nutrition; Nutrition Disorders; Lipids

24. (U) Tech Objective: Basic studies in nutrition and on the metabolism of nutrients to enable a better understanding of their role in health and disease. Of particular interest is the development of techniques and investigations that may provide further knowledge as to the metabolism, utilization, or functions of the major nutrients, including lipids, carbohydrates, proteins and minerals.

25. (U) Approach: For each of the nutrients, the investigations will be largely concerned with subcellular and enzyme techniques involving radioactive tracers to develop and follow metabolic pathways and interactions. The studies will involve primarily the use of laboratory animals and microorganisms. Enzyme purification and isolation will be required in certain instances to establish metabolic pathways. These studies are often tedious and unpredictable. Major effort in the area of minerals will be devoted to calcium and phosphorus metabolism as related to bone chemistry and cartilage calcification. Efforts will be directed towards developing techniques to study trace elements, including the more recently recognized essential elements, and their interaction with other dietary nutrients and hormones.

26. (U) Progress: Animals with high calcium-low phosphorus rickets have no impairment in their ability to transport calcium as evidenced by normal levels in blood of rachitic animals. Both calcium and phosphorus levels in blood are increased in response to vitamin D. Radiocalcium and radiophosphorus reveal the extent of absorption and distribution between blood and bone.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71P	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. 1 Investigate the relationship between the synthesis and utilization of glycogen when cartilage changes from non-calcifying (rachitic) to calcifying (healing rickets) cartilage

STUDY NO. 2 Assess the role of vitamin D in promoting the absorption of calcium across the wall of the rat's small intestine

STUDY NO. 3 The effect of amino acid deficiencies on rat liver polysomes and amino acid incorporation

STUDY NO. 4 Procedure for degrading radioactive aldoses

1. Utilizing glucose-U-^{14}C incorporation and measuring the amount of glycogen in rachitic and healing cartilages, it was determined that there was no significant difference in the synthesis of glycogen or glycogen content. The change from non-calcifying cartilage, as in rickets, to calcifying cartilage, as in healing rickets, does not appear to depend on differences in the synthesis or utilization of glycogen.

2. The mechanism of calcium and phosphorus absorption across the wall of the small intestine of the rat is being studied using actinomycin-D as a specific inhibitor for the synthesis of "calcium translocase." The relation of vitamin D to this enzyme is one of the aims of this investigation.

3. Studies have been initiated to investigate the effect of amino acid deficiencies on rat liver polysomes and amino acid incorporation.

4. A procedure was developed for determining the radioactivity of individual carbon atoms in aldopentoses and aldohexoses labeled with carbon-14.

BODY OF REPORT

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

STUDY NO. 1 — Investigate the relationship between the synthesis and utilization of glycogen when cartilage changes from non-calcifying (rachitic) to calcifying (healing rickets) cartilage.

PROBLEM:

Literature reports have established glycogen as being involved in calcification. In addition to acting as a substrate for glycolysis in cartilage, the amounts of glycogen have been shown to be low in rachitic cartilage, to increase in the zone of calcification just prior to calcification, and to decrease as calcification begins. It seemed possible that one of the metabolic pathways required to change the rachitic cartilage to that of calcifying cartilage was the one involved in the synthesis or utilization of glycogen, or both.

RESULTS AND DISCUSSION OF THE RESULTS:

After measuring the glycogen concentration in cartilage, as well as the specific activity of this glycogen when glucose-U-^{14}C was administered, it was expected that the individual enzymes of glycogen synthesis and utilization would be assayed for activity. Upon finding that both rachitic cartilage and healing rachitic cartilage had the same concentration of glycogen with the same specific activities, it was apparent that: (a) while glycogen was a necessary substrate for calcification of cartilage, rachitic cartilage was able to synthesize and utilize glycogen; (b) activities of the enzymes which synthesize or utilize glycogen were apparently the same in rachitic and healing cartilage.

CONCLUSIONS:

Glycogen synthesis and utilization do not appear to control the initiation of calcification.

PUBLICATIONS:

1. Ziporin, Z. Z., K. Sirinit, J. Chambers and P. P. Waring. Enzyme levels in rachitic, healing and normal rat cartilages (manuscript prepared).

WORK UNIT NO. 060 - BODY OF REPORT

STUDY NO. 2 — Assess the role of vitamin D in promoting the absorption of calcium across the wall of the rat's small intestine

PROBLEM:

It has been shown that Actinomycin-D administered intraperitoneally 18-24 hours before feeding vitamin D can interfere with the absorption of calcium. The vitamin itself can increase the absorption of calcium and phosphorus in a rachitic rat with high calcium-low phosphorus rickets. Thus, it has been postulated that Actinomycin-D, an inhibitor of the DNA-mRNA link, acts to prevent the synthesis of calcium translocase and that the function of vitamin D, therefore, is to initiate the synthesis of this enzyme in the wall of the small intestine. From the fact that the vitamin D causes a significant increase of both calcium and phosphorus in the blood of our high calcium-low phosphorus rachitic animals, it appeared worthwhile to investigate the possibility that the vitamin may induce a "calcium-phosphorus translocase" in the small intestine.

RESULTS AND DISCUSSION OF THE RESULTS:

Rachitic animals were injected intraperitoneally with Actinomycin-D followed by an oral dose of vitamin D 2 hours later. After 24 hours, the animals were fed a dose of ^{45}Ca and ^{32}P by oral intubation. One hour later the animals were anesthetized and blood drawn from the abdominal aorta. The animals were sacrificed by excess ether and radii and tibiae removed. The radii were stained by the von Kossa silver nitrate method, while the tibiae were treated to remove the head from the shaft so that each could be used for percentage ash and radioactivity measurements for ^{45}Ca and ^{32}P.

Preliminary data show that blood calcium drops from 10 mg% in the rachitic animal not treated with actinomycin, and to 6.6 mg% in the treated animal, while the blood phosphorus increased from 0.8 mg% in the former animal to 5 mg% in the latter animal. Radioactivity evidence indicates that the blood picture for inorganic phosphorus and calcium does not correlate well with the per cent of administered dose found circulating in the blood. For example, rachitic animals (no vitamin D, no Actinomycin-D) have 10.2 mg% calcium in their blood containing 6.2% of the administered radiocalcium 1 hour after intubation. At the same time, rachitic animals with vitamin D and with Actinomycin-D have 6.6 mg% calcium in their blood containing

6.7% of the administered radiocalcium at the same interval as the previous animals. Thus, there is 40% less calcium circulating with no apparent differences in capacity for absorbing calcium.

Also, despite the short interval of time between the administration of the nuclides and the time of sacrifice (1 hour), the heads and shafts of the tibiae were active in their uptake of the calcium and phosphorus, with indications pointing to the uptake of phosphorus before that of calcium. This confirms previous work reported in the literature, but more accurate measurements are now possible.

CONCLUSIONS:

The specific function of vitamin D in the initiation of calcium translocase synthesis in the small intestine remains to be clarified. It appears probable that vitamin D also may promote the absorption of phosphorus either by stimulating phosphorus uptake and transfer via the mitochondria of the small intestine or by inducing a "phosphorus translocase" as well in the small intestine.

PUBLICATIONS:

None

STUDY NO. 3 — The effect of amino acid deficiencies on rat liver polysomes and amino acid incorporation

PROBLEM:

The amount of protein in the liver is considerably influenced by the quantity and quality of dietary protein (Allison et al., J. Nutr., 84: 383, 1964) and, thus, the supply of amino acids may affect the mechanisms of protein synthesis. Nutritional studies of the effect of protein intake on body protein metabolism have now advanced to the point where it is possible to examine the impact of amino acid supply on subcellular components engaged in protein synthesis. The polysomes of the hepatic cell are formed by ribosomes attached to strands of Messenger RNA (mRNA) coding for the amino acid sequences of proteins and are the sites of polypeptide chain formation. As it is possible that amino acid availability may alter the rate of protein synthesis, and thereby the speed of attachment and detachment of ribosomes on the messenger strand (Noll et al., Nature, 198: 632, 1963), the lack of one amino

acid required in the peptide sequence could alter polysome stability (Villa-Trevino et al., J. Biol. Chem., 239: 3826, 1964). For example, it has been shown that liver polysomes dissociate within a 2-hour period after rats are force-fed an amino acid mixture devoid of tryptophan (Wunner et al., Biochem. J., 101: 417, 1966). It is hoped that the nature of this effect can be better understood by extending the previously mentioned studies to include amino acid mixtures devoid or deficient in amino acids other than tryptophan, and also to see what effects these mixtures may have on the polysomes by feeding them for various lengths of time (e.g., 2 hours to 1 week). Other factors associated with polysome integrity such as polyamines (e.g., 1-3 diamino-propane, putrescine, cadavarine, spermine) and divalent cations (e.g., Mg^{++}) will also be investigated at the subcellular level in vivo, and if differences are observed, in vitro studies will be carried out using liver polysome preparations to determine how these substances affect the ability of polysomes to incorporate labeled amino acids into protein. Similar types of investigations are planned to elucidate the effect of hormones (e.g., insulin, growth hormone, cortisone, hydrocortisone) upon polysome preparations.

RESULTS AND DISCUSSION OF THE RESULTS:

Complete amino acid mixtures and mixtures devoid of tryptophan have been force-fed to male white rats following an 18-hour fast in preliminary investigations of the effect on liver polysome patterns. Two hours after force-feeding, the rats were decapitated, the livers removed and polysomes were prepared according to the procedures of Wunner et al. (Biochem. J., 101: 417, 1966). Difficulties have been encountered in the resolution of clear polysome patterns although the system used to continuously monitor the O.D. of constant velocity sucrose gradients on which the polysome samples are analyzed has been worked out to a large degree. Further studies are needed to determine more accurately what size of liver sample and what magnitude of dilutions will be required to obtain repeatable results, after which a more thorough examination of previously mentioned factors can be carried out.

CONCLUSIONS:

Studies have been initiated to investigate the effect of amino acid deficiencies and imbalances on rat liver polysomes and amino acid incorporation. Preliminary efforts have been directed towards establishing techniques and facilities needed to analyze polysome patterns prepared on constant velocity sucrose gradients.

WORK UNIT NO. 060 - BODY OF REPORT

PUBLICATIONS:

None

STUDY NO. 4 — Procedure for degrading radioactive aldoses

PROBLEM:

The use of ^{14}C and ^{3}H radioactive tracers is widespread in biochemistry. The degradation of labeled molecules by a systematic, unambiguous reaction sequence for the determination of radioactivity in each carbon and/or carbon-bound hydrogen atom is a necessary tool for many biochemical studies. The objective of these studies was to develop a method which would allow the total degradation of any aldopentose or aldohexose for location of ^{14}C radioactivity in each carbon atom independently.

RESULTS AND DISCUSSION OF THE RESULTS:

A procedure was developed for determining the radioactivity of individual carbon atoms in aldopentoses and aldohexoses labeled with ^{14}C. Each position is obtained independently from 0.7 millimole of starting methyl glycoside. The methyl glycoside is oxidized with periodic acid yielding C-3 as formic acid and a dialdehyde which was oxidized with Br_2 to strontium methoxydiglycolate. Formic acid was obtained for counting by sublimation of its ammonium salt. The crude strontium salt was hydrolyzed to glyoxylic and glyceric (or glycolic) acid, and the latter mixture separated quantitatively on a Dowex-1 acetate column. The glyoxylic acid was isolated as its sodium salt (C-1 + C-2), which was decarboxylated with sodium periodate to give carbon dioxide (C-2) and formic acid (C-1). Glyceric acid was isolated as its calcium salt (C-4 + C-5 + C-6), which was oxidized with sodium periodate to give C-4 as CO_2, C-5 as formic acid and C-6 as formaldehyde. Glycolic acid, obtained from pentoses, was isolated as its calcium salt (C-4 + C-5) and was degraded with lead tetraacetate to yield C-4 as CO_2 and C-5 as formaldehyde. The method was tested with four different methyl glycosides possessing a 20-fold range in specific activity with satisfactory results. The procedure produces four white, crystalline derivatives and two carbon dioxides trapped in ethanolamine-ethyleneglycol monomethyl ether for direct liquid scintillation counting.

WORK UNIT NO. 060 - BODY OF REPORT

CONCLUSIONS:

A procedure was developed for determining the radioactivity of individual carbon atoms in aldopentoses and aldohexoses labeled with carbon-14.

PUBLICATIONS:

1. Russ, P. L. and R. D. Bevill. A general procedure for degrading radioactive aldoses (manuscript prepared for publication).

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6341	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 07 66)	RPT U WRK U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER CODE
61145011 3A014501B71P 01 061	None

11. TITLE:
(U) Mineral Metabolism (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry	07 66	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	1	20
		CURRENT FY 68	1	20

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME	
ADDRESS Headquarters U.S. Army Med Rsch & Dev Comd Washington, D. C. 20315	NAME / ADDRESS U.S. Army Med Rsch & Nutr Lab Fitzsimons General Hospital Denver, Colo. 80240
RESP. INDIV. Davis, T. E., MAJ	INVESTIGATORS PRINCIPAL ASSOCIATE Johnson, H.L., Consolazio, C.F.
TEL. 202 OXford 6 5472	TEL 303 366 5311 X25222 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
NA	None

23. KEYWORDS Trace mineral nutrition; Dietary interrelationships of selenium; Biochemical role of selenium; Radioisotopes; Vitamin E; Mineral requirements

24. (U) Technical Objective: To determine if selenium is an essential or beneficial element and if it is, to determine its role in the biochemistry of the body.

25. (U) Approach: The initial studies will involve the study of interrelationships of selenium with other nutrients such as, methionine or cystine, vitamin E, and dietary fat levels. The next phase of research will be the study of various metabolic enzyme systems. The direction of these studies will be dependent upon the results of the initial studies. Another phase of the studies will be regarding the metabolism of selenium in the animal using the radioisotope.

26. (U) Progress: Five animal studies have been completed in the past two years using albino rats. Data from these studies indicated that either vitamin E or selenium would prevent mortality and promote normal growth in the animal fed a torula yeast-stripped lard diet. The addition of methionine to the diet, in the absence of added selenium or vitamin E, would prolong survival but eventually the rats succumbed to the deficiency. By combining results from two of the studies, it appeared that the male rat was more susceptible to the deficiencies; however, this may be attributable to the faster growth rate of the male since the animals appeared to die when they attained a certain weight range and these weight ranges increased as the dietary level of methionine was increased. The fifth study was a balance study to observe the efforts of various dietary levels of selenium upon nitrogen balance and food efficiency. The analyses of the samples and data have not been completed as yet.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C, WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A014501B71P Basic Research in Support of Military Medicine

TASK NO. 01

WORK UNIT NO. 061 Mineral Metabolism

The initial studies of this series established the dietary requirements to produce a selenium deficiency in the rat and some of the interrelationships existing among sex of the animal, methionine in the diet, and dietary selenium. The primary lesion observed was the necrosis and hemorrhaging of the liver which appears to develop quite rapidly after three weeks on the deficient diet. At this stage the animals die within three days. Supplemental dietary methionine delayed the onset of necrosis but did not appear to alleviate the syndrome after it was initiated. Female rats appeared to be more resistant to necrosis but this may be due to the slower growth of the female since the animals would die at about the same body weight.

BODY OF REPORT

WORK UNIT NO. 061 Mineral Metabolism

PROBLEM:

Most selenium deficiency syndromes involve the necrosis of various organs, i. e., liver in rats, gizzard in turkeys, muscle in sheep, and blood vessels in chickens. In certain cases in the treatment of kwashiokor, a positive nitrogen balance could only be achieved after treatment with selenium and in these instances, the serum levels of selenium were about 50% of that found in unaffected children. These observations along with others suggest that methionine delays the onset of necrosis in rat liver, the exudative diathesis in chickens, and the gizzard atrophy in turkey poults, could indicate that selenium deficiency results in some defect in protein metabolism.

The primary objectives of this study are to assess the effects of a deficiency of selenium upon the urinary nitrogen excretion of the rat, and the effects of methionine upon the excretion pattern. The urinary nitrogen excretion should give some indication if protein metabolism is affected and the metabolic pathways involved.

RESULTS AND DISCUSSION OF THE RESULTS:

Five animal studies have been completed in the past two years using albino rats. Data from these studies indicated that either vitamin E or selenium would prevent mortality and promote normal growth in the animal fed a torula yeast-stripped lard diet. The addition of methionine to the diet, in the absence of added selenium or vitamin E, would prolong survival but eventually the rats succumbed to the deficiency. By combining results from two of the studies, it appeared that the male rat was more susceptible to the deficiencies; however, this may be attributable to the faster growth rate of the male since the animals appeared to die when they attained a certain weight range and these weight ranges increased as the dietary level of methionine was increased. The fifth study was a balance study to observe the effects of various dietary levels of selenium upon nitrogen balance and food efficiency. The analyses of the samples and data have not been completed as yet.

CONCLUSIONS:

The data from five animal studies (albino rats) indicate that

Mineral Metabolism (Cont'd)

either vitamin E or selenium would prevent mortality and promote normal growth in animals fed a torula yeast-stripped lard diet. Methionine in the absence of either selenium or vitamin E would prolong survival time. In general, the male rat is more susceptible to the deficiencies.

A balance study recently completed was to observe the effects of various nutrient levels of selenium upon nitrogen balance and food efficiency.

RECOMMENDATIONS:

Evaluation of the data on the five studies. These studies should also be continued.

PUBLICATIONS:

None at the present time.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6338	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 07 66)	U RPT U WRK	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER/CODE
61145011 3A014501B71P 01 062	None

11. TITLE:

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

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry	07 66	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 67	0	11
		CURRENT FY 68	1	35

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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RESP. INDIV: Davis, T. E., MAJ	INVESTIGATORS PRINCIPAL ASSOCIATE: Herman, R. H., LTC
TEL: 202 OXford 6 5472	TEL 303 366-5311 X10221 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Medical Problems in General	

23. KEYWORDS

Blood cell metabolism; Red blood cell enzymes, Red blood cell membrane

24. (U) Technical Objective: Red blood cells contain a pentose phosphate pathway and an Embden-Meyerhof metabolic pathway. This pathway can be studied in red cells without the complicating features of pyruvate oxidation or mitochondrial metabolism and without the complications of protein synthesis. The pentose phosphate pathway of red blood cells can be influenced by a variety of circumstances which gives us a means of studying the factors which are able to control the various glycolytic and pentose phosphate pathways.

25. (U) The effect of a variety of oxidative reductive substances on red blood cells will be studied. These include pyridine nucleotides and their analogues, riboflavin, ascorbic acid, folic acid and vitamins E and K.

26. (U) In the study of normal and G6PD deficient red blood cells, we have found that various riboflavin compounds are able to stimulate the pentose phosphate pathway. Certain analogues are potent stimulators of the pentose phosphate pathway. Stimulation of the pentose phosphate pathway occurs only to a limited degree in glucose-6-phosphate dehydrogenase deficient red cells. This indicates that the riboflavin effect on the pentose phosphate pathway is mediated via a TPNH shuttle mechanism. Blockage of the pentose phosphate pathway by N-ethyl maleimide is overcome by means of a riboflavin analogue.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34 SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71P	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

Riboflavin compounds including riboflavin, flavin mononucleotide, flavin adenine dinucleotide, galactoflavin, and isoriboflavin stimulate the pentose phosphate pathway of human normal and glucose-6-phosphate deficient red blood cells in hemolysates and in the intact state in a dose-response fashion. Isoriboflavin is the most potent of the flavin compounds tested. Isoriboflavin is able to reverse the N-ethyl maleimide inhibition of the pentose phosphate pathway. The data suggests that the stimulatory effect of the various flavin compounds is due to an increased turnover rate of TPN from TPNH generated by the pentose phosphate pathway. Further, flavin mononucleotide, it is suggested, is interlocked with glutathione in the formation of TPN from TPNH.

BODY OF REPORT

WORK UNIT NO. 062

Haemopoietic Metabolism as Related to Nutrition, Genetics and Metabolic Diseases

DESCRIPTION:

The human red blood cell serves as a convenient model system for the study of the control of metabolic pathways. It is a tissue that is easy to obtain, relatively simple to purify, it is resistant to manipulative procedures, it is devoid of mitochondria and protein synthetic mechanisms, and thus contains the glycolytic pathways and pentose phosphate pathways. We have found in past studies that the pentose phosphate pathway of human red blood cells is stimulated by various pyridine nucleotides, by glutathione, and various combinations thereof. Riboflavin was discovered in the early 1930's in red blood cells and found to be necessary for the oxidation of glucose in the presence of triphosphopyridine nucleotide long before the pentose phosphate pathway was known to exist. Because of the possible implication of riboflavin in the regulation of the pentose phosphate pathway a study was initiated to determine what the effect of riboflavin compounds might be on the pentose phosphate pathway of human red blood cells. In addition to normal human red blood cells the red blood cells from a patient with glucose-6-phosphate dehydrogenase deficiency were also used. The pentose phosphate pathway was tested by measuring the amount of $^{14}CO_2$ recovered after incubating the erythrocytes with 1-^{14}C-glucose in the intact and hemolyzed state. The effect on the amount of $^{14}CO_2$ recovered of various riboflavin compounds with normal and glucose-6-phosphate dehydrogenase deficient red blood cells was then measured.

PROGRESS:

The pentose phosphate pathway of normal red blood cells is stimulated to produce increased amounts of $^{14}CO_2$ from 1-^{14}C-glucose by flavin adenine dinucleotide (FAD), riboflavin, and flavin mononucleotide (FMN). The stimulatory effect is greatest in hemolysates as compared to intact red blood cells. A dose-response effect can be demonstrated. This indicates that the red cell membrane limits the amount of flavin compounds that can enter into the red blood cells. Glucose-6-phosphate dehydrogenase (G-6-PD) deficient red blood cells are stimulated only to a slight extent by the various riboflavin compounds indicating that these compounds stimulate the pentose phosphate pathway by triphosphopyridine nucleotide (TPN). The riboflavin analogues, isoriboflavin and galactoflavin also stimulate the pentose phosphate pathway. Galactoflavin stimulates to a greater extent in hemolysates than with intact red blood cells. Isoriboflavin is by far the most potent compound both with hemolysates and with intact red blood cells. Isoriboflavin also stimulates the pentose phosphate pathway of G-6-PD deficient red cells though to a lesser degree than normal red blood cells. The sulfhydryl inhibitor, N-ethyl maleimide (NEM), suppresses the activity of the pentose phosphate

pathway presumably by inhibiting glutathione metabolism which is necessary for the regeneration of TPN from reduced TPN (TPNH). Isoriboflavin is able to reverse to a partial extent NEM inhibition of the pentose phosphate pathway of normal red blood cells. These data suggest that the pentose phosphate pathway generates TPNH which in turn reduces glutathione (GSSG) to reduced glutathione (GSH) using the enzyme glutathione reductase. FMN is also reduced to $FMNH_2$ using the so-called old yellow enzyme. In the presence of oxygen $FMNH_2$ is oxidized to FMN by old yellow enzyme and hydrogen peroxide is formed. The hydrogen peroxide reacts with GSH and glutathione peroxidase to reform GSSG. As GSSG and FMN are reduced the TPNH is oxidized to TPN. As one increases the amount of riboflavin the rate of oxidation of TPN increases with the resultant increase in the rate of the pentose phosphate pathway.

SUMMARY:

Riboflavin compounds including riboflavin, flavin mononucleotide, flavin adenine dinucleotide, galactoflavin and isoriboflavin are able to stimulate the pentose phosphate pathway of normal and G-6-PD deficient red blood cells with isoriboflavin being the most potent compound. Isoriboflavin is able to reverse N-ethylmaleimide inhibition of the pentose phosphate pathway to a partial degree. The data imply an interlocking mechanism for the regeneration of TPN from TPNH involving glutathione and flavin mononucleotide.

PUBLICATIONS:

1. Herman, R. H., Herman, Y. F., Sauberlich, H. E., and Plunket, D. Effect of Riboflavin Compounds on the 1-14C-Glucose Metabolism of Human Red Blood Cells. Fed. Proc. 26:305, 1967 (Abstract No. 279).

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6311	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U RPT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER CODE
61145011 3A014501B71P 07 030	None

11. TITLE:

(U) Performance Physiology (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
005900 Environmental Bio 012900 Physiology 016200 Stress Phys	06 63	NA	OTHER IDA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18 RESOURCES EST.	a PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	1	30
		CURRENT FY 68	1	25

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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ADDRESS Headquarters U.S. Army Med Rsch & Dev Comd Washington, D. C. 20315	ADDRESS U.S. Army Med Rsch & Nutr Lab Fitzsimons General Hospital Denver, Colo. 80240
RESP. INDIV. TEL. Davis, T. E., MAJ 202 OXford 6 5472	INVESTIGATORS PRINCIPAL ASSOCIATE Consolazio, C.F., Matoush, L.O. Johnson, H. L.
	TEL 303 366 5311 X25222 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Human Job Performance	None

23. KEYWORDS

Exercise; Exhaustion; Work; Fatigue; Performance; Antifatigue

24. (U) Tech Objective: To develop a better understanding of the basic aspects of physical performance in relation to age.

25. (U) Approach: Specific problems of the physiology of exercise are to be studied with noncatheterized and catheterized humans working on a bicycle ergometer. Environmental conditions, i.e., temperature, altitude, and food deprivation are being studied as influencing factors on physical performance in man to help elucidate adaptive mechanisms related to fatigue and exhaustion.

26. (U) Progress: 1. In a study at Fort Carson, Colorado, maximal performance (V_{O_2}) was completed on 112 men in 8 age groups between 17-50+ years. The maximal V_{O_2} decreased with an increase in age.

2. At Fort Huachuca, Arizona and Fort Campbell, Kentucky, maximal treadmill performance was completed on men and women between the ages of 18-50+ years.

3. At high altitude (two scientific journal papers, Fed. Proc. 25:1380, 1966 and J. Appl. Physiol. 21:1732, 1966), submaximal and maximal work was compared to sea level values in two studies (3475 and 4300 meters). No significant changes were observed in the submaximal work at either of the high elevations. However, maximal V_{O_2} was significantly decreased by 17 and 20% at 3475 and 4300 meters, respectively.

4. Two published Laboratory Reports (Nos. 298, 299) on starvation for 10 days as related to moderate and maximal work show performance not to be significantly impaired during caloric restriction.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1333) REPLACES DD FORMS 613 & 613C, WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71P	Basic Research in Support of Military Medicine
TASK NO.	07	Pharmacology
WORK UNIT NO.	030	Performance Physiology

The physiology of work and exercise in animals and man is being studied to ascertain methods of measuring and improving the performance and fitness of soldiers of all ages working in extreme environments. Methodology developed includes: (a) the continuous measurement of heart rate, ventilation rate and volume, oxygen uptake during stable or increasing and exhaustive workloads on the bicycle ergometer, and the motor driven treadmill on humans; and (b) the repetitive measurements of cardiac output, arterial and venous pressures, blood gas tensions and pH, and other physiologic and biochemical parameters in chronically-catheterized, treadmill-exercised dogs.

Data is now being analyzed on studies relating age and body composition to bicycle and treadmill work performance on enlisted men and women. Further improvements have been made in the continuous oxygen uptake analysis system. All signals have been fed into a single recorder and are also fed into an "analog to digital data conversion" system with punched paper tape output compatible with the USAMRNL RCA 301 digital computing system. Studies on the effects of grade walking and load carrying on the treadmill at altitude are being evaluated.

BODY OF REPORT

WORK UNIT NO. 030 — Performance Physiology

STUDY NO. 1 — Maximal and Submaximal Performance in Relation to Age and Body Composition

PROBLEM:

Work performance in relation to age and body composition have been conducted during the: (a) Nutrition Surveys at Fort Carson, Colorado, Fort Huachuca, Arizona, and Fort Campbell, Kentucky; (b) the two high altitude studies at 3475 and 4300 meter elevations; and (c) the three 10 day studies including complete starvation and caloric restriction.

Physiological measurements included a maximal and submaximal work performance test on a bicycle ergometer during the Fort Carson nutrition survey and the two high altitude studies. At Fort Huachuca and Fort Campbell, and during the 10 day starvation and caloric restriction studies, work performance was conducted on a treadmill. Metabolic and pulse rates were measured continuously before, during, and after the exercise. Other measurements included maximum breathing capacity and the one and two second vital capacity. Body composition measurements included deuterium oxide for body water, volumetry for body fat, plasma, blood and red cell volume (using Evans Blue Dye), and K^{40} counting for lean body mass (using a total body counter). Urine samples were also collected for the measurement of creatinine and creatine excretion. Electrocardiograms were obtained on every subject prior to the physical work test.

RESULTS AND DISCUSSION OF THE RESULTS:

A total of nine studies have been conducted on maximal and submaximal work performance in relation to age. The following investigations have been conducted under this work unit:

1. Fort Carson, Colorado - Nutrition Survey: Data is being computed and evaluated on the bicycle performance data as it relates to age, for publication as a scientific report.

2. Fort Huachuca, Arizona - Nutrition Survey: The third in the current series of annual nutrition surveys was conducted at Fort Huachuca in March and April 1966. Oxygen uptakes during

sitting rest, maximal grade walking, and recovery were measured on 120 men in 8 age groups, 20 obese men, and 40 women (WACs). The work performance was on the treadmill (based on Balke's test with increasing grade) and included measurements of total ventilation, total walking time, and maximal and recovery pulse rates. Physical fitness index scores were also calculated using the sum of the recovery pulse rates as follows:

$$PFI = \frac{\text{Duration of Exercise (Time)} \times 40}{\text{Sum of 3 Recovery Pulses in Minute}}$$

Information on body composition in relation to age and food intake was obtained and is now being analyzed and evaluated for correlations with work performance.

3. Fort Campbell, Kentucky - Nutrition Survey: (March 1967) The fourth in the series of nutrition surveys was conducted on basic trainees during their first, fifth, and eighth week of basic training. Data on maximal performance and body composition was also gathered on troops above the age of 25. In all, 183 individuals were studied between the ages of 17 - 50+.

4. Effects of High Altitude Performance: Maximal work capacity ($\dot{V}_{O_2}$), on the bicycle ergometer at altitude, was published in two scientific journals (Fed. Proc. 25:1380, 1966 and J. Appl. Physiol. 21:732, 1966). See last years Annual Report for summary. Information has been gathered on grade walking and load carrying at 4300 meters. Sixteen sea level Medical Training Center troops have been utilized to evaluate walking on the level on a 4 and 8% grade on the treadmill with and without a 20 kg pack. Data is being evaluated.

5. Starvation for 10 Days: Recent emphasis on the importance of combat situations involving prolonged combat patrol activities, air drop operations, limited amphibious operations, and other operations where resupply is difficult, have created new problems in providing sufficient food and water for combat personnel to maintain adequate performance. The soldier in combat or on combat patrol for periods of 0 - 10 days must carry his pack, radio equipment, weapons, and an adequate supply of food and water. This constitutes a heavy and bulky load. The question has repeatedly arisen, as to the minimal food intake necessary to permit the individual soldier to effectively perform his duties for periods of 3, 7, and 10 days. The primary

objective of this preliminary study was to gather information on some of the problems encountered during a 10 day starvation period. This study included evaluation of submaximal and maximal work performance, changes in body compartment, nitrogen, fluid, and mineral balances. The results are summarized in two USAMRNL Laboratory Reports (Nos. 299 and 300).

6. Semi-Starvation for 10 Days: In two studies now being prepared for publication using 400 and 500 Calories for 10 days, with and without mineral supplementation, it was observed that physiological and psychological performance was not impaired during caloric restriction.

CONCLUSIONS:

The relationship between maximal work performance (oxygen uptake in liters/minute) and age was studied during three Nutrition Surveys at Fort Carson, Colorado, Fort Huachuca, Arizona, and Fort Campbell, Kentucky, by measuring oxygen uptake in 112, 120, and 220 men, respectively, in 8 age groups between 17 - 50+ years.

During the Fort Huachuca study, 20 obese men and 40 WACs were measured on the treadmill, and at Fort Campbell, 50 additional men including 26 obese and 24 special training troops were measured. These data are presently being analyzed.

In the starvation study, six healthy adult males between the ages of 21 and 52 years fasted for a period of 10 days with water available ad libitum. Basal oxygen uptakes ($\dot{V}_{O_2}$ in ml/minute and ml/kg body weight/minute) were significantly decreased during the fasting period. In submaximal work on the treadmill, $\dot{V}_E$ BTPS and $\dot{V}_{O_2}$ STPD in ml/minute and ml/kg/minute were significantly decreased during the fasting and rehabilitation periods, indicating a training effect. The maximal oxygen uptakes in ml/kg/minute were not significantly lowered during the fasting period. Other maximal work measurements ($\dot{V}_E$ BTPS, pulse rates, and kg meters of work/minute) were also lower when compared to the control values, again suggesting a training effect. EKGs were significantly abnormal during the fasting phase and considered to reflect a severe stress. One subject developed an abnormal EEG and was immediately removed from the study. Although physical performance was not significantly impaired during the 10 day starvation period, complete fasting without mineral supplementation is not recommended for

troops on combat patrol. Preliminary studies in the two semi-starvation studies for 10 days, indicate that physiological and psychological performance are not significantly changed during caloric restriction.

RECOMMENDATIONS:

The following studies are recommended:

1. Continue performance at altitude to include the energy expenditure of various military tasks.

2. Continue surveys to include studies in extremely hot and cold environments.

3. Performance of field study in a jungle environment (Panama) to evaluate the minimal requirements of troops on simulated combat patrol.

PUBLICATIONS:

1. Consolazio, C. F., L. O. Matoush, and R. A. Nelson: Energy metabolism in maximum and submaximum performance at high altitude. Fed. Proc. 25:1380, 1966 (Abstract).

2. Johnson, H. L., L. O. Matoush, H. J. Krzywicki, and C. F. Consolazio: The effects of caloric restriction upon performance. Fed. Proc. 26:474, 1967 (Abstract).

3. Consolazio, C. F., R. A. Nelson, L. O. Matoush, and J. E. Hansen: Energy metabolism at high altitude (3475 m). J. Appl. Physiol. 21:1732, 1966.

4. Consolazio, C. F.: Nutritional variation in world populations and performance potential. Ann. N. Y. Acad. Sci., 134:585, 1966.

5. Consolazio, C. F., R. A. Nelson, H. L. Johnson, L. O. Matoush, H. J. Krzywicki, and G. J. Isaac: Metabolic aspects of human starvation in normal humans. Performance and cardiovascular evaluation function. USAMRNL Laboratory Report No. 298, September 1966.

WORK UNIT NO. 030

Performance Physiology

STUDY NO. 2

Development and Application of a Continuous Direct Reading Instrument for Measuring Oxygen Consumption During Maximal and Submaximal Exercise

PROBLEM:

The original problem was to develop an instrument for the continuous monitoring of expiratory gas volume, expiratory oxygen and carbon dioxide concentrations, pulse rate, respiration rate, and body temperature of humans during rest and exercise.

RESULTS AND DISCUSSION OF THE RESULTS:

The "continuous oxygen consumption analysis sytem" has been rebuilt into a new, inclined panel type console which has allowed a shortening of the sample tubing and thus an improvement in the response of the instrument as well as a better control over sample mixing and integration. Instrumental measurement of environmental relative humidity has been added and all the signals have been fed to a single recorder, eliminating the need for handling three strip chart records for each measurement. The same signals which are fed to the multichannel recorder, are also fed to an "analog to digital data conversion" system with punched paper tape output compatible with the USAMRNL RCA 301 digital computing system.

Computer programming necessary for the automatic use of this collected data is broken down into a group of subroutines which will eventually carry the data from the "analog to digital data conversion" system to final oxygen consumption data without manual intervention.

CONCLUSIONS:

The new model of the continuous oxygen uptake system has been utilized in the recent starvation study and the recent Fort Huachuca, Arizona and Fort Campbell, Kentucky surveys, for the measurement of maximal work performance, pulse rates, and total expiratory volumes. Data from the strip chart records and the automatic systems are now being compared and evaluated.

Performance Physiology (Cont'd)

RECOMMENDATIONS:

Completion of final report.

PUBLICATIONS:

None at present.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOV'T ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6318	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U RPT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 02 055	None

11. TITLE:
(U) Experimental Surgery in Support of Medical Research (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
012900 Physiology 005900 Environ Biol 016200 Stress Phys	08 54	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	1	13
		CURRENT FY 68	1	20

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME Headquarters	NAME U.S. Army Med Rsch & Nutr Lab
ADDRESS U.S. Army Med Rsch & Dev Cmd Washington, D. C. 20315	ADDRESS Fitzsimons General Hospital Denver, Colorado 80240
RESP. INDIV Davis, T. E., MAJ	INVESTIGATORS PRINCIPAL ASSOCIATE Merrill, R. H., CPT
TEL. 202 OXford 6 5472	TEL. 303 366-5311 X26122 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Human Job Performance	None

23. KEYWORDS Surgical Techniques; Vascular Surgery; Vascular Prosthesis; Cardiac Denervation; Chemoreceptor Denervation; Blood Flowmeters; Shock

24. (U) Technical Objective: (1) To provide surgical support for research projects carried on by this laboratory. (2) To provide surgical support for research programs carried on by personnel of Fitzsimons General Hospital. (3) To conduct research for new and improved surgical procedures. (4) To provide training in surgical techniques for students of the Clinical Specialists Course.

25. (U) Approach: Efforts are being made to chronically catheterize the heart and implant flow probes and myocardial strain gauges. Studies of blood electrolytes during irreversible shock and tissue response to Marlex mesh are being supported. Support is also being given to research in cochlear-microphonics. A program for student training in surgical techniques is currently being offered.

26. (U) Progress: All above studies and support programs are currently in process. Progress is being made in all areas. Experimental results have not been evaluated. All programs and studies mentioned are expected to continue during FY 68.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY + 1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	Research in Biomedical Science
TASK NO.	02	Internal Medicine
WORK UNIT NO.	055	Experimental Surgery in Support of Medical Research

Surgical work in support of research carried on by USAMRNL has consisted of: (1) chronic catheterization of the heart; (2) surgical implantation of flow-probes on the aorta and coronary arteries; (3) surgical implantation of myocardial strain gauges; (4) aortic body denervation and; (5) catheterization of the thoracic lymph duct.

Surgical support of research carried on by personnel of Fitzsimons General Hospital has consisted of: (1) the study of blood electrolytes during irreversible shock; (2) tissue reaction to the synthetic prosthetic material - Marlex; (3) methods of determining the efficiency of inner ear structure prostheses.

Surgical training has also been provided to the Clinical Specialists' Training Course. Instruction in sterile technique, basic surgical principles and suture techniques is given.

BODY OF REPORT

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

PROBLEM:

The objectives of this work unit have continued to be primarily two-fold: (1) surgical support for physiological and metabolic studies and; (2) surgical support of teaching and clinical research activities of Fitzsimons General Hospital. Both include work toward improving present techniques as well as an extensive effort toward developing new ones when required.

DISCUSSION:

Chronic catheterization of the heart has continued this year as standard procedure. Approximately 30 dogs have been so prepared and utilized for high altitude research.

Continued efforts are being made to chronically implant flow probes. Moderate success has been achieved with coronary artery flow probes; aortic flow probes still present a problem of post-surgical rupture. This problem may be prevented in future procedures, however, by the use of Ivalon sponge.

Surgical placement of myocardial strain gauges has proved a relatively easy procedure. Further use of this procedure is anticipated in the evaluation of the force of contraction of the ventricles following coronary occlusion.

Denervation of the aortic body has been done in the past year although on only three dogs. Difficulties with post-operative care and time involved with the procedure have made it impractical as an experimental procedure for use in a large population of animals.

Much work has been done recently in an attempt to chronically catheterize the thoracic lymph duct. Efforts have been made to direct the duct outside the thoracic cavity and then redirect it back into the azygos vein. This will allow chronic measurements on lymph flow. To date, many problems have been encountered. The catheter material, clotting properties of the lymph, and length of the shunt system all appear to be factors in occlusion of the system by the second to third post-operative day.

Support to the staff of Fitzsimons General Hospital is being given, at present, to three major studies: (1) The blood gases, pH and lactic acid levels are being measured in dogs that have been bled to 30 mm. Hg. The effects of $NaHCO_3$ administered at this time are being investigated. The study has not yet been completed,

and therefore, results will be forthcoming; (2) Continued work is being carried on investigating Marlex as a possible prosthesis for diaphragmatic hernias. At present, it is being implanted around the esophagus and in the subcutaneous tissues of the abdomen. Presently, we have four dogs with such prostheses awaiting necropsy and histological evaluation; (3) Recently, work is being done to assess the auditory response of dogs by cochlear microphonics. Having established a means to assess this response, further work will be done to test prostheses for inner ear structures, particularly the malleus.

This year, the surgery section has initiated a training program for students of the Clinical Specialists' Training Program at Fitzsimons General Hospital. Students are given instruction in sterile technique, gowning procedures, basic surgical techniques and principles of skin closure. This program appears to be very valuable in the student's training, and it is anticipated that it will continue.

CONCLUSIONS:

Work involving standard techniques developed here is being carried out routinely as the need arises. New techniques, and improvements on old techniques are constantly being sought. Surgical support to research programs carried on by Fitzsimons General Hospital personnel is being given. Shortly, results will be available for effects of $NaHCO_3$ during irreversible shock, tissue response to Marlex mesh, and effects of a lengthening-type prosthesis for the malleus. Student training has been very successful and will continue.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6320	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U RPT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER/CODE
61145011 3A014501B71R 02 057	None

11. TITLE:
(U) Maintenance of Animals and Study of Pathology of Animals Utilized in Research (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002600 Biology	01 58	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	a. DATE b. NUMBER NA c. TYPE d. AMOUNT	PRIOR FY 67	2	103
		CURRENT FY 68	2	73

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME Headquarters	NAME U.S. Army Med Rsch & Nutr Lab
ADDRESS U.S. Army Med Rsch & Dev Comd Washington, D. C. 20315	ADDRESS Fitzsimons General Hospital Denver, Colorado 80240
RESP. INDIV Davis, T. E., MAJ	INVESTIGATORS PRINCIPAL ASSOCIATE Bucci, T. J., MAJ Stedham, M. A., MAJ, Jones, L. D.
TEL. 202 OXford 6 5472	TEL 303 366-5311 X23230 TYPE DA

21. TECHNOLOGY UTILIZATION	22 COORDINATION
Basic Life Sciences	None

23. KEYWORDS
Histopathology; Experimental animals; Histology; Clinical pathology; Staining technics

24. (U) Technical Objective: To maintain animals used by any division of the laboratory and to detect and study diseases of laboratory animals.

25. (U) Approach: 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 alterations of diseases in laboratory animals. Some technics used are light and electron microscopy, histochemistry and enzyme histochemistry, clinical examinations, clinical laboratory tests, and photographic recording of lesions.

26. (U) Progress: Approximately 6000 animals were maintained: 3100 rats, 1500 mice, and others including guinea pigs, rabbits, hamsters, dogs, cats, frogs, snakes, squirrels, and monkeys. 873 cases were accessioned (558 complete necropsies) which generated 774 bags of wet tissue, 5503 tissue blocks, 23,000 H&E stained slides, 7000 special stained slides, and 1974 feet of 35 mm. film strip used in serial sectioning. 315 kodachromes were taken. Punch card coding of the photographic file was accomplished. Clinicopathologic examinations were routinely performed on dogs admitted to the colony and on other animals as deemed necessary. Experimental work was completed for papers "Degenerative Myopathy in Turkeys", by Maronpot, Bucci, Stedham, to be presented at the July 1967 AVMA meeting and "Sexual and Asexual Aspergillus Forms in an Egret with Tuberculosis", by Stedham, Maronpot, and Bucci, to be submitted.

27. COMMUNICATIONS SECURITY	28.	29 OSD CODE	30 BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32 PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34 SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 67 498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	Research in Biomedical Science
TASK NO.	02	Internal Medicine
WORK UNIT NO.:	057	Maintenance of Animals and Study of Pathology of Animals Utilized in Research

PURPOSE OF WORK:

This work unit covers the division's studies in diseases of laboratory animals, the maintenance of animals used by all divisions, the conduct of a training program in Veterinary Pathology, and provision of pathology service to the other laboratory divisions.

METHODS BY WHICH ACCOMPLISHED:

Routine and special pathologic and clinicopathologic technics are used to elucidate cause, pathogenesis, and pathologic alterations of diseases in laboratory animals or of changes produced by experiment. Some specific technics used are light and electron microscopy, histochemistry and enzyme histochemistry, clinical examinations, clinical laboratory tests, and photographic recording of lesions. 873 cases were accessioned (558 complete necropsies) which generated 774 bags of wet tissue, 5503 tissue blocks, 23,000 H&E stained slides, 7000 special stained slides, and 1974 feet of 35 mm. film strip used in serial sectioning. 315 kodachromes were taken. Punch card coding of the photographic file was accomplished. These same materials were used in the training program, designed to raise the level of competence of assigned Veterinary Corps Officers in the performance of veterinary pathology, and also to provide more trained veterinary pathologists to AMEDS.

RESULTS:

Approximately 6000 animals were maintained: 3100 rats, 1500 mice, and others including guinea pigs, rabbits, hamsters, dogs, cats, frogs, snakes, squirrels, and monkeys. Animals are housed, exercised, and fed in accordance with experimenters' directions and humane principles.

Experimental work was completed for papers "Degenerative Myopathy in Turkeys', by Maronpot, Bucci, Stedham, to be presented at the July 1967 AVMA meeting and "Sexual and Asexual Aspergillus Forms in an Egret with Tuberculosis", by Stedham, Maronpot, and Bucci, to be submitted Both are largely descriptive accounts, the first is a spontaneous disorder which may be nutritional; the second is the first description of a sexual phase of this organism in tissue to be reported.

BODY OF REPORT

WORK UNIT NO. 057 — Maintenance of Animals and Study of Pathology of Animals Utilized in Research

PROBLEM:

1. To detect, diagnose and study diseases occurring in this laboratory's animal colony and to initiate studies designed to elucidate the nature, pathogenesis and pathology of diseases of laboratory animals.

2. To maintain animals used by all divisions of the laboratory.

3. To provide pathology service to the laboratory. This function also supplies case material and support for the Preceptorship in Veterinary Pathology, a program designed to train VC Officers in animal pathology.

RESULTS AND DISCUSSION OF THE RESULTS:

1. Spontaneous disease in the laboratory's animal colony was rare, limited to small numbers of rats with chronic murine pneumonia and occasional undiagnosed deaths in rabbits.

Characterization of a spontaneous myopathy in turkeys, chiefly the light microscopy and enzyme histochemistry of the lesions, has resulted in a presentation of this material at a national gathering of poultry scientists, with subsequent publication.

Discovery of a sexual form of a species of the fungus Aspergillus in an egret dead of avian tuberculosis (from the Denver Zoological Park) will result in a publication; this form has previously not been described in tissue.

A small transmission study was carried out, inoculating healthy cats with ascitic fluid from cats having a newly-described disease, Feline Infectious Peritonitis. The ascitic fluid was obtained from private veterinary practitioners. The object of the study was to attempt transmission to other laboratory animal species once the virulence of the inoculation was established in cats. The inoculated cats remained well.

An enzyme histochemistry survey of the complete gastrointestinal tract of the boa constrictor is underway. This serves a two-fold purpose: 1) to provide some insight into this animal's ability to digest whole animals nearly quantitatively; 2) to provide a variety of enzymes with which to develop additional histochemical procedures.

Maintenance of Animals and Study of Pathology of Animals Utilized in Research (Cont'd)

2. Animals were maintained for the laboratory's investigation in numbers which are totalled in the abstract. A typical daily census would yield approximately 600 each of rats and mice, 60-80 each of rabbits and guinea pigs, 25-50 dogs, several cats, smaller numbers of frogs, squirrels, monkeys, snakes.

The division's facilities for holding dogs is limited to 50 small dogs, fewer large ones. This is an insufficient supply; a nearby kennel is under contract and provides board for an additional 25-50 dogs on a year-round basis. Though not an ideal arrangement, this is a workable compromise and is used chiefly for larger dogs requiring long-term observation.

This work unit supports the salaries of 8 civilian (Wage Board) Animal Caretakers, as well as providing funds for the outside boarding contract. The 8 men provide 7-day care and in addition, mix to specification and feed on schedule special diets required by investigators. Forty different diets were prepared during the year; a typical day would include the feeding of some 15-30 different diets and a control diet, representing perhaps 10 different studies. Scrupulous cleanliness is demanded in the diet kitchen and animal quarters, since subtle dietary effects can be masked by conditions arising from poor husbandry.

3. Pathology Service:

Input includes submission from investigators outside of the Pathology Division, intradivisional research, and special cases solicited from the Denver Zoological Park, private veterinary practitioners, and Animal Service of the University of Colorado Medical Center. The special cases provide varied species and disease conditions for the training program.

A 350-case backlog exists in the laboratory, with some 10-15 additional cases accumulating weekly. Present technical staff is working to capacity.

Services provided are listed in the abstract. An MSC electron microscopist was assigned in March, 1967 and he is contributing greatly to our capability in that area. He is hampered by a lack of technical help and by inadequate laboratory space but is making admirable strides in building a reference library of photographs of normal ultrastructure of animal tissues. He also participates in the study of animals on experiment.

Maintenance of Animals and Study of Pathology of Animals Utilized in Research (Cont'd)

The training program is intended to increase the level of competence in veterinary pathology of the assigned VC officers. During this fiscal year the Division lost four 2-year officers. Of the four replacements, three were new graduates, and one had a year's postgraduate civilian training. A fifth officer will complete his tour in July 1967 and will be replaced by a new graduate. The training is necessary for these new men to enable them to provide the Division's mission of pathology service. Additionally, the training program is being "formalized" as the "Preceptorship in Veterinary Pathology", along with similar programs in other AMEDS laboratories, to provide long-range (5-year) OJT of officers remaining in the VC. The goal of this latter program is to provide AMEDS with veterinary pathologists, a specialty in critical supply.

This program involves 8 hours of didactic lectures per week for all concerned, usually in the form of microslide seminars presented by the trainees according to a schedule drawn by the Division Chief. Each man also spends an additional hour of duty time and more during off-duty hours preparing for the seminars. Current case material and that from the files are used as instructional material and include x-rays, photographs, clinicopathologic workups, and microslides. Special stains, enzyme, histochemistry and occasionally electron microscopy are included. Study sets of normal tissues or classical examples of disease are prepared. Extra slides and photographs used for training are included in the totals listed in the abstract. TDY is utilized where appropriate, e.g. special conferences on animal disease.

For more expeditious accessioning and retrieval of completed cases, the present computer program is to be replaced, utilizing a "Natural Language" system in place of the current restrictive codified one. This operation is time-consuming; two officers have completed RCA's two-week computer programmer course in preparation for the task.

Space for filing of case reports, storage of slides, blocks, and wet tissue, and desk space for assigned officers are all limited and becoming critical.

RECOMMENDATIONS:

Since the conduct of research investigations requires detailed familiarity with subject matter and technique, it has been difficult to accomplish studies in depth with a succession of 2-year officers. This is particularly true when the work involves histopathology and other techniques in pathology for evaluation of changes in animals. Newly assigned VC officers are not able to become extremely proficient in both areas in the

period of their assignment. It is therefore recommended that VC officers with 3-year obligations be assigned whenever possible, and these be men with expressed interest and academic proficiency in pathology.

To alleviate the backlog and decrease the delay in service, it is recommended that the full authorized complement of enlisted personnel (8) be assigned this division - only 6 are presently assigned - and that all enlisted men be of the MOS 92B (Medical Laboratory Specialist).

Approximately 2/3 of one enlisted man's time and approximately I hour/day of an officer's time are occupied by the photography function - from exposing through processing to mounting (if transparencies) and filing. It is recommended that steps be taken to consolidate all the laboratory's photographic requirements and employ a full-time medical illustrator.

Boarding approximately 25-50 dogs/day at a commercial kennel will cost approximately $15-18,000 during FY 68, cost approximately $10,000 during FY 67. As a long-range investment it is recommended that appropriate facilities be constructed integral to this laboratory. This would also permit closer surveillance of these dogs, as well as much improved access and greater utilization.

The shortage of adequate space is becoming a critical factor - the electron microscopy function is hampered and will soon be limited; officers are cramped at their desks (e.g. 3 in an area 8' X 14'), detracting from their ability to concentrate and study; wet tissues and tissue embedded in blocks are stored in areas so hot the embedding material melts and blocks fuse and are ruined - even this space is inadequate. It is, therefore, recommended that more footage be provided this division. One means of accomplishing this would be to relocate all electron microscopy functions to another area, and by provision in that area of suitable storage for case material (slides, blocks, tissue); the vacated area could thus be used for additional desk space, etc.

CONCLUSIONS:

The laboratory's requirement for well-kept animals is being met in a highly satisfactory manner with respect to small laboratory animals. Space for sufficient number of dogs is inadequate, and the requirement is being met by boarding dogs at a commercial kennel; this is an unsatisfactory arrangement which can be alleviated only by new construction or by modification of the present laboratory space.

Maintenance of Animals and Study of Pathology of Animals Utilized in Research (Cont'd)

Study of the spontaneous myopathy in turkeys revealed its cause may be nutritional; studies are underway to establish the relation between the diet and the disease.

Newly assigned Veterinary Corps officers have usually been new graduates requiring considerable training to perform well in pathology service and in laboratory investigation of animal diseases. Newly assigned officers should be personnel with 3-year obligation and with expressed interest in pathology.

Pathology Service would be improved considerably by assignment of the full complement of authorized personnel, especially if all enlisted men are of MOS 92B.

Electron Microscopy as a service and as a research technique will be unable to realize its full potential even with existing manpower, until more suitable laboratory space is available.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6321	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U RPT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 02 058

10b PRIOR NUMBER/CODE: None

11. TITLE:
(U) Nutritional and Metabolic Adaptations and Interrelationships (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry; 002600 Biology; 012900 Physiology	05 65	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C.In-House	b. NUMBER NA	PRIOR FY 67	3	64
		CURRENT FY 68	4	78

19. GOV'T LAB/INSTALLATION/ACTIVITY
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RESP. INDIV.
TEL. Davis, T. E., MAJ
202 OXford 6 5472

20. PERFORMING ORGANIZATION
NAME
ADDRESS: U.S. Army Med Rsch & Nutr Lab
Fitzsimons General Hospital
Denver, Colorado 80240
INVESTIGATORS PRINCIPAL ASSOCIATE: Sauberlich, H. E.
TEL 303 366 5311 X24214 TYPE DA

21. TECHNOLOGY UTILIZATION
Nutrient utilization and requirements

22. COORDINATION
None

23. KEYWORDS: Periodicity of eating; meal-eating; nibbling; adaptation; enzymes; lipogenesis; fatty acid; carbohydrates; elastin; aging; triblycerides

24. (U) Technical Objective: To determine the mechanisms and significance of biochemical adaptations in the mammal exposed to varied patterns and levels of macronutrient and micronutrient intakes. To obtain information about dietary and environmental factors that may affect changes in proteins in relation to the aging process.

25. (U) Approach: To induce adaptive changes, primarily by dietary means, and to elucidate the biochemical basis for such changes. Initial approaches will involve investigations on alterations in lipid metabolism or in elastin properties.

26. (U) Progress:

1. Dietary medium chain triglyceride (MCT) signifiantly owered serum cholesterol levels and cholesterol synthesis from acetate-1-^{14}C; fatty acid elongation was markely enhanced.

2. High carbohydrate diets cause increased lipogenesis as measured by the incorporation of various ^{14}C-labeled substrates into fatty acid by liver slices of rats fed the respective diets. Feeding of high fructose diets results in elevated serum triglycerides. A mechanism for these effects has been proposed.

3. Vitamin B_6-deficient rats had increased lipogenesis in kidney and adipose tissues when compared to pair-fed control rats.

4. Techniques have been developed for application to the elastin studies.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	Research in Biomedical Science
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. 1	Investigate the effect of the ingestion of medium chain triglyceride on lipid metabolism in the rat and chick
STUDY NO. 2	Mechanism of adaptation for elevated lipogenesis in rats fed high carbohydrate diets
STUDY NO. 3	Effect of pyridoxine deficiency on lipid metabolism in the rat
STUDY NO. 4	Effect of specific amino acids and vitamins on the formation of cross-linkages in aortal elastin during aging
STUDY NO. 5	Studies on threonine metabolism

1. Rats ingesting medium chain triglyceride (MCT), a triglyceride containing approximately 75% caprylic (C_8) and 25% capric (C_{10}) acids, had depressed plasma and liver cholesterol and total lipids, as compared to corn oil-fed rats. In vitro cholesterol synthesis from acetate-1-^{14}C was lower in rats consuming MCT than in similar rats receiving corn oil. These observations suggest that MCT ingestion results in depressed hepatic cholesterol synthesis and, consequently, depressed serum cholesterol levels in the rat.

2. In a series of in vitro studies, it was established that MCT feeding resulted in an enhancement of the ability of rat liver to desaturate and elongate fatty acids. Although adipose tissue from MCT-fed rats converts acetate-1-^{14}C to fatty acids at a greater rate than tissue from corn oil-fed animals, evidence is presented to indicate that this enhancement represents de novo synthesis.

3. In chicks fed MCT, plasma total lipids and cholesterol were elevated and liver total lipids were depressed as compared to corn oil-fed chicks; however, MCT ingestion did not alter the plasma-liver cholesterol pool, as compared to animals fed corn oil.

4. In vitro cholesterol and fatty acid synthesis were elevated in MCT-fed as compared to corn oil-fed chicks, as measured by the incorporation of acetate-1-^{14}C into the various lipid components. A series of in vitro studies indicated that de novo fatty acid synthesis, chain elongation and desaturation mechanisms were elevated in the livers of MCT-fed chicks.

5. The substitution of fructose for glucose in a high carbohydrate diet resulted in elevated serum and liver triglycerides in the rat.

6. The concentration of hepatic pyruvate, acetyl-CoA and malic acid was increased in fructose-fed as compared to glucose- or chow-fed rats.

7. In vitro incorporation of fructose-^{14}C into fatty acids was the highest in the fructose-fed group. The conversion of fructose-^{14}C into fatty acids was higher than the incorporation of glucose-^{14}C into fatty acids in all groups tested. This observation could be explained by the fact that the activity of hepatic fructokinase was greater than the activity of glucokinase or hexokinase for all dietary groups.

8. It was postulated that the increased synthesis of fatty acids was a result of the increased availability of the substrate, acetyl-CoA, in the fructose-fed group. More acetyl-CoA was available from fructose than from glucose because fructose utilization does not proceed through the phosphofructokinase step (a rate limiting step) but rather via the fructokinase pathway.

9. Rats fed a high fructose diet and atromid had a significantly lower amount of serum triglycerides, cholesterol and total lipids. Similar observations have been reported for humans; this drug may be used for heart patients and appears to be quite effective. Future studies are being designed to elucidate its mode of action on lowering serum lipids.

10. Preliminary studies of the effect of pyridoxine deficiency on lipid metabolism indicated that pyridoxine was somehow involved in lipogenesis. The incorporation of acetate-1-^{14}C into fatty acids was enhanced in adipose, kidney and spleen of rats during various stages of deficiency.

11. Serum total lipids, cholesterol and lipid phosphorus were elevated during vitamin B_6 deficiency when compared to pair-fed controls. Serum triglyceride and free fatty acids, on the other hand, were depressed during deficiency in these same animals. Liver lipids were not affected by vitamin B_6 deprivation.

12. Liver glutamic pyruvic transaminase (GPT) activities were used to monitor the stage of vitamin B_6 deficiency. The ratio of deficient vs. sufficient liver GPT was suggested as a means for relating the deficiency state to alterations in lipid metabolism.

13. Experiments were initiated to study the effect of dietary vitamin deficiencies on the formation of cross-linkages in aortic elastin of rats.

14. Procedures are being developed to determine the predominant pathway of threonine metabolism in the rat.

BODY OF REPORT

WORK UNIT NO. 058	Nutritional and Metabolic Adaptations and Interrelationships
STUDY NO. 1	Investigate the effect of the ingestion of medium chain triglyceride (MCT) on lipid metabolism in the rat and chick

PROBLEM:

The objective of this work was to investigate the effect of the ingestion of medium chain triglyceride (MCT), a triglyceride containing approximately 75% caprylic (C_8) and 25% capric (C_{10}) acids, on lipid metabolism in the rat and chick.

RESULTS AND DISCUSSION OF THE RESULTS:

The studies presented in the present report were concerned with the alterations in lipogenic activity of adipose tissue and liver from rats and chicks fed MCT.

1. Influence of Dietary Lipids on Rat Plasma and Liver Lipids. Lower plasma and liver total lipid and cholesterol levels were observed in rats fed MCT as compared to corn oil- or coconut oil-fed animals. This depression in plasma and liver cholesterol was reflected in the plasma-liver cholesterol pool (sum of total plasma + liver cholesterol).

In rats fed cholesterol-cholic acid supplemented diets, MCT, or coconut oil, in comparison to corn oil, did not influence plasma cholesterol or total lipid levels; however, the rats fed coconut oil or MCT had lower liver cholesterol and total lipid levels and the plasma-liver cholesterol pool was decreased.

2. Effect of Ingested Fat on Fatty Acid Composition. The plasma and liver from rats on MCT, coconut oil, or corn oil were analyzed for fatty acid composition. The most striking difference was the high level of linoleate (C_{18}:2) in plasma and liver of corn oil-fed rats. The lower level of linoleate observed in the MCT-fed group as compared to the corn oil-fed group was offset by higher palmitate (C_{16}:0) levels. Of significance was the absence of high levels of short chain fatty acids in the plasma and liver lipids of MCT-fed rats, in spite of the ingestion of large quantities of these fatty acids. This observation suggests that the different mode of absorption, i.e., via the portal system, transported to the liver where they are presumably rapidly

oxidized or elongated, could account for the low level of the fatty acids in plasma and liver lipids. In general, except for the C_8 and C_{10} fatty acids noted above, the fatty acid patterns of rat hepatic and serum lipids reflect the fatty acid pattern of the dietary lipid.

3. Utilization of Acetate-1-^{14}C by Adipose and Liver Tissue In Vitro. The ingestion of coconut oil or MCT significantly increased the ability of isolated rat epididymal adipose tissue to incorporate acetate-1-^{14}C into fatty acids, whereas MCT or coconut oil ingestion did not alter the ability of rat liver slices to incorporate acetate-1-^{14}C into fatty acids. Acetate incorporation into cholesterol by rat liver slices was depressed by MCT ingestion.

4. Fatty Acid Composition of Adipose Tissue. The adipose tissue of the MCT-fed rat shows very little increase in short chain fatty acids. This could reflect the efficiency of the liver in removing and metabolizing the shorter chain fatty acids, with little of the unmodified acid reaching the adipose tissue.

5. Evaluation of Chain Elongation Pathway. The fatty acid chain elongating activity was tested in liver slices and adipose by determining the relative incorporation of ^{14}C from acetate-1-^{14}C into the carboxyl carbon of fatty acids, and by the ability of the tissue to convert palmitate-^{14}C to C_{18} fatty acids.

The data from these experiments suggest that chain lengthening activity was increased in liver but not in adipose tissue of rats fed MCT as compared to corn oil-fed animals.

6. Fatty Acid Desaturation Activity. The ability of liver slices to desaturate fatty acids was determined by incubating slices in a buffer containing stearate-2-^{14}C and determining the radioactivity in gas chromatographic peaks relating to oleate (C_{18}:1) and linoleate (C_{18}:2).

Liver slices from rats fed coconut or MCT had an enhanced ability to desaturate fatty acids.

7. Influence of Dietary Lipids on Chick Hepatic and Plasma Lipids. Chicks, in contrast to the rats, were found to have elevated plasma cholesterol and total lipid levels as a result of MCT feeding compared to corn oil-fed animals. However, MCT feeding significantly lowered hepatic cholesterol levels in the chick. The inverse effects of MCT on the plasma and liver cholesterol levels essentially balance each other, as the plasma-liver cholesterol pool was identical for corn oil-, coconut oil- and MCT-fed chicks.

MCT ingestion depressed total liver and serum lipids and cholesterol in chicks fed cholesterol-supplemented diets. These observations suggest that the effect of dietary MCT on circulating lipids in the chick differs in animals fed cholesterol-free or -supplemented diets. The plasma-liver cholesterol pool was depressed 36% by feeding MCT as compared to corn oil-fed chicks supplemented with cholesterol.

8. In Vitro Utilization of Acetate-1-^{14}C by Chick Liver Slices. MCT ingestion depressed overall metabolic activity as measured by the oxidation of acetate-1-^{14}C to $^{14}CO_2$ by chick liver slices.

9. Fatty Acid Elongation and Desaturation by Chick Liver Slices. Liver slices of chicks fed MCT demonstrated an increased capacity for de novo fatty acid synthesis, fatty acid chain elongation and desaturation activity.

CONCLUSIONS:

These experiments on the influence of MCT ingestion on lipid metabolism have demonstrated the following:

1. MCT ingestion depresses rat serum and liver total lipids and cholesterol. However, when MCT and cholesterol are ingested together, plasma cholesterol levels are not altered but liver cholesterol levels are depressed as compared to corn oil-fed rats. On the other hand, the ingestion of MCT by chicks resulted in elevated plasma total lipids and cholesterol and depressed liver total lipids and cholesterol as compared to corn oil-fed animals. The chick plasma-liver cholesterol pool was not influenced by the different dietary fats fed. When cholesterol was included in the diet, MCT depressed chick liver and plasma total lipids and plasma cholesterol when compared to corn oil.

2. In vitro cholesterol synthesis from acetate-1-^{14}C was lower in liver slices of rats consuming MCT than similar preparations from corn oil-fed rats. In vitro cholesterol synthesis was elevated in liver slices of chicks consuming MCT as compared to similar slices from corn oil-fed chicks.

3. In vitro fatty acid synthesis from acetate-1-^{14}C was elevated in liver slices of rats or chicks being fed MCT compared to corn oil. This increased incorporation of acetate-1-^{14}C was determined to be due to enhanced ability to elongate fatty acids, and not de novo synthesis, in the rat liver preparation, whereas the chick liver slice increased incorporation was due to an increased de novo synthesis. Chain elongation was also elevated in chick liver slices from MCT-fed chicks.

4. Both rat and chick liver slices from animals fed MCT, as compared to corn oil, had an enhanced ability to desaturate fatty acids.

PUBLICATIONS:

1. Leveille, G. A. In vitro lipogenesis in adipose tissue of fed and fasted rats: mechanism of pyruvate stimulation. Life Sci., 5: 421, 1966.

2. Leveille, G. A. The reversal of glycolysis in rat epididymal adipose tissue. Life Sci., 6: 803, 1967.

3. Hanson, R. W. and Z. Z. Ziporin. Pyridine nucleotide synthesis by rat adipose tissue in vitro. J. Lipid Res., 8: 30, 1967.

4. Leveille, G. A., R. S. Pardini and J. A. Tillotson. Influence of medium chain triglycerides on lipid metabolism in the rat. Lipids (in press).

5. Leveille, G. A. Lipogenesis in adipose tissue of meal-fed rats: A possible regulatory role of a-glycerophosphate formation. Can. J. Physiol. Pharmacol. (in press).

6. Leveille, G. A. Influence of dietary fat and protein on metabolic and enzymatic activities in adipose tissue of meal-fed rats. J. Nutr. (in press).

7. Leveille, G. A. and R. W. Hanson. Metabolic aspects of periodicity of eating in rat adipose tissue. Proc. VIIth Int. Cong. Nutr., Hamburg, Germany (in press).

8. Leveille, G. A. Glycogen metabolism in meal-fed rats and chicks and the time sequence of lipogenic and enzymatic adaptive changes. J. Nutr. (in press).

9. Leveille, G. A. Amino acid utilization by isolated adipose tissue of meal-fed rats. Proc. Soc. Exp. Biol. Med. (in press).

10. Leveille, G. A. Influence of dietary fat level on the enzymatic and lipogenic adaptations in adipose tissue of meal-fed rats. J. Nutr. (in press).

11. Leveille, G. A., R. S. Pardini and J. A. Tillotson. Influence of medium chain triglycerides on lipid metabolism in the chick. Lipids (submitted).

WORK UNIT NO. 058 - BODY OF REPORT

STUDY NO. 2 — Mechanism of adaptation for elevated lipogenesis in rats fed high carbohydrate diets

PROBLEM:

The purpose of this study was to study the mechanism of adaptation for elevated lipogenesis in rats fed high carbohydrate diets. The differential effects of dietary fructose, glucose and lab chow were determined.

RESULTS AND DISCUSSION OF THE RESULTS:

Fructose-fed rats had the highest level of serum and liver triglyceride, whereas the chow-fed animals had the lowest. Serum cholesterol concentration was unaffected by the dietary regimen employed. Rats maintained on the high fructose or high glucose diets had higher hepatic cholesterol and serum lipid phosphorus than similar animals maintained on lab chow. Fructose feeding resulted in lower concentrations of hepatic lipid phosphorus.

1. Effect of Dietary Carbohydrate on the Concentration of Glycolytic and Tricarboxylic Acid Cycle Intermediates. Fructose-fed rats had depressed hepatic concentrations of glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-diphosphate when compared to glucose- or chow-fed animals. This is indicative of the pathway of fructose phosphorylation which involves fructokinase and the formation of fructose-1-phosphate.

Hepatic dihydroxyacetone phosphate was the same for all experimental groups; however, hepatic a-glycerol phosphate concentration was the lowest in the fructose-fed group and the highest in the chow-fed group. Hepatic pyruvate and malate levels were elevated in the fructose-fed group when compared to similar animals fed glucose or chow. Hepatic citrate and acetyl-CoA concentrations were elevated in the fructose-fed group.

2. In Vitro Utilization of Glucose-^{14}C and Fructose-^{14}C by Liver Slices. The incorporation of ^{14}C-fructose into fatty acids and CO_2 was always greater than the incorporation of ^{14}C-glucose in all dietary groups. The incorporation of ^{14}C-fructose or ^{14}C-glucose was elevated in the fructose- or glucose-fed animals when compared to the lab chow-fed animals.

3. Activity of Hepatic Glycolytic and Lipogenic Enzymes. Liver glucokinase activity was 2 to 3 times higher in the glucose-fed rats as compared to the fructose- or chow-fed animals. Hexokinase and fructokinase activity

was not affected by the dietary regimen employed. It is of interest to note that the activity of fructokinase was considerably greater than the combined activities of glucokinase and hexokinase.

NADP-malic citrate cleavage and acetyl-CoA carboxylase enzyme activities were greater in the fructose- or glucose-fed than the lab chow-fed animals.

It was concluded that the mechanism for the differential effects of high carbohydrate diets on hepatic fatty acid synthesis depends on the higher rate of acetyl-CoA formation as indicated by the elevated pyruvate and acetyl-CoA concentrations in the fructose-fed rats. The difference in the activity of the glucose and fructose phosphorylating enzymes could account for the different rates at which fructose and glucose were metabolized to acetyl-CoA. A secondary effect is that either a diet high in fructose or glucose increased hepatic acetyl-CoA carboxylase activity.

4. Initial Observations on the Effect of Feeding Atromid (Clofibrate)* on Rats Receiving High Fructose Diets. Atromid feeding resulted in depressed serum and liver total lipids, cholesterol, lipid phosphorus and triglyceride when compared to similar animals not receiving atromid.

The conversion of ^{14}C-glucose or ^{14}C-fructose to glycogen or cholesterol was depressed in liver slices from rats fed clofibrate as compared to liver slices from animals fed the same diet without clofibrate.

These studies are in a preliminary stage, and further research will be directed at determining the mechanism of action of clofibrate in lowering serum lipids.

CONCLUSIONS:

1. Lipogenesis is increased in liver slices of rats fed a high fructose diet. This effect is reflected in the elevated serum triglyceride levels in fructose-fed rats.

2. Elevated hepatic concentrations of acetyl-CoA, pyruvic acid and depressed α-glycerol phosphate in the fructose-fed rats indicate that fructose is more readily converted to acetyl-CoA and pyruvic acid. The depressed

*Clofibrate obtained from Oyerst Laboratories.

α-glycerol phosphate levels may be related to the increased turnover due to increased triglyceride formation.

3. ^{14}C-fructose was incorporated into fatty acids and CO_2 at a faster rate than ^{14}C-glucose by liver slices from fructose-, glucose-, or chow-fed rats.

4. Fructokinase activity in all groups was substantially higher than glucokinase activity.

5. The ready availability of fructose via the fructokinase reaction for pyruvate and acetyl-CoA formation causes fructose-fed animals to synthesize more fatty acid and triglyceride than glucose-fed animals whose acetyl-CoA availability is controlled by phosphofructokinase activity.

6. Atromid feeding results in depressed serum total lipids, cholesterol, triglycerides and lipid phosphorus in fructose-fed animals. The incorporation of ^{14}C-fructose or ^{14}C-glucose into cholesterol and glycogen was depressed in clofibrate-fed rat liver.

PUBLICATIONS:

1. Zakim, D., R. S. Pardini, R. H. Herman and H. E. Sauberlich. The relation of hepatic α-glycerophosphate concentration to lipogenesis in rat liver. Biochim. Biophys. Acta, 137: 179, 1967.

2. Zakim, D., R. Pardini, R. Herman and H. Sauberlich. Relation of high carbohydrate diets to fatty acid synthesis in rat liver. Abstr. 7th Int. Cong. Biochem., Tokyo, Japan, 19-27 August 1967.

3. Zakim, D., R. S. Pardini, R. H. Herman and H. E. Sauberlich. Mechanism for the differential effects of high carbohydrate diets on lipogenesis in rat liver. Biochim. Biophys. Acta (in press).

STUDY NO. 3 — Effect of pyridoxine deficiency on lipid metabolism in the rat

PROBLEM:

The objective of this study was to investigate the effect of pyridoxine deficiency on lipid metabolism in the rat. It includes in vitro utilization of acetate-1-^{14}C and ^{14}C-labeled amino acids, enzyme determinations and the analysis of various lipid classes in the serum and liver.

WORK UNIT NO. 058 - BODY OF REPORT

RESULTS AND DISCUSSION OF THE RESULTS:

Vitamin B_6 deficiency resulted in elevated serum total lipids, cholesterol and lipid phosphorus, and depressed triglycerides and free fatty acids; however, the liver lipids were not appreciably altered.

In order to establish deficiency, liver glutamic pyruvic transaminase (LGPT) activity was determined and ratio of deficient to sufficient LGPT activities calculated to monitor the stage of deficiency. The ratio varied between 0.3 and 0.8 in the different experiments. Since the sufficient LGPT values had very little variation between studies, the main differences were in the deficient values; hence, the LGPT ratio truly reflects the stage of deficiency.

In all of the studies employed, the conversion of acetate-1-^{14}C into fatty acid was elevated in kidney, adipose and spleen from the vitamin B_6-deficient rats, as compared to similar rats receiving vitamin B_6, except for the most severely deficient group (LGPT ratio of 0.3), in which case this observation was reversed.

The ratio of deficient to sufficient liver glutamic pyruvic transaminase values was used to monitor the stage of deficiency of the animals from different experiments.

These observations indicate that the effect of pyridoxine on lipid metabolism depends on the stage of deficiency of the animals under investigation, a fact too frequently overlooked in the literature. The use of LGPT ratio and perhaps serum glutamic pyruvic transaminase activity could be a useful tool in future research concerning the role of vitamin B_6 deficiency in lipid metabolism.

Preliminary results suggest that the pattern of incorporation of ^{14}C-label from amino acids follows that of acetate-^{14}C. In future experiments, the utilization of amino acids will be studied in order to establish whether the decrease in transaminase activity in vitamin B_6 deficiency has a direct or indirect effect on lipogenesis.

CONCLUSIONS:

1. Lipogenesis is enhanced during the early and middle stages of vitamin B_6 deprivation, as measured by the incorporation of acetate-1-^{14}C into fatty acids.

2. Pyridoxine deficiency resulted in elevated serum total lipids, cholesterol and lipid phosphorus, and depressed serum triglyceride and free fatty acids. Liver lipids were not affected by vitamin B_6 deprivation.

PUBLICATIONS:

1. Pardini, R. S. and H. E. Sauberlich. The effect of pyridoxine deficiency on lipid metabolism. Fed. Proc., 26: 412, 1967 (abstract).

STUDY NO. 4 — Effect of specific amino acids and vitamins on the formation of cross-linkages in aortal elastin during aging

PROBLEM:

Elastin, the insoluble constituent protein of elastic fibers occurring in the intercellular spaces of connective tissue, has some unusual properties and is distinct from other vertebrate structural proteins. There seems to be no major secondary structure but a tertiary structure of a three-dimensional network formed by the covalent cross-linking of randomly kinked polypeptide chains, involving bonds other than disulfide since elastin contains very little cystine.

The structure of the cross-linking compounds has recently been elucidated. However, the reaction mechanism and the cellular control mechanism for their formation are not known. Cross-linkages have been associated with biological aging.

There have been suggestions and evidence the last few years that relate copper, β-aminopropionitrile and oxidative enzymes in the cross-linking of elastin. The involvement of a copper-containing enzyme similar to the monoamine oxidases has been suggested. The literature makes it quite clear that the formation of cross-linkages in elastin does not occur at random. Instead, it is a highly controlled biological process, the mechanism of which is still unknown.

RESULTS AND DISCUSSION OF THE RESULTS:

The present studies are designed to investigate the effect of vitamin E deficiency on the formation of cross-linkages in aortic elastin of rats since

the formation of the desmosine and isodesmosine appears to involve an oxidative deamination at the epsilon amino group of lysine residues in the peptide chains of elastin. The structures of desmosine and isodesmosine suggest that they could arise from condensation of four lysine residues pre-existing in straight-chain elastin precursors. It has been hypothesized that the side-chains of three lysine residues are deaminated and oxidized at some point after or during the synthesis of a soluble pro-elastin in the cell. The three pro-elastins then migrate to the site of synthesis and condense with a fourth unmodified lysine side-chain. There is an appreciable time lag between the incorporation of lysine into the elastin and the transformation of lysine into cross-links. A third amino acid which apparently is a cross-linking amino acid in elastin is lysinonorleucine, [N-(5-amino-5-carboxypentanyl)-lysine].

Thirty-six rats were divided into two groups of 18 each. One group received a vitamin E-deficient diet and the other served as a control. Each rat from both groups received a total of 25 μc of U-^{14}C-lysine intraperitoneally over a 4-day period.

Techniques for removal of elastin from the aortic tissue have been worked out and the analytical procedures have been developed. The aortas were freed from connective tissue, washed exhaustively with 0.15 M NaCl to remove serous proteins, dehydrated in acetone, treated with 0.1 N NaOH at 98° C for 20 minutes to remove collagen, treated six times by boiling for 5 minutes with water, and dried by treatment with ethanol and ether (1:1). This yielded a purified elastin. The purified elastin is hydrolyzed by 6 N HCl at 110° C for 22 hours in a heat-sealed test tube after purging the sample with nitrogen. The HCl is removed under vacuum at 40° C and the hydrolyzate residue dissolved in pH 2.2 citrate buffer to give a final concentration of 1 mg/ml. One ml is applied to a column (135 cm x 0.6 cm) packed with Biorad Aminex A-4 beaded resin, and elution follows the standard Technicon procedure for automatic amino acid analysis.

Primary interest will be focused on the cross-linking amino acids, desmosine, isodesmosine and lysinonorleucine; however, attention will also be given to the changes that occur in amino acid composition of elastin as a result of vitamin E deficiency since this vitamin may affect fat oxidation in vivo. Primary or even secondary effects of free radicals produced from peroxidizing fat may affect structural proteins and the aging process.

Attempts will be made to study the regulatory mechanism of conversion of pro-elastin to the cross-linked elastin. Rats will be injected intraperitoneally with various levels of desmosine and with U-^{14}C-lysine similar to

that described above to determine whether or not a feedback mechanism of control is involved in the formation of the desmosines in elastin fibers. Analysis will follow that already described.

Experiments involving the effect of vitamin E on cross-linkage may be extended to aortic tissue of chick embryo and growing chicks.

The nature of further experimentation will depend upon results obtained.

CONCLUSIONS:

Studies were initiated designed to investigate effects of specific vitamins and amino acids on the formation of cross-linkages in aortic elastin of experimental rats as may relate to aging.

PUBLICATIONS:

None

STUDY NO. 5 Studies on threonine metabolism

PROBLEM:

In mammalian liver, threonine can be catabolized by three different pathways. The enzymes involved and their control have been reported, but the predominant pathway and what hormonal or dietary factors may affect their relationship have not been studied. Many techniques are available to establish the particular pathway taken in the use of specifically labeled substrates; however, these results do not necessarily produce definitive results in the case of threonine.

RESULTS AND DISCUSSION OF THE RESULTS:

From preliminary experiments, it was found that in vitro incubations of tissue slices yield insufficient quantities of metabolites to test the labeling technique. It was concluded that in vivo procedures should be developed with the use of orally administered labeled substrates and the isolation of metabolic products from the intact organs.

WORK UNIT NO. 058 - BODY OF REPORT

CONCLUSIONS:

Preliminary studies have been initiated on an investigation on the metabolic pathways of threonine in the rat.

PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6317	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	6. SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (27 02 67)	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 02 061

10b. PRIOR NUMBER CODE: None

11. TITLE: (U) Work Performance and Body Composition as Related to Environment and Nutritional Status (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002600 Biology 012900 Physiology 003500 Clinical Medicine	07 56	NA	OTHER I DA

16. PROCURE. METHOD	17. CONTRACT GRANT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	a. DATE b. NUMBER NA c. TYPE d. AMOUNT	PRIOR FY 67	1	65
		CURRENT FY 68	1	70

19. GOV'T LAB/INSTALLATION: ACTIVITY
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INVESTIGATORS PRINCIPAL ASSOCIATE: Krzywicki, H. J., Chinn, K.S.K.
Consolazio, C. F.
TEL 303 366 5311 X25222 TYPE DA

21. TECHNOLOGY UTILIZATION: Civilian Medicine
Acturial Studies Animal Husbandry

22. COORDINATION: None

23. KEYWORDS: Anthropometry; Body Composition; Height; Weight; Water; Fat; Protein; Mineral; Density; Potassium Counting

24. (U) Tech Objective: To standardize a simple method to accurately and reliably define the major components of the human body.

25. (U) Approach: Densitometric estimation of body fat by water displacement, further developed at this Laboratory, is simple and as accurate as underwater weighing techniques. Comparative studies of K^{40} whole body counting, with body volumeter data, total body water, blood volume, extra-cellular fluid, and selected anthropometric techniques are being evaluated. These body components (in rats and humans) are compared to creatinine and creatine excretions, oxygen uptake and potassium content, in an effort to further define the active metabolizing tissue mass of the body.

26. (U) Progress: Body composition was studied during 10 days of caloric restriction on 2 groups of 4 men fed either 400 Calories carbohydrate or 500 Calories/day of carbohydrate plus protein diets with and without mineral supplementation. Body components were studied in 66 basic trainees aged 17 - 24, plus 104 soldiers aged 25 - 50 years; 24 basic trainees undergoing special training so to pass the Army physical fitness test; and 26 obese individuals 19 - 49 years of age. The general population showed increased body fatness with age but not body weight related. Analyses of the data is in progress.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	Research in Biomedical Medicine
TASK NO.	02	Internal Medicine
WORK UNIT NO.	061	Work Performance and Body Composition as Related to Environment and Nutritional Status

The assessment of that most widely variable aspect of body composition, i. e., body fat, still proves elusive as does the gasless body volume. Complete radioisotope dilution techniques are well adapted and accepted for the clinical patient as described by Moore (Body Cell Mass, Saunders, 1963), but time and method requirements do not lend these techniques to survey type studies. The use of tritium for estimation of total body water is an exception, however, provided permission for waste disposal is granted by the AEC.

Continued studies of minimal nutritional requirements of the combat patrol soldier resulted in two studies on two groups of four men each -- one fed 400 Calories of carbohydrate and the second 500 Calories of a mixture of carbohydrate-protein with and without mineral supplementation for 10 days. The group fed the 500 Calories of the mineral supplemented mixture exhibited the greatest loss of body fat and the lowest loss of dry body protein.

The Nutrition Survey at Fort Campbell, Kentucky showed population trends of body fatness observed at Fort Carson, Colorado and Fort Huachuca, Arizona to be similar, i. e., increasing fat burdens with age that are not body weight related.

BODY OF REPORT

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

PROBLEM:

The vertebrate body, consisting of water, fat, mineral, and protein, is in a dynamic state easily altered in direction and kind by internal and external environment. No single technique of accurately estimating human body compartments exist, yet several methods for approximating any one compartment are available. It is of continuing necessity to seek an accurate, reliable, and valid method to measure all body compositional aspects. Present investigation includes further verification and application of a simple volume measurement of the human body by water displacement, with adequate correction for contained air and gas, whereby the "residual mass" (body less its bone mineral, water, and fat) represents an active metabolizing mass principally composed of muscle tissue. This active metabolizing mass will provide a real basis for correlation to various physiologic functions. Total body potassium is now being counted using a NaI crystal to compare body potassium as an index of "active metabolizing mass" with estimates as calculated from body volumes and selected anthropometry. Total body water, using deuterium oxide dilution and the gas chromatograph, are being determined with some success. Deuterium concentrations in blood and urine are being compared to determine the validity of the technique.

RESULTS AND DISCUSSION OF THE RESULTS:

Two studies were performed as a continuation of the need for determining those minimal essential nutrients required to sustain the combat patrol soldier for 10 days. Body composition was studied using the aforementioned techniques and preliminary results in terms of density, fat, and dry protein mass showed that four subjects fed only 400 Calories as carbohydrate lost 5.7 kg of body weight which was 1.6 kg less than subjects deprived of food for 10 days. The 400 Calorie mineral supplemented group lost only 4.2 kg of body weight which appeared to represent some water retention. Both mineral free and mineral supplemented groups of subjects lost 1.0 kg and 0.8 kg fat, and 1.0 kg and 0.8 kg of dry protein, respectively. During the starvation study, the men lost an

average of 3.6 kg of fat and 0.8 kg of dry protein.

In the 500 Calorie study, the same approximate body weight losses were observed in those four subjects deprived of essential minerals and those four subjects receiving the mineral supplement. However, the group lacking minerals lost 3.6 kg of body fat (similar to body fat losses with total starvation) while the mineral supplemented group lost 2.7 kg of fat. In both groups, only 0.4 kg of dry protein loss was observed, which was half the amount lost during starvation or when the subjects were fed 400 Calories of carbohydrate, with or without the mineral supplement. Essentially, the greatest body fat utilization was observed in the 500 Calorie mineral supplemented diet. The loss of body dry protein is not desirable; however, future testing of this diet in greater caloric density may alleviate this condition.

A nutritional survey at Fort Campbell, Kentucky was completed on 30 March 1967. Preliminary direct water displacement densitometric data shows this population to also demonstrate the decrease of body density with an increase of body fat which is not age related. A comparison of the results of the 1966 Fort Huachuca, Arizona survey on 20 - 40 year olds with that of the Fort Campbell, Kentucky data shows this group as well as similar groups at Fort Carson, Colorado (1963) and Fort Huachuca, Arizona (1966) to have a fat free body weight of approximately 59.1 kg. Similar results have been observed by other investigators over the past two decades.

Further analysis of anthropometric and body composition data is to be accomplished by automatic data processing so to relate body composition to nutriture and work performance.

CONCLUSIONS:

Comparisons of body composition changes were made on two groups of men fed a 400 Calorie carbohydrate diet and a 500 Calorie protein-carbohydrate diet, with and without mineral supplementation. It was observed that the greatest loss of body fat occurred on the 500 Calorie mineral supplement diet. Body protein (dry) was decreased to a loss of only 0.4 kg, which indicated that a greater calorie intake may eliminate this deficiency in the diet.

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

Several groups of soldiers studied at Fort Campbell, Kentucky (basic trainees, special trainees, support personnel, and obese individuals) showed the fat free mass of individuals aged 20 - 40 years, to be 59.1 kg, as has been observed in similar populations.

RECOMMENDATIONS:

Continue to compile and evaluate the body composition data for a near future scientific publication.

Evaluate body compartment measurements during altitude exposure of 4300 meters.

PUBLICATIONS:

1. Hannon, J. P. and K. S. K. Chinn: Effect of altitude on body fluid volumes. Fed. Proc. 26:719, 1967 (Abstract).

2. Krzywicki, H. J., L. O. Matoush, C. F. Consolazio, and H. L. Johnson: Changes in human body composition with caloric restriction. Fed. Proc. 26:474, 1967 (Abstract).

3. Krzywicki, H. J. and C. F. Consolazio: Changes in human body fat and protein during restricted intake. J. Colorado-Wyoming Acad. Sci., April 1967 (Abstract).

4. Chinn, K. S. K.: Effect of diet and altitude in body compartments of rats. J. Colorado-Wyoming Acad. Sci., April 1967, (Abstract).

5. Krzywicki, H. J. and K. S. K. Chinn: Body composition of a military population, Fort Carson, Colorado, 1963. I. Body density, fat, and potassium ^{40}. USAMRNL Report #296, December 1966.

6. Krzywicki, H. J. and K. S. K. Chinn: Human body density and fat of an adult male population as measured by water displacement. USAMRNL Report #297, December 1966.

7. Krzywicki, H. J., C. F. Consolazio, L. O. Matoush, and H. L. Johnson: Metabolic aspects of acute starvation. Body composition changes. USAMRNL Report #303, December 1966

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

8. Chinn, K. S. K.: Urinary creatinine excretion as an index of the fat-free mass composition in rats. USAMRNL Report # 305, 1967.

9. Chinn, K. S. K.: Effects of diet and altitude on body composition in rats. USAMRNL Report #306, 1967.

10. Chinn, K. S. K., R. Burlington, J. P. Hannon, G. J. Klain, and J. L. Shields: Effects of diets and altitudes in growth and food intake in rats. USAMRNL Report #307, 1967.

11. Chinn, K. S. K.: Content and distribution of potassium and creatine in rats. USAMRNL Report #310, 1967.

12. Chinn, K. S. K. and J. P. Hannon: The relationship of muscle protein to other components of the fat-free mass. Am. J. Physiol. 211:993, 1966.

13. Chinn, K. S. K.: Urinary creatinine and total body potassium as indices of muscle and non-muscle protein in rats. J. Nutr. 90:323, 1966.

14. Surks, M. I., K. S. K. Chinn, and L. O. Matoush: Alterations in body composition in man after acute exposure to high altitude. J. Appl. Physiol. 21:1741, 1966.

15. Krzywicki, H. J. and K. S. K. Chinn: Human body density and fat of an adult male population as measured by water displacement. Am. J. Clin. Nutr. 20:305, 1966.

16. Krzywicki, H. J. and K. S. K. Chinn: Body composition of a military population. I. Body density, fat, and potassium[40]. Am. J. Clin. Nutr. (in press).

17. Krzywicki, H. J., C. F. Consolazio, L. O. Matoush, and H. L. Johnson: Metabolic aspects of acute starvation. Body composition changes. Am. J. Clin. Nutr. (in press).

18. Krzywicki, H. J. and C. F. Consolazio: Body composition methodology in military nutrition surveys. Natl. Acad. Sci., National Research Council (in press).

19. Krzywicki, H. J.: Changes in quantities of fat and protein in humans during caloric restriction. Presented at Colorado Conference on Body Composition at Colorado State University, 24 May 1967.

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

20. Chinn, K. S. K.: Shifts in intra and extracellular water at altitude. Presented at Colorado State University, Conference on Body Composition, 24 May 1967.

21. Krzywicki, H. J. and C. F. Consolazio: Body composition methodology in military nutrition surveys. Presented by invitation at NAS-NRC sponsored public conference on Body Composition, University of Missouri, Columbia, Missouri, 6 May 1967.

RESEARCH AND TECHNOLOGY RESUME	1	2 GOVT ACCESSION	3 AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6334	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6 SECURITY	7 REGRADING	8 RELEASE LIMITATION	9 LEVEL OF RESUME
01 07 67	D. Change (03 03 67)	U U	NA	NL	A. WORK UNIT

10a CURRENT NUMBER/CODE	10b PRIOR NUMBER CODE
61145011 3A014501B71R 02 062	None

11. TITLE: (U) Muscle metabolism as related to exercise, serum electrolytes, diet, the influence of steroids in normal man and disease (06)

12. SCIENTIFIC OR TECH. AREA	13 START DATE	14 CRIT COMPL DATE	15 FUNDING AGENCY
003500 Clinical Medicine 002300 Biochemistry	11 65	NA	OTHER | DA

16 PROCURE. METHOD	17 CONTRACT GRANT		18 RESOURCES EST	a PROFESSIONAL MAN-YEARS	b FUNDS (In thousands)
C. In-House	a. NUMBER NA	a. DATE	PRIOR FY 67	0	18
	c. TYPE	d. AMOUNT	CURRENT FY 68	0	35

19 GOV'T LAB INSTALLATION ACTIVITY	20 PERFORMING ORGANIZATION
NAME	NAME
ADDRESS Headquarters U.S. Army Med Rsch & Dev Cmd Washington, D. C. 20315	ADDRESS U.S. Army Med Rsch & Nutr Lab Fitzsimons General Hospital Denver, Colorado 80240
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TEL 202 OXford 6 5472	TEL 303 366-5311, X10221 TYPE DA

21 TECHNOLOGY UTILIZATION	22 COORDINATION
NA	None

23. KEYWORDS: Muscle; Metabolism; Exercise; Electrolytes; Diet; Steroids; Disease

24 (U) Technical Objective: Exercise depends upon the flux of sodium and potassium ions in and out of the muscle cell. This is thought to control the binding of calcium which in turn controls the contraction of the muscle fibrils. By studying patients with a variety of muscle diseases we are able to determine the mechanism whereby electrolytes control muscle contraction.

25 (U) Approach: Normal human subjects under regulated conditions of diet and conditioning exercise on a metabolic ward will undergo exercise and the change in serum electrolytes will be measured. A variety of patients with varying types of muscle disease will be compared to the response of normal individuals. The effect of various agents in altering the potassium tolerance test in selected patients and normal subjects will be studied.

26 (U) Progress: Varying sodium and potassium diets have very little influence on serum electrolytes in exercise. Exercise leads to changes in serum magnesium. The significance of this is as yet unknown. We have measured the normal potassium tolerance in man. In patients with a variety of myotonic states we have determined that there are marked abnormalities in the potassium tolerance test. This is not altered by quinine. The abnormalities in the potassium tolerance test are consistent in all patients studied. The degree of abnormality of the potassium tolerance test seems correlated with the severity of disease.

27 COMMUNICATIONS SECURITY	28	29 OSD CODE	30 BUDGET CODE
[ ] a. COMSEC OR COMSEC RELATED [X] b. NOT RELATED		BR	1

31 MISSION OBJECTIVE	32 PARTICIPATION
NA	NA

33 REQUESTING AGENCY	34 SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	Research in Biomedical Sciences
TASK NO.	02	Internal Medicine
WORK UNIT NO.	062	Muscle Metabolism as Related to Exercise, Serum Electrolytes, Diet The Influence of Steroids in Normal Man and Disease

Potassium given orally to normal man causes a rise in serum potassium levels with a maximum at about 90 to 120 minutes with a return to normal by 180 minutes. Thus, it is possible to perform a potassium tolerance test in much the same way as a glucose tolerance test. Patients with myotonia dystrophica and myotonia congenita have abnormal potassium tolerance tests as compared to normal human subjects. Treatment of patients with myotonia congenita with quinine reduces the myotonia but has no effect on the potassium tolerance test which remains abnormal. In one patient with myotonia dystrophica who did not respond clinically to quinine therapy the potassium tolerance test became less normal. The potassium tolerance tests in two patients with myotonia dystrophica who did respond to quinine therapy are pending. It is planned to study normal human subjects with potassium tolerance tests to determine which of several agents will alter serum potassium levels after the ingestion of oral potassium. The agents which lower serum potassium in normal subjects will then be tested in patients with muscle disease after the ingestion of oral potassium.

BODY OF REPORT

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

DESCRIPTION:

During exercise serum electrolytes change rapidly. Serum potassium rises while serum sodium and calcium may rise or remain normal. Normal muscle contraction depends upon cation flux across muscle cell membranes. In certain muscle diseases there is pathological derangement of cation movement in and out of muscle cell. In hyperkalemic periodic paralysis the elevation of serum potassium levels causes a flaccid paralysis which can be reversed by the injection or ingestion of a glucose solution. In familial hypokalemic periodic paralysis exercise followed by a large carbohydrate meal results in paralysis which can be reversed by potassium. In the study of a patient with familial hypokalemic periodic paralysis it was found that a high sodium diet protected the individual from paralytic episodes and appeared to affect the level of serum electrolytes during exercise (M. K. McDowell, R. H. Herman, and T. E. Davis, Metabolism 12:388, 1963). Further studies now completed in normal individuals have shown that dietary levels of potassium and sodium have little effect on serum electrolyte changes during and shortly after exercise. This implies that normal individuals are able to change serum electrolytes during exercise to the same degree despite changes in tissue cation content induced by dietary means. Since muscle function depends on cation flux and in in vitro preparations of muscle contraction ceases after cation depletion reaches a certain level it has been proposed that "muscle fatigue" is related to the depletion of muscle electrolytes incurred during exercise. To study this aspect of muscle function the ability of patients with certain muscle disease to handle an oral potassium load has been tested. Normal individuals will be tested also with an oral potassium load and the effects of various agents in reversing electrolyte changes induced by an oral potassium load will be studied. The measures that are potent in lowering serum potassium levels after oral ingestion of potassium in normal subjects then can be used to alter serum potassium levles in patients with muscle disorders in which there is an abnormal potassium metabolism and in normal individuals undergoing severe exercise.

PROGRESS:

We have studied six patients with myotonia dystophica, three patients from one family with myotonia congenita, and one patient with a sporadic form of myotonia congenita. All of these patients have an abnormal potassium tolerance test. Even though serum potassium rises to abnormal levels as compared to normal subjects electrocardiograms remain unchanged to a remarkable degree. The three patients with familial myotonia congenita responded well to treatment with quinine sulfate but despite the clinical improvement the potassium tolerance test remained abnormal and unchanged. The patient with sporadic myotonia congenita responded poorly to quinine

and later became markedly worse when a diabetic state developed. It is interesting that oral sulfonylurea improved his diabetes and myotonia. This point will be investigated since abnormal glucose tolerance has been noted in certain patients with muscle disease. The patients with myotonia dystrophica had quite abnormal potassium tolerance tests and this seemed to correlate approximately with the severity of their disease although this would be difficult to quantitate precisely. In one patient who was given quinine who did not respond clinically the potassium tolerance test became less abnormal. The results of potassium tolerance tests in two individuals who did respond clinically to quinine are pending. As an extension of the present studies we will study potassium tolerance in normal subjects and test the ability of various agents in lowering the serum potassium after the oral ingestion of potassium. It is then planned to test the most effective agents in patients with certain muscle disorders after the oral ingestion of potassium and finally in normal subjects during exercise.

SUMMARY:

Potassium tolerance tests in patients with myotonia dystrophica and myotonia congenita are abnormal. Quinine which is effective therapeutically in patients with myotonia congenita had no effect on the potassium tolerance test. In one patient with myotonia dystrophica quinine caused the potassium tolerance test to be less abnormal but had no effect clinically.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2 GOVT ACCESSION	3 AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6328	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6 SECURITY RPT	WRK	7 REGRADING	8 RELEASE LIMITATION	9 LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U	U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER CODE
61145011 3A014501B71R 02 063	61130011 3A013001A91C 01 043

11. TITLE
(U) Studies in Microbial Metabolism (06)

12. SCIENTIFIC OR TECH AREA	13 START DATE	14 CRIT COMPL DATE	15 FUNDING AGENCY
010100 Microbiology	10 64	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT GRANT	18 RESOURCES EST	a PROFESSIONAL MAN-YEARS	b FUNDS (In thousands)
C. In-House	a DATE b NUMBER NA c TYPE d AMOUNT	PRIOR FY 67	2	46
		CURRENT FY 68	2	82

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21. TECHNOLOGY UTILIZATION
All medical laboratories

22 COORDINATION
None

23. KEYWORDS
Mycoplasma; Staphylococci; Mycobacteria; Metabolism; Environment

24. (U) Tech Objective: To define metabolic pathways of drug-susceptible and drug-resistant Mycobacterium tuberculosis with the objective of improving isolation and identification techniques, as well as providing a base for future chemotherapeutic study.

25. (U) Approach: Metabolic pathways in drug-susceptible and drug-resistant M. tuberculosis are mapped by determining their enzyme complement and by their conversion of carbon-14 labeled compounds to metabolic intermediates.

26. (U) Progress: A paper describing the synergistic effect of various carbohydrates upon the inhibition of growth of INH-resistant M. tuberculosis by hydrogen peroxide has been accepted for publication in the American Review of Respiratory Diseases. The loss of catalase with the acquisition of resistance to INH makes this organism particularly susceptible to peroxides. A number of enzymes including glucose-6-phosphate dehydrogenase, fructose-1, 6-diphosphatase, aldolase, NADH oxidase, menadione reductase, 2,6-dichlorophenolindophenol reductase, and iodonitrotetrazolium violet reductase were assayed in extracts prepared from INH-resistant and INH-susceptible M. tuberculosis. Differences were noted between the two organisms in their tetrazolium reductase content. Electrophoretic separation of the reductases in extracts from the two organisms showed distinctly different patterns. As corollary to the enzyme assays the dissimilation of glucose labeled in the 1 and 6 positions with carbon-14 was followed with washed cell suspensions of INH-susceptible and resistant M. tuberculosis. The amount of radioactivity appearing in carbon dioxide, various cell fractions, and suspending medium was determined. One significant result of this work has been the demonstration that a considerable portion of the glucose is converted to trehalose.

27. COMMUNICATIONS SECURITY	28	29 OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32 PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34 SPECIAL EQUIPMENT

35. EST FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C, WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A0i450iB7iR Military Internal Medicine

TASK NO. 02

WORK UNIT NO. 063 Studies in Microbial Metabolism

During the past report period work has continued within areas related to the achievement of our defined objective of studying the metabolism, growth physiology, and nutrition of drug-susceptible and drug-resistant Mycobacterium tuberculosis. The purpose of these studies is to improve techniques employed in the mycobacteriological examination of clinical specimens and to further knowledge of the metabolic activities of these medically important organisms. Experimentation has followed three main avenues of approach: (1) Growth studies involving variation in basal media and environmental factors; (2) The use of cell suspensions to determine the dissimilation of carbon-14 labeled metabolites; (3) Assay of enzymes present in cell free extracts.

Previously reported findings, which demonstrated the synergistic effect of glucose in the growth suppression of an INH-resistant M. tuberculosis by peroxides, have been extended by considering the ability of other carbohydrates and polyhydric alcohols to act in a similar manner. In addition to glucose, it has been found that glycerol, fructose, galactose, and mannose also act with hydrogen peroxide to suppress growth. These results have provided new insight into reported growth failures of the INH-resistant organism in autoclaved basal medium.

Studies concerned with the dissimilation of carbon-14 labeled glucose by drug-susceptible, INH-resistant, and streptomycin resistant M. tuberculosis have progressed to the point that tentative maps have been prepared illustrating the flow of carbon into such major cell fractions as cold trichloroacetic acid extracts, lipids, hot trichloroacetic acid extracts, protein, and carbon dioxide. A major portion of glucose was found to be converted to trehalose which is extracted in the cold trichloroacetic acid fraction.

In conjunction with the above studies, the slow-growing M. tuberculosis has been obtained in sufficient quantities at regular intervals to allow examination of the enzyme content of INH-susceptible and INH-resistant strains. It has been found that cells grown in Middlebrook's 7H10 media contain reduced levels of glucose-6-phosphate dehydrogenase and elevated levels of aldolase as compared to cells grown in modified Proskauer-Beck media. Also observed were differences in the ability of extracts of INH-susceptible and resistant M. tuberculosis to reduce iodonitrotetrazolium violet. By strip electrophoresis this reductase activity has been resolved into a family of enzymes which occur in the two organisms in distinctly different patterns.

BODY OF REPORT

WORK UNIT NO. 063 Studies in Microbial Metabolism

PROBLEM:

The present investigations were instituted to help remedy the lack of information pertaining to the metabolic activities of drug-resistant and drug-susceptible Mycobacterium tuberculosis. The pathogenic Mycobacteria have never received the concerted study that other bacteria have enjoyed. Undoubtedly their exteremely slow rate of growth as well as their highly virulent nature has contributed to this situation. Much of the knowledge of mycobacterial metabolism has been derived from studies with saprophytic species which in the final analysis leaves many questions unanswered. Therefore it would seem that the currently reported investigations offer the opportunity of making a needed and significant contribution to microbiologic knowledge.

RESULTS AND DISCUSSION OF RESULTS:

In recently completed studies dealing with the supplementation of minimal basal media with various carbohydrates as utilizable sources of carbon it was discovered that a synergistic relationship existed between various carbohydrates and hydrogen peroxide in suppressing growth of INH-resistant M. tuberculosis. The essential point of these findings is that growth of the INH-resistant organism proceeds in an apparently normal manner in the absence of glucose, for example, while with glucose present growth is almost totally inhibited by 0.5 µg of hydrogen peroxide per ml. In addition to glucose, glycerol, galactose, fructose, and mannose acted to increase the susceptibility of the cell to hydrogen peroxide, while mannitol had no effect. It is interesting that glucose, glycerol, mannose, galactose and fructose have been shown to be oxidized by constitutive or adaptive enzymes present in M. tuberculosis. In general these results serve to emphasize how the loss of catalase with the acquisition of resistance to INH can exert an influence on growth of INH-resistant M. tuberculosis.

Considerable attention has been given to the manner in which drug-resistant and drug-susceptible M. tuberculosis metabolize glucose. Employing the technique of incubating cell suspensions with glucose labeled in the 1 and 6 positions with carbon-14 an attempt has been made to estimate the extent of metabolism occurring through the oxidative pentose pathway as compared to the classical Embden-Meyerhof pathway. As a working hypothesis it was assumed that these well known pathways are operating in M. tuberculosis, although as knowledge increases it may be that variations of these pathways will emerge. Balance studies have been conducted with susceptible, INH-resistant, and streptomycin resistant strains. With 50% of labeled glucose utilized 26% to 35% of the radioactivity has been recovered in more or less defined fractions such as

respiratory CO_2, cold trichloroacetic acid (TCA), lipid, hot trichloroacetic acid, and protein. The streptomycin resistant organism converted more of the glucose-C^{14} to these defined products (35%), than the susceptible (26%) or INH-resistant (29%) strains. The streptomycin resistant organism also formed more $C^{14}O_2$ from glucose-1-C^{14} in comparison to that formed from glucose-6-C^{14} than the susceptible or INH resistant strains. The streptomycin resistant organism converted more radioactivity into the hot TCA extract or nucleic acid fraction. A considerable portion of the total utilized radioactivity was found in the cold trichloroacetic acid fraction. By paper chromatography it was shown that the major portion of this radioactivity gave an Rf value identical to standard trehalose. The presence of trehalose was further demonstrated by eluting the cold TCA fraction from a Dowex-1 column (borate form) with borate buffer. Some 40% of the radioactivity present in the cold TCA extract eluted from the column with known, added, trehalose. The large accumulation of trehalsoe points out the rather unique role trehalose plays in the nutrition of M. tuberculosis, occurring as a diester of mycolic acid (cord factor) and as fatty acid esters in neutral fat and waxes.

An important aspect of our work has been the examination of enzymes present in cell free extracts prepared from drug susceptible or drug resistant Mycobacterium tuberculosis. To do this it has been necessary to obtain the organisms in quantity. This problem has been met through the use of large capacity fermenters, although our output of cells is still limited by the extremely slow growth of Mycobacterium tuberculosis. In practice the cells are harvested in an aerosol free refrigerated centrifugation system. The recovered cells are washed, sonicated for 30 minutes and the sonicate centrifuged at 20,000 x g and 105,000 x g. The last centrifugation removes particulate NADH and NADPH oxidases which interfere with enzyme assays. To date, extracts have been prepared and examined from INH-resistant and susceptible M. tuberculosis for glucose-6 phosphate dehydrogenase, aldolase, fructose-1, 6-diphosphatase, NADH oxidase, menadione reductase, 2,6-dichlorophenolindophenol reductase, and iodonitrotetrazolium violet reductase. From these studies it has become apparent that different levels of enzymes are present in cells grown in modified Proskauer-Beck medium as compared to cells from the richer Middlebrook 7H10 basal medium. Cells from the modified Proskauer-Beck medium have much more glucose-6-phosphate dehydrogenase while the levels of aldolase is increased in cells grown in 7H10 medium. It may be noted that the 7H10 media contains glucose which is known to play a role in suppressing certain enzyme pathways. With the iodonitrotetrazolium violet reductase it was found what appears to be a real difference between the INH-resistant and susceptible strains. Subjecting extracts to strip electrophoresis, the reductase activity has been resolved into a family of enzymes. These enzymes show a different pattern in extracts from INH-resistant as compared to susceptible strains.

CONCLUSIONS:

1. A group of carbohydrates and polyhydric alcohols were shown to greatly increase the susceptibility of INH-resistant M. tuberculosis to hydrogen peroxide.

2. The metabolism of glucose by suspensions of drug-susceptible and drug-resistant M. tuberculosis was found to result in the synthesis of considerable amounts of trehalose.

3. Levels of aldolase and glucose-6-phosphate dehydrogenase were elevated or suppressed depending on the type of basal medium used to grow M. tuberculosis.

4. Differences in iodonitrotetrazolium violet reductase content have been noted between INH-resistant and INH-susceptible M. tuberculosis.

RECOMMENDATIONS:

It would seem reasonable to consider that the rather unique relationship of trehalose to the nutrition of M. tuberculosis might justify the synthesis of trehalose derivatives as possible chemotherapeutic agents.

PUBLICATIONS:

"Interaction of Hydrogen Peroxide with Carbohydrates and Polyhydric Alcohols in the Growth Suppression of Isoniazid-Resistant Mycobacterium Tuberculosis." 1966. Thomas P. O'Barr and Melvin C. Eagle. Accepted for publication in American Review of Respiratory Diseases.

RESEARCH AND TECHNOLOGY RESUME	1	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6355	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (14 04 67)	U RPT U WRK	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 02 064

10b. PRIOR NUMBER CODE: None

11. TITLE: (U) Bio-medical Information Systems Design (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
Digital Computer	04 67	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	0	2
		CURRENT FY 68	0	8

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21. TECHNOLOGY UTILIZATION: Hospitals: Laboratories

22. COORDINATION:

23. KEYWORDS: Digital Computer; Medical Information; Statistics; Patient's Record

24. (U) Technical Objective: To design and develop a bio-medical information system programmed for a digital computer, capable of processing medical records under fully automatic controls. The computer system will process all information requirements for data storage, retrieval, analysis, display and presentation. It will serve to expand the computer effort beyond the provisions of Work Unit 067.

25. (U) Approach: Materials serving as a basis for development of the bio-medical information system will be derived from clinical and laboratory environments at FGH and USAMRNL. This will produce a number of studies under the work unit. The studies will involve individual areas within the hospital and laboratory to provide for unique characteristics of their operations and services. The requirements of the computer systems will be determined through an interdisciplinary team approach with members of the Computer Div., hospital physicians and laboratory investigators. The methods of computer programming data preparation and display required to effect the information processing in support of medical decision making will be researched and developed by the Computer Division.

26. (U) Progress: This project was initiated on 1 March 67, through a cooperative effort with the Dept. of Clinical Research and the Ob-Gyn Service, Fitzsimons General Hospital. The area of research involves the evaluation of long-term use of oral contraceptives. Data collection formats have been designed and are prepared for computer input, via punched paper tape, on a weekly basis. A system for storage, retrieval and presentation of the information has been programmed for the RCA 301 digital computer. At present 500 cases have been entered into the computerized data banks.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	Research in Biomedical Sciences
TASK NO.	02	Internal Medicine
WORK UNIT NO.	064	Bio-medical Information Systems Design

The following investigations have been conducted under this work unit:

STUDY NO. 1 The Evaluation of Long-Term Use of Oral Contraceptives

This effort approaches the problem of developing bio-medical information system programmed for a digital computer, for selected medical, surgical and laboratory activities. It will serve to expand the research effort to automate the soldier's medical record beyond the scope provided by the existing computer project (Work Unit 067) "The Computer Classification of Pulmonary Disability."

Selected medical, surgical and laboratory activities will be utilized as prototype models of definitive bio-medical information systems. Ultimately each of these selected project areas will function as a component of an integrated bio-medical system for servicing the needs of the Army Medical Service.

It is becoming increasingly clear that the volume, scope and complexity of information processing confronting clinical physicians and laboratory investigators is beyond the response capabilities of manual systems. Electronic data processing systems programmed for fully automated functions under digital computer controls are required. Only the electronically based system is capable of maintaining voluminous historical files with adequate response characteristics, providing swift input of new information, processing a complete numerical analysis system, deriving qualtitative estimates on qualitative variables, transmitting and receiving medical and laboratory data from remote stations and exercising fully automatic controls over "user's interrogations."

BODY OF REPORT

WORK UNIT NO. 064 — Bio-Medical Information Systems Design

STUDY NO. 1 — The Evaluation of Long-term Use of Oral Contraceptives

PROBLEM:

There is a need to study and evaluate the effects of long-term use of oral contraceptives and to derive a systematic classification of patient populations through mathematical methods for ordering the data. To meet the data processing requirements, an information system must be designed and programmed for a digital computer. This system must meet all requirements for data storage, retrieval, tabulation, analysis and presentation.

RESULTS AND DISCUSSION OF THE RESULTS:

This project was initiated on 1 March 1967. Today a complete set of data formats has been designed which includes all items of information required to evaluate the stated medical problem. This was accomplished through close coordination between Ob-Gyn Service, Fitzsimons General Hospital and Computer Division, U. S. Army Medical Research and Nutrition Laboratory.

The data formats are completed in the Family Planning and Post-Partem Clinics, Ob-Gyn Service, FGH. They are sent to the data preparation section, Computer Division, U. S. Army Medical Research and Nutrition Laboratory where they are punched to paper tape for computer input. Master files of the patient data have been established. Computer programming for storage, analysis and presentation of the data is being authored.

A mathematical method which attempts to measure similarity of the data will be utilized to order the data. The method of the classification technique will be based upon the data representing the clinical cases and their associated properties or attributes. The data is derived from selections made by the principal investigators. The presence or absence of the attributes is represented by a rectangular table consisting of "Ø's and "1's". This provides a standard representation for the data of the classification problem. The basic principle of the analysis method will be a pair-wise comparison of any two cases or attributes. The process for ordering or classifying the data on a numerical basis will be achieved

through the use of derived similarity coefficients, ramification order and hierarchial power as measures of relatedness of cases and attributes.[1] This is the basic logic being followed in Work Unit No. 067, "Computer Classification of Pulmonary Disability."

CONCLUSIONS:

Completion of the classification process will permit greater ease in handling and thinking about the data for accomplishing objectives in furthering treatment of the data.

The two broad categories represented by this problem include: 1) Signal transmission handling; and 2) Information processing within the digital computer of derived digital output in clinical measurement and concentration units.

[1] Tanimoto, T. and Lomis, R. G., The Application of Computers to Clinical Medical Data. IBM Medical Symposium, June 15-17, 1959.

RESEARCH AND TECHNOLOGY RESUME	1	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6312	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 05 080

10b PRIOR NUMBER CODE: None

11. TITLE: (U) High Altitude Bioenergetics (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT COMPL DATE	15. FUNDING AGENCY
016200 Stress Physiology 005900 Env Biology 013400 Psychology	08 63	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	18. RESOURCES EST	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	a. DATE b. NUMBER NA c. TYPE d. AMOUNT	PRIOR FY 67	2	45
		CURRENT FY 68	1	20

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21. TECHNOLOGY UTILIZATION: Mountaineering; Mining

22. COORDINATION:

23. KEYWORDS: Hypoxia; Stress; Performance decrement; Work; Balance-metabolic; Blood gas analysis; Psychological tests; Spirometry

24. (U) Tech Objective: Locate and quantitate the human performance decrements to be expected in military operations at 10-18,000 feet; to measure the extent of and rates of acclimatization; to investigate the physiology, biochemistry, and pharmacology of the organ systems causing the decrements; and to ascertain how to minimize the decrements by selection, conditioning, previous environmental exposure, nutrition, drugs, or other variables.

25. (U) Approach: A. Measure and compare symptoms; food, nitrogen, mineral, and water balance; rest, mild, and maximal work on the bicycle ergometer with pulse and O_2 consumption in soldiers brought to 11,400 feet for three weeks from sea level or 5200 feet. B. Measure pulmonary, cardiovascular, and metabolic changes during rest, mild, moderate, and exhausting work and recovery in 16 sea level soldiers before, during, and after four weeks at altitude, as affected by physical conditioning and abruptness of ascent; symptoms and psychometrics; food, nitrogen, and water balance and other parameters. C. Measure physiology of other mammals in chamber and altitude environments in multiple experiments. D. Participate in field study with performance measurements of load carrying and grade walking at 14,100 feet.

26. (U) Progress: In addition to two scientific journal publications on performance at high altitude (J. Appl. Physiol. 21:1732, 1966 and Fed. Proc. 25:1380, 1966), Laboratory Reports were completed on "Respiratory Function at Altitude" and "The Caloric, Nitrogen and Water Requirements at Altitude".

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	Environmental Medicine
TASK NO.	05	
WORK UNIT NO.	080	High Altitude Bioenergetics

This work unit is a portion of the Laboratory program investigating the nutritional, biochemical, and physiological consequences of exposing humans to high terrestrial environments. The problems which have been and are still being investigated include: (a) acute mountain sickness, its prevention and treatment; (b) the effects of high altitude exposure upon incidence of pulmonary edema; (c) adaptation to altitude and methods of increasing the rate of adapting; and (d) the effects of high altitude upon protein and water metabolism. Changes in water and nitrogen balance have been observed in acute exposure of humans to altitude but the mechanism of these changes are not known. Further work in this area is required in order to gain insight into these effects. The mechanisms of these changes could be best elucidated through animal experimentation.

BODY OF REPORT

WORK UNIT NO. 080 High Altitude Bioenergetics

PROBLEM:

A decrement in human performance after acute exposure to altitude, which would seriously impair the combat effectiveness of the individual soldier, has been repeatedly demonstrated. Some of the medical problems involved include nausea, vomiting, anorexia, and negative nitrogen and water balances. The effects of hypohydration upon physical performance have been previously demonstrated. Animal experimentation is needed in order to understand some of these altitude effects upon water and nitrogen balance so that we can gain insight into the prevention of these effects upon humans and thereby attempt to alleviate the decrement in performance.

Our objectives are to study nitrogen and water balances in animals before, during, and after exposure to altitude. These studies will include digestion and absorption of proteins and protein synthesis in organs of the animals. Water balance will include intake, excretion, and the water content of the various spaces in the body.

RESULTS AND DISCUSSION OF THE RESULTS:

Exposure to altitudes of 4300 meters resulted in a negative nitrogen balance in protein intakes of 0.8 gm/kg body weight (60 gm/day). Sea level controls, consuming the same quantity of protein, were in positive balance. The negative nitrogen balance at high altitude may be due to decreased protein utilization or decreased protein synthesis.

Large weight losses were observed during the first few days of altitude exposure which could only be explained by negative water balances. Much of this weight loss is rapidly regained upon return to sea level which is consistent with the hypothesis that altitude exposure causes hypohydration.

Data are now being evaluated on the effectiveness of the consumption of a high carbohydrate diet as a means of relieving mountain sickness symptoms.

CONCLUSIONS:

Altitude exposure results in negative water and nitrogen balances in humans and these effects may be related to the decreased

ability to perform physical work at altitude. Preliminary information suggests that protein utilization or synthesis may be impaired.

RECOMMENDATIONS:

Water and protein metabolism during altitude exposure should be investigated further. These studies should be extended to animals so that information upon the mechanisms of the changes can be elucidated and this information can then be applied to human studies.

DPQ 70084

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6336	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 05 66)	U (DEPT) U (WORK)	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 05 081	None

11. TITLE:
(U) Cardiovascular and Pulmonary Responses at High Altitude (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
00590 Environmental Biology 012900 Physiology	05 66	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 67	1	52
		CURRENT FY 68	1	18

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Hannon, J. P. PhD, Carson, R P, CPT
TEL 303 366-5311 X22119 TYPE DA

21. TECHNOLOGY UTILIZATION Medical problems in populations at high altitude

22. COORDINATION
None

23. KEYWORDS
Stress, High Altitude, Adaptation, Cardiovascular System

24. (U) Technical Objective: The purpose of this work unit is to describe the effects of hypoxia, particularly that induced by high altitude exposure, on cardiovascular function. The basic aspects of cardiovascular physiology will be emphasized including the changes in hemodynamics, regional blood flow, blood composition, myocardial function, etc. The relationships and/or influence of the cardiovascular function on other body systems or functions will also be investigated.

25. (U) Approach: These studies will be conducted almost entirely on laboratory animals, including rats, guinea pigs, rabbits and dogs. Occasionally, in comparative studies wild animals, e.g., thirteen-lined squirrels, will be utilized. For the most part, standard physiological and biochemical procedures will be used, depending upon the particular functional aspect which is being investigated. The studies will be conducted under various hypoxic gas mixtures, in environmental test chanbers, or at actual high altitude field sites. The latter sites will be used primarily for long-term, or acclimatization studies.

26. (U) Progress: During the past year the research effort under this work unit was directed toward describing the anatomical, pathological and hematological changes in dogs transported from low to high altitude; alterations in cardiovascular anatomy during altitude acclimatization; neuroendocrine control of cardiovascular function; the role the spleen plays in the hemodynamic response to hypoxia.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY + 1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1121) REPLACES DD FORM 613 & USC WHICH ARE OBSOLETE

ABSTRACT

PROJECT NO.	3A014501B71R	Research in Biomedical Sciences
TASK NO.	05	Environmental Medicine
WORK UNIT NO.	081	Cardiovascular and Pulmonary Responses at High Altitude

During the past year, four studies have been conducted under this work unit:

STUDY NO. 1: Neuroendocrine control of cardiovascular responses of conscious dogs to high altitude.

STUDY NO. 2: The role of the spleen in blood volume regulation at high altitude.

STUDY NO. 3: Effects of prolonged altitude exposure on the anatomy and physiology of the cardiovascular system.

STUDY NO. 4: Sea level control comparisons for studies of anatomy, pathology and hematology of high altitude exposure.

These studies were conducted at Denver, Edgewood, and Pikes Peak and in all instances dogs were utilized as experimental animals. In the hemodynamic studies, catheterized animals and conventional physiological recording systems were employed while in anatomical and pathological studies, the animals were sacrificed for autopsy examination and subsequent study. Due to the departure of the principal investigator from the laboratory, the data from these studies have been only partially analyzed. We know, for example, that the spleen plays a major role in elevating the red cell content of canine blood during acute altitude exposure. Also, certain dogs, i.e., beagles, exhibit a reduced heart size during chronic altitude exposure. In this respect, they differ from most other mammals.

BODY OF REPORT

WORK UNIT NO. 081 — Cardiovascular and Pulmonary Responses at High Altitude

STUDY NO. 1 — Neuroendocrine Control of Cardiovascular Responses of Conscious Dogs to High Altitude

PROBLEM:

Dogs, like humans and other species, respond to high altitude with an acute increase in cardiac output which reaches a maximum value after one to three days' exposure. Thereafter, output declines again, reaching low altitude values after a week or ten days. At the present time, we do not know the cause of this initial increase in cardiac output. It could involve an increase in sympathetic activity to the myocardium and/or a decrease in parasympathetic activity. It could involve a direct action of hypoxia, hypocapnea or other metabolites on the myocardium. Or, it could involve any combination of the foregoing.

RESULTS AND DISCUSSION:

Several groups of dogs were exposed acutely (about a week) to high altitude on Pikes Peak. They were subjected to various treatment procedures including: myocardial denervation; adrenergic and cholinergic blocking agents; and catecholamine injections. Due to the departure of the principal investigator, most of the experimental data remain to be analyzed and reported. Hopefully, this will be accomplished during the coming year. At present, we know that denervation of the myocardium prevents the increase in cardiac output, as do adrenergic blocking agents. Presumably, therefore, the increase in cardiac output is attributable, at least in part, to an increase in sympathetic activity.

RECOMMENDATIONS:

These studies should be further pursued in both dogs and other species. Species differences in response to altitude; the relationship of degree of hypoxia to cardiac output; the cause of the subsequent reversion of the cardiac output to low altitude levels; and measurements of catecholamine metabolism during hypoxia would seem desirable areas for future investigation.

PUBLICATIONS:

1. J. A. Vogel, J. E. Hansen and J. P. Hannon. Hemodynamic alterations in humans and animals during chronic high

altitude exposure. Proceedings of the Ann. Army Res. Conf., West Point, New York, 1966.

2. J. A. Vogel and J. P. Hannon. Cardiovascular and metabolic responses of dogs to exercise at high altitude. J. Applied Physiol., 21: 1959-1601, 1966.

STUDY NO. 2 — The Role of the Spleen in Blood Volume Regulation at High Altitude

PROBLEM:

Certain species, such as the dog, have a relatively large spleen which can serve as a major body store for erythrocytes. Discharge of these erythrocytes during acute high altitude exposure could lead to a rapid increase in both the oxygen carrying capacity as well as the volume of blood in the body. To what extent this source of red cells affects the blood volume of dogs at high altitude and how this compares to similar observations made by others in humans represents an unanswered question.

RESULTS AND DISCUSSION:

Groups of splenectomized and normal dogs were exposed to high altitude on Pikes Peak. The normal dogs exhibited a much more rapid hemoconcentration than the splenectomized dogs. Both groups, however, exhibited a decrease in plasma volume which is probably attributable to an actual plasma loss, not dehydration. Major errors in blood volume measurements utilizing erythrocyte labeling are to be expected in normal dogs exposed to altitude. Generally, the tagged red cells are rapidly, but to varying degrees, taken up by the spleen of altitude-exposed animals. This may lead to the erroneous conclusion that canine blood volume increases markedly at altitude.

RECOMMENDATIONS:

These studies should be extended to other species.

PUBLICATIONS:

1. J. A. Vogel, G. W. Bishop, R. L. Genovese and T. L. Powell. Hematological and blood volume responses of dogs exposed to high altitude. J. Applied Physiol. (Submitted)

STUDY NO. 3 — Effects of Prolonged Altitude Exposure on The Anatomy and Physiology of the Cardiovascular System

PROBLEM:

The primary purpose of this study is to study the anatomical and physiological responses of various breeds of dogs to acute and chronic high altitude exposure and to compare their responses to those observed in other species. In short, is the dog a typical mammal insofar as his responses to high altitude are concerned?

RESULTS AND CONCLUSIONS:

Groups of beagle and mongrel dogs were chronically exposed to high altitude on Pikes Peak. Control groups were maintained in Denver. At the end of chronic exposure (three months) the animals were catheterized and hemodynamic studies were conducted under unanesthetized conditions. The data from these measurements are currently being analyzed and prepared for publication by the principal investigator. In addition, anatomical and pathological studies were conducted on these animals subsequent to their sacrifice and autopsy. One of the most startling findings was a reduction in the heart size of chronically-exposed beagles. This is quite unlike other species, e.g., rats and humans, which exhibit an enlargement of the myocardium following prolonged exposure or residency at high altitude.

CONCLUSION:

Beagles are probably not desirable for high altitude studies, at least those where information pertinent to the understanding of human responses is desired.

STUDY NO. 4 — Sea Level Control Comparisons For Studies of Anatomy, Pathology and Hematology of High Altitude Exposure

PROBLEM:

Animals and humans living in Denver are exposed to an elevation considerably above sea level and this, in many instances, has an ameliorating effect on their subsequent response to high altitude. This study is designed to describe the differences in anatomy, pathology and hematology between Denver and sea level, especially as subsequent exposure to high altitude affects these variables.

Cardiovascular and Pulmonary Responses at High Altitude (Cont'd)

RESULTS AND DISCUSSION:

A group of dogs were studied in detail at Edgewood, Maryland and compared to a similar group living in Denver. The data from this study are still being analyzed and will probably be reported during the coming year.

RECOMMENDATIONS:

The differences between sea level and Denver or other moderate altitudes (up to 10,000 ft.) on the subsequent responses to high altitude should be investigated in various species, including man.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6339	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (13 03 67)	U RPT U WORK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 05 082	61145011 3A014501B71R 05 080

11. TITLE:
(U) Metabolic Effects of Altitude (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
016200 Stress Physiology	07 66	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	a. DATE	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA		PRIOR FY 67	3	60
	c. TYPE	d. AMOUNT	CURRENT FY 68	3	55

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME Headquarters	NAME U.S. Army Med Rsch & Nutr Lab
ADDRESS U. S. Army Med Rsch & Dev Cmd Washington, D. C. 20315	ADDRESS Fitzsimons General Hospital Denver, Colorado 80240
RESP. INDIV. Davis, T. E., MAJ	INVESTIGATORS PRINCIPAL ASSOCIATE Klain, G.J.,Whitten,B.K.,Chinn K.S.,Hannon,J.P.,Shields,J.L.
TEL. 202 OXford 6 5472	TEL. 303 366-5311 X26212 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Environmental Factors Affecting Life and Health	None

23. KEYWORDS Altitude; Adaptation, Physiological; Endocrine; Biochemistry; Stress; Physiology

24. (U) Technical Objective: Research under this work unit will be directed toward describing the various metabolic defects produced either directly or indirectly by hypoxic exposure in laboratory animals. It will also be concerned with describing the pattern and extent of metabolic adaptations associated with hypoxic exposure, both acute and chronic. Attention will be given to the basic physiological and biochemical mechanisms which underlie the defects and adaptations which are observed.

25. (U) Approach: Laboratory animals will be subjected to actual and simulated high altitude environments and various physiological, radioisotope and biochemical measures will be applied to describe the metabolic defects associated with hypoxia. These studies will be largely concerned with the changes in tissue, cellular and intermediary metabolism. Where feasible, these investigations will be conducted in two stages: the first will be concerned with the acute or "stress" response to hypoxia; while the second will be concerned with the nature, rate and extent of adaptation to the defects observed.

26. (U) Progress: Altitude exposure resulted in decreased growth rate, growth efficiency, body fat, muscle protein and extracellular space, while nonmuscle protein and intracellular space were increased. Total body protein and body water, however, were unaltered. Activities of high altitude exposed rats were reduced by 50% as compared to the control rats. Complete water, electrolyte, nitrogen, and nutrient balances and carcass analyses are still in progress.

Publications: Comp. Biochem. & Physiol. 20:275, 1967; USAMRNL Report 307, 1967, USAMRNL Lab Report 305, 1967.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY + 1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	Research in Biomedical Sciences
TASK NO.	05	Environmental Medicine
WORK UNIT NO.	082	Metabolic Effects of Altitude

The following investigations have been conducted under this work unit:

STUDY NO. 1: Effects of Diets and Altitudes on Growth and Food Intake in Rats

STUDY NO. 2: Effects of Diet and Altitude on the Body Compartments of Rats

Two studies were conducted to examine the effects of diet and altitude on growth, voluntary food intake, and alterations in body composition of growing rats. Altitude exposed animals were kept at Climax, Colorado (altitude 11,400 ft.) and Pikes Peak, Colorado (altitude 14,110 ft.) while the control animals were kept at Denver, Colorado (altitude 5,280 ft.). The animals were fed either high-carbohydrate, high-fat or high-protein diets ad libitum for 21-24 days. Daily measurements included body weight gain, food consumption, water intake, and urine output. Growth rate and efficiency of food utilization were calculated for all rats. Animals exposed to an altitude of 11,400 feet were sacrificed for body composition analysis at the end of the study.

Animals exposed to both altitudes (11,400 ft. and 14,110 ft.) had depressed growth rate and reduction in efficiency of food utilization. Furthermore, body fat accumulation was significantly less in comparison with the control rats. The fat-free mass composition of the rats exposed to high altitude was unaltered; however, there was a significant decrease in the amount of muscle protein with a corresponding increase in nonmuscle protein. Moreover, there was a significant shift between intracellular space and extracellular space of rats exposed to both altitudes.

BODY OF REPORT

WORK UNIT NO. 082 — Metabolic Effects of Altitude

STUDY NO. 1 — Effects of Diets and Altitudes on Growth and Food Intake in Rats

PROBLEM:

Observations of men on high altitude expeditions indicate that, in general, body weight loss occurs, appetite is depressed and dietary preference for carbohydrate is increased. Similarly, altitudes above 12,000 feet have been shown to reduce the growth rate and growth efficiency of laboratory rats. On the basis of the above observations, it seems reasonable to postulate that the composition and amount of diet consumed at high altitude is an important factor in growth and maintenance. Accordingly, the effects of high-carbohydrate, high-fat and high-protein diets on growth and voluntary food consumption at moderate altitude (11,400 ft.) and high altitude (14,110 ft.) were studied in young growing rats.

Male Holtzman albino rats were exposed to an altitude of 11,400 feet and 14,110 feet while the corresponding controls were kept in Denver (altitude 5,280 ft.). They were divided into three groups and housed in individual metabolic cages with food and water ad libitum. The animals in a given group were fed either a high-fat, high-carbohydrate or high-protein diet. Daily weight and food consumption were recorded. At the end of 21-24 days, growth rate and efficiency of food utilization were calculated for each animal.

RESULTS AND DISCUSSION:

Regardless of dietary treatments, growth was slightly depressed at 11,400 feet and markedly ($P<0.001$) depressed at 14,110 feet. Rats appeared to grow better at both altitudes when fed a high-fat diet. The type of diet, however, is not a major factor affecting growth at altitude. Food consumption was significantly ($P<0.001$) depressed at 11,400 feet but no differences in food utilization were observed. On the other hand, food consumption was decreased ($P<0.05$) at 14,110 feet in rats fed a high-protein or high-carbohydrate diet but not in rats fed a high-fat diet. However, food utilization was significantly ($P<0.001$) decreased. It appears likely the depressed growth at high altitude can be traced to metabolic derangement resulting from the combined stress of diet and altitude.

STUDY NO. 2 Effects of Diet and Altitude on the Body Compartments of Rats

PROBLEM:

It has been suggested that when a specific stress is placed upon an animal, the lean body composition changes in accordance to the stress. It is reasonable, therefore, to presume the combined stress of diet and altitude not only triggered the responses of the animal but also altered the proportions of body fat, water and protein that accompany growth. Accordingly, the present investigation was undertaken to examine two parameters which affect the body composition of growing rats.

Forty-eight growing Holtzman rats were divided into three groups of 16 rats each and were fed a high-carbohydrate, high-fat, or high-protein diet for one week at Denver (altitude 5,280 ft.). After one week of preliminary control, one-half of each dietary group was transported to Climax, Colorado (altitude 11,400 ft.) and kept there for 24 days. They were housed in individual metabolic cages and offered food and water ad libitum. Daily body weight, food intake, water intake and urine output were recorded. At the end of the study, all rats were sacrificed for analysis. Analysis included total body fat, water, protein, mineral, potassium, chloride, urinary creatinine excretion, and the partition of total body water into intracellular and extracellular spaces and total body protein into muscle and nonmuscle protein.

RESULTS AND DISCUSSION:

The data indicate that growing rats exposed to a high altitude accumulated significantly ($P<0.001$) less body fat in comparison to the control rats. Rats fed a high-fat diet have considerably greater fat load than rats fed a high-carbohydrate or high-protein diet. No changes in the fat-free composition were observed between the two altitudes nor among the three dietary groups. Muscle protein decreased significantly at 11,400 feet altitude with a corresponding increase in nonmuscle protein. The effect of diet on the amount of muscle protein at both altitudes, listed in order of the diets are: protein > carbohydrate > fat. Although total body water was not affected by altitude exposure, a significant shift between the intra- and extracellular space was increased with a corresponding decrease in the percentage of extracellular space.

SUMMARY AND CONCLUSIONS:

Exposure of animals to altitude above 11,000 feet results in depressed rate and efficiency of food utilization. The role of these changes in the adaptation of animals to the stress of altitude is likely due to decreased voluntary food intake, disturbances in nutrient digestion and intestinal absorption. In view of the fact that normal hydration (73%) of the fat-free mass exists in humans and animals exposed to high altitude, the increased weight loss in humans and animals cannot be due to high pulmonary water loss and/or polyuria.

Marked decreases in total body fat and muscle protein indicate that these two body components are utilized for energy purposes during the process of adaptation to the stress of high altitude.

The significant increase in intracellular space and the corresponding decrease in extracellular space of human and animals are highly altitude dependent. Significant correlations between altitude and intracellular space ($r = 0.927$) and extracellular space ($r = -0.927$) were obtained in these studies.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6340	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / MRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (13 03 67)	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 05 083

10b. PRIOR NUMBER/CODE: 61145011 3A014501B71R 080

11. TITLE: (U) Physiological and Psychological Aspects of Performance at Altitude (06)

12. SCIENTIFIC OR TECH. AREA: 013400 Psychology; 016200 Stress Physiology

13. START DATE: 07 66

14. CRIT. COMPL. DATE: NA

15. FUNDING AGENCY: OTHER | DA

16. PROCURE. METHOD: C. In-House

17. CONTRACT/GRANT — a. DATE; b. NUMBER: NA; c. TYPE; d. AMOUNT

18. RESOURCES EST.

	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 67	1	30
CURRENT FY 68	1	25

19. GOV'T LAB/INSTALLATION/ACTIVITY
NAME: Headquarters
ADDRESS: U.S. Army Med Rsch & Dev Cmd
Washington, D. C. 20315
RESP. INDIV: Davis, T. E., MAJ
TEL: 202 OXford 6 5472

20. PERFORMING ORGANIZATION
NAME: U.S. Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240
INVESTIGATORS PRINCIPAL ASSOCIATE: Evans, W. O., MAJ; Carson, R. P., CPT
TEL: 303 366-5311 X22119 TYPE: DA

21. TECHNOLOGY UTILIZATION: Effects of environment on human performance

22. COORDINATION: None

23. KEYWORDS: Altitude; Physiology; Stress; Human Factors; Drug Effects; Physiological and Psychological Correlates; Performance Decrement

24. (U) Technical Objective: Research on this work unit will be directed toward a description of the deterioration of different types of human capacities to perform psychomotor, physical and mental tasks which are produced by a rapid transition from low altitudes to those ranging between 10,000 to 18,000 feet. In particular, attention will be directed to various types of cognitive functioning and considerations of small group performance.

25. (U) Approach: The performance capacities of subjects on tasks of memory, concept formation, and motivation will be studied utilizing the theoretic paradigms of experimental psychology. An attempt will be made not only to determine the crude estimates of overall decrement in these areas, but rather a fine-grain analysis of the behaviors. In addition to a study of the cognitive functions of the single individual, preliminary work will be instigated on the performance capacity, solidarity, and communications effectiveness of small groups. Initial studies in this area will utilize the Baylis interaction category processes as a scoring implement.

26. (U) Progress: Since the initiation of this program the grosser aspects of physical work performance, psychomotor and mental tasks have been studied in human subjects. It has been found that the decrements in the different types of activities vary as a function of the specific type of task, the rate at which a subject progresses to high altitude, the chemotherapeutic treatment of the subject and the length of time of exposure to the high altitude environment. In addition, initial cross correlation with physiologic data has demonstrated that the different types of performance decrement can be found to be associated with different physiologic subsystems of the organism. It is upon this information concerning the response of individuals to high altitude that finer-grained analyses and the analysis of group behavior will be initiated.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST FUNDS (In thousands)	36.
CPY 1	

DD FORM 1 AUG 64 1498 — Items 1 to 28 identical to NASA Form 11287. NSPL AEGIS DD FORMS 613 & 613C UNION ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	Research in Biomedical Sciences
TASK NO.	05	Environmental Medicine
WORK UNIT NO.	083	Physiological and Psychological Aspects of Performance at Altitude

During the last fiscal year, research studies were carried out to investigate the effects of high altitude and simulated high altitude environments on basic psychological functioning. Types of tests that were included to survey the potential psychological decrements associated with high altitude were: psychomotor activities, intellectual functioning, physical work performance and subjective symptomatology. Descriptions of the decrements in performance associated with 2, 11, 14 and 15,000 feet were described. In general, the findings noted that different types of psychologic and behavioral performance were differentially affected by the various high altitude environments.

BODY OF REPORT

WORK UNIT 083 — Physiological and Psychological Aspects of Performance at Altitude

STUDY NO. 1

PROBLEM:

A study was conducted in a simulated high altitude of 2, 11, and 15,000 feet in a high altitude chamber. In this study, the subjective symptomatology of the subjects was noted at the different altitudes. The study was conducted on a double-blind basis. As a result of this study, it was found that adjectives, which the subject can use to describe their own symptomatology can demonstrate the quantitative relationship to the altitude of exposure and to the period of exposure to that altitude. In addition, during this study, intellectual functioning was measured using the Digit Symbol Substitution Test of Wechsler. It was found that decrements in the performance of this test could be noted. This would suggest that quantitative evidence for a failure of some types of intellectual functioning can be found at high altitude.

STUDY NO. 2

PROBLEM:

In this study, troops were taken from a sea level site in one day to the top of Pikes Peak, Colorado, an altitude of 14,110 feet. Again their subjective symptomatology was measured using a self-report adjective check list. Once again this test demonstrated that subjects can accurately quantify their own subjective symptomatology. Intellectual functioning was studied using a series of tests drawn from the Educational Testing Service. These groups of tests are supposed to represent relatively noncorrelated aspects of intellectual functioning. Although practice effects were not controlled in this study, which would tend to eliminate any decrements in performance, still tests of rote memory were found to be significantly decremented at high altitude. Trends also indicated that a study which was properly controlled for practice effects might show decrements in the areas of originality and creativity and logical reasoning. In this study, physical fitness was measured using the Fleishman battery of tests which are designed to represent different aspects of physical performance capacity. In this series of tests, the only type of performance which tended to be decremented by the high altitude were those which utilized explosive bursts of energy. Finally, a specific hypothesis in regard to the adequacy of brain functioning was tested using the Continuous Performance Test of Rosvold and the Digit Symbol Substitution Test of

Wechsler. Previous evidence from drug studies has shown that these tests are differentially affected by different types of drugs. The Continuous Performance Test is most affected by drugs which cause a simple decline in alertness; whereas, the Digit Symbol Substitution Test is most affected by intoxicating substances. These tests are also associated with specific changes in electroencephalographic functioning: the Continuous Performance Test decrements are associated with slow-wave activity; whereas, the greatest decrement in the Digit Symbol Substitution Test is associated with spindle-burst activity. Performance on these tests was measured both at sea level and at the mountain site in conjunction with electroencephalographic recordings. On the behavioral side, it was noted that the Digit Symbol Substitution Test was markedly affected by high altitude; whereas, the Continuous Performance Test of Rosvold was not so affected. The electroencephalographic recordings which were taken concurrent to this testing performance are still being analyzed. As soon as the analysis of these tests is completed, the brain wave patterns will be correlated to the degree of performance decrements on these tests that were induced by the high altitude.

RESULTS AND CONCLUSIONS:

In general, the information accrued during the last field work indicates that many subtle factors of the behavioral performance of the subject change at high altitudes. We now know that we will not be able to generalize across different types of physical performance, psychomotor performance, or different types of intellectual tasks. Changes produced by high altitude have selective effects on certain types of behavior. These data are going to require that future studies perform a much finer grain analysis, looking for a specific subfactor affected by high altitude. In addition, future studies will require a counterbalance of the testing in order to overcome the practiced effects. In addition, the information that specific functions are selectively affected by high altitude leads to the speculation as to what would happen to group performance. The individual changes of functioning and previous studies on the effects of groups from a stressor, could lead to the prediction that under conditions of high altitude, group solidarity would be weakened. Future tests in this area will have to analyze the effect of the individual decrements as they are related to group structure and performance. Even

though individual decrements up to 35% within the individual have been shown as low as 14,000 feet, the group variables may be far more sensitive to functions since they require the conjoined activities of a number of individuals which are decremented in some aspects of their activity.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION: DA OA 6342	REPORT CONTROL SYMBOL: CSCRD 103
4. DATE OF RESUME: 01 07 67	5. KIND OF RESUME: B. Terminated (01 07 66)	6. SECURITY: U RPT / U WRK	7. REGRADING: NA	8. RELEASE LIMITATION: NL	9. LEVEL OF RESUME: A.WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 05 084

10b. PRIOR NUMBER CODE: None

11. TITLE:
(U) Microbial Flora of Human Subjects: Possible Effects of Altitude and/or Drugs (06)

12. SCIENTIFIC OR TECH. AREA: 005900 Environmental Biology; 910100 Microbiology 016200 Stress Physiology

13. START DATE: 07 66

14. CRIT. COMPL. DATE: NA

15. FUNDING AGENCY: OTHER | DA

16. PROCURE. METHOD: C.In-House

17. CONTRACT/GRANT: a. DATE; b. NUMBER: NA; c. TYPE; d. AMOUNT

18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 67	0	10
CURRENT FY 68	0	0

19. GOV'T LAB/INSTALLATION/ACTIVITY
NAME
ADDRESS: Headquarters
U.S. Army Med Rsch & Dev Cmd
Washington, D. C. 20315
RESP. INDIV.
TEL: Davis, T. E., MAJ
202 OXford 6 5472

20. PERFORMING ORGANIZATION
NAME
ADDRESS: U.S. Army Med Rsch & Nutr Lab
Fitzsimons General Hospital
Denver, Colo. 80240
INVESTIGATORS PRINCIPAL ASSOCIATE: Morse, W. C., COL, MSC
Weiser, O. L.
TEL 303 366-5311 X25223 TYPE DA

21. TECHNOLOGY UTILIZATION: Mining, Agriculture, Aeronautics

22. COORDINATION: None

23. KEYWORDS: Altitude; Blood Cell Count; Microbiology; Skin, Feces; Temperature; Humidity

24. (U) Tech Objective: Man's physiological and psychological reaction to a defined environment and altitude has been the subject of numerous studies. The effect of altitude on the indigenous microbiota of man, however, has not been investigated in depth. In this study, three parameters interacting with man and his indigenous microflora were investigated: (1) Environment, which included altitude, temperature, relative humidity and intensity of light; (2) drugs used to suppress or reduce symptoms of altitude
25. exposure,such as carbonic anhydrase inhibitors; (3) idiopathic or altitude-induced ilness distinguished from infectious diseases.
25. (U) Approach: To study the effect of environment and/or specific drugs on the indigenous microflora, the following approach was used: Skin cultures for isolation of Micrococci, yeasts and general aerobic flora; throat cultures for normal flora, Mycoplasma and viruses; stool cultures to detect any overt changes in the normal flora and venous blood for drug level determinations, hematology, and electrophoretic patterns to determine evidence of significant antibody changes. All of the previously listed samples were monitored for evidence of infectious disease agents. Subjects were selec-
26. ted from volunteers at low altitude training centers where baselines were established. These subjects were then exposed to high altitudes and changes from baselines studied and evaluated. Subjects indigenous to high altitudes will be studied and compared to comparable economic and vocational groups at various altitudes.
26. (U) Progress: None - Terminate and transfer to 62156011 3A025601A827 00 074 Microbiological Flora of Human Subjects: Possible Effects of Altitude and/or Drugs.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A014501B71R	RESEARCH IN BIOMEDICAL SCIENCES ENVIRONMENTAL MEDICINE
TASK NO.	05	
WORK UNIT NO.	084	Microbial Flora of Human Subjects: Possible Effects of Altitude and/or Drugs

The study was activated to gain information on man's physiological and psychological reaction to a defined environment and was conducted by the Physiology Division of USAMRNL. A concurrent study by the Microbiology Division was conducted to gain information on the effect of environment on the indigenous microbiota of man. This study was transferred to Project No. 3A025601A827 00 074 and is being reported under this number.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6356	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 03 67)	U RPT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 05 085

10b PRIOR NUMBER/CODE: None

11. TITLE:
(U) Effects of Altitude on Myocardium of Animals (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
005900 Environmental Biology 016200 stress physio.	04 67	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 67	0	15
		CURRENT FY 68	1	11

19. GOV'T LAB/INSTALLATION/ACTIVITY
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Dean, W. D., CPT, VC
TEL 303 366-5311 X26122 TYPE DA

21. TECHNOLOGY UTILIZATION
Medical and Paramedical Sciences

22. COORDINATION
None

23. KEYWORDS Altitude; Animal Kingdom; Vertebrates; dog; rat; rabbit; Cardiovascular system; Biological Sciences;Veterinary Pathology; Physiological Functions

24. (U) Technical Objective: To attempt to further elucidate heart size changes and the causes of their changes, describe their histological and chemical nature and attempt to determine whether these changes can be considered pathlogical or merely desirable physiological adaptive processes.

25. (U) Approach: Validate our past data on dogs and compare them with species known to develop cardiac hypertrophy. Describe the effect of increasing degrees of altitude on this phenomena and determine, if possible, the critical altitude for its development. Determine the time required for development of this condition at several altitudes. Determine the specific morphologic changes taking place in the muscle fibers during altitude exposure by routine histology as well as electron microscopy. Relate histological changes to chemical composition changes in the myocardium during altitude exposure.

26. (U) Progress: All of the animals are on location at 5,280, 11,000 and 14,100 feet. Initial radiographic and hematologic examinations have been performed.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.:	3A014501B71R	Research in Biomedical Science
TASK NO.:	05	Environmental Medicine
WORK UNIT NO.:	085	Effects of Altitude on Myocardium of Animals

STUDY NO. 1: The Effect of Altitude, Exposure, Time and Species on the Development of Hypoxic Cardiac Hypertrophy

PURPOSE:

Right heart enlargement as the result of exposure to reduced atmospheric pressure has been demonstrated in man and several animal species. The most popular theory to account for its occurrence is that it is a compensation for the increase in pulmonary vascular resistance usually observed at high altitude. Pulmonary arterioles undergo vasoconstriction due to change in vasomotor tone and/or anatomical changes of the medial layer of the vessel wall. The right ventricle must therefore pump against greater resistance which results in an increase in the force per unit cross-sectional area of the muscle. The heart then attempts to return the force per unit to normal by increasing the muscle area. This process is evidenced by myocardial hypertrophy.

Work at this laboratory during the summer of 1966 showed that dogs taken from sea level to 14,110 feet altitude did not show cardiac enlargement. In fact they showed somewhat smaller hearts, despite a recorded increase in pulmonary artery pressure. Only one other report exists on the dog showing the absence of hypertrophy after intermittent exposure to 18,000 feet. It is therefore apparent that species differences do exist in respect to hypoxia induced cardiac hypertrophy. Whether the dog possesses a cardiac reserve for teleological reasons, and this is related to the absence of hypertrophy, cannot be concluded at this time. It is known, however, that the dog is quite resistant to work hypertrophy as well as to right heart failure when compared to many other species.

The general objective of this study is to attempt to further elucidate the cause of any heart size changes, describe their histological and chemical nature, and attempt to determine whether these changes can be considered pathological or merely desirable physiological adaptive processes.

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

Specific objectives are as follows: (1) Validate our past data on dogs and compare them with species known to develop cardiac hypertrophy (2) Describe the effect of increasing degrees of altitude on this phenomena and determine, if possible, the critical altitude for its development (3) Determine the time required for development of this condition at several altitudes (4) Determine the specific morphologic changes taking place in the muscle fibers during exposure (5) Relate histologically changes to chemical compositional changes in the myocardium during altitude exposure.

METHODS BY WHICH ACCOMPLISHED:

1. Three animal species will be utilized in the study. The dog will be closely observed to confirm or disprove findings in this laboratory during the summer of 1966. The cardiac changes will be studied chemically and histologically in an attempt to explain them.

The rat will also be studied. That cardiac hypertrophy occurs with altitude exposure in this species is well documented. These animals will be used as a "positive control" for comparison. Also chemical and histologic changes in the myocardium will be observed.

Rabbits will be the third species utilized. Very little work has been done with the rabbit, although some studies have been conducted which indicate a cardiac hypertrophy. The rabbit will be compared to the dog and rat and chemical and histologic studies will be conducted to attempt to answer how the cardiac muscle responds.

2. Four altitudes will be utilized in the study. An equal number of animals will be located at sea level, 5,280 feet, 11,000 feet and 14,110 feet.

3. The primary system observed in the study will be the cardiovascular system. In studying the heart, the total ventricular weight will be closely observed as will the right ventricular to total heart ratio, left ventricular to total heart ratio and septum to total heart ratio. Also, the right ventricular to total body weight, left ventricular to total body weight, septum to total body weight and total heart to total body weight will be observed.

At intervals throughout the study, the mass of the heart of the dogs will be approximated by dorso-ventral radiographs and ECG. Also, hematologic studies will be conducted on the dogs and possibly the other species.

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

At termination of each subject the heart will be studied histologically by light microscopy and electron microscopy, to determine morphologic alterations. Also a complete necropsy will be performed with special emphasis on thoracic organs.

Pulmonary artery pressures will be taken in each species at the end of the exposure period by catheterization of the pulmonary artery.

Chemical analysis of the heart will also be studied. Water, protein and lipid composition of the myocardium will be determined.

SUMMARY OF RESULTS:

To date (1 June 1967) all the animals are located at sea level, 5,280, 11,000 and 14,110 feet. Initial radiographic, electrocardiographic and hematologic examinations have been performed.

BODY OF REPORT

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

STUDY NO. 1 — The Effect of Altitude, Exposure, Time and Species on the Development of Hypoxic Cardiac Hypertrophy

PROBLEM:

1. Right heart enlargement as the result of exposure to reduced atmospheric pressure has been demonstrated in man and several animal species. The most popular theory to account for its occurrence is that it is a compensation for the increase in pulmonary vascular resistance usually observed at high altitude. Pulmonary arterioles undergo vasoconstriction due to change in vasomotor tone and/or anatomical changes of the medial layer of the vessel wall. The right ventricle must therefore pump against greater resistance (an increased pressure head) which results in an increase in the force per unit cross-sectional area of the muscle. The heart than attempts to return the force per unit to normal by increasing the muscle area as evidenced by the myocardial hypertrophy.

2. Work at this laboratory during the summer of 1966 showed that dogs taken from sea level to 14,100 foot altitude did not show cardiac enlargement, in fact, showed somewhat smaller hearts, despite an increase in pulmonary artery pressure. Only one other report exists on the dog, this also showing the absence of hypertrophy after intermittent exposure to 18,000 feet. It is therefore apparent that species differences do exist in respect to hypoxia induced cardiac hypertrophy. Whether the dog possesses a cardiac reserve for teleological reasons, and this is related to the absence of hypertrophy, cannot be concluded at this time. It is known, however, that the dog is quite resistant to work hypertrophy as well as right heart failure as compared to many other species.

3. In species which readily develop cardiac enlargement, little is known about the nature of this change. It has been suggested that it is related to an actual fiber thickening but adequate data in this regard is lacking. Adequate information on water or protein changes also does not exist.

4. It appears worthwhile to continue this area of research in order to determine whether these anatomical changes are truly a desirable

adaptative mechanism to hypoxemia or whether it possibly could be, in some species at least, a deleterious pathologic consequence. A possibly atrophy associated with reduced stroke volume in dogs indicates that this problem has not yet been answered.

5. The general objective of this study is to attempt to further elucidate the cause of any heart size changes, describe their histological and chemical nature, and attempt to determine whether these changes can be considered pathological or merely desirable physiological adaptative processes.

RESULTS AND DISCUSSION OF RESULTS:

Study not completed.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6326	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	C.Terminated(30 06 66)	U NPT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER CODE
61130011 3A013001A91C 00 041	None

11. TITLE:
(U) Symbiosis and Intestinal Flora in Nutrition (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15 FUNDING AGENCY
002300 Biochemistry; 002600 Biology; 010100 Microbiology	01 64	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18 RESOURCES EST.	# PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C.In-House	b. NUMBER NA	PRIOR FY 67	2	36
		CURRENT FY 68	0	0

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME	NAME
ADDRESS U.S. Army Med Rsch & Nutr Lab Fitzsimons General Hospital Denver, Colorado 80240	ADDRESS U.S. Army Med Rsch & Nutr Lab Fitzsimons General Hospital Denver, Colorado 80240
RESP. INDIV. TEL. Syner, J. C., COL 303 366-5311 X21108	INVESTIGATORS PRINCIPAL ASSOCIATE Raica, N., Jr. Baker, E.M., LTC TEL 303 366-5311 x24214 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Medicine (infections and malabsorption); nutrition	None

23. KEYWORDS Symbiosis; intestinal microorganisms; intestinal diseases; germfree life; laboratory animals

24. (U) Technical Objective: Develop germfree animal facilities and techniques and apply such to various nutrition studies and related investigations.

25. (U) Approach: The necessary equipment will be obtained and installed. Consultation will be made with institutions possessing germfree animal facilities for information pertaining to equipment, use of equipment and techniques. Upon development of satisfactory techniques, germfree animal studies will be initiated. The studies will be related to nutritional and medical problems where the intestinal flora may be involved or interrelated.

26. (U) Progress: Germfree (GF) facilities and techniques have been established. GF rats have a higher riboflavin requirement for growth but are more resistant to riboflavin deficiency. GF rats, with or without antibiotics, develop thiamine deficiency at the same rate as conventional rats, but degenerate at a faster rate. Changes in intestinal microflora occur in conventional rats when placed on various vitamin-deficient diets. Conventional rats were observed not to digest cellulose when ^{14}C-labeled cotton (Avicel form) was fed. Further studies under this title will be incorporated and continued under the work unit entitled, "Nutritional and Metabolic Aspects of Nutrients."

Publication: Riboflavin Requirements of Germ-free and Pathogen-free Rats. Bact. Proc. 68, 1967.

27. COMMUNICATIONS SECURITY	28	29 OSD CODE	30 BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32 PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34 SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A013001A91C In-House Laboratory Independent Research

TASK NO. 01

WORK UNIT NO. 041 Symbiosis and Intestinal Flora in Nutrition

The following investigations have been conducted under this work unit:

STUDY NO. 1 Cellulose digestion in rats

STUDY NO. 2 Conversion of tryptophan to indoleacetic acid in gnotobiotic rats

STUDY NO. 3 Riboflavin nutrition in the germfree rat

STUDY NO. 4 Effect of antibiotics on thiamine nutrition in the germfree rat

1. Although it is generally assumed that the nonruminant mammal cannot digest and utilize cellulose, a number of reports have presented data to suggest that 20% or more of ingested cellulose is digested by rats and humans. To test this problem, pure cotton cellulose labeled with carbon-14 was intubated into conventional rats. Over 98% of the administered dose was recovered in the feces. No carbon-14 was detected in the urine, organs, or expired CO_2. After 3 or 5 days, only traces were recovered from the lower GI tract. The rats used in this study were tail-cupped to prevent coprophagy and to facilitate the collection of urine uncontaminated with feces. These data support the conclusions that the rat cannot digest and utilize pure cellulose. Previous experiments that indicated that there was digestion of plant leaf or wood cellulose preparations could be explained as being the result of non-cellulose contaminants which were degradable by cecal microflora.

2. The urinary excretion of indoleacetic acid by the rat has been attributed to microbial conversion of tryptophan. In vivo studies have shown that increased levels of indoleacetic acid are excreted by vitamin B_6-deficient rats following a tryptophan load. In vitro studies with rat liver preparations have also demonstrated the production of indoleacetic acid from tryptophan. In order to establish the role of intestinal microflora in the tryptophan/indoleacetic acid pathway, germfree rats were depleted of vitamin B_6 and then injected intraperitoneally with D- or L-tryptophan-methylene-^{14}C plus D- or L-tryptophan, respectively. Expired CO_2 was monitored for 24 hours and urine collected for the same period. There was increased urinary excretion of carbon-14 in vitamin B_6 deficiency with either D- or L-tryptophan. However, there was less

$^{14}CO_2$ expired in deficiency with L-tryptophan, but no change in deficiency when D-tryptophan was administered. Indoleacetic acid excretion has not been determined as of this reporting period.

3. The riboflavin requirement of germfree (GF) and pathogen-free (PF) rats was determined. Concurrently, the effect of diets with and without riboflavin on the fecal microflora of the PF rats was investigated. Male GF and male PF rats were fed a 20% casein riboflavin-deficient diet until weights became constant. Not exceeding 2 weeks at this constant weight, GF and PF rats, still maintained on the riboflavin-deficient diet, were repleted daily intraperitoneally with 10, 30 and 60 µg of riboflavin for a period of 8 weeks. The riboflavin requirement for the GF rat under these experimental conditions was determined to be more than 10 but less than 30 µg. The 10 µg of riboflavin apparently was sufficient as a minimal requirement for the PF rat approaching adulthood. PF rats receiving 30 or 60 µg of riboflavin injections gained more weight on the riboflavin-deficient diet than the control PF rats on the supplemented 20% casein diet. The microflora of the PF fecal specimens, prior to the riboflavin depletion period, consisted of a mixed population of Escherichia, Aerobacter and Streptococcus species. Change in the microflora genera occurred very rapidly following the consumption of the riboflavin-deficient diet, so that just before intraperitoneal injections of riboflavin were initiated, Escherichia coli was the only predominant genus isolated. On riboflavin repletion of the host, the PF fecal microflora population reverted to the original microflora genera. The riboflavin-deficient diet had an effect on the fecal microflora of the PF rat, as well as on the host itself. Due to this microbial presence, the PF rats as weanlings depleted faster and their weight gain per day was much less than that of the GF rats.

4. To study thiamine (vitamin B_1) nutrition of the germfree (GF) and the pathogen-free (PF) rats, penicillin or succinyl sulfathiazole was added to a 20% casein basal diet with and without vitamin B_1. The animals were fed these diets until a vitamin B_1 deficiency developed, as observed in a dramatically sudden loss of weight and characteristic vitamin B_1 deficiency symptoms. At this point the animals were sacrificed. Red blood cells (RBC) were collected and assayed for transketolase activity and the metabolism of 1-^{14}C-glucose by GF and PF rodent RBCs was measured. Tissues were fixed for light microscopy. PF rat fecal specimens were examined during the course of the depletion period to observe the effect of the antibiotics on the intestinal microflora. GF rats, with or without antibiotics, developed thiamine deficiency at the same rate as the PF rats, but degenerated at a faster rate.

BODY OF REPORT

WORK UNIT NO. 041 Symbiosis and Intestinal Flora in Nutrition

STUDY NO. 1 Cellulose digestion in rats

PROBLEM:

In general, cellulose is not considered to be digested by the nonruminant mammal. However, the literature contains data to suggest that while cellulose may not be nutritionally available, it is altered or degraded during passage through the GI tract so that it is not assayed as cellulose.

Previous studies from this laboratory with rats (R. B. Johnson, D. A. Peterson and B. M. Tolbert. J. Nutrition, 72: 353, 1960) fed tobacco or soybean cellulose labeled with carbon-14 showed that about 20% of the carbon-14 was recovered in the expired CO_2. Human studies employing microcrystalline cellulose (Avicel, American Viscose Corp.) demonstrated a comparable loss of ingested cellulose when assayed in the feces by the method of Crampton and Maynard (Canham et al. Fed. Proc., 24: 314, 1965). Similar data were also obtained by E. Evrard et al. (Brit. J. Exp. Pathol., 45: 409, 1964) in conventional rats when feces were assayed by the method of Kurschner and Hanak. No losses of ingested cellulose were found in germfree rats.

Because the existing data on cellulose digestion and availability are not definitive, and because of the possible contamination of plant structural cellulose preparations with non-cellulose matter, it was decided to utilize uniformly labeled cotton cellulose-^{14}C to explore this problem further.

RESULTS AND DISCUSSION OF THE RESULTS:

Cotton plants were raised to boll set at which time they were injected with glucose-^{14}C. The labeled cotton was harvested, defatted and converted to colloidal microcrystalline cellulose (Avicel-R) by American Viscose Corp. The activity of the wet cake (60% moisture) was 5 μc/gram. Particle size was less than 10 microns. An aqueous colloidal suspension was prepared for intubation. Activity of this suspension was 1.3 μc/ml.

Two Charles-River CDF young adult females and one Holtzman mature female were used. Diet fed was a casein-starch laboratory ration, which contained 20% cotton-derived Avicel-R. This diet was fed for 4-6 weeks prior to testing with the labeled cellulose preparation. Animals were fasted for 24 hours prior to intubation. Administered dose was 6.24 μc for the young adult rats and 9.75 μc for the mature rat.

The fasted rats were tail-cupped, intubated with cellulose-^{14}C and then placed in a glass metabolism-respiration chamber. Expired CO_2 was continuously monitored with a recording Cary vibrating reed electrometer. Feces and urine were collected every 24-hour period. After 3-5 days, the animals were sacrificed, organs and GI tract removed. Feces, organs and GI tract were lyophilized and digested by wet oxidation. The evolved CO_2 was trapped and counted in a liquid scintillation counter. Urine was counted directly.

The pooled data for the three rats are shown in Table I. There was no detectable carbon-14 in the expired CO_2, urine, or organs. Traces of activity were found in the lower gut, primarily in the cecum and its contents as well as in the colon. Most of the administered dose was recovered in the feces during the first 24 hours. Recovery of carbon-14 was in excess of 98%. (Individual recoveries were 91%, 108% and 95%.)

TABLE I

Pooled Carbon-14 Excretion

% of Dose[1]

24-Hour Period	Feces	CO_2	Urine	Organs	GI Tract
1	74.3	0	0	-	-
2	20.9	0	0	-	-
3	1.4	0	0	0	Trace
4 and 5	1.4	0	0	0	Trace

[1]Total μc administered for 3 rats: 22.23 μc of cotton cellulose-^{14}C (Avicel-R).

These data suggest that the conventional rat does not digest and utilize cellulose when coprophagy is prevented. The reasons for the seeming disagreement with previously reported data are several. The primary reason may be in the purity of the cellulose used in this study. Plant leaf-derived cellulose is very difficult to purify and commercial preparations of cellulose fibers may contain non-cellulose contaminants. Both of these cellulose preparations may contain fractions which may be degraded by the cecal microflora. This is made evident from the data of Johnson et al. in which no utilization was found in

cecectomized rats, and from the data of Evrard et al. in which no apparent loss of ingested cellulose was found in germfree animals. Coprophagy was not prevented in these cited studies. The possibility that continuously recirculated cecal degraded "cellulose" can be eventually utilized must be considered. However, even if cecal microflora could eventually degrade pure cellulose to cellobiose, the intestinal cellobiose activity is very low and it would seem very unlikely that 20% or more of the cellulose could be utilized in this manner.

CONCLUSIONS:

It is therefore concluded that the rat cannot digest and utilize pure cellulose.

PUBLICATIONS:

None

STUDY NO. 2 — Conversion of tryptophan to indoleacetic acid in gnotobiotic rats

PROBLEM:

Indoleacetic acid and indoleaceturic acid observed in mammalian excretory products have generally been assumed to be produced from tryptophan by the intestinal flora. More recent studies suggest that indoleacetic acid may be a normal metabolic product in mammalian tryptophan metabolism. Intraperitoneal injections of DL-tryptophan-^{14}C produced ^{14}C-labeled indoleacetic acid. The excretion of indoleacetic acid was much greater in vitamin B_6-deficient rats after a load of DL-tryptophan-^{14}C. Indoleacetic acid has also been recovered from rat liver preparations incubated with DL-tryptophan.

The objectives of this study were to determine, with germfree rats, whether or not in vitamin B_6 deficiency indoleacetic acid is a normal metabolic product and whether or not a difference toward this product is shown by D- and L-tryptophan-methylene-^{14}C.

WORK UNIT NO. 041 - BODY OF REPORT

RESULTS AND DISCUSSION OF THE RESULTS:

Germfree rats were fed a vitamin B_6-deficient diet until growth plateaued. Only four rats were used -- 2 deficient and 2 normal. Rats were injected intraperitoneally with D- or L-tryptophan-methylene-^{14}C (24 or 20 μc, respectively) and D- or L-tryptophan (17 mg/100 g body weight). Immediately after the injection of the tryptophan, the animals were tail-cupped and placed in a respiration-metabolism chamber for monitoring the expired $^{14}CO_2$. Samples were collected for 24 hours. Aliquots of the urine were removed for counting and the remaining urine was lyophilized and stored for subsequent analysis.

TABLE II

Twenty-four Hour Carbon-14 Excretion (% of Dose) by Rats Injected Intraperitoneally with ^{14}C-labeled Tryptophan

		Urine	CO_2
L-tryptophan	$-B_6$	36.1	23.1
	$+B_6$	6.4	40.6
D-tryptophan	$-B_6$	59.3	13.0
	$+B_6$	11.5	15.2

It is seen from Table II that there is about a 5-fold increase in urinary output of carbon-14 in vitamin B_6 deficiency regardless of tryptophan form. However, in vitamin B_6 deficiency there is about a 50% decrease in the expired $^{14}CO_2$ with the L-tryptophan load, but little or no change with D-tryptophan in the expired $^{14}CO_2$ regardless of vitamin B_6 nutriture.

Data on indoleacetic acid excretion have not been obtained. The methodology for the indoleacetic acid-glycine conjugate (indoleaceturic acid), the form in which most of the indoleacetic acid is excreted, is being developed.

CONCLUSIONS:

The metabolism of ^{14}C-labeled D- and L-tryptophan was studied in normal and vitamin B_6-deficient germfree rats.

WORK UNIT NO. 41 - BODY OF REPORT

PUBLICATIONS:

None

This study is a cooperative project with Dr. R. K. Gholson, Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma.

STUDY NO. 3 Riboflavin nutrition in the germfree rat

PROBLEM:

Since it was observed in a previous study on riboflavin (vitamin B_2) nutrition in the female germfree (GF) and female pathogen-free (PF) rat that 30 µg of riboflavin by intraperitoneal injection per day were not adequate for good growth in the GF rats but were adequate for PF rats, the riboflavin requirements of GF and PF rats were determined. Concurrently, the effect of diets with and without riboflavin on the fecal microflora of the PF rats was studied.

RESULTS AND DISCUSSION OF THE RESULTS:

Thirty-two male rats, strain CDF, of which sixteen were axenic (germfree), were received as 4-week-old weanlings. On arrival, they were transferred in groups of eight into sterile environmental plastic chambers and placed individually into a numbered wire-floored cage. Each animal was weighed, then weighed twice weekly thereafter. Food and water were given ad libitum and the food efficiency calculated for each animal.

The basal diet was a heat-sterilized ration containing 20% "vitamin-free" casein, 67% cornstarch, 4% Crisco, 3% Alphacel non-nutritive bulk (ground cellulose), 4% salt mixture (U.S.P. XIV, 1950) and 1% corn oil. The complete diet (supplemented with riboflavin) was prepared by mixing 6.201 grams of vitamin mix (Table III) with 3.799 grams cornstarch and adding to each kilogram of basal diet. The riboflavin-deficient diet was prepared by omitting the riboflavin from the vitamin mix. Both types of diet were microbiologically assayed before heat-sterilization for riboflavin content and after heat-sterilization for content, as well as destruction, if any, and to what extent. Lactobacillus casei ATTC #7469 was employed as the test organism.

TABLE III

Vitamin Mix

	g/kg Basal Diet	
Riboflavin	0.03	
Thiamine HCl	0.06	
Biotin	0.001	
Folic Acid	0.010	
Nicotinamide	0.05	
Nicotinic Acid	0.05	
Vitamin B_{12} (0.1% trit.)	0.003	
DL Ca Pantothenate	0.30	
Inositol	1.00	
Ascorbic Acid	2.00	
Choline Chloride	2.00	
Pyridoxine HCl	0.02	
Pyridoxamine $(HCl)_2$	0.005	
PABA	0.050	
Menadione K	0.10	
Vitamin E (α-tocopherol)	0.5	(1 mg = 1.1 IU)
Vitamin A	0.02	(10,000 IU)
Vitamin D	0.002	(1,000 IU)
TOTAL	6.201	(add per kg of diet)

Fresh fecal samples were collected from all animals. For routine weekly microbiological monitoring of both the GF and PF rats' environmental chambers, diets, water and feces, fluid thioglycolate medium, trypticase soy broth and Sabouraud liquid medium were used. These were incubated at 37° C and room temperature and kept for at least 3 weeks before being termed negative. For

examining the PF fecal specimens, the primary plating media employed for initial isolation were MacConkey, EMB, SS and Brillian Green agars, Azide blood agar base, APT medium and Staphylococcus medium No. 110. Culturally characteristically different colonies were selected from all plates and subcultured on slants of TSI (Triple Sugar Iron) and Kliger iron agars. Pure cultures were obtained and these were inoculated to secondary differential media: SIM medium (deep stab), Urea Broth and Simmons Citrate agar (slant). PF fecal specimens were examined weekly and any other time when a change was made in the riboflavin dietary regimen.

Experimentation on the animals was begun between 4 to 6 days after their arrival and they had acclimatized to the environment and the complete 20% casein (riboflavin-supplemented) diet. Twelve GF and twelve PF rats were placed on the riboflavin-deficient diet. Four animals of each group continued to receive the riboflavin-supplemented diet and served as controls. When weights became constant, the twelve GF and the twelve PF rats in groups of four, still maintained on the riboflavin-deficient diet, were repleted daily intraperitoneally with 10, 30 and 60 µg of riboflavin, respectively, for a period of 8 weeks.

Table IV summarizes the results during riboflavin depletion. When the twelve GF rats (average weight, 86.7 g) and the twelve PF rats (average weight, 70.4 g) were placed on the riboflavin-deficient diet, the GF rats exhibited growth for a longer length of time (14.5 days) before attaining a constant average weight of 126 grams, which they maintained for 10.5 days, for a total of 25 days. The PF rats gained weight for a shorter period (7 days) and plateaued at a constant average weight of 85.6 grams, which was maintained for 14.5 days for a total of 21.5 days on the riboflavin-deficient diet. It was demonstrated that the PF rats depleted faster, presumably due to the added burden of supplying the intestinal microflora with its riboflavin requirements from the minute amount present in the riboflavin-deficient diet.

The average weight gain per day during the riboflavin depletion period for the GF rats was 1.6 g/day; for the PF rats, 0.74 g/day. There was a statistically significant difference in weight gain per day between the two groups at the 0.1% level. Control animals of both groups fed the riboflavin-supplemented diet gained 2.607 g/day for the GF rats and 2.604 g/day for the PF rats. No statistically significant difference was found between the GF and PF controls. Statistical analysis of the effect of the riboflavin-deficient diet and the complete diet within each group showed a statistically significant response at the 0.1% level in both the GF and PF group of animals. The riboflavin-deficient diet consumed by the GF rats was an average of 8.4 g/day; the riboflavin-supplemented diet, 9.3 g/day. The PF rats consumed an average of 7.9 g/day

of the riboflavin-deficient diet; 8.6 g/day of the supplemented diet. It can be seen that the GF rats on the riboflavin-deficient diet were more efficient in food utilization, and possibly in the absorption of nutrients, due to the absence of any intestinal microflora.

TABLE IV

Comparison of Growth Between Male Germfree (GF) and Male Pathogen-free (PF) Rats During Riboflavin (B_2) Depletion on a 20% Casein Diet

20% Casein Diet	Germfree Rats		Pathogen-free Rats	
	Riboflavin		Riboflavin	
	Omitted	Added	Omitted	Added
Weight gain	39.3	65.0	15.8	63.3
No. of days	25	25	21.5	21.5
Weight gain/day	1.6	2.607	0 74	2.604
No riboflavin vs. plus riboflavin	$t = 5.35$; $P < 0.001$		$t = 10.3111$; $P < .001$	
Diet consumed/day	8.4	9.3	7.9	8.6
	GF ($-B_2$) vs. PF ($-B_2$)		$t = 7.2820$; $P < .001$	
	GF ($+B_2$) vs. PF ($+B_2$)		$t = 0.0225$; n.s.	

Table V summarizes the results during intraperitoneal riboflavin repletion. Thirty and 60 µg of riboflavin repletion intraperitoneally caused a statistically significant effect at the 2.5% level between the riboflavin-deficient and supplemented diets consumed by the PF rats. However, their weight gain per day was greater on the riboflavin-deficient diet. GF rats did not display a similar statistically significant weight gain per day, although the 60 µg intraperitoneal repletion indicated a slightly similar trend. Contrarily, the 10 µg

of riboflavin intraperitoneal repletion caused a statistically significant effect at the 0.5% level between the riboflavin-deficient and the supplemented diets consumed by the GF rats but not significant for the PF rats. These data statistically demonstrated that the 10 µg of riboflavin intraperitoneal repletion was not sufficient for the GF rats while on a riboflavin-deficient diet and that their minimal riboflavin requirement would be more than 10 but less than 30 µg. For the PF rats, 10 µg of riboflavin intraperitoneally apparently met their minimal requirement as young adults. There was a statistically significant difference between the two groups when they received 30 or 60 µg On the riboflavin-supplemented diet, the GF and PF control rats showed no statistically significant difference in weight gain per day.

TABLE V

Comparison of Growth Between Male Germfree (GF) and Male Pathogen-free (PF) Rats During 10, 30, 60 Micrograms Intraperitoneal Riboflavin (B_2) Repletion on a 20% Casein Diet

20% Casein Diet	Germfree Rats		Pathogen-free Rats	
	Riboflavin		Riboflavin	
	Omitted	Added	Omitted	Added
Weight gain/day 60 µg IP	2.22	1.73	2.53	2.05
$-B_2$ vs. $+B_2$	n.s.		$P < .025$	
Weight gain/day 30 µg IP	1.93	1.73	2.45	2.05
$-B_2$ vs. $+B_2$	n.s.		$P < .025$	
Weight gain/day 10 µg IP	0.89	1.70	1.80	1.83
$-B_2$ vs. $+B_2$	$P < .005$		n.s.	
	GF ($-B_2$) vs. PF ($-B_2$)		n.s.	
	60 µg IP / 30 µg IP GF ($-B_2$) vs. 60 µg IP / 30 µg IP PF ($-B_2$)		$P < .005$	
	10 µg IP GF ($+B_2$) vs. 10 µg IP PF ($+B_2$)		n.s.	

WORK UNIT NO. 041 - BODY OF REPORT

Prior to the placing of the twelve PF rats on the riboflavin-deficient diet, techniques were employed to culture, enumerate, differentiate and selectively isolate their fecal microflora. The microflora consisted of a mixed population of Escherichia, Aerobacter and Streptococcus species in equal proportions. There was a very rapid change in the microflora genera when the riboflavin-deficient diet was consumed so that just before riboflavin intraperitoneal injections were initiated, Escherichia coli was the only predominant genus isolatable. During the riboflavin repletion period, a mixed population of Aerobacter, Escherichia and Streptococcus species again fluorished along with the Escherichia coli, and no difference was seen in population number or kind of the fecal microflora genera even though the PF rats received varying levels of riboflavin injections.

CONCLUSIONS:

1. The riboflavin requirement for the male GF rat under these experimental conditions was determined to be more than 10 but less than 30 micrograms of riboflavin intraperitoneally.

2. The 10 micrograms of riboflavin intraperitoneally apparently were sufficient as a minimal requirement for the male PF rat approaching adulthood while still maintained on a riboflavin-deficient diet.

3. Male PF rats receiving 30 or 60 micrograms of riboflavin injections gained more weight on the riboflavin-deficient diet than the male PF control rats on the riboflavin-supplemented 20% casein diet.

4. The riboflavin-deficient diet did have an effect on the fecal microflora of the male PF rat, as well as on the host itself. A mixed population of Escherichia, Aerobacter and Streptococcus species in the PF fecal specimens diminished to Escherichia coli only when the host was placed on the riboflavin-deficient diet.

5. Due to this microbial presence, the male PF rats as weanlings during their growth period depleted faster on the riboflavin-deficient diet, and their weight gain per day was much less than that of the male GF rats.

RECOMMENDATIONS:

Germfree animals are a good tool with which to evaluate nutritional and medical problems where the intestinal flora may be involved or interrelated.

WORK UNIT NO. 041 - BODY OF REPORT

PUBLICATIONS:

1. Herman, Y. F., H. E. Sauberlich and N. Raica. Riboflavin requirement of germ-free and pathogen-free rats. Bact. Proc., 68, 1967 (Abstract).

STUDY NO. 4 — Effect of antibiotics on thiamine nutrition in the germfree rat

PROBLEM:

A study to evaluate and to compare the effects of antibiotics, penicillin and succinyl sulfathiazole, on the thiamine (vitamin B_1) nutrition and thiamine utilization of the germfree (GF) and pathogen-free (PF) rats.

RESULTS AND DISCUSSION OF THE RESULTS:

Sixty-four male rats, strain CDR, thirty-two of which were axenic (germ-free), were placed as follows on the various diet combinations:

1. Six GF and six PF rats on the $+B_1$ diet.
2. Six GF and six PF rats on the $-B_1$ diet.
3. Five GF and five PF rats on the $+B_1$ diet with penicillin.
4. Five GF and five PF rats on the $-B_1$ diet with penicillin.
5. Five GF and five PF rats on the $+B_1$ diet with succinyl sulfathiazole.
6. Five GF and five PF rats on the $-B_1$ diet with succinyl sulfathiazole.

The basal diet was the same 20% "vitamin-free" casein diet which was used in a previous study on riboflavin (vitamin B_2) requirement of GF and PF rats (Study No. 3), except for the addition of 3 grams of DL-methionine/g of basal diet. Five-tenths per cent of succinyl sulfathiazole was incorporated into the basal diet before heat-sterilization, but the 0.01% procaine penicillin was added to the heat-sterilized aseptically. The thiamine-supplemented diet was composed of the basal diet plus the vitamin mix (Table III, Study No. 3) and the thiamine-deficient diet was prepared by omitting 0.06 gram of thiamine hydrochloride from the vitamin mix. The PF rats were not housed in the sterile environmental plastic chambers as in the riboflavin study, but were placed in individual wire-floored cages in a separate room adjacent to the room with the environmental chambers. Animals were weighed twice weekly and were observed daily for any physical changes.

WORK UNIT NO. 041 - BODY OF REPORT

The weight gain (average g/day) in 2 weeks' time, 14 days, is tabulated as follows:

Diet Addition	GF		PF	
	$+B_1$	$-B_1$	$+B_1$	$-B_1$
None	4.0	3.5	3.5	2.7
Penicillin	4.3	3.5	3.9	2.9
Sulfa.	3.9	3.2	3.3	2.8

During the end of the third week both groups of animals on the thiamine-deficient diet, with and without antibiotics, developed vitamin B_1 deficiency and were losing weight. Weight loss was reflected more severely with the GF rats on the vitamin B_1-deficient diet with the added succinyl sulfathiazole. Both groups on the thiamine-supplemented diet, with and without antibiotics, continued to gain weight. Experimentation with animals on the vitamin B_1-deficient diets was terminated during the fourth week, when tissues were collected and transketolase activity was measured on the red blood cell hemolysates. The 1-^{14}C-glucose uptake was determined on intact red blood cells, as well as on the hemolysates.

CONCLUSIONS:

That GF rats, with or without antibiotics, developed thiamine deficiency at the same rate as the PF rats, but degenerated at a faster rate, was clearly demonstrated.

PUBLICATIONS:

None

Future studies in this area will be reported under the appropriate work units employing germfree animals, techniques, and the Gnotobiotic Laboratory.

GPO 778804

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6331	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	C. Terminated (30 06 66)	U U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 00 046

10b. PRIOR NUMBER/CODE: None

11. TITLE: (U) Development of a Means for Measurement of Work Decrement in the Rat (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
012900 Physiology	11 64	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	c. TYPE NA	PRIOR FY 67	1	2
		CURRENT FY 68	0	0

19. GOV'T LAB/INSTALLATION/ACTIVITY

NAME: U.S. Army Med Rsch & Nutr Lab
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Denver, Colorado 80240

RESP. INDIV: Syner, J. C., COL
TEL. 303 366-5311, X 21108

20. PERFORMING ORGANIZATION

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

INVESTIGATORS PRINCIPAL ASSOCIATE: Evans, W. O., MAJ
TEL. 303 366-5311, X 26112 TYPE DA

21. TECHNOLOGY UTILIZATION: Antifatigue Drug Screening

22. COORDINATION: None

23. KEYWORDS: Rats; Fatigue; Drug Effects; Endurance; Work Decrement; Work

24. (U) Technical Objective: The objective is to develop a means of measuring decrement in the work capacity of rats performing heavy physical exertion which does not have the limitations of the swimming test or other animal methods for the analysis of the effects of drugs, environments, etc., on work capacity.

25. (U) Approach: It is based on the "titration" concept of measurement in which the animals work to avoid a nociceptive stimulus. The more work performed by the animal, the less electric shock it receives. Thus, the discomfort developed by continuous heavy work is placed in opposition to the noxious qualities of the shock. The advantages of this procedure are: The situation is ecologically valid in that the animal can vary its work rate; the entire performance decrement curve may be studied; and only a few animals will be required to obtain reliable results.

26. (U) Progress: This work unit was terminated due to inadequate number of personnel.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY + 1	

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A013001A91C	In-House Indepent Laboratory Research
TASK NO.	01	
WORK UNIT NO.	046	Development of a Means for Measurement of Work Decrement in the Rat

The objective of this work unit is to develop a means of measuring the decrement in the work capacity of rats performing heavy physical exertion which does not have the limitations of the swimming test or other animal methods for the analysis of the effects of drugs, environments, etc., on work capacity. It is based on the "titration" concept of measurement in which the animal works to avoid a nociceptive stimulus. The more work performed by the animal, the less electric shock it received. Thus, the discomfort developed by continuous heavy work is placed in opposition to the noxious qualities of the shock. The advantages of this procedure are: the situation is ecologically valid in that the animal can vary its work rate; the entire performance decrement may be studied; and only a few animals will be required to obtain reliable results.

BODY OF REPORT

WORK UNIT NO. 046 — Development of a Means for Measurement of Work Decrement in the Rat

PROBLEM:

The purpose of this research study is to develop a method of measurement of work capacity as it changes due to continued physical exertion, environmental factors, physiological status or due to drug action in small animals. It must meet the criteria of being reliable for small numbers of animals, sensitive to small changes in environment or physical condition of the subject and be ecologically valid.

To accomplish this intent, a running wheel is being developed for use with rats. The basic novelty of the approach stems from the use of a "titration" schedule with the wheel. The titration schedule entails apparatus and training of the animal so that the faster an animal runs in the wheel, the less electric shock it will receive to its feet. The animal that fails to run at maximum speed received a punishing shock; whereas, maximum speed running yields minimum electrical stimuli. The animal thus "titrates" the amount of shock it receives by adjusting its running speed. Previous experiments have shown this principle to yield reliable, sensitive and ecologically valid results in dogs and humans.

As performance in this situation continues, the nociceptive stimuli due to fatigue, tends to signal the animal to slow down; however, slowing down will produce more shock. The animal, therefore, must constantly balance two aversive sets of stimuli. The resulting behavior of running speed is directly charted for a time period, to some predetermined percent of decrement in running speed from the original maximum.

RESULTS:

This project has been terminated for the time being since the personnel involved are more urgently needed for other work units.

Prior to the termination of the project, the apparatus for the procedure was completed.

RECOMMENDATIONS:

When an adequate number of personnel are available, this project may be reintroduced. Pilot work demonstrated the possibility of training a rat in this particular type of schedule.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6346	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (21 03 67)	RPT U WRK	NA	NL	A WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER CODE
61130011 3A013001A91C 01 049	

11. TITLE:

(U) The Mechanism of Body Temperature Control by Adrenal Steroids (06)

12. SCIENTIFIC OR TECH. AREA	13 START DATE	14 CRIT COMPL DATE	15 FUNDING AGENCY
002300 Biochemistry 003500 Clinical Medicine 012900 Physiology	07 66	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18 RESOURCES EST	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 67	1	39
		CURRENT FY 68	1	45

19. GOV'T LAB/INSTALLATION/ACTIVITY	20 PERFORMING ORGANIZATION
NAME	
ADDRESS Headquarters U.S. Army Med Rsch & Nutr Lab Fitzsimons General Hospital Denver, Colorado 80240	NAME U.S. Army Med Rsch & Nutr Lab ADDRESS Fitzsimons General Hospital Denver, Colorado 80240
RESP. INDIV. Syner, J. C., COL	INVESTIGATORS PRINCIPAL ASSOCIATE Herman, R. H., LTC
TEL. 366-5311 X21108	TEL 303 366-5311, X10221 TYPE DA

21. TECHNOLOGY UTILIZATION	22 COORDINATION
Medicine	None

23. KEYWORDS

Temperature; Fever; Steroids

24. (U) Technical Objective: It has been found that in a patient with a persistent fever his temperature could be altered by the use of dexamethasone and the use of a variety of uronic acids. This has led to the idea that etiocholanolone is a substance which may control body temperature by regulating metabolic rates in the liver. By regulating the ratio between etiocholanolone glucosiduronate it may be possible to regulate body temperature.

25. (U) Approach: Patients with periodic fever and normal subjects will be studied with respect to their response to etiocholanolone and a variety of similar steroids and the effect of uronic acids and a variety of other antipyrogenic substances, such as dexamethasone. The factors which can modify intrahepatic etiocholanolone will be studied and their effect on patients with fever will be noted.

26. (U) Progress: Normal subjects develop fever after the intramuscular injection of etiocholanolone. This can be controlled better with prednisone than by dexamethasone, which is the reverse of what we have found in certain patients with periodic fever. Uronic acids have no effect on etiocholanolone-induced fever in normal subjects. A variety of mechanisms have been tested with regard to etiocholanolone induction of fever but the results are pending at this time and no firm conclusions can be drawn.

27. COMMUNICATIONS SECURITY	28.	29 OSU CODE	30 BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32 PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A013001A91C	In-house Laboratory Independent Research
TASK NO.	00	
WORK UNIT NO.	049	The Mechanism of Body Temperature Control by Adrenal Steroids

Investigation of a patient of Lebanese ethnic Origin who had life-long familial persistent fever showed that he responded to dexamethasone and various uronic acids which are beta-glucuronidase inhibitors. He had no evidence of infection, neoplasm, collagen-vascular disease, allergy, neurological disease, hepatic disease, or malingering. He did not respond to antibiotics, antipyretics, or prednisone. His sweat mechanism and response to killed typhoid bacilli was normal. His adrenal, thyroid, and pituitary function were normal. Urinary etiocholanolone and serum beta-glucuronidase were normal. It is postulated that this patient has an abnormal beta-glucuronidase that is not inhibited by the usual endogenous levels of intra-hepatic beta-glucuronidase inhibitors or is unduly sensitive to normal levles of intra-hepatic etiocholanolone. Normal individuals rendered febrile with intra-muscular injections of etiocholanolone are not protected with oral glucuronic acid but are protected by pre-treatment with desamethasone and better with prednisone. The study of several patients with various types of periodic fever has shown that glucuronic acid has no appreciable effect. No abnormality of beta-glucuronidase has been found in these patients. The nature of the defect in periodic fever, the mechanism of action of etiocholanolone, and the reason why prednisone inhibits etiocholanolone-induced fever is still unknown.

BODY OF REPORT

WORK UNIT NO. 049 The Mechanism of Body Temperature Control by Adrenal Steroids

DESCRIPTION:

A patient with persistent pyrexia (102°F.) was investigated and found to have no evidence of the usual causes of persistent fever. This patient gave a history of life-long fever in himself and a twin brother. There was no evidence of infection, neoplasm, collagen-vascular disease, allergy, neurological disease, hepatic disease, or malingering. His sweat mechanism and response to killed typhoid bacilli was normal. He had no diurnal variation of his temperature. Therapeutic trials with antibiotics, antipyretics, and prednisone had no effect on his fever. Further studies were undertaken to understand the nature of this patients febrile state. Since the patient was of Lebanese ethnic origin and apparently had a familial congenital condition it was postulated that his disease was related to Mediterranean Familial Fever which is one of a group of periodic fevers.

PROGRESS:

The patient's fever did not respond to a low fat or a low protein diet. His adrenal function was entirely normal and urinary etiocholanolone levels were normal. However, his fever was decreased by oral dexamethasone. On dexamethasone suppression ACTH and intra-muscular and oral etiocholanolone had no effect on his fever. The administration of glucuronic acid, 10 grams, orally, decreased his fever to normal levels and then gradually his temperature rose to its usual high levels over the next four days. The administration of various uronic acids and precursors to glucuronic acid such as myo-inositol and glucuronamide also decreased his fever. These effective agents are known to be inhibitors or inhibitor-precursors of beta-glucuronidase. It is of importance that the non-physiological uronic acid, galacturonic acid which is a beta-glucuronidase inhibitor, also decreased the patient's fever. It is postulated that the patient has either an abnormal beta-glucuronidase which cannot be inhibited by the usual endogenous levels of uronic acids so that it produces relatively increased levels of intra-hepatic etiocholanolone which is known to be pyrogenic, or is unduly sensitive to normal levels of intra-hepatic etiocholanolone. In the latter case beta-glucuronidase inhibitors would block the formation of free etiocholanolone and thus reduce the level of the intra-hepatic etiocholanolone to which the patient is sensitive. Serum beta-glucuronidase levels in the patient were normal. Fever was induced in normal human subjects with the intra-muscular injection of etiocholanolone. This etiocholanolone-induced fever was inhibited by pre-treatment with dexamethasone but even more so by pre-treatment with prednisone with what is considered to be equivilent therapeutic doses. Glucuronic acid given orally or intravenously either with etiocholanolone or during the fever had no effect on the fever occurrence.

The study of six pateints with various types of periodic fever have so far shown no effect of glucuronic acid in alleviating the fever. No abnormality of serum beta-glucuronidase has yet been detected. One of the patients has elevated urinary etiocholanolone and can be considered to have what is called etiocholanolone fever. She also has the associated problem of edema. Another patient has hepatic granulomata associated with his febrile state. Several of these patients seem more resistant than normal subjects to intra-muscular etiocholanolone. The nature of the defect of the patient with the persistent fever is still being investigated. The pathogenesis of the fever in the patients with periodic fever is still obscure b.t several theoretical approaches are being studied.

It appears that man has a hitherto unappreciated temperature-producing mechanism which seems to be located within the liver related to etiocholanolone and beta-glucuronidase. Derangements of this system can result in the most rare case as persistent fever but more commonly as periodic fever. The mechanism whereby etiocholanolone is pyrogenic and how various antiinflammatory steroids can block this fever is still unknown.

SUMMARY:

The nature of the fever in a patient with persistent fever seems to be related to etiocholanolone and beta-glucuronidase. This patient's fever was resistant to antibiotics, antipyretics, and prednisone but responded to dexamethasone and various uronic acids which are beta-glucuronidase inhibitors. Normal subjects with etiocholanolone-induced fever did not respond to glucuronic acid but fever was prevented with dexamethasone and even better with prednisone. Patients with periodic fever of various types did not respond to glucuronic acid. The nature of periodic fever, the nature of the pyrogenic action of etiocholanolone, and the mechanism of action of steroids in blocking etiocholanolone-fever is still unknown.

PUBLICATIONS:

1. Herman, R. H., Overholt, E. L., and Hagler, L. Effect of Dexamethasone and Uronic Acids on a Patient with Persistent Fever. Clin. Res. 15:320, 1967. Presented at the National Meeting of the American Federation for Clinical Research, April 30, 1967, in Atlantic City, New Jersey.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 0332	CSGRD-1R3

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / MRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 08 66)	U / U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE: 6113011 3A013001A91C 00 050

10b. PRIOR NUMBER CODE: None

11. TITLE: (U) Clinical and Laboratory Examinations of Mature German Shepherd Dogs Fed Rice Base Diets (06)

12. SCIENTIFIC OR TECH AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
006500 Food	08 66	NA	OTHER DA

16. PROCURE METHOD	17. CONTRACT GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	1	8
		CURRENT FY 68	1	16

19. GOVT LAB INSTALLATION ACTIVITY

NAME

ADDRESS: U. S. Med Rsch & Nutr Lab
Fitzsimons General Hospital
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RESP INDIV: Syner, J. C., COL

TEL: 303 366-5311 X21108

20. PERFORMING ORGANIZATION

NAME

ADDRESS: U.S. Med Rsch & Nutr Lab
Fitzsimons General Hospital
Denver, Colorado 80240

INVESTIGATORS PRINCIPAL ASSOCIATE: Pope, C. R., CPT
Stedham, M.A., MAJ

TEL: 303 366-5311 X26122 TYPE: DA

21. TECHNOLOGY UTILIZATION: Dog Food

22. COORDINATION: None

23. KEYWORDS: Dogs, Diet; Food; Rice; Veterinary Medicine; Laboratory Diagnosis

24. (U) Technical objective: To document the clinical and laboratory findings in active mature German Shepherds fed rice diets. Formulate and test a balanced rice diet using mineral and vegetable nutritional supplements. The information gathered would pertain to the advisability of using rice-base diets for the military dog and give an estimate of how long a military dog will be workable while fed a rice diet. Supplementation of diets with animal protein will be avoided. An acceptable and practical
25. balanced rice diet would reduce logistical load and expense. The acceptability of a rice diet to dogs accustomed to other foods can be critical and especially so if these animals have to be worked immediately. This could be negated by utilizing a rice-based diet under normal conditions for feeding of sentry dogs programmed into areas where emergency feeding could be encountered.

25. (U) Approach: Two diets will be used. The experimental diets will consist of parboiled rice with the following supplements: rice bran, rice polish and bone meal. Protein and fat supplementation as needed will be derived from peanut oil meal and/or soybean oil meal. Other supplements, if needed, will be taken from the Republic of
26. Viet Nam Nutrition Survey. The control diet will consist of the commercial dog food "Prime". All dogs will receive measured water ad libitum and daily exercise. Included will be daily physical examinations, diet analysis, nitrogen and mineral balance studies, daily body weights, food and water intake and excretion measurements, urinalysis, complete blood count, and sedimentation rate measurements. Blood chemistry will include total protein, total cholesterol, Ca, P, K, Na, Cl, SGPT, SGOT, Alkaline phosphatase, CPK, BUN, Creatinine, sugar, and serum electrophoretic measurements.

26. (U) Progress: We have received 9 mature male German Shepherds of variable weight and age. The exercising device is near completion and estimated operational date is 1 July 67. The initial ingredients for the test and control diets have beenreceived.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
a. COMSEC OR COMSEC RELATED [X] b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CY:	

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to NASA Form 1121) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.:	3A013001A91C	In-House Laboratory Independent Research
TASK NO.:	01	" " "
WORK UNIT NO.:	050	Clinical and Laboratory Examinations of Mature German Shepherd Dogs Fed Rice Base Diets

PURPOSE OF WORK:

To document the clinical and laboratory findings in active mature German Shepherds fed rice diets. Formulate and test a balanced rice diet using mineral and vegetable nutritional supplements. The information gathered would pertain to the advisability of using rice-base diets for the military dog and give an estimate of how long a military dog will be workable while fed a rice diet. Supplementation of diets with animal protein will be avoided. An acceptable and practical balanced rice diet would reduce logistical load and expense. The acceptability of a rice diet to dogs accustomed to other foods can be critical and especially so if these animals have to be worked immediately. This could be negated by utilizing a rice-based diet under normal conditions for feeding of sentry dogs programmed into areas where emergency feeding could be encountered.

METHODS BY WHICH ACCOMPLISHED:

Two diets will be used. The experimental diets will consist of parboiled rice with the following supplements: rice bran, rice polish and bone meal. Protein and fat supplementation as needed will be derived from peanut oil meal and/or soybean oil meal. Other supplements, if needed, will be taken from the Republic of Viet Nam Nutrition Survey. The control diet will consist of the commercial dog food "Prime". All dogs will receive measured water ad libitum and daily exercise. Included will be daily physical examinations, diet analysis, nitrogen and mineral balance studies, daily body weights, food and water intake and excretion measurements, urinalysis, complete blood count, and sedimentation rate measurements. Blood chemistry will include total protein, total cholesterol, Ca, P, K, Na, Cl, SGPT, SGOT, Alkaline phosphatase, CPK, BUN, Creatinine, sugar, and serum electrophoretic measurements.

We have received 9 mature, male, German Shepherds of variable weight and age. The exercising device is near completion and estimated operational date is 1 July 67. The initial ingredients for the test and control diets have been received.

Clinical and Laboratory Examinations of Mature German Shepherd Dogs Fed Rice Base Diets

Delay in the start of the project has occurred due to the difficulty in obtaining mature German Shepherds with close body signalment, and late completion of the exercising device.

BODY OF REPORT

WORK UNIT NO.: 050 — Clinical and Laboratory Examinations of Mature German Shepherd Dogs Fed Rice Base Diets

STUDY NO.: 1 — Formulate and Test a balanced Rice Diet without Protein Supplementation

PROBLEM:

1. Military dogs play an important role in security for the Armed Forces. Mature German Shepherds weighing 65 pounds and over are used. They have proved their effectiveness as scout and sentry dogs.

2. In Viet Nam nutritional problems were originally ones of supply, but the improvement of supply channels has largely solved these. There is still a potential supply problem in the more isolated areas. When the standard bagged or canned ration was not available diets were fed that were composed of rice and various supplements. Results with these diets were inconclusive.

RESULTS AND DISCUSSION OF THE RESULTS:

1. We are interested in developing and testing a practical rice-base diet without animal protein supplementation for the following reasons that are not necessarily in the order of importance: 1) If a satisfactory rice-base diet for mature German Shepherds could be developed, this would reduce logistical load and expense in that less dog food would have to be sent from the United States. 2) A portion of indigenous rice and that which is exported from the U.S. has the potential of being used as food for the military dog. 3) The acceptability of a rice diet to dogs accustomed to other foods can be critical and especially so if these animals have to be worked immediately. 4) If supply problems should arise in a major rice-producing country where military dogs are located, how would they fare on a rice base diet? 5) Would a rice base diet be practical? 6) What are the most practical and satisfactory vegetable supplements for such a diet for military dogs in Viet Nam?

2. Animal protein supplementation will be avoided since in underdeveloped countries animal protein is at a premium for the human populace.

Clinical and Laboratory Examinations of Mature German Shepherd Dogs Fed Rice Base Diets (Cont'd)

3. The experimental diets will consist of parboiled rice with the following supplementations: Rice bran, rice polish and bone meal. Protein and fat supplementation as needed will be derived from peanut oil meal and/or soybean oil meal.

4. Other supplements, if needed, will be taken from the Republic of Viet Nam Nutrition Survey. A report by the Interdepartmental Committee on Nutrition for National Defense, July 1960.

5. The control diet will consist of the commercial dog food "Prime". All dogs will receive measured water ad libitum and daily exercise. The exercise device is a motorized playground merry-go-round. This is being used because potentially 6 to 8 dogs can be exercised simultaneously whereas only one can be exercised at a time on a tread mill. Included will be daily physical examinations, diet analysis, nitrogen and mineral balance studies, daily body weights, food and water intake and excretion measurements, urinalysis to include specific gravity, pH, protein, glucose, acetone, volume, blood; urobilinogen; complete blood counts, and sedimentation rate measurements. Blood chemistry will include total protein, total cholesterol, Ca, P, K, Na, Cl, SGPT, SGOT, Alkaline phosphatase, CPK, BUN, creatinine, sugar, and serum electrophoretic measurements.

6. We have received 9 mature, male, German Shepherds of varied weights and ages. The exercising device is near completion and estimated operational date is 1 July 67. The initial ingredients for the test and control diets have been received.

7. Delay in the start of this project has occurred due to the difficulty in obtaining mature German Shepherds with close body signalment and late completion of the exercising device.

RESEARCH AND TECHNOLOGY RESUME

2 GOVT ACCESSION	3 AGENCY ACCESSION	REPORT CONTROL SYMBOL
	DA OA 6353	CSCRD 103

4 DATE OF RESUME	5 KIND OF RESUME	6 SECURITY	7 REGRADING	8 RELEASE LIMITATION	9 LEVEL OF RESUME
01 07 67	D. Change (01 02 67)		NA	NL	A.WORK UNIT

10a CURRENT NUMBER/CODE: 61130011 3A013001A91C 00 051

10b PRIOR NUMBER CODE: None

11 TITLE: (U) The Evaluation of the Natriuretic Property of 16-Alpha-Hydroxyprogesterone in Human Subjects (06)

12 SCIENTIFIC OR TECH AREA	13 START DATE	14 CRIT COMPL DATE	15 FUNDING AGENCY
012600 Pharmacology; 012900 Physiology	02 67	NA	OTHER | DA

16 PROCURE METHOD	17 CONTRACT/GRANT a. DATE b. NUMBER c. TYPE d. AMOUNT	18 RESOURCES EST	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	0	3
		CURRENT FY 68	1	15

19 GOVT LAB/INSTALLATION ACTIVITY
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20 PERFORMING ORGANIZATION
NAME: U.S. Army Med Rsch & Nutr Lab
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Denver, Colorado 80240
INVESTIGATORS PRINCIPAL: Janoski, A. H., CPT
ASSOCIATE: Herman, R. H., LTC
TEL: 303 366-5311 X22119 TYPE: DA

21 TECHNOLOGY UTILIZATION: Medical and Paramedical Sciences

22 COORDINATION: None

23 KEYWORDS: Adrenal Cortex Hormones; Renal Physiology; Endocrine Physiology; Sodium Metabolism; Water-Electrolyte Balance; Aldosterone Antagonists; Diuresis

24 (U) Tech Objective: 16-alpha hydroxyprogesterone is produced by the normal human adrenal gland. Natriuretic properties have been attributed to 16-alpha hydroxyprogesterone in various reports. The actual blood concentration necessary to produce a response in humans is unknown. The objective of this study is to establish the dose response necessary to induce sodium diuresis in human subjects by the intravenous administration of known quantities of 16-alpha hydroxyprogesterone.

25 (U) Approach: After constant electrolyte balance is attained in human subjects, a known concentration of 16-alpha hydroxyprogesterone will be administered intravenously over a timed interval. Frequent blood and urine samples will be collected to evaluate the changes in electrolyte balance and aldosterone excretion. The dose of the steroid infused and the changes incurred will determine the potency of 16-alpha hydroxyprogesterone as a natriuretic agent.

26 (U) Progress: Chemical reagents have been ordered. A small quantity of 16-alpha hydroxyprogesterone was synthesized using the precursor 4-pregnen 16 alpha, 17 alpha-epoxy-3, 20 dione to test the method. The results are satisfactory and larger quantities will be synthesized for the study in the near future.

27 COMMUNICATIONS SECURITY	28	29 DDC CODE	30 BUDGET CODE
a. COMSEC OR COMSEC RELATED [X] b. NOT RELATED		BP	1

31 MISSION OBJECTIVE	32 PARTICIPATION
NA	NA

33 REQUESTING AGENCY	34 SPECIAL EQUIPMENT

35 EST FUNDS (In thousands)	36
CPY	

DD FORM 1498

ABSTRACT

PROJECT NO.	3A013001A91C	In-House Laboratory Independent Research
TASK NO.	01	
WORK UNIT NO.	051	The Evaluation of the Natriuretic Property of 16-Alpha-Hydroxyprogesterone in Human Subjects

16-alpha-hydroxyprogesterone is an adrenal steroid compound which is produced by the normal adrenal gland in vivo in small quantities. The steroid has been implicated as a natriuretic agent in human and animal studies. The studies of the natriuretic property of this compound in man, however, have been incomplete. The purpose of this study is to determine the dose response of intravenously administered 16-alpha-hydroxyprogesterone in human subjects with regard to sodium excretion and aldosterone production. Knowledge of the role of the adrenal gland in salt and water metabolism is essential in understanding the factors responsible for alterations in man under varying stresses.

16-alpha-hydroxyprogesterone has been synthesized in this laboratory from commercially available 4-pregnen-16 α-epoxy-3, 20 dione. Human studies are pending final approval of the protocol.

BODY OF REPORT

WORK UNIT NO. 051 The Evaluation of the Natriuretic Property of 16-Alpha-Hydroxyprogesterone in Human Subjects

STUDY NO. 1

PROBLEM:

Water balance in individuals varies significantly with respect to environment, performance and disease states. Knowledge of the factors that regulate salt and water metabolism is urgently needed in order to understand the changes that occur in man under varying stresses. The steroid, 16-alpha-hydroxyprogesterone has been implicated as a natriuretic agent in animals and man. This compound is produced by the normal human adrenal gland in vivo in small quantities. The objective of this study is to study the dose response of intravenously administered 16-alpha-hydroxyprogesterone in human subjects with regard to sodium excretion and aldosterone production.

16-alpha-hydroxyprogesterone will be synthesized from 4-pregnen-16 α, 17 α-expoxy-3, 20 dione by the method of Julian and Cole. Chemical purity will be established by determination of the melting point, mixed melting point with reference standard and infrared absorption spectroscopy.

RESULTS:

The methodology for the synthesis of 16-alpha-hydroxyprogesterone has been tested on a small scale and has been successful. Final approval of the protocol for human study is pending.

GPO 772804

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6354	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 02 67)	U (NPT) U (WRK)	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 00 052

10b. PRIOR NUMBER/CODE: None

11. TITLE: (U) Coronary Blood Flow Studies (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
012600 Pharmacology; 012900 Physiology	02 67	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 67	1	3
		CURRENT FY 68	1	11

19. GOV'T LAB/INSTALLATION/ACTIVITY

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RESP. INDIV: Syner, J. C., COL
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20. PERFORMING ORGANIZATION

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INVESTIGATORS PRINCIPAL ASSOCIATE: Carson, R. P., CPT
Merrill, R. H., CPT
TEL: 303 366-5311 X22119 TYPE: DA

21. TECHNOLOGY UTILIZATION: Clinical Medicine

22. COORDINATION: None

23. KEYWORDS: Coronary Vessels; Blood Flow; Drug Effects; Cold Exposure; Altitude Exposure; Experimental Myocardial Infarction; Xenon-133; Electromagnetic Flowmeters

24. (U) Technical Objective: To determine qualitative and quantitative effects of drugs, environmental stress, and experimental coronary occlusion on coronary blood flow in the closed-chest dog.

25. (U) Approach: Coronary blood flow will be measured by precordial clearance rate of radioactivity following selective coronary injection of Xenon-133 in saline or by electromagnetic flowmeters chronically implanted onto coronary arteries. Initial studies will determine changes in coronary flow produced by: (1) administration of pharmacological agents; (2) breathing cold air; (3) exposure to high altitude; (4) experimental coronary occlusion. Subsequent studies will attempt to delineate: (1) modifying effects of drugs on coronary flow responses to breathing cold air and to coronary occlusion; (2) modifying effect of high altitude on pharmacological responses.

26. (U) Progress: This work unit was initiated with preliminary experience gained in chronic coronary artery implantation of electromagnetic flow transducers in dogs. Action has been taken to correct technical problems encountered with the available flowmeter system. Several methods are under study to improve our ability to maintain patent indwelling vascular catheters. A study comparing the effects of nitroglycerin and amyl nitrate in anesthetized and awake animals has been started.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY + 1	

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to AMSAA Form 1128) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A013001A91C	In-House Laboratory Independent Research
TASK NO.	01	
WORK UNIT NO.	052	Coronary Blood Flow Studies

The following investigation has been initiated under this work unit:

STUDY NO. 1: Effects of Nitroglycerin and Amyl Nitrate on Coronary Blood Flow in Intact Dogs

This work unit was initiated to investigate the effects and interrelationships of environmental stress, pharmacological agents and experimental cardiac pathology on the coronary circulation of the intact dog. Coronary blood flow is measured by selective intracoronary injection of Xenon-133 in saline in anesthetized dogs and by chronically implanted coronary artery electromagnetic flow transducers in awake animals. In the four months since this project began, we have been involved primarily with acquiring equipment and supplies, working out radioisotope and surgical techniques and becoming familiar with the operation of the equipment. A study comparing the effects of nitroglycerin and amyl nitrate in anesthetized and awake dogs has recently been started; however, data is insufficient at this time for any conclusions to be reached.

BODY OF REPORT

WORK UNIT NO. 052 — Coronary Blood Flow Studies

STUDY NO. 1 — Effect of Nitroglycerin and Amyl Nitrate on Coronary Blood Flow in Intact Dogs

PROBLEM:

Until recent years, information concerning the responses of the coronary circulation has been derived primarily from isolated heart or heart-lung preparations of experimental animals or from post-mortem studies in man. Unfortunately, the data obtained from these experimental situations, excluded from normal metabolic support, thermal regulation and autonomic nervous system and respiratory influences is not satisfactory for predicting hemodynamic responses in the intact animal or in man. Recently, however, several methods have been developed which allow the measurement of coronary blood flow in the intact, closed-chest dog. This permits the study of the coronary circulation within a physiologically intact cardiovascular system.

The first study under this work unit has as its objective the determination of changes in coronary blood flow induced by nitroglycerin and amyl nitrate. Previous studies with these drugs, particularly with regard to the former, have been performed principally in surgical preparations and/or with extremely large doses of the drugs. The present study will examine responses in intact dogs both in anesthetized and awake states, utilizing doses more equivalent to those used in man. A comparison of these two drugs was chosen because of the fact that although chemically similar, they have been shown to have some dissimilar effects on peripheral hemodynamics.

RESULTS:

Preliminary endeavors have allowed us to gain experience with radioisotope techniques and equipment and surgical methods of chronic flow transducer implantations. Mechanical difficulties with our flowmeter system has hampered the investigation, but the study has been initiated using the radioisotope method. Observations have not been completed and, therefore, not yet subjected to data analysis.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6300	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	6. SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U	U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 00 065

10b PRIOR NUMBER/CODE: None

11. TITLE: (U) Microbiological Research in Tuberculosis (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
010100 Microbiology	08 59	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	a. DATE	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA		PRIOR FY 67	2	39
	c. TYPE	d. AMOUNT	CURRENT FY 68	2	35

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
NAME: U. S. Army Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240
INVESTIGATORS PRINCIPAL ASSOCIATE: Morse, W. C., COL: Sproat, E.F.; Tull, A. H.
TEL 303 366-5311 X25223 TYPE: DA

21. TECHNOLOGY UTILIZATION: Hospitals; Sanitariums

22. COORDINATION: None

23. KEYWORDS: Drug Susceptibility, Mycobacteria, technic pathogenicity, drug assay; chemotherapy, antigens, fluorescence antibody, serologic diagnosis, tuberculosis, immunity

24. (U) Tech Objective: To improve methods for isolation, identification and drug susceptibility of mycobacteria recovered rom patients. To correlate drug dosage, serum drug concentrations and microbe susceptibility with response to treatment. To develop the use of an indirect fluorescent antibody (SAFA) method for measuring antibodies produced in human mycobacterial infection, and experimental infection in animals.

25. (U) Approach: Continuing modifications of procedures in specimen preparation, medium composition, incubation environment, drug susceptibility technics, biochemical aids in identification are being computerized for combinational analysis with clinical data. Rates of bacterial conversion to negative will be correlated with drugs, dosage, serum concentrations achieved, microbial drug susceptibility and to extensive clinical background information. Antibodies will be measured by the SAFA method in human and monkey serum using precisely identified antigens.

26. (U) Progress: Results of studies on improved mycobacteriology methods are being incorporated in a new revision of USAMRNL, Report #283. The current tuberculosis chemotherapy protocol is correlating, for computer combinatorial analysis, drugs, dosage, serum concentrations, and precise microbial susceptibility with complete clinical files of information. Promising results are being obtained with the application on a new quantitative soluble antigen, fluorescent antibody measurement of humoral antibodies in tuberculosis patients, and in rhesus monkeys challenged with M. tuberculosis. Preliminary results suggest that this technic may become a valuable diagnostic tool and useful in following patients on chemotherapy. Precise identification of the antigen of choice suggest that the RNA moiety of Youman's ribosomal antigen will be used for serologic diagnosis, and may also be used for immunization.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A025601A822 00 065	Military Internal Medicine
TASK NO.	00	
WORK UNIT NO.	065	Microbiological Research in Tuberculosis

Study No. 1

Modifications of the composition of the isolation medium and of incubation environment permit rapid isolation and identification of the various mycobacterial species capable of causing tuberculosis. A recent medium modification, the addition of L-asparagine, stimulates rate of growth of all mycobacteria and increases the niacin production of typical human tubercle bacilli. A new niacin test makes easy the separation of M. tuberculosis from all other mycobacteria. Sophistication of laboratory procedures include twice weekly isolation attempts from new patients until bacteriologic conversion to negative is achieved, precise drug susceptibility titrations on isolated mycobacteria and the measurement of patient's serum plus drug content to inhibit growth of his own mycobacterial strain. Results of studies are being computerized for combinatorial analysis with complete computerized master files of clinical data.

Study No. 2

Improved mass culture, harvesting, fractionation and separation procedures have led to isolation and purification of antigenic substances which may be directly related to active disease and immunity responses in tuberculosis. A new quantitative Soluble Antigen Fluorescent Antibody (SAFA) method shows excellent promise of being a valuable aid in studying specific antigen-antibody relationships in tuberculosis. The ribonucleic acid from tubercle bacilli, when studied by the SAFA method on the serum of rabbits challenged with M. tuberculosis strain H37Ra and Rhesus monkeys "immunized" with mycobacterial fractions and challenged with virulent M. tuberculosis, suggests that this moiety may measure the development of active disease and immunity. Continued studies will include qualitation and quantitation of the antibody globulins involved in the response to antigenic stimulus.

BODY OF REPORT

WORK UNIT NO. 065 — Microbiologic Research in Tuberculosis

PROBLEM: (Study No. 1)

The microbiologic study of clinical material from tuberculous patients is now complicated by the knowledge that there are several species of mycobacteria capable of causing tuberculosis. An important point of this is that these mycobacterial species are usually resistant to the in vivo and in vitro inhibiting effect of most of the classical antituberculosis drugs. Too, there is a small percentage of patients (approximately 10%) who have acquired a drug-induced infection with resistant mycobacteria and who are, then, chemotherapy problems. In addition, though most tuberculosis patients whose disease is caused by drug-susceptible typical tubercle bacilli and who are treated with conventional dosages of the major drugs, streptomycin, isoniazid and p-amino salicylic acid convert to a bacteriologically negative state in a very few months; a small number do not and the reason is not known.

It is of utmost importance, therefore, that laboratory personnel have the knowledge and technics which will permit easy identification of the mycobacterial species involved and provide rapid drug susceptibility-resistance patterns. Also of importance are the studies which measure the metabolic rate of the drugs administered by performance of chemical and biologic assays of concentrations of the drugs in the patient's serum.

RESULTS AND DISCUSSION OF RESULTS:

Continued efforts have been made by personnel of this division to improve the growth promoting qualities of the 7H10, OA agar isolation medium used to recover mycobacteria from clinical material. A report from the Baltimore VA Hospital indicated that the addition of 0.2 to 0.5 percent l-asparagine to this medium increased the rate of growth of mycobacteria and increased niacin production in those strains which are capable of synthesizing this substance. Studies in this division confirmed these observations. Too, the niacin test, which distinguishes typical human type tubercle bacilli (M. tuberculosis) from nearly all other mycobacteria, was changed from a report presented from the Communicable Disease Center, Atlanta, Georgia. This valuable biochemical test for aiding identification of mycobacteria may now be applied in routine hospital laboratories. Prior to the introduction of the new niacin test, the reaction reagents included cyanogen bromide and a reaction product was toxic hydrocyanic acid which necessitated that the test be performed in a chemical hood. The reagents now used produce no toxic reaction products.

To offer more definitive laboratory information to clinicians responsible for the tuberculosis treatment program and to accumulate more precise data for combinatorial computer analyses with master files of clinical information, a

sophistication of mycobacteriologic laboratory procedures has been initiated. The mycobacteria recovered from all patients are being precisely titrated for susceptibility to the drugs being administered. Drug levels attained in serum at various hours after administration are being performed by biological and chemical procedures. The patient's serum, plus drug content, is being biologically assayed to determine its capability in inhibiting the multiplication of the patient's own mycobacterial species. To accurately identify the time of bacteriologic conversion to negative, material from patients with pulmonary tuberculosis is being cultured at twice weekly intervals.

CONCLUSIONS:

Results of sophisticated mycobacteriology methods show promise of providing data which may be of value in aiding the clinical service in the management of tuberculosis patients.

RECOMMENDATIONS:

The collaborative study, performed under approved protocol should continue indenfinitely.

PUBLICATIONS:

None.

PROBLEM: (Study No. 2)

Humoral antibodies which may be related to active tuberculosis or immunity have not been demonstrated. Indeed, many tuberculo-immunologists serologists believe that cell and tissue antibody is responsible for developing immunity to tuberculosis and that tissue "resistance" developes concommitant with, though not directly related to, the development of tuberculin hypersensitivity. Vaccination to develope immunity against tuberculosis is primarily accomplished using viable whole cells of an attenuated mycobacteria strain. Currently, there is no serologic way to measure the protection attained by vaccination and the reasons are many. Mycobacteria are tremendously complex organisms comprised of myriads of immunogenic chemical configurations capable of eliciting an antibody response. Methods of sorting and identifying the many antigens and methods for measuring the antibodies have not had sufficient specificity nor sensitivity to relate antigen to antibody to state of disease or immunity.

Improved mass culture, collection, fractionation and component separation procedures now permit isolation, purification and concentration of antigenic fractions of mycobacterial cells. Too, the development of a new Soluble Antigen Fluorescent Antibody (SAFA) method for antigen-antibody measurements has provided an objective, quantitative procedure for serologic-immunologic study.

RESULTS AND DISCUSSION OF RESULTS:

Briefly the SAFA method of antigen-antibody analysis utilizes cellulose acetate paper discs to which antigen is adsorbed. Serum is added and specific antibody, if present, binds to antigen. After appropriate washings, etc., fluorescein-tagged anti-antibody is added and, if the reaction is complete, a three-way complex is formed, e.g., antigen-antibody and anti-antibody. Specific and quantitative fluorescence is then measured in a fluorometer and recorded. The projected success of this SAFA method is based upon the selection of soluble purified and identified antigens.

Six months of experimentation with the SAFA technic using complexes of culture filtrate antigens which were tested against serum from rabbits "immunized" with whole cells of the attenuated M. tuberculosis strain H37Ra and Rhesus monkeys challenged with pathogenic M. tuberculosis, strain 5159, and against serum from tuberculosis patients served to develope the SAFA methodology to where it was a highly sensitive and quantitative laboratory tool. Early results clearly indicated that with purified and identified single antigens the SAFA technic would offer investigators methodology for distinguishing between active and arrested tuberculosis and possibly assess a measurement of immunity.

One antigen of promise is the ribosomal antigen of Youmans (J. Bact., 80:1291, 1965) the injection of which into mice and guinea pigs has conferred a significant immunity. Of extreme importance is the fact that immunity is established without developing tuberculin hypersensitivity. Youman's ribosomal antigen was prepared in the reporting laboratory and was successfully used as the SAFA method antigen. A comparison with ribosomal antigen supplied by Youmans gave results which showed that the ribosomal antigen produced by this unit was identical. Chemical studies divided the antigen into three major fractions: i.e. RNA, DNA and unknown protein. By use of the SAFA technic a quantitative relationship was established which indicated that the RNA moiety of the ribosomal antigen is probably responsible for serologic and immunologic specificity.

Current plans include a joint effort with Dr. Youmans of Northwestern University, who will prepare the ribosomal antigen for immunogenic study in Rhesus monkeys, Dr. Schmidt, National Primate Center for Biology, University of California, who will provide the animals, and by personnel of this unit who will provide serologic measurement of antibodies formed and determine which antibody globulin or globulins are involved in protection.

CONCLUSIONS:

None at this time.

RECOMMENDATIONS.

The continuation of studies using the SAFA technic suggests that a valuable diagnostic antigen for separating active from arrested tuberculosis will be soon available and that humoral antibodies play a significant role in immunity to tuberculosis.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6301	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D.Change (30 06 66)	U	U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER/CODE
62156011 3A025601A822 00 066	None

11. TITLE:
(U) Miscellaneous Microbiological Clinical Research and Support (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
010100 Microbiology	10 64	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	a. DATE b. NUMBER NA c. TYPE d. AMOUNT	PRIOR FY 67	1	45
		CURRENT FY 68	1	47

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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TEL 303 366-5311 X25223 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Hospitals, sanitariums	None

23. KEYWORDS Mycoplasma, viruses, culture media, serology, agar diffusion, antigens, fluorescent antibody, Mycoses, tuberculosis

24. (U) Tech Objective: To evaluate the role of Mycoplasma species and bacterial L forms as etiologic agents of acute and chronic disease. To improve methodology for the isolation and identification of these microorganisms. To study and apply fluorescent antibody technics with purified antigens from fungi.

25. (U) Approach: Patients are being selected by the Medical Service, Fitzsimons General Hospital. Specimens of exudates, sputum, urine, blood swabs and biopsied tissue are cultured on special selective media for the presence of Mycoplasma species or L forms. Cause and effect relationship between the isolated organisms and disease entities are being evaluated by: Isolation of Mycoplasma or L forms from culture specimens; clinical and bacteriologic response to antibiotic therapy; sequential serum antibody titers - using a soluble antigen fluorescent antibody technic quantitation of humoral antibody is being measured employing coccidioidin.

26. (U) Progress: An accumulative total of 73 patients have been studied in the L form, Mycoplasma protocol to date. No evaluation or conclusions have been made. A report of Mycoplasma antibodies in newborn has been published. Scarcity of patients with coccidioidomycosis at this installation has limited the accumulation of statiscally creditable information. As positive sera become available, a more critical evaluation of this method will be conducted.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A02560IA822	Military Internal Medicine
TASK NO.	00	
WORK UNIT NO.	066	Miscellaneous Microbiological Clinical Research and Support

The role of Mycoplasma species and bacterial L forms as etiologic agents of acute and chronic disease is being studied. Patients for this study are being selected by the Medical Service, Fitzsimons General Hospital and specimens of exudates, sputum, urine, blood, swabs and biopsied tissue are cultured on special selective media for the presence of Mycoplasma species and/or L forms. The cause and effect relationship of these organisms and disease entities are being evaluated by: Isolation and identification of Mycoplasma or L forms from the culture specimens; clinical and bacteriologic response to antibiotic therapy; and sequential serum antibody titers. Concurrently studies to improve methodology for the isolation and identification of these organisms is in progress. To date (June 1967) 73 patients have been studied under the L form, Mycoplasma protocol. Mycoplasma sp. have been isolated from 8 patients and L forms from 13. Mycoplasma species isolated have been Mycoplasma hominis, Mycoplasma fermentans, and Mycoplasma salivarium. Bacterial L forms of Pseudomonas, alpha streptococci, Staphylococci and Aerobacter have been reverted and identified. With the exception of the alpha streptococci, no evaluation or conclusions have been made of the results.

BODY OF REPORT

WORK UNIT NO. 066 — Miscellaneous Microbiological Clinical Research and Support

PROBLEM:

To evaluate the possible role of bacterial L forms and Mycoplasma species as the eitiology of various diseases.

Collagen vascular disease, lymphomas, infectious mononucleosis, malabsorptive disease, ulcerative colitis, idiopathic pleural effusions, sarcoidosis, and histiocytosis X are all diseases or groups of diseases whose etiology has not been clearly defined. Each of these has been studied repeatedly, without success, by conventional bacteriologic and serologic techniques in an effort to prove bacterial etiology. Logically, it has been concluded that these entities are not bacterial in origin.

Knowledge gained in recent years reveals the existence of bacterial forms which differ from the parent organism and have been referred to as L forms. The latter differ from the parent form in absence of a cell wall, small size, fastidious culture requirements, and special stains necessary for demonstration. L forms can differ antigenically from the parent form and/or can be changed antigenically by a certain influence like bacteriophage. Under suitable circumstances, L forms can and do revert to the parent form.

Knowledge of the existence of L forms and Mycoplasma raises the possibility that some or all of the above-mentioned diseases are due to these microorganisms. The purpose of this study is to investigate this possibility.

All patients (adult and pediatric) on the Medical Service who have a proved or clinical diagnosis of any of the following entities are being included in the study:

a. Collagen vascular disease to include rheumatic fever, glomerulonephritis, disseminated lupus erythematosis, periarteritis nodosa and its variants (Wegner's Granulomatosis), and rheumatoid arthritis and its variants (Reiter's disease, palindromic rheumatism, intermittent hydrathrosis).

b. Lymphomas to include Hodgkin's disease, reticulum cell sarcoma, giant follicular lymphoma, lymphosarcoma and chronic lymphatic leukemia.

c. Infectious mononucleosis.

d. "Idiopathic" pleural effusions.

e. Malabsorptive diseases to include tropical sprue, regional ileitis and Whipple's diseases.

f. Ulcerative colitis.

g. Sarcoidosis.

h. Histiocytosis X to include Letterer Siwe disease, Hans Schuller Christian disease and eosinophilic granuloma.

All patients selected are being studied microbiologically as follows:

a. Conventional, Mycoplasma, and L form cultures on blood (x3) and urine (x 1) initially and then weekly throughout hospitalization.

b. Conventional, Mycoplasma, and L form culture of any and all biopsy specimens.

c. Initial serum specimen for antibody study and weekly thereafter throughout hospitalization.

d. Dependent upon the clinical or proved diagnosis, patients will have additional studies as follows:

(1) Rheumatic Fever - conventional, Mycoplasma, and L form throat culture initially and weekly thereafter.

(2) Arthritides - conventional, Mycoplasma, and L form culture of joint effusions, conjunctional exudates, and urethral discharges if present.

(3) Glomerulonephritis - conventional, Mycoplasma, and L form culture of urine three times weekly.

(4) Lymphomas - conventional, Mycoplasma, and L form culture of marrow, effusions if present, and sputum if lung involvement is present.

(5) Mononucleosis - conventional, Mycoplasma, and L form throat culture initially and weekly thereafter.

(6) Malabsorptive states and ulcerative colitis - conventional, Mycoplasma, and L form culture of stool initially and twice weekly thereafter.

(7) Idiopathic pleural effusions - conventional, Mycoplasma, and L form culture of pleural fluid and pleura (if needle biospy is done). Sputum will be similarly cultured initially and weekly thereafter.

(8) Sarcoidosis - conventional, Mycoplasma, and L form culture of sputum initially and weekly therafter if lung involvement is present.

Cause and effect relationship between isolated L forms and disease entities is being evaluated by the following:

a. Isolation of L forms or Mycoplasma from culture specimens.

b. Clinical and bacteriologic response to antibiotic therapy.

c. Sequential serum antibody determinations against isolated L forms and Mycoplasma.

Concurrently,, published bacteriologic methods are being evaluated and studies to improve methodology for the isolation and identification of these microorganisms is in progress.

RESULTS AND DISCUSSION OF RESULTS:

To date, 73 patients have been studies under the L form, Mycoplasma protocol. Mycoplasma sp. have been isolated from 8 patients and L forms from 13 . The species of Mycoplasma isolated have been Mycoplasma hominis, Mycoplasma salivarium, and Mycoplasma fermentans. Bacterial L forms of Pseudomonas, alpha streptococci, Staphylococci, and Aerobacter have been reverted to the parent bacterial species. With the exception of the alpha streptococci, no evaluation or conclusions have been made.

CONCLUSIONS:

None at this time.

RE COMMENDATIONS:

That the project be continued.

PUBLICATIONS:

Blair, E. B., Emerson, J. S. and Tull, A. H.: A new medium, Salt Mannitol Plasma Agar, for the isolation of Staphylococcus Aureus, Am. Jour. Clin. Path. 47, 30-39, 1967.

Organic, Avrum B. and Resnick, Abraham: Indirect Hemagglutination Test for Mycoplasma Pneumoniae Employing a Commercially Available Antigen and Adapted to the Microtiter* Technic. USAMRNL, Report 301, Dec. 1966.

Weiser, O. L., Higaki, H. H., and Nolte, L. B.: Mycoplasma Pneumoniae: Complement Fixation Titers at Birth: Am. Jour. Clin. Path. 47, 1967.

RESEARCH AND TECHNOLOGY RESUME		2 GOVT ACCESSION	3 AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6302	CSCRD 103

4 DATE OF RESUME	5 KIND OF RESUME	6 SECURITY	7 REGRADING	8 RELEASE LIMITATION	9 LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U U	NA	NL	A.WORK UNIT

10a CURRENT NUMBER/CODE: 62156011 3A025601A822 00 067

10b PR OR NUMBER CODE: None

11 TITLE: (U) Computer Classification of Pulmonary Disability (06)

12 SCIENTIFIC OR TECH AREA	13 START DATE	14 CMPT COMPL DATE	15 FUNDING AGENCY
003500 Clinical Medicine 002400 Bioengineering, 009800 Med & Hosp Equip	03 61	NA	OTHER | DA

16 PROCURE METHOD	17 CONTRACT/GRANT		18 RESOURCES EST	a PROFESSIONAL MAN YEARS	b FUNDS (In thousands)
	a NUMBER	c DATE			
C. In-House	NA		PRIOR FY 67	1	56
	b TYPE	d AMOUNT	CURRENT FY 68	2	60

19 GOVT LAB INSTALLATION ACTIVITY
NAME
ADDRESS: Headquarters
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TEL: 202 OXford 6 5472

20 PERFORMING ORGANIZATION
NAME
ADDRESS: U.S. Army Med Rsch & Nutr Lab
Fitzsimons General Hospital
Denver, Colorado 80240

INVESTIGATORS PRINCIPAL ASSOCIATE: Syner, J. C., COL

TEL: 303 366-5311 X25130 TYPE: DA

21 TECHNOLOGY UTILIZATION: Computer Industry, Clinical Med., Manpower Disability Compensation

22 COORDINATION: None

23 KEYWORDS: Computer; Medicine; Hospitals; Information Storage; Retrieval; Simulation; Classification; Statistics; Symbolic Logic; Programmed

24 (U) Technical Objective: To design, program for a digital computer, and test run on a production basis a system for classifying disability in patients with pulmonary diseases.

25 (U) Approach: Patient data enters the system from laboratories, clinics and wards serving the evaluation of patients admitted to the Pulmonary Disease Service, FGH. The items of information collected are selected on the basis of their pertinence to the evaluation of diseases of the chest and physical fitness. The information flows into an integrated "computer programming" network which executes all commands for the storage, retrieval, analysis (mathematical and logical) and presentation of information. The execution of the reasoning foundations essential to decision making in classification (numerical taxonomy) is attempted within the computer programming.

26 (U) Progress: An initial classification technique has been programmed and its results are being compared with the actual decision processed by the clinical method. The preliminary evaluation indicates a need for revision in the computer programming to achieve a more approximate simulation of the clinical situation.

27 COMMUNICATIONS SECURITY	28	29 OSD CODE	30 BUDGET CODE
( ) * COMSEC OR COMSEC RELATED (X) NOT RELATED		AR	1

31 MISSION OBJECTIVE	32 PARTICIPATION
CDOG 1412 a	NA

33 REQUESTING AGENCY	34 SPECIAL EQUIPMENT

35 EST FUNDS (In thousands)	36
CPFF:	

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE

ABSTRACT

PROJECT NO.	3A025601A822	Military Internal Medicine
TASK NO.	01	Bio-Medical Investigations
WORK UNIT NO.	067	Computer Classification of Pulmonary Disability

A medical information system is being designed and programmed for a general purpose digital computer. The basic components (sub-systems) of the Master System are the relevant items of clinical information, laboratory data and medical reasoning foundations utilized by physicians in providing health care services to patients with pulmonary diseases.

A mathematical-logical process for ordering the data is being programmed for the digital computer. This process is the classification technique. It has produced an initial classification of disability in patients with pulmonary diseases. The computer derived classification is now being compared with the medical decisions formulated by the clinical system for evaluating patients with pulmonary diseases.

BODY OF REPORT

WORK UNIT NO. 067 Computer Classification of Pulmonary Disability

PROBLEM:

To derive a classification of disability severity in patients with pulmonary diseases which is processed under fully automatic controls in a general purpose digital computer. The classification technique must be based upon a mathematical-logical process which provides objectivity (free of external bias) and standardization to the profiling process.

RESULTS AND DISCUSSION OF THE RESULTS:

The basic reasoning foundation which guides the approach to the problem is that a total information system for classifying disability in patients with pulmonary diseases is one of enormous complexity. Therefore, it is clearly necessary to subdivide the problem that it represents into several parts. The method adopted for this problem is that the total information system can be viewed as made up of parts which, for design purposes, can be viewed as independent, elementary units. Within this concept we view the structure and functioning of each elementary unit individually and independently. The summary phase of the problem will involve the design of "logical-linkages" which organize the elements into a whole such that the functioning of the whole is a natural expression of its elements.

The following components (sub-systems) of a central medical information system required to evaluate patients with pulmonary diseases have been designed as systems procedures and authored into computer programs for the RCA 301 digital computer:

1. Data collection input from clinics and laboratories generating information pertinent to the evaluation of patients with pulmonary diseases.

2. A file maintenance system for updating clinical information and laboratory data on patients which is processed on a daily basis under fully automated computer controls.

3. The production of Clinical Information Report Forms, Medical Summaries and Patient Data Records for feed-back to physicians and visual edit and review.

4. A Population System which prepares data into prescribed sets and classes for clinical and statistical analysis.

5. A Statistical Analysis System which prepares and processes data for prescribed numerical analyses and presents the results in report forms for feedback to physicians and investigators.

Computer Classification of Pulmonary Disability (Cont'd)

6. A Natural Language System to read and write Medical Summaries.

7. A Classification Techniques which prepares data for mathematical ordering through two basic numbers of enumeration; M (A,B) and N (A,B), which represent counts of ∅, 1 sequences according t the configurations (1, 1) and (∅, 1) respectively. These provide a measure of similarity.[1]

8. A remote control data linkage system between USAMRNL, Denver, Colorado and Valley Forge General Hospital, Phoenixville, Pa. Transmission - receiving terminals will be located at the medical facilities in Colorado and Pennsylvania. The initial phase of the project will involve the following categories of information: Spirometry measurements; the lung volume and its subdivisions; Arterial Blood Gases, Lung diffusing capacities; Respiratory Disease Questionnaires; Smoking Histories; Physical examination; Respiratory History.

These listed components are currently in various stages of operational processing and debugging. Component No. 1 is nearly completed in terms of inclusive coverage of the items of information required to evaluate patients with pulmonary diseases. Component No. 2 must be regarded as "open-ended" from the viewpoint that the perfect system is never realized, and the existing process can always be corrected and improved upon. Component No. 3 requires considerable expansion, but is currently providing a number of report forms to physicians at FGH on a daily basis. Component No. 4 has been significantly improved and has been brought under more fully automatic controls through the design and definitive authoring of computer programming. Component No. 5 has been expanded to include additional procedures for numerical analysis and the simultaneous handling of quantitative and qualitative data through the Kronecker Statistic. Component No. 6 is a very difficult sub-system to express freely in computer programming; it has, however, been improved in its capability to author more expressive English syntax and achieve more meaningful semantics. Component No. 7 has produced an initial ordering of disability severity in patients with pulmonary diseases and this computer (internal) derived classification is now being compared with medical decisions formulated by the existing manual hospital (external) system for evaluating patients with pulmonary diseases. Component No. 8 has been designed, the computer systems programming authored, and the study scheduled for experimental implementation in FY 1968.

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[1] Tanimoto, T., and Lomis, R. G., The Application of Computers to Clinical Medical Data. IBM Medical Symposium, June 15-17, 1959.

Computer Classification of Pulmonary Disability (Cont'd)

CONCLUSIONS:

The design and programming of a computer processed medical information system to provide a classification of disability severity in patients with pulmonary diseases has progressed in a sequential manner. Additional actions of the Pulmonary Disease Service, FGH have been phased into daily operational computer runs and provide an improved interface between the clinical environment and the computer technology. The availability of the total information system, operating on a daily basis to provide assistance to physicians in meeting the requirements of health care services, is beginning to fall within "vision's range", and the time of fruition is approaching.

ABSTRACT

PROJECT NO.	3A025601A822	Military Internal Medicine
TASK NO.	01	Bio-Medical Investigations
WORK UNIT NO.	068	Computer Instrument Linkage

The following investigations have been conducted under this work unit:

STUDY NO. 1 Continuous Oxygen Consumption Under Steady State Exercise

STUDY NO. 2 Xenon linear scanner measurement of regional ventilation perfusion relationships of the lung

An automated system to effect analoge to digital linkages of instruments utilized in clinics and laboratories at Fitzsimons General Hospital and the U. S. Army Medical Research and Nutrition Laboratory is under study and development.

BODY OF REPORT

WORK UNIT NO. 068 — Computer Instrument Linkage

STUDY NO. 1 — Continuous Oxygen Consumption Under Steady-State Exercise

PROBLEM:

To develop the electronic systems and digital computer programming required to place under fully automatic controls the processing of information derived from energy expenditure studies of subject exercising on a motor driven treadmill.

RESULTS AND DISCUSSION OF THE RESULTS:

This "analoge to digital data conversion" system has been established within the Bioenergetics Division and has been field tested on a ten channel "continuous oxygen consumption analysis system" in parallel with the normal strip chart recording. Considerable progress has been made in developing sub systems which will eventually carry the data from the recording instruments to final oxygen consumption data without manual intervention. These sub systems include: 1) Quality Control procedures; 2) Storage of multichannel recorded data; 3) Retrieval, conversion and scaling of derived digital data; 4) Averaging of read-outs over time; 5) Calculation of oxygen consumption, carbon dioxide production; and related paramenters required to describe energy expenditure; 5 Master file formats for storage of computed values; and 6) Signal transmission handling systems.

Due to the absence of the principal investigator to attend advanced university training and education for a one-year period, this project has been carried on a status quo processing asis.

CONCLUSIONS:

Two broad categories of expansion are required: 1) Signal transmission handling; 2) Digital Computer programming to effect information processing.

STUDY NO. 2 — Xenon linear scanner measurement of regional ventilation perfusion relationships of the lung

PROBLEM

To develop equipment for the study of pulmonary ventilation perfusion relationships and effect its utilization in the evaluation of patients with pulmonary disease. To effect "analoge to digital conversion systems" for linkage to a digital computer.

Computer Instrument Linkage (Cont'd)

RESULTS AND DISCUSSION OF THE RESULTS:

The Xenon linear scanner measurement of regional ventilation perfusion relationships of the lung has progressed satisfactorily. Fifteen patients with varying pulmonary diagnoses have been examined on the linear scanner and good data have been obtained in these persons. In an equal number of patients the data was not acceptable for inclusion in this study due to the patient's inability to cooperate or due to machine failure. Nine patients with thyroid carcinoma have been studied with the whole body scanner in an attempt to exploit some of the unique advantages of this scanning system. Initial results look encouraging but more definitive work is yet required particularly in the areas of technique and data presentation-reduction. Twenty-three patients have been studied using SR^{85} and serial whole body profile scans. This particular technique is well developed and has proved very useful as a screening procedure in localizing metastatic bone lesions prior to detailed scanning.

During the past year a small-scale limited purpose analoge computer was obtained. The instrument greatly facilitates data handling and the computation of final results

In the past 6 months discussions with investigators at McGill University, University of Kentucky and Barnes Hospital, St. Louis, Mo., have introduced some doubt as to the validity of the single breath method of measuring ventilation perfusion ratios. Other investigators using similar equipment to ours, particularly McGill University, have demonstrated that it is very difficult to achieve reproducible results using a linear scanner and the single breath method. The linear scanner single breath method is more difficult to use than the multiprobe method, but with careful attention to detail it can be used and meaningful information obtained.

CONCLUSIONS:

The general techniques of data acquisition, recording and presentation have been standardized. Development of an automatic digital computer linkage remains a problem.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6305	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	RPT U WRK U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER/CODE
62156011 3A025601A822 00 070	None

11. TITLE:
(U) The Effects of INH on Animal Histology (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
016800 Toxicology 002600 Biology, 012600 Pharmacology	04 63	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	18. RESOURCES EST.	a PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	a. DATE b. NUMBER NA c. TYPE d. AMOUNT	PRIOR FY 67	1	30
		CURRENT FY 68	1	31

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME ADDRESS Headquarters U.S. Army Med Rsch & Dev Cmd Washington, D. C. 20315 RESP. INDIV. Davis, T. E., MAJ TEL 202 OXford 6 5472	NAME ADDRESS U.S. Army Med Rsch & Nutr Lab Fitzsimons General Hospital Denver, Colorado 80240 INVESTIGATORS PRINCIPAL ASSOCIATE Jones, L. D., Morse, W. C. COL Stedham, M. A. MAJ TEL 303 366-5311 X23230 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Tuberculosis therapy	None

23. KEYWORDS Lung; mice; Oncology; Isoniazid; Tuberculosis; Pathology; Carcinogenicity Tumor, toxicity

24. (U) Technical Objective: To ascertain the effect of isoniazid (INH) on murine pulmonary histology, principally to investigate the potential pulmonary carcinogenicity of isoniazid in mice.

25. (U) Approach: Reports of studies made indicated that INh is a pulmonary carcinogen when administered to mice for long periods. Two strains of mice, both sexes, and in sufficient numbers to provide statistical significance, were used. Normal saline or INH was given parenterally to BalbC and Strong-A mice at varied dose levels. In other tests the drug was added to the diet and an equal number maintained as controls. Necropsies and thorough histopathologic examinations included serial sections of lungs to evaluate the incidence of lung neoplasia.

26. (U) Progress: Phases 1 and 2 were completed. Phase 3 (examinations, study, and evaluation of results) is in process. Preliminary and partial results indicated confirmation of the early reports. A significant increase in lung tumor incidence was seen in mice receiving INH by all routes and amounts over an equal number of controls, and a dose-incidence relationship was indicated. INH was not well accepted when given in the ration as indicated by weight performance. This was not influenced by experimental additions of glycine or of glutamic-acid-arginine. INH produced severe reactions and deaths in BalbC mice believed due to biological and strain variation. Supplemental pyridoxine intensified the results and appeared to potentiate sensitivity or toxicity. In one test of mice given INH there was a significant increased incidence of lung tumors in the females over the males.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.:	3A025601A822	Military Internal Medicine
TASK NO.:	01	Bio-Medical Investigations
WORK UNIT NO.:	070	The Effects of INH on Animal Histology

PURPOSE:

To ascertain the effect of isoniazid (INH) on murine pulmonary histology, to evaluate the potential pulmonary carcinogenicity of isoniazid in mice.

METHODS BY WHICH ACCOMPLISHED:

Reports of previous studies indicated that INH is a pulmonary carcinogen when administered to mice for long periods. Two strains of mice, both sexes, and in sufficient numbers to provide statistical significance, were used. Normal saline or INH was given parenterally to BalbC and Strong-A mice at varied dose levels. In other tests the drug was added to the diet and an equal number maintained as controls. Necropsies and thorough histopathologic examinations included serial sections of lungs to evaluate the incidence of lung neoplasia.

SUMMARY OF RESULTS:

Phases 1 and 2 were completed. Phase 3 (examinations, study, and evaluation of results) is in process. Preliminary and partial results confirm the early reports. A significant increase in lung tumor incidence was seen in mice receiving INH by all routes, over an equal number of controls. A dose-incidence relationship was indicated. INH was not well accepted when given in the ration as indicated by weight performance. This was not influenced by the addition of glycine or of glutamic-acid-arginine. INH produced severe reactions and deaths in BalbC mice believed due to biological and strain variation. Supplemental pyridoxine intensified the results and appeared to potentiate sensitivity or toxicity. In one test of mice given INH there was a significantly increased incidence of lung tumors in the females over the males.

BODY OF REPORT

WORK UNIT NO.: 070 The Effects of Isonicotinic Acid Hydrazide (INH) on Animal Histology

PROBLEM:

1. Isonicotinic acid hydrazide (INH) is one of the drugs of choice in the treatment of tuberculosis in man and animals. Its antituberculosis activity is comparable to streptomycin. Its virtues include easy administration, availability, and low cost. The therapeutic use of INH is not without certain perils. Like all other antituberculosis drugs, it must be employed continuously throughout long periods of time. It has been recognized as a pyridoxine antagonist and as such it is a neurotoxic drug capable of producing peripheral neuritis, convulsive seizures, and toxic psychoses. It has also been implicated in the production of toxic hepatitis, jaundice, and generalized allergenic toxicities. Despite these and other lesions INH is by far the most useful antituberculosis drug of the day both economically and therapeutically.

2. Reports by other investigators credit this chemical with a high order of pulmonary carcinogenicity in mice when administered orally or parenterally for prolonged periods. In all the experiments only relatively small numbers of mice were used and only one sex (female) was employed. Furthermore, study of the reports reveals that the weight performance of the mice was not good in those experiments where the drug was administered orally by incorporation into the diet. This study was designed to more critically evaluate the production, pathogenesis, and incidence of pulmonary neoplasia in mice given INH. In comparison with those reports in the literature this study was enlarged to include both sexes and a large number of experimental animals, with an equal number of controls for the purpose of securing sound statistical design. Two strains of mice were included for strain susceptibility studies. In consideration of the pathogenesis, comparisons will be made of the different modes of administration, and the various aspects and circumstances will be studied in the search for contributing and influencing factors.

The study was divided into three main phases:

I. Breeding of the required number and strains of mice.
II. Administration of test and test control materials, and
III. Study and evaluation of the results.

RESULTS AND DISCUSSION OF RESULTS:

1. Phase I and II have been completed. In conducting the tests in Phase II the mice were 7-8 weeks of age and weighed 20-25 grams when put on

experiment. Test and control materials were administered for a period of 18 weeks. Parenteral administrations were made every other day. Physiological saline (0.9% sodium chloride) solution (PSS) was given the test control animals. Oral administrations were accomplished continuously by incorporating test materials into the diet. The test control animals in these cases received the identical diets minus test materials. Following the test period, the mice were held for 12 weeks for observation. Body weights were obtained one or two times each week throughout the test and observation periods. Following the observation period, the survivors were killed, a complete necropsy performed, and histopathological studies were made of all organs. Particular attention was paid to the lungs. Those lungs without gross evidence of a neoplasm were completely serially sectioned at 10 microns, mounted on film strips, and stained with hematoxylin and eosin for histopathological study. About every 10th section was mounted on glass slides for similar processing. Neoplasms of the lung which were observed at necropsy were subjected to routine histopathologic examination.

2. There were six foundation tests outlined in the basic protocol as follows:

Test 1.	A. to Strong-A mice: 0.2 ml. 1% INH sol.	subcut.
	B. to Strong-A mice: 0.2 ml. PSS	"
Test 2.	A. to BalbC mice: 0.2 ml. 1% INH sol.	"
	B. to BalbC mice: 0.2 ml. PSS	
Test 3.	A. to Strong-A: 0.2 ml. 0.25% INH sol.	"
	B. to Strong-A: 0.2 ml. 0.125% INH sol.	"
	C. to Strong-A: 0.2 ml. PSS	"
Test 4.	A. to BalbC: 0.2 ml. 0.25% INH sol.	"
	B. to BalbC: 0.2 ml. 0.125% INH sol.	"
	C. to BalbC: 0.2 ml. PSS	
Test 5.	A. to Strong-A: (in food) INH	orally
	B. to Strong-A: (in food) No INH	
Test 6.	A. to BalbC: (in food) iNH	"
	B. to BalbC: (in food) No INH	

The Effects of Isonicotinic Acid Hydrazide (INH) on Animal Histology (Cont'd)

3. Phase III. Examinations and studies have been completed on all the tissues of the mice on the following tests:

Test 1. A. INH
B. PSS

Test 2. A. INH
O. No treatment: environmental controls

Test 3. C. PSS
D. (added to replace 3. A and 3. B) This test duplicated Test 1 employing Strong-A mice, but they were given 0.2 ml. 0.2% INH solution in the same manner.
O. No treatment: environmental controls

Test 4. Omitted because of difficulties encountered in Test 2 owing to sensitivity to BalbC mice to the drug.

Test 5. A. INH
B. No treatment
A. 1. INH plus arginine and glutamic acid supplement
A. 2. INH plus glycine supplement.

4. Evaluation and analysis of results are in progress. Partial results were published in the last previous report.

SUMMARY AND CONCLUSIONS:

Studies by several investigators indicate that INH can induce a high incidence of pulmonary neoplasm in susceptible animals. This is especially true in mice when INH is administered orally or parenterally for long periods. Incomplete analyses of the results of these tests indicate (1) a drug dose-tumor incidence relationship, (2) mouse strain differences in drug sensitivity, and (3) possibly sex variation in drug-tumor production. Standard mouse food with added INH was very poorly accepted thus creating an unfavorable experiment for comparison studies with the parenteral route of administration.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY RESUME	1	2 GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6306	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U RPT U WRK	NA	NL	A.WORK UNIT

10a CURRENT NUMBER/CODE	10b PRIOR NUMBER CODE
62156011 3A025601A822 00 071	None

11. TITLE

(U) Intravenous Fat Emulsions (06)

12. SCIENTIFIC OR TECH AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
003500 Clinical Medicine 002300 Biochemistry, 012600 Pharmacology	06 53	NA	OTHER | DA

16. PROCURE METHOD	17. CONTRACT GRANT a. DATE b. NUMBER c. TYPE d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	1	18
		CURRENT FY 68	1	31

19. GOV'T LAB INSTALLATION ACTIVITY	20. PERFORMING ORGANIZATION
NAME ADDRESS Headquarters U.S. Army Med Rsch & Dev Comd Washington, D. C. 20315 RESP INDIV Davis, T. E., MAJ TEL 202 OXford 6 5472	NAME ADDRESS U. S. Army Med Rsch & Nutr Lab Fitzsimons General Hospital Denver, Colorado 80240 INVESTIGATORS PRINCIPAL ASSOCIATE Jones, L. D. DVM TEL 303 366-5311, X 26122 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Clinical Medicine, Pharmaceutical Industry	None

23. KEYWORDS **Fat Emulsions; IV Fat; Lipids; Oils; Fatty acids; Balance-Metabolic; Toxicology; Lipid Metabolism; Parenteral Feedings; Phosphatide; Pathology; Electron microscopy**

24. (U) Technical Objective: To produce a non-toxic intravenous preparation which will provide a relatively high caloric intake per milliliter of solution for patients unable to assimilate sufficient nourishment.

25. (U) Approach: The high caloric density and the absence of osmotic effect of fat emulsions offer a number of theoretical advantages for intravenous nutrition not possessed by carbohydrate or amino acid solutions. Various experimental and commercial emulsions and their components are being evaluated by animal testing. Observations include changes in the clinical status, renal function, hepatic function, and hematological state following emulsion administration plus detailed necropsy and histopathological examination including histochemical and electron microscopy studies of liver and spleen tissues.

26. (U) Progress: Detailed clinical and histopathological toxicity studies were conducted on six newly developed experimental fat emulsions prepared commercially or experimentally for intravenous administration. These emulsions were administered to both rabbits and rats. This laboratory continued to perform histopathological and electron microscope examinations of tissues from animals utilized in various parenteral nutrition studies performed at other institutions. Studies have failed to establish any relationship with any specific emulsion component, or methods of their preparation, and the development of the intravenous fat pigment deposition following multiple infusions of fat emulsions. Most pigment was found in tissues from test animals given cottonseed oil emulsions. Parenteral tocopherol has been added to the experimental dietary regimen; evaluation of its effect is not complete.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. CONSEC OR CONSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A025601A822	In-House Laboratory Independent Research
TASK NO.	02	Metabolism and Nutrition
WORK UNIT NO.	071	Intravenous Fat Emulsions

PURPOSE OF WORK:

To produce a non-toxic intravenous preparation which will provide a relatively high caloric intake per milliliter of solution for patients unable to assimilate sufficient nourishment by oral feeding.

METHODS BY WHICH ACCOMPLISHED:

The high caloric density and the absence of osmotic effect of fat emulsions offer a number of practical advantages for intravenous nutrition not possessed by carbohydrate or amino acid solutions. Various experimental and commercial emulsions and their components are being evaluated by animal testing. Observations include changes in the clinical status, renal function, hepatic function, and hematological state following emulsion administration. Detailed necropsy and histopathological examination including histochemical and electron microscopy studies of liver and spleen tissues have been accomplished.

SUMMARY OF RESULTS AND CONCLUSIONS DERIVED:

Detailed clinical and histopathological toxicity studies were conducted on 3 newly developed experimental fat emulsions prepared experimentally for intravenous administration. These emulsions were administered to both rabbits and rats. This laboratory continued to perform histopathological and electron microscope examinations of tissues from animals utilized in various parenteral nutrition studies performed at other institutions. Studies have failed to establish any relationship with any specific emulsion component, or methods of their preparation, to the development of the intravenous fat pigment deposition following multiple infusions of fat emulsions. Most pigment was found in tissues from test animals given cottonseed oil emulsions. Parenteral tocopherol has been added to the experimental dietary regimen, but evaluation of its effect is not complete.

BODY OF REPORT

WORK UNIT NO.:	071	Intravenous Fat Emulsions
STUDY NUMBER:	1	To Produce a Non-toxic Intravenous Preparation Which will provide a Relatively High Caloric Intake per Milliliter of Solution for Patients Unable to Assimilate Sufficient Nourishment

PROBLEM:

The treatment of malnutrition from illness and disease includes battle casualties, burn patients, radiological casualties, and certain surgical and medical cases requiring maintenance of adequate nutrition to support therapeutic measures and healing processes is in progress. The patients incapable of ingesting their required nutrients present an important problem. The goal of the Surgeon General's Intravenous Nutriment Program is to secure a high caloric, protein-sparing, stable, liquid product for intravenous administration. Todate emulsions of fat offer the most promise towards fulfilling the critical requirements necessary for support of these patients. In support of this program, this laboratory has conducted a research program to ascertain the toxicity of experimental intravenous emulsions. The work includes studies of clinical, histopathological, ultrastructural, and chemical changes in test animals, and those aspects of lipid transport and metabolism which influence, or are influenced by, infusions of fat emulsions.

RESULTS AND DISCUSSION OF THE RESULTS:

1. Major areas in which work was accomplished during the past year include:

(1) Toxicity testing in animals of experimental emulsions and emulsion components.

(2) Histopathological studies of tissues from animals involved in the toxicity testing program. This is a support function provided to the investigator members of the I. V. Fat Toxicity Testing Group, and it has been utilized by investigators at (a) Vanderbilt University, Nashville, Tennessee, (b) Karolinska Institute of Health, Stockholm, Sweden, (c) Vitrum AB, Stockholm, Sweden, and (d) this laboratory.

(3) Physical and chemical studies to identify the previously described intravenous fat pigment.

Intravenous Fat Emulsions (Cont'd)

2. The testing of experimental emulsions has been performed utilizing rabbits and rats. Test emulsions were infused intravenously with 15 ml/kg body weight once daily for five days each week for three weeks. An appropriate pre-infusion adaptation period was observed. Before, during, and after the infusion weeks, extensive observations and clinical pathological tests were made to evaluate response status. During the third post-infusion week a detailed necropsy was performed followed by histopathological studies of tissues. Utilizing these procedures, the following products have been tested:

(1) SR-207 - Prepared by W. S. Singleton of U.S.D.A. Southern Utilization Research and Development Division, New Orleans, Louisiana, containing 20% adsorbent fractionated cottonseed oil, and 0.6% egg yolk phosphatide in distilled water. The product was subjected to oxidation procedures and covered with nitrogen gas.

(2) SR-208 - The same as above but exposed to a higher degree of oxidation procedures and covered with nitrogen gas.

(3) SRRL-Batch 101 - Prepared in the same laboratory and containing 10% decolorized cottonseed oil, 1.5% egg lecithin, and 2.0% glycerol in distilled water, and covered with nitrogen gas.

The results of the tests conducted were charted for study. During the course of the infusions those rabbits on SR-207 and SR-208 lost weight during the first infusion week while those on SRRL emulsion gained a slight amount of weight. During the remainder of all the test periods average body weight gains were suppressed to a very low order of magnitude during the infusion periods. The major portion of the gains in weight were accomplished during the two-day weekend rest periods. In all cases food consumption dropped during the infusion period and in general corresponded with the weight performances. Mild to moderate thermogenic responses were produced by the infusions but all returned to normal levels by the following morning. One rabbit died 14 days after receiving the last infusion of SRRL. None of the products tested produced significant alterations in the packed cell volume, total red blood cell counts, and total hemoglobin of rabbits and rats to which they were administered. Administration of the emulsions to rats did not produce any recognizable adverse clinical results.

Histopathological studies of tissues from the test animals receiving fat emulsions revealed the presence of intravenous fat pigment and associated lipoid granulomas in organs in association with their reticuloendothelial components. The amount of pigment and severity of lesions

paralleled results obtained in previous trials employing similar products utilizing cottonseed oil. The studies are not completed on the tissues from animals which received emulsion SRRL.

Tissues received from Karolinska Institute and Vitrum AB of Stockholm, Sweden were from 8 dogs which had received varying amounts of Intralipid of various composition, age, and supplementation with tocopherols. Evaluation of these tissues has not been completed.

Tissues received from Vanderbilt University were from rats which had received Intralipid supplemented with vitamin E or choline. Histopathological studies of these tissues did not establish any fixed relationship between the various supplements employed and the deposition of intravenous fat pigment with associated lesions. In connection with these studies a large number of tissues were recut, processed, and studied for reevaluation.

CONCLUSION:

1. Seven different emulsions of either cottonseed or soybean oil were administered to rats, rabbits, or dogs. Three were administered to both rats and rabbits. In one study, the soybean oil emulsion also contained vitamin E, or choline. Vitamin E was also present in one of the cottonseed emulsions.

Histopathologically, lipid granulomas and intravenous fat pigment were present in the tissues of all animals receiving the emulsions; the degree of involvement was less in those receiving the soybean preparations.

2. No correlation could be made between either concentration of lipid in the emulsion, or presence of additives (vitamin E, choline) and presence of granulomas or pigment.

3. Future studies in this division will be directed toward the role of auto-oxidizable lipids and the production of pigment, and the effect of supplemental parenteral tocopherol.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6307	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (15 01 67)	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER CODE
62156011 3A025601A822 00 072	None

11. TITLE:
(U) Studies in Human Nutrition (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
000800 Agri. Economics; 002300 Biochem.; 003500 Clin. Chem	03 56	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	1	65
		CURRENT FY 68	1	82

19. GOV'T LAB/INSTALLATION/ACTIVITY
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INVESTIGATORS PRINCIPAL ASSOCIATE Sauberlich, H. E.
Baker, E. M. LTC

TEL 303 366-5311 X24214 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Medicine, Agriculture	None

23. KEYWORDS Nutrition, medicine, nutr.disorders, survey, nutr.surveys, vitamins, deficiency diseases,protein,protein deficiency,thiamine,riboflavin,vit.C,pyridoxine,folacin,vit.A

24. Technical Objective: To study the nutritional status of populations, either domestic or foreign, for the primary purpose of appraising and improving the nutritional status of the population concerned. To further study tne metabolism and requirements of vitamins B-1, B-6, C and B-2 in the human, as well as ascertaining interrelationships between the various vitamins and attempting to find a state of balance of optimal vitamin intake.

25. (U) Approach: To participate as members of a team organized for the on-site gathering of information pertaining to the nutritional status of a country or a population group. Data are then analyzed, compiled into reports and recommendations for the improvement of the nutrition of the population studies are made. The study of vitamin metabolism and requirement will be accomplished by the use of either carbon-14 or tritium-3 labeled vitamins in order to determine turnover, pool size, catabolic fate and requirement.

26. (U) Progress: During the past year, a study has been completed in conjunction with the University of Iowa on human vitamin C metabolism and requirement using carbon-14 labeled L-ascorbic acid. Daily minimal and optimal vitamin C requirements have been established for the adult male. Many other biochemical parameters were studies and will be reported in detail. Additionally, several human vitamin B-1 and B-6 metabolism and requirement studies have been completed. A second long-term vitamin C depletion-repletion study will be undertaken with the University of Iowa, commencing on 1 September 1967. Research assistance in Thailand, Honduras and Panama was provided.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A025601A822 Military Internal Medicine

TASK NO. 01

WORK UNIT NO. 072 Studies in Human Nutrition

The following investigations have been conducted under this work unit:

STUDY NO. 1 Ascorbic acid metabolism and requirement in the human male

STUDY NO. 2 Ascorbic-4-^{3}H acid metabolism in man

STUDY NO. 3 Further studies on the vitamin B_6 requirements of young adult male humans

STUDY NO. 4 Thiamine metabolism and requirements

STUDY NO. 5 Metabolism of ^{14}C-labeled cotton cellulose by man

STUDY NO. 6 Other nutrition activities, national and international

1. The preliminary phase of a study on the production of experimental human vitamin C deficiency is completed. The objective of the study was to induce scurvy in healthy men under controlled conditions in an attempt to evaluate the human requirement for this vitamin and to study its metabolism and dietary interrelationships. Numerous observations have been reported as a result of this initial study.

2. Vitamin C metabolism in man was also studied with the use of L-ascorbic-4-^{3}H acid. It was observed that the radioactive label does not enter the body water pool but, instead, is excreted as organic-bound tritium. The results of these studies indicate the presence of more than one kinetically distinguishable pool in the metabolism of ascorbic acid. The unknown metabolites observed are possibly derivatives of L-threose or L-threonic acid.

3. The results of a recent experiment in a series of studies on the human requirement for vitamin B_6 demonstrated that there is no apparent effect of increased caloric utilization upon vitamin B_6 requirement. Under the conditions of this experiment, the minimum daily requirement of the young adult male human for vitamin B_6 is 1.25 milligrams.

4. Additional studies were conducted on thiamine metabolism and requirements. The yeast method for measuring some of the metabolites of thiamine in urine appears to offer a specific assay for thiamine nutriture in the human. The effect of caloric intake and exercise on the thiamine requirement of the human

has been investigated in a study employing adult males. Preliminary evaluation of the data indicates that increased caloric expenditure, employing carbohydrate as the source of calories, increases the human requirement for thiamine.

5. ^{14}C-labeled cotton cellulose was modified to form microcrystalline ^{14}C-cellulose (Avicel-R). This material has been fed to a volunteer human subject in an attempt to determine whether or not the human is capable of utilizing cellulose. Although the study is not entirely completed, evidence would indicate that the human cannot utilize cellulose to any significant degree.

6. Continuing support was provided national and international nutrition agencies and their basic or applied nutrition programs and training activities.

BODY OF REPORT

WORK UNIT NO. 072 — Studies in Human Nutrition

STUDY NO. 1 — Ascorbic acid metabolism and requirement in the human male

PROBLEM:

The preliminary phase of a study on human scurvy was completed on 31 May 1967. This study, a joint project between the USAMRNL Chemistry Division and the Metabolic Ward at the University Hospitals, University of Iowa, has led to exciting new concepts of vitamin C requirements and metabolism in man. Dr. R. E. Hodges of the Department of Internal Medicine, University Hospitals, is the chief medical investigator for this study. The objectives of these studies are as follows:

1. To induce in healthy men (prison volunteers) a deficiency of ascorbic acid.

2. To label their ascorbic acid pools with L-ascorbic-1-^{14}C acid in order to study (a) total body pool size; (b) rate of depletion; (c) minimal requirements for vitamin C; (d) the relationship between symptoms and signs of scurvy and levels of the vitamin body pool; (e) the amount of ascorbic acid necessary to replete the body pool and to alleviate signs and symptoms of deficiency; and (f) theories concerning the physiologic functions of ascorbic acid based on metabolites in blood and urine.

RESULTS AND DISCUSSION OF THE RESULTS:

The pilot study began with six healthy men (prison volunteers from Iowa State Penitentiary) who were housed on the Metabolic Ward of University Hospitals and were fed a diet totally deficient in vitamin C, but adequate in all essential nutrients and containing sufficient calories to maintain body weight. This diet, a formula composed of vitamin-free casein, purified carbohydrates and fats, was fed in three equal portions daily through a naso-gastric tube. The formula was continued throughout depletion and for the first 15 days of the repletion period. On the 99th day, repletion with vitamin C was commenced, and on the 115th day the diet was changed to solid foods. This diet, composed of a soybean food, casein products, fats and carbohydrates, was found by thin-layer chromatographic assay to furnish only 2.5 mg of ascorbic acid daily.

WORK UNIT NO. 072 - BODY OF REPORT

Throughout the study, detailed clinical, biochemical and radioisotopic studies were performed. Photographs were taken of the eyes, mucous membranes and skin at regular intervals.

To date, only a small portion of these results is available for interpretation, but those which are available are shown below:

1. Depletion Period. Following the administration of 1.3 mg of L-ascorbic-1-^{14}C acid containing 39.77 μc to each subject, the ^{14}C-ascorbate metabolites excreted were followed daily. From the total cumulative plot of the daily radiocarbon excretion, it appeared that more than 97% of the ^{14}C-labeled ascorbate was absorbed and retained in a deep "physiologic pool" by all subjects. Once the incorporation of the ^{14}C-labeled ascorbate had taken place, the rate of urinary ^{14}C-labeled organic metabolites of ascorbate occurred as a logarithmic function at a rate of one-half of one per cent of the total pool of ascorbic acid per day. This implies that the catabolism of ascorbic acid from the "physiologic pool" is a first order exponential process; therefore, the summation of all metabolic products derived from this pool must also be a first order process.

Estimates were made of the initial pool size and the rate of utilization of vitamin C in each man. By the 99th day of depletion, the average man had reduced his pool size to approximately 170 mg, with a utilization rate of 2.5 mg daily. Had the project been continued for an additional 32 days, the vitamin C pool size would have been depleted to less than 100 mg total, with a daily utilization of 1 mg; at that point, frank and possibly dangerous scurvy might have ensued.

2. "Physiologic Ascorbate Pool." The exact chemical nature of this pool is as yet unknown. We do know that the organic ^{14}C-labeled material is not L-ascorbic acid or any of its commonly recognized derivatives. The urinary metabolites of this physiologic ascorbate pool are water soluble and chemically stable. We have been able to isolate, on chromatographic columns, some of these materials. Thus far, it appears there are two major metabolites and three minor ones. Work is in progress to isolate and identify the chemical nature of these compounds. The two major metabolites have been found to be reproducibly homogeneous in three different solvent systems, thus indicating that they constitute the major metabolites of this pool. Even though chemical identification of these compounds in the urine has not yet been accomplished, we believe that characterization of them will not explain the physiologic functions of vitamin C. Identification of these compounds will, however, direct attention to other systems which must be studied in order to understand the physiological functions of vitamin C.

3. Repletion Period. After a depletion period of 99 days, during which the diet contained no ascorbate, each of the men was repleted with labeled ascorbic acid in amounts of 4, 8, 16, or 32 mg daily. The administered ascorbic acid had a specific activity of 0.05 μc/mg, and permitted tabulation of a cumulative material balance for each subject throughout the repletion period. The subject who received the 8 mg daily supplement did so for a period of 57 days, and then was placed on an intake of 64 mg daily for a period of 20 days. The rate of repletion in all subjects was a zero order linear function of the intake of ascorbate. The data indicated that the rate of incorporation into the "deep pool" occurred at the rate of 80-90% of the administered dose within the range of 4-64 mg intake daily. Repletion continues until the "deep pool" becomes fully saturated. During the repletion phase, there was no free ascorbic acid excreted in the urine until the "deep pool" had been repleted fully. Once this occurred, urinary spilling of ascorbic acid did take place and the rate of incorporation of ascorbic acid into the deep pool fell accordingly. Once urinary spillage of ascorbic acid occurred in any of the subjects, labeled ascorbate supplementation was discontinued and the subject then received the same level of ascorbate supplementation as "cold" ascorbate.

4. Minimal and Optimal Ascorbic Acid Requirements. Preliminary data indicate, as of the 97th day of repletion, that the minimal daily requirement of ascorbic acid necessary to prevent scurvy is approximately 3.8 mg (as compared with the British estimate of 3 to 4 mg daily). The data further imply that the optimal daily intake would be 10 mg if we assume that the physiologic pool is already saturated. This is in accord with the observation that the rate of depletion is an exponential process occurring at the rate of 0.5% daily and that the average subject has a saturated pool size of 2,000 mg. Thus, the normal rate of catabolism would be 10 mg daily.

It was of interest to note that simple counting of radiocarbon-labeled products in the urine each day provides the best indication of a physiologic, metabolic, or emotional stress as these influence vitamin C catabolism. As an example, one of the subjects underwent a 21-day period of severe emotional stress. His emotional stress was clearly reflected in an increased rate of excretion of ascorbate metabolites, an observation that strongly suggested an increased requirement for ascorbate under these conditions.

5. Other Vitamin Interrelationships. Additional interesting observations have been noted that relate to other essential nutrients. It was observed that the level of vitamin A in serum of these men (who were receiving supplements of 5,000 IU of vitamin A daily) fell approximately 20 to 30% during the deficiency period and began to rise again during repletion with vitamin C. In

addition, there were changes in the nonpolar lipids of the erythrocytes which suggest the possibility of an effect on vitamin E metabolism resulting from an ascorbic acid deficiency (despite an intake of 80 mg of alpha-tocopherol daily). Also, there may be a relationship of vitamin C to pyridoxine metabolism, since the subjects were found to have a three-fold increase in the rate of urinary excretion of "free" pyridoxine during an ascorbic acid deficiency. These subjects were on a constant daily intake of 1.7 mg of pyridoxine during the entire experiment.

6. Clinical Observations. During the deficiency period, three of the four men complained of fatigue and mild malaise. All four developed some degree of follicular hyperkeratosis. Hemorrhagic manifestations included the appearance of a positive Rumple-Leeds test (petechiae of the skin following venous occlusion) in three men and bleeding of the gums and minor bruising tendencies in all four. A puzzling and possibly new observation was the appearance of tiny triangular hemorrhages in the bulbar conjunctiva of the eye. These appeared near the end of the deficiency period or early in the repletion period, and the severity of their occurence was inversely proportionate to the dose of vitamin C given. Thus, they were most severe in the subject given only 4 mg of ascorbic acid; less severe in the man receiving 8 mg; mild in the subject who received 16 mg; and absent in the person who received 32 mg of ascorbic acid daily. Despite these evidences of hemorrhagic tendencies, blood studies performed failed to show any evidence of impairment of any of the blood clotting mechanisms, which are measurable by modern techniques.

Physiologic tests, including basal metabolic rates and electroencephalograms, failed to demonstrate any significant departure from normal. Electrocardiograms showed minor changes, which are yet to be interpreted.

Throughout the study, each of the men was required to walk for an estimated 10 miles daily. Three miles of this was provided by an escorted walk. The men wore pedometers and were instructed to walk an additional 7 miles, for a total of 10 miles. Although this was an imprecise measure of exercise, they did maintain a rather high degree of energy expenditure, as evidenced by the fact that their weight remained constant on a caloric intake designed to meet this level of energy expenditure.

7. Infections. One man had three episodes of infection of the external ear canals with accompanying fever and egional lymphadenopathy, yet there was no apparent change in his rate of utilization of vitamin C.

8. Wound Healing. At the beginning of the repletion period, each of the men was subjected to a surgical laceration on the lateral aspect of the thigh, extending through the dermis and the fascia. One week later, a punch biopsy was taken from the healing wound margin and processed for electron microscopic examination, standard hematoxylin and eosin histologic examination and ^{14}Carbon vitamin C content. A second punch biopsy was similarly processed at the end of 2 weeks. The rate of healing of these wounds appeared to be directly proportional to the dose of vitamin C administered, yet all of the wounds eventually healed satisfactorily.

CONCLUSIONS:

1. It would appear that the physiologically functional pool of ascorbic acid is not vitamin C as we chemically identify it, but rather a stable derivative of L-ascorbic acid.

2. When subjects are placed on a zero vitamin C intake, the extent ascorbate pool is catabolized exponentially at a very low rate (27 day half-time), and this rate of catabolism is a function of the pool size.

3. The rate of repletion of ascorbic acid in deficient subjects is a zero order linear function of the intake of ascorbate. The rate of incorporation into the physiologic pool occurs at the rate of 80-90% of the administered dose within the range of 4-64 mg intake daily. Repletion continues until the ascorbate pool becomes fully saturated. During the repletion phase, there is no free ascorbic acid excreted in the urine. Once the pool has been fully repleted, urinary spilling does occur, and the rate of incorporation of ascorbate into the physiologic pool falls accordingly.

4. The clinical responses pertaining to hemorrhagic manifestations, physiologic tests, stress, infections and wound healing have been discussed.

PUBLICATIONS:

1. Baker, E. M., J. C. Saari and B. M. Tolbert. Ascorbic acid metabolism in man. Am. J. Clin. Nutr., 19: 371-378, 1966.

2. Saari, J. C., E. M. Baker and H. E. Sauberlich. Thin-layer chromatographic separation of the oxidative degradation products of ascorbic acid. Anal. Biochem., 18: 173, 1967.

3. Baker, E. M. Vitamin C metabolism in stress. Am. J. Clin. Nutr. (in press).

WORK UNIT NO. 072 - BODY OF REPORT

STUDY NO. 2 Ascorbic-4-^{3}H acid metabolism in man

PROBLEM:

Recently, 4-^{3}H-labeled ascorbic acid has been prepared, having a specific activity of 9.5 μc/mg. This material was prepared for metabolic studies in both animal and man.

RESULTS AND DISCUSSION OF THE RESULTS:

The kinetic and metabolic fate of ascorbic-4-^{3}H acid have been studied in a human subject. The radioactive label does not enter the body water pool but, instead, is excreted as organic bound tritium. The excretion products were found to be ascorbic acid and immediate oxidation products, and unknown organic compound(s). Kinetic analysis of the data shows half-times of 2 days and 46 days for turnover of the labeled ascorbic acid and the unknown compound, respectively. These results, combined with previous ascorbate-1-^{14}C studies, indicate that the unknown metabolite(s) are probably derivative(s) of L-threose or L-threonic acid.

CONCLUSIONS:

The metabolism of L-ascorbic-4-^{3}H acid has been studied in man, with the following results:

1. The radioactive label does not enter the body water pool but, instead, is excreted as organic bound tritium.

2. These data indicate the presence of more than one kinetically distinguishable pool in the metabolism of ascorbic acid.

3. The unknown metabolite(s) are probably derivative(s) of L-threose or L-threonic acid.

PUBLICATIONS:

1. Tolbert, B. M., A. W. Chen, E. M. Bell and E. M. Baker. Metabolism of L-ascorbic-4-^{3}H acid in man. Am. J. Clin. Nutr., 20: 250, 1967.

2. Tolbert, B. M., S. C. March, D. B. Karr, W. Scharf and E. M. Baker. Ascorbate function: donor of a four carbon moiety? Fed. Proc., 26: 854, 1967 (abstract).

WORK UNIT NO. 072 - BODY OF REPORT

STUDY NO. 3 — Further studies on the vitamin B_6 requirements of young adult male humans

PROBLEM:

In the course of a previous study, the question was raised as to whether or not the level of exercise affected the human requirement for vitamin B_6. Further, at recent NAS-NRC Food and Dietary Allowances Committee meetings, the question was raised as to whether there is a caloric effect upon the vitamin B_6 requirement of the human. In order to answer these questions, the following study was proposed in an effort to determine if there is any relationship between vitamin B_6 requirement and caloric utilization.

RESULTS AND DISCUSSION OF THE RESULTS:

A study was performed to determine if there was any relationship between vitamin B_6 requirement and caloric utilization. Eight subjects were placed on a vitamin B_6-free formula diet providing daily 100 g protein and 2800 calories for a period of 2 weeks. That a vitamin B_6 deficiency was produced was demonstrated by increased urinary xanthurenic acid excretion following a 5 g L-tryptophan load and by reduced urinary excretion of vitamin B_6.

Following depletion, the subjects were divided into two groups. Group A was given a processed natural diet that provided 100 g protein, 0.90 mg vitamin B_6 and 2800 calories per day. Group B received the same basic diet, but at the level of 3600 calories per day. All subjects received a pyridoxine supplement to provide a total controlled daily intake of 1.25 mg of vitamin B_6. All subjects were exercised to constant weight. The subjects were maintained on the respective diets for 6 weeks. During this period, urinary excretion of vitamin B_6 and of xanthurenic acid following L-tryptophan loads was studied. The results demonstrated that (a) there is no apparent effect of increased caloric utilization upon vitamin B_6 requirement; and (b) the daily minimum requirement of vitamin B_6 is 1.25 mg under the conditions employed.

CONCLUSIONS:

The results of a recent experiment in a series of human vitamin B_6 requirement studies demonstrated that (a) there is no apparent effect of increased caloric utilization upon vitamin B_6 requirement; and (b) the daily minimum requirement, in young adult male humans, of vitamin B_6 is 1.25 mg under the conditions employed in the experiment.

WORK UNIT NO. 072 - BODY OF REPORT

PUBLICATIONS:

1. Baker, E. M., Y. F. Herman and H. E. Sauberlich. Vitamin B_6 requirement of young adult male humans. Fed. Proc., 26: 413, 1967 (abstract and presentation).

2. Canham, J. E., E. M. Baker, N. Raica and H. E. Sauberlich. Vitamin B_6 requirement of adult men. Proc. VIIth Int. Cong. Nutr., Hamburg, Germany (in press).

3. Tillotson, J. A., H. E. Sauberlich, E. M. Baker and J. E. Canham. Use of carbon-14 vitamins in human nutrition studies: Pyridoxine. Proc. VIIth Int. Cong. Nutr., Hamburg, Germany (in press).

STUDY NO. 4 Thiamine metabolism and requirements

PROBLEM:

Various techniques are under study to investigate the human metabolism and requirement for thiamine. Urinary metabolites and ^{14}C-labeled thiamine are employed in addition to human volunteer studies. For example, a method has been published describing a procedure whereby metabolites of thiamine may be measured in human urine. The procedure depends upon the ability of yeast to synthesize thiamine from thiamine-related compounds. Following the synthesis, the urine is analyzed for thiamine by the conventional thiochrome procedure. The increase in thiamine following incubation with yeast is taken as the amount of thiamine metabolites in the urine.

In a recent study with human subjects on a restricted intake of thiamine, it was shown that the excretion of thiamine reached such low levels in 12 days as to be undetectable by the thiochrome assay. Yet, at the same time, the metabolite excretion increased to a constant level during the deficiency. This increase has led to speculation that the yeast was synthesizing substances which acted like thiamine in the yeast assay but were not directly related to thiamine. Thus, the validity of the method was in doubt.

In order to identify the thiamine-related compounds obtained from the incubation of urine with yeast, the following standards were synthesized: pyrimidine sulfonic acid, pyrimidine carboxylic acid, thiochrome, N-methylnicotinamide, thiazole and hydroxyethylthiamine. With the development of a thin-layer chromatographic procedure which could separate the

above compounds from each other and from thiamine, it is now possible to identify the compounds being measured as fluorescent substances in the thiochrome assay following the incubation of human urine with yeast.

RESULTS AND DISCUSSION OF THE RESULTS:

Preliminary results indicate that the fluorescent substances found in the thiochrome assay are, in fact, thiochrome. If future work should confirm this finding, it would establish the specificity of the yeast method in measuring thiamine derivatives in the urine. This would assure those interested in assessing thiamine nutriture in various populations that the use of this method is valid for measuring thiamine metabolites in the urine.

No additional metabolic studies have been performed during the past year using ^{14}C-labeled thiamine. However, ^{14}C-pyrimidine labeled thiamine has been obtained, and it is projected that a human study utilizing this material will take place this fall.

The effect of caloric intake and exercise on thiamine requirement has just been completed in a study employing young adult healthy male human subjects. The data are being processed. Preliminary evaluation of the findings indicates that increased caloric expenditure, employing carbohydrate as the source of calories, increases the human requirement for thiamine.

CONCLUSIONS:

1. The yeast method for measuring some of the metabolites of thiamine in urine appears to offer a specific assay for thiamine nutriture in humans.

2. Results indicate that increased caloric expenditure, employing carbohydrate as the source of calories, increases the human requirement for thiamine.

PUBLICATIONS:

1. Sauberlich, H. E. Biochemical alterations in thiamine deficiency -- their interpretation. Am. J. Clin. Nutr. (in press).

2. Waring, P. P., W. C. Goad and Z. Z. Ziporin. The use of thin-layer chromatography to separate thiamine and related compounds as well as N-methylnicotinamide and related compounds (manuscript in preparation).

WORK UNIT NO. 072 - BODY OF REPORT

STUDY NO. 5 Metabolism of ^{14}C-labeled cotton cellulose by man

PROBLEM:

The objective of these studies was to determine whether or not the human subject is capable of digestion of cellulose and, if so, to what extent and what factors may influence such an ability.

RESULTS AND DISCUSSION OF THE RESULTS:

Cotton labeled with carbon-14, grown at USAMRNL, and nonlabeled cotton were converted into microcrystalline cellulose, "Avicel-R," by the American Viscose Corporation. Avicel-R is similar to the Avicel preparation which was previously used at USAMRNL, with the exception that it is of a smaller and more uniform particle size. The emulsification and other properties of Avicel-R gels are also more superior to the earlier product.

A human volunteer subject was placed on a 15-day preconditioning control period during which time he was fed a total of 150 g (dry weight) of nonlabeled microcrystalline cellulose per day in two equal portions. The cellulose was consumed in the form of milk shakes or sherbets. After the control period, he was then fed the ^{14}C-labeled cellulose (approximately 30 μc) without any nonlabeled cellulose as carrier added. Following the ingestion of the ^{14}C-labeled cellulose, 24-hour fecal and urine collections were obtained until such time as a complete ^{14}C-material balance was obtained. During the same experimental period, expired $^{14}CO_2$ was monitored daily.

The subject received the ^{14}C-labeled cellulose on 19 May 1967. Thus far, there has been no detectable $^{14}CO_2$ expired via the lung. Further, there has been no measurable ^{14}C activity in the urine over the past 11 days. At this time, the amount of ^{14}C activity excreted in the feces has not been ascertained. This information will be forthcoming. There is no reason not to believe that in excess of 98% of the ^{14}C-labeled cellulose will be excreted via the feces, as was the case in the animal studies.

CONCLUSIONS:

Although the study is not entirely completed, available evidence indicates that the human cannot utilize cellulose to any significant degree.

WORK UNIT NO. 072 - BODY OF REPORT

PUBLICATIONS:

None

STUDY NO. 6 — Other nutrition activities, national and international

PROBLEM:

Assistance and cooperation are provided in support of the mission of USAMRNL to extend nutritional and medical research, recommendations and training to U.S. military and civilian groups and to civil and military populations of other countries as judged important and appropriate.

RESULTS AND DISCUSSION OF THE RESULTS:

Members of the Chemistry Division have assisted in the training program of the Reserve Officers' groups and of individual foreign officers assigned to USAMRNL for special programs or training. Similarly, assistance has been provided in research, training and consulting to regional, national and international educational or government institutes, including FAO of the United Nations. Locally, cooperative support has been provided the University of Colorado, University of Colorado Medical School and Colorado State University. Assistance has been provided the Office of International Research, Nutrition Section, of the National Institutes of Health, as requested, in support of nutrition programs in Central America and South East Asia. Recently, a biochemist from USAMRNL spent 3 months at the Institute of Nutrition of Central America and Panama (INCAP), located in Guatemala, in support of the nutrition survey of Honduras. Blood samples from the recent nutrition survey of Panama were sent to USAMRNL for transketolase assay to assist in thiamine nutriture evaluation. Consulting and analytical services have been provided nutrition programs in Thailand, Malaysia and Indonesia. Currently, the Advanced Research Program Agency (ARPA) is being assisted in an evaluation of Thai military rations. Assistance was provided in conducting the nutrition survey of Ft. Campbell, Kentucky. Professional assistance has been provided by staff members by serving as members of editorial boards of nutrition journals and as members of various scientific committees, including NAS-NRC Committee on Dietary Allowances. Currently, additional studies have been proposed for Iran, Panama and Thailand and are pending approval.

WORK UNIT NO. 072 - BODY OF REPORT

CONCLUSIONS:

Continuing support was provided national and international nutrition agencies and their basic or applied nutrition programs and training activities.

PUBLICATIONS:

1. Sauberlich, H. E. World nutrition problems. Proc. Western Section of the Am. Soc. of Animal Sci., 1966.

2. "Requirements of Vitamin A, Thiamine, Riboflavine and Niacin," Report of a Joint FAO/WHO Expert Group. Rome, Italy, 1967.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6308	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	RPT U WRK U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER/CODE
62156011 3A025601A822 00 073	None

11. TITLE:
(U) Applied Nutrition Studies of Military Populations (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry 012900 Physiology 006500 Food Management	08 63	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	2	47
		CURRENT FY 68	2	48

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
NAME
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INVESTIGATORS PRINCIPAL ASSOCIATE Consolazio, C.F.,, Matoush, L. O.
Sauberlich, H.E.
TEL 303 366 5311 X25222 TYPE DA

21. TECHNOLOGY UTILIZATION Nutrition; Nutritional Status; Nutrition Surveys; Food Technology

22. COORDINATION
None

23. KEYWORDS Nutrition surveys; Performance evaluation; Energy metabolism; Food; Diet; Rations; Body composition; Anthropometry; Biochemistry (Clinical & Food); Envir.

24. (U) Tech Objective: To evaluate the nutritional status, work performance, body composition, and work capacity of the soldier and to insure that military performance is not impaired by improper nutrition.

25. (U) Approach: Studies have been designed to evaluate the above problems. The first study evaluated the nutritional adequacy and acceptability of a variety of high caloric density rations, under non-resupply conditions. The second includes a number of surveys to ascertain the soldier's dietary intake, the clinical and biochemical status of the men, the body composition, and the work capacity. Extensive studies have already been conducted at Fort Carson, Colorado, Fort Benning, Georgia (Rangers), Fort Huachuca, Arizona, and Fort Campbell, Kentucky. Two additional studies on caloric restriction to evaluate minimal food intakes to effectively permit the soldier to perform his duties for 10 days were completed, one using 400 Calories of carbohydrate and the second using 500 Calories/day of a protein-carbohydrate mixtur.

26. (U) Progress: The Fort Bragg ration study (USAMRNL Report No. 288) and the Fort Benning Ranger study (USAMRNL Report No. 291) were completed. A report of the food intake and nutritional adequacy of the ration were completed on three units at Fort Huachuca, Arizona. The field phase of the survey at Fort Campbell, Kentucky was completed in March 1967. The nutritional adequacy of the ration was evaluated in basic trainees during their first, fifth, and eighth week of training. .Two papers concerning the metabolic aspects of human starvation (for 10 days) were completed and accepted by the American Journal of Clinical Nutrition.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A025601A822	Military Internal Medicine
TASK NO.	01	
WORK UNIT NO.	073	Applied Nutrition Studies of Military Populations

The following investigations have been conducted under this work unit:

STUDY NO. 1: A number of surveys are planned to evaluate longitudinally the nutrient intake and nutritional status of soldiers living under a variety of duty requirements and environmental conditions. Current, newly developed, and experimental rations are to be evaluated for nutritional adequacy. At the present time, four surveys have been completed at Fort Carson, Colorado, Fort Benning, Georgia (Rangers), Fort Huachuca, Arizona, and Fort Campbell, Kentucky, to help answer some of the above problems. These studies were designed to ascertain the soldiers dietary intake, the clinical and biochemical status of the men, the body composition, and their physical work capacity.

In each camp, three units were studied to evaluate the adequacy of the present day garrison ration. At Fort Huachuca, a unit of WACs were surveyed. The surveys were completed for Fort Benning, Georgia (Rangers), and Fort Huachuca, Arizona and published in Laboratory Report Nos. 291 and 309, respectively.

STUDY NO. 2: Three studies were completed to evaluate the minimal food intake necessary to permit the individual soldier to effectively perform his duties for periods of 3, 7, and 10 days. These studies included 10 days of complete starvation and two studies of caloric restriction (400 Calories of carbohydrate and 500 Calories of a protein-carbohydrate mixture) each for a 10 day period. In the caloric restriction studies, 8 men were divided into two groups, 4 receiving mineral supplementation and 4 others receiving no supplementation.

Information on 10 days of starvation on 6 normal healthy adults are summarized as follows. The major problems encountered, under conditions of no mineral supplementation, were: (a) highly negative water balances resulting in body hypohydration; (b) negative nitrogen balances, showing that body protein was

being catabolized; and (c) the large mineral and urea losses. These undesirable factors could eventually lead to physical and psychological inefficiency.

In addition, significantly abnormal EKGs and EEGs were observed at the end of the fasting period. The men were in fairly poor condition both physically and mentally. There appeared to be increased fatigue and frequent lapse of memory. They were not mentally alert, were extremely tired, pale and haggard and showed some indications of muscle cramps.

Although performance was not greatly impaired, it is suggested that complete fasting without mineral supplementation should not be recommended for a soldier on combat patrol.

BODY OF REPORT

WORK UNIT NO. 073 Applied Nutrition Studies of Military Populations

STUDY NO. 1

PROBLEM:

Army post surveys will be conducted over a minimum of 5 years to evaluate the adequacy of the Army diet under varied climatic conditions in terms of established recommended allowances. Biochemical diet analysis will also include essential nutrients for which recommended dietary allowances have not been established. Clinical evaluation of the nutritional and physical status of military personnel is essential in addition to biochemical evaluations of blood and urine samples. A special effort will be made to evaluate body composition, work performance, and cardiopulmonary measurements in terms of dietary intake, habit, and nutritional status.

RESULTS AND DISCUSSION OF THE RESULTS:

Fort Huachuca, Arizona - Nutrition Survey: In the third in a series of Nutrition Surveys being conducted in an Army camp at Fort Huachuca, food intakes were measured in three military units (men) and in one WAC Company. Total food intakes averaged 3881, 3886, and 3731 Calories/day for the three groups of men, and 2846 Calories/day for the WAC Company. These intakes were considerably above the minimal allowances prescribed in AR 40-5. The edible waste averaged 15.3, 13.5, and 9.3% for the three groups of males, and 21.9% for the WAC Company. This study was published as USAMRNL Laboratory Report No. 309, April 1967.

Fort Campbell, Kentucky - Nutrition Survey: Information on food intake at three mess halls, biochemical evaluation, body compartment measurements, and maximal work performance on 220 men between the ages of 17 - 50+ years are now being compiled and analyzed for future publication.

CONCLUSIONS:

Fort Huachuca, Arizona - Nutrition Survey: The primary purpose of this study was to conduct a Nutrition Survey on a representative sample of U. S. Army personnel, and to provide information

necessary for execution of AMEDS responsibilities under AR 40-5. This was the third in a series of surveys and was conducted at Fort Huachuca, Arizona in FY 1966. Food intakes and food wastes were evaluated in three military messes (men) and one WAC Company. Food intake from all sources averaged 3881, 3886, and 3731 Calories/day for the men in Groups I, II, and III. The WAC Company averaged 2846 Calories/day. All of these intakes were considerably above the minimal allowances prescribed in AR 40-5. Total edible wastes from all sources averaged 15.3, 13.5, and 9.3% for the same groups of men. The WACs waste was very high, averaging 21.9% of the total food served.

Fort Campbell, Kentucky - Nutrition Survey: Nutrition Surveys were conducted on three units of basic trainees (during their first, fifth, and eighth week). Information was also gathered on maximal work performance on the treadmill, on various physical training tests, and on the body composition of these trainees. In addition, 26 obese individuals and 24 special training troops were also studied completely.

RECOMMENDATIONS:

Continue the nutrition surveys to include food consumption of troops in extremely cold and hot environments.

PUBLICATIONS:

1. Consolazio, C. F., L. O. Matoush, H. L. Johnson, R. A. Nelson, and H. J. Krzywicki: Nutrition Survey, Fort Huachuca, Arizona, March-April 1966. USAMRNL Laboratory Report No. 309, April 1967.

WORK UNIT NO. 073 Applied Nutrition Studies of Military Populations

STUDY NO. 2

PROBLEM:

Due to changing needs of the military, there is a continuing necessity to evaluate the capability of current and newly developed rations (both freshly prepared and as altered by varied storage conditions) to provide adequate nutrition to the soldier under a variety of duty requirements and environmental situations. The nutritional basis of our present ration system is adequate for garrison training duty on a current basis but may not be optimum for the total military life of the soldier. Longitudinal evaluations of the nutritional status, the body composition, and the work performance and capacity of the soldier during his duty career is required to ensure that they are not impaired by improper nutrition. Such impairment could limit the capability of the Army's cadre at a time when instant readiness is mandatory. Previous studies, though helpful, provide only some of the necessary answers.

RESULTS AND DISCUSSION OF THE RESULTS:

A 10 day complete starvation study on 6 men between the ages of 20 - 52 was completed and published as Laboratory Report No. 299, dated September 1966. Three undesirable problems related to fasting were encountered: (a) the great body water loss resulting in body hypohydration; (b) the fairly large urinary nitrogen excretion showing that body protein was being catabolized; and (c) the fairly large mineral losses concomitant with the large body water loss. Also, abnormalities in electrocardiograms and one electroencephalogram were observed during the latter stages of starvation. These factors, reflecting a severe stress, could eventually lead to greatly impaired mental and physical inefficiency.

At the end of 10 days of starvation, the men were in very poor condition both physically and mentally. There appeared to be increased weakness and apathy toward mental and physical work. They had frequent lapse of memory, were not at all mentally alert, were slow to respond to questions, were tired, pale, and haggard, and had indications of muscle cramps, which may have been due to salt restriction and body stores depletion.

No problems were observed during rehabilitation. The men

ate all desired food items and although practically no bowel movements were observed during starvation, the men all had normal movements within 6 - 8 hours of the first meal.

It is suggested that complete fasting without mineral or vitamin supplementation should not be recommended either for a soldier on combat patrol or for a reducing diet. Although the men appeared to be in fairly good physical condition at the end of the 3rd day of fasting, starvation will not be recommended for this period of time since there might be an occasion when the period would be extended.

CONCLUSIONS:

Starvation Study: The soldier in combat or on combat patrol for periods of 1 - 10 days must carry his full field pack, weapons, radio equipment, and an adequate supply of food and water. The question has arisen repeatedly as to the minimal food intake necessary to permit the individual soldier to effectively perform his duties for periods of 3, 7, and 10 days. A 10 day study was performed on 6 normal adult males between the ages of 20 and 52 years to evaluate whether an individual could perform his duties adequately while on complete starvation. Water, coffee, tea (without cream or sugar), and no calorie carbonated soft drinks were available ad libitum. No vitamin or mineral supplements were taken during the study.

The major problems encountered, under conditions of no mineral supplementation, were: (a) highly negative water balances resulting in body hypohydration; (b) negative nitrogen balances showing that body protein was being catabolized; and (c) the large mineral and urea losses. These undesirable factors could eventually lead to physical and psychological inefficiency.

The 10 day body weight losses averaged 16.1 pounds which was equivalent to 9.47% of the initial total body weight. Within two days of rehabilitation on a normal intake, the men regained 4.0 kg (8.8 lbs).

In addition, significantly abnormal electrocardiograms and electroencephalograms were observed at the end of the fasting period. The men were in fairly poor condition both physically and mentally. They were not mentally alert, were extremely tired, pale, and haggard, and showed some indications of muscle cramps.

Although performance was not greatly impaired, it is suggested that complete fasting without mineral supplementation should not be recommended for a soldier on combat patrol.

Two studies on caloric restriction using 400 Calories of carbohydrate and 500 Calories of a protein-carbohydrate mixture for periods of 10 days have been completed. Preliminary observations indicate that these diets without mineral supplementation are practically the same as complete starvation except for the ketosis. The addition of protein with or without supplementation did not help the great body nitrogen loss.

RECOMMENDATIONS:

Conduct field study to evaluate the minimal food requirements during simulated combat patrol in a jungle environment.

PUBLICATIONS:

1. Consolazio, C. F., L. O. Matoush, R. A. Nelson, G. J. Isaac, and J. E. Canham: Comparisons of nitrogen, calcium, and iodine excretion in arm and total body sweat. Am. J. Clin. Nutr. 18:443, 1966.

2. Consolazio, C. F.: Nutrient requirements of troops in extreme environment. U. S. Army Research and Development Newsmagazine, pp 24-27, November 1966.

3. Consolazio, C. F.: Paper in "The Encyclopedia of Biochemistry" edited by R. J. Williams and E. M. Lansford, Jr., "Caloric intake and caloric requirements", pp 164-169, Reinhold Publishing Corp., New York, N. Y., 1967.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6345	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 07 66)	U RPT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER/CODE
62156011 3A025601A822 00 074	62156011 3A025601A822 01 075

11. TITLE:
(U) Nutritional and Metabolic Aspects of Nutrients (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry; 003500 Clinical Medicine	07 66	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	18 RESOURCES EST.	a PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	a. DATE b. NUMBER NA c TYPE d AMOUNT	PRIOR FY 67	1	55
		CURRENT FY 68	2	82

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
NAME ADDRESS Headquarters U.S. Army Med Rsch & Dev Cmd Washington, D. C. 20315 RESP. INDIV TEL Davis, T. E., MAJ 202 OXford 6 5472	NAME ADDRESS U.S. Army Med Rsch & Nutr Lab Fitzsimons General Hospital Denver, Colorado 80240 INVESTIGATORS PRINCIPAL ASSOCIATE Raica, N., Baker, E. M., LTC Sauberlich, H. E. TEL 303 366-5311 X24214 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Nutrition; Clinical Medicine; Food Preservation	None

23. KEYWORDS
Food Preservation; Food Technology; Nutrition; Radiation; Radiation Biochemistry; Metabolism; Nutrients; Malabsorption; Vitamins; Enzymes

24. (U) Tech Objective: The objectives are (a) to provide additional information pertaining to the wholesomeness and nutritional adequacy of foods sterilized with ionizing radiation and factors that may influence nutrient losses associated with this form of food preservation; (b) investigate requirements for nutrients, particularly vitamins, and factors that may alter these requirements, including malabsorption and infectious diseases; (c) study the metabolic aspects of nutrients; and (d) develop techniques

25. for evaluating nutritional adequacy.
25. (U) Approach: The studies will involve mainly animal and microbial experimentation for later application to human situations. With the availability of a ^{60}Cobalt radiation source, ionizing radiation effects on nutrients under specific controlled conditions will be investigated. Nutrients of primary interest are lipids, vitamins and amino acids. Continued assistance will be provided the OTSG in evaluating the wholesomeness of irradiated foods. Isotopically labeled vitamins will be employed to study their metabolism, pool size, turnover rates,etc. in animals under various dietary situations. Of interest will be the influence of intestinal flora and infections on

26. nutrient utilization. Controlled experimental conditions will be approximated with the use of germ-free and pathogen-free animals. Techniques such as the measurement of specific blood enzymes or vitamins or of urinary metabolites will be employed or developed for evaluating nutritional adequacy.
26. (U) Progress: A ^{60}Cobalt source is installed and operating. OTSG irradiated food interests continue to be provided for. Germ-free studies reported elsewhere. Extensive work in elucidating the metabolic pathways of ascorbic acid metabolism as well as tissue distribution of vitamin C in the rat and guinea pig using carbon-14 and tritium-3 labeled ascorbic acid has been completed. Similar studies are in progress employing ^{14}C-pyridoxine.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A025601A822 Military Internal Medicine

TASK NO. 01

WORK UNIT NO. 074 Nutritional and Metabolic Aspects of Nutrients

The following investigations have been conducted under this work unit:

STUDY NO. 1 Malabsorption, diarrhea, steatorrhea and nutritional deficiency syndrome

STUDY NO. 2 Nutritional and wholesomeness aspects of irradiated foods

STUDY NO. 3 In vitro lipase hydrolysis of irradiated triglycerides

STUDY NO. 4 Vitamin B_6 metabolism; microbiological assays

STUDY NO. 5 Chemistry and biochemistry of ascorbic acid

1. In the conventional animal, nutrient absorption and utilization are complicated by the presence of intestinal flora. The study of malabsorption syndromes, whether of infectious or of nutritional origin, may therefore be studied to advantage in the germfree animal. This problem will be approached by the following procedures: (a) testing of toxic food components on intestinal mucosa; (b) inoculations with fecal and other samples from tropical sprue patients; (c) study of nutrient absorption and utilization in deficiency states; and (d) combinations of these approaches. These studies have just been initiated, but some data have been obtained. Sub-toxic levels of cyanide or autoxidized oils do not produce lesions in the intestinal mucosa of conventional rats. Germfree rats can utilize β-carotene as efficiently as conventional rats. Irradiation-sterilized diets will be tested. This procedure has been reported to be superior to heat-sterilization for germfree animal studies.

2. Liaison and assistance to Medical R&D regarding the wholesomeness aspects of irradiated foods has continued. Although the contractural feeding studies are complete, information and data are being gathered for the preparation of petitions to FDA. The compilation of a single volume containing an expanded summary of all progress reports pertaining to wholesomeness is in progress. Meetings and conferences were attended with participation.

3. Irradiated natural fats have been reported to reduce lipase hydrolysis rates both in vivo and in vitro. The objectives of this study are to determine the inhibition of lipase by various irradiated pure triglycerides and to determine the active factors if inhibition is found. Irradiated (5 Mrad) trimyristin,

tricaprin, or tripalmitolein do not inhibit in vitro lipase (steapsin) hydrolysis rate when emulsified by sonication in an ox bile solution at pH 8.8 and 37° C. Emulsions of the irradiated triglycerides tested are comparable in degree of dispersion and stability to nonirradiated triglycerides. Because most of the emulsion particles were less than 1 micron in diameter, particle size distribution could not be conveniently determined with a Coulter counter. Future studies will include investigations of dose relationships to lipase activity with polyunsaturated triglycerides.

4. Investigations were initiated using ^{14}C-labeled pyridoxine to study the cellular and organ distribution, turn-over rate and metabolites of vitamin B_6. Microbiological assay services were provided investigators and cooperating agencies for vitamin B_6, folic acid, vitamin B_{12}, thiamine, riboflavin and niacin.

5. Additional studies were conducted on the chemistry and biochemistry of ascorbic acid in an attempt to elucidate the function of this vitamin. In this respect, the NMR spectra of xyloascorbic acid, isoascorbic (araboascorbic) acid, dehydroxyloascorbic acid and dehydroisoascorbic acid have been determined. The tissue distribution pattern in the rat of radioactivity from administered ^{14}C- and ^{3}H-labeled ascorbic acid has been obtained, and the data indicate that several 6-carbon metabolites, as well as a possible 4-carbon metabolite, are present. Similarly, the urinary metabolites of guinea pigs administered ^{14}C- and ^{3}H-labeled ascorbate have been investigated. Of the four or five derivatives of ascorbic acid found in the urine, all but one were double-labeled. The sub-cellular levels of radioactivity of selected tissues of guinea pigs who had been labeled with ^{14}C- and ^{3}H-ascorbic acid have been measured. In order to obtain additional information on the metabolism of vitamin C, attempts are underway to synthesize L-ascorbic-6-^{14}C acid from glucose-1-^{14}C.

BODY OF REPORT

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

STUDY NO. 1 — Malabsorption, diarrhea, steatorrhea and nutritional deficiency syndrome

PROBLEM:

The presence of "normal" intestinal microflora, pathogenic and nonpathogenic, complicates the study of nutrient absorption and utilization in the normal or diseased animal. The objectives of this study are to study the absorption and utilization of nutrients in the gnotobiotic and conventional rat and to determine the effect of nutritional deficiencies, toxic food components and/or microorganisms on the pathology of the intestinal mucosa and malabsorption syndromes. One approach will be an attempt to induce a tropical sprue syndrome in germfree rats by inoculation with crude fecal homogenates or cell-free fecal filtrates from patients with tropical sprue. Other studies will utilize radiolabeled nutrients and vitamin deficiencies to determine absorption and utilization in the normal and diseased germfree animal.

RESULTS AND DISCUSSION OF THE RESULTS:

Effect of Dietary Components on Intestinal Mucosa. Studies have been initiated in conventional rats to determine the effect of cyanide or autoxidized oils on the intestinal mucosa. Levels of cyanide (0.65 g/kg and 2.0 g/kg diet) did not affect the growth, food consumption, or histopathology of the intestinal mucosa. Twelve-week feeding studies with diets containing 20% oxidized oil (76 or 226 me/kg oil) have been completed. Histopathologic data are not available for this reporting period. Cyanide or autoxidized fats do not affect the pathology of the conventional rat's intestinal mucosa. These substances have been suggested as possible mucosal toxic agents in the tropics. Some tropical fruits contain nitriles. Autoxidation of oils has been a problem in some areas. If these substances are active in the human, they are not in the conventional rat.

Arrangements have been made to obtain fecal and other samples from tropical sprue patients for the inoculation studies with germfree rats. Vitamin A absorption studies are also planned.

Diet Sterilization and Composition for Germfree Animal Studies. The usual procedure for sterilizing diets for germfree studies is autoclave sterilization. This procedure is very drastic and, in addition to nutrient destruction,

"browning" reaction products are formed, particularly if sugars are present. Existing studies of limited scope suggest that irradiation-sterilized diets (3-5 Mrads) are superior to heat-sterilized. In order that the nutritional and absorption studies can be made more meaningful and versatile in regard to diet compositions, studies with irradiation-sterilized diets will be initiated. A gamma (^{60}Co) source is available for these studies. These studies have just been initiated and data are not available.

The Availability of β-carotene in the Germfree Rat. The intestine of the conventional rat is an efficient converter of β-carotene to vitamin A. The utilization of β-carotene has not, however, been determined in the germfree rat. Diets are generally fortified with vitamin A to allow for sterilization losses and, therefore, carotene utilization, even with natural-type diets, is not of concern. For planning future studies concerned with carotene utilization and microflora relationships, it was essential that data be obtained in the germfree animal regarding its efficiency of carotene utilization. Female germfree rats were fed a vitamin A-deficient diet until growth plateau was reached. They were then supplemented orally daily for 12 days with 30 μg β-carotene or 15 μg vitamin A acetate in cottonseed oil. Oil solutions were sterilized by filtration. At the termination of the supplement period, the rats were sacrificed and the livers and cecums were removed, frozen in dry ice and stored for subsequent vitamin A and carotene assay.

Analysis of the livers for vitamin A shows that β-carotene is as efficiently utilized by germfree animals as by conventional animals, assuming a 50% efficiency. The data (Table I) for liver stores and growth are comparable for the carotene and vitamin A-supplemented rats. Cecal contents have not been assayed.

TABLE I

Response of Vitamin A-depleted Female Rats to Carotene or Vitamin A

Weight[1]		Supplement[2]	Total
Initial	Gain		Liver Vitamin A
g			μg
170	"0"	None	"0"
174	22	Carotene	71
166	18	Vitamin A	85

[1] Average of three animals.

[2] Orally in oil; daily for 12 days. Carotene, 30 μg; vitamin A, 15 μg.

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CONCLUSIONS:

In the conventional animal, nutrient absorption and utilization are complicated by the presence of intestinal flora. The study of malabsorption syndromes, whether of infectious or of nutritional origin, may therefore be studied to advantage in the germfree animal. Studies were initiated to study the problem, using various approaches. Progress thus far has demonstrated that subtoxic levels of cyanide or autoxidized oils do not produce lesions in the intestinal mucosa of conventional rats. Germfree rats have been observed to utilize β-carotene as efficiently as conventional rats.

PUBLICATIONS:

None

STUDY NO. 2 — Nutritional and wholesomeness aspects of irradiated foods

PROBLEM:

The wholesomeness of irradiated food program sponsored by the Office of The Surgeon General, and an important part of the U.S. Army irradiated food program, has been essentially completed. However, through the continued efforts of the U.S. Army and Atomic Energy Commission with irradiated foods and preparation of petitions for the Food and Drug Administration, this laboratory has continued active liaison, technical support and participation in the program.

RESULTS AND DISCUSSION OF THE RESULTS:

In addition to the normal requirements of assisting in the Medical R&D irradiated food interests, a compilation of the wholesomeness studies has been initiated. The purpose for this compilation is to have available in concise form a summary in depth of all wholesomeness data in one volume. At the present time, with the exception of the detailed progress reports and scattered literature publications, only brief summaries or reviews of the entire program are available. To supplement the compilation, the progress reports will be microfilmed for more ready accessability to data details, results and conclusions.

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In support of the irradiated food interests, the following meetings and conferences were attended with participation:

1. Joint meeting: Committee on Radiation Preservation of Food and U.S. Army Surgeon General's Advisory Committee on Nutrition. NAS-NRC. Boston, Massachusetts, 9 March 1967.

2. American Institute of Chemical Engineers Symposium on Progress in Food Irradiation. Paper read: "Wholesomeness Testing of Irradiated Foods." Atlantic City, New Jersey, 18-21 September 1966.

3. Canadian Dietetics Association. Paper read: "The Nutritional Evaluation of Irradiated Foods." Ottawa, Canada, 13-15 June 1967.

CONCLUSIONS:

Liaison and assistance to Medical R&D regarding the wholesomeness aspects of irradiated foods has continued. Although the contractural feeding studies are complete, information and data are being gathered for the preparation of petitions to FDA. The compilation of a single volume containing an expanded summary of all progress reports pertaining to wholesomeness is in progress. Meetings and conferences were attended with participation.

PUBLICATIONS:

1. Raica, N. and D. L. Howie. Review of the U.S. Army Wholesomeness of Irradiated Food Program. In "Food Irradiation." IAEA, Vienna, August 1966.

2. Raica, N. Wholesomeness testing of irradiated foods. Proc. Am. Inst. Chem. Eng. (in press).

STUDY NO. 3 — In vitro lipase hydrolysis of irradiated triglycerides

PROBLEM:

The digestibility rate of irradiated natural oils and fats has been shown to be reduced both in vivo and in vitro. Although the digestibility rate was reduced, the net utilization was not significantly reduced. In some of these

studies, the oils and fats had very elevated peroxide numbers. Peroxides may have been the active lipase inhibitors. Other studies reported that irradiated emulsions or emulsions formed from irradiated fats are unstable.

The objectives of this study were to determine the effect of irradiation (5 Mrads) on the in vitro lipase hydrolysis rate of pure triglycerides, and to determine the active component(s) if there was a reduced rate of hydrolysis.

RESULTS AND DISCUSSION OF THE RESULTS:

Pure triglycerides were purchased from the Hormel Institute. These triglycerides were irradiated with gamma rays from ^{60}Co to 5 Mrads at 0° C. Aliquots were emulsified by sonication in an ox bile preparation. The emulsion was diluted with a saline-desoxycholine-calcium chloride solution, and after the addition of a lipase (steapsin) suspension, titrated with base for 2 minutes with a radiometer pH stat at pH 8.8 and 37° C. The volume of base per unit time (1 min.) was arbitrarily selected as the initial velocity, and the weight of fat per aliquot titrated was selected as the substrate concentration.

Attempts were made to size and count the emulsion particles with a Coulter counter. Because most of the particles were less than 1 micron in diameter, it was not possible to obtain a reliable size distribution.

Data have been obtained with tripalmitolein, tricaprin and trimyristin. Tristearin emulsions could not be hydrolyzed with lipase to obtain reproducible velocities. Bennzonana and Desnuelle (Biochim. Biophys. Acta, 105: 121, 1965) had shown that a plot of 1/v vs. 1/surface area of emulsion particles was linear. When 1/v was plotted vs. 1/grams of substrate, a linear relationship was obtained with greater velocities for the emulsion containing the greater surface area per unit weight of substrate; however, Vmax was constant regardless of surface area within the tested range of 0.95 to 2.9 m^2/gram of substrate. For this reason, lipase activity was calculated on the basis of weight of substrate per total hydrolysis volume since preliminary determinations on particle size distribution showed that irradiated triglycerides were emulsified to the same degree as nonirradiated triglycerides, and formed stable emulsions.

Preliminary gas chromatography data of the triglycerides did not reveal any loss of fatty acids due to irradiation.

Titration data are shown in Table II. Calculations were made so that a plot of v vs. v/w, which seems to correspond to v vs. v/[S], can be made. This correspondence has not been critically evaluated, but it does seem to exaggerate

TABLE II

In Vitro Lipase Hydrolysis of Irradiated and Nonirradiated Triglycerides

Volume[4]	Trimyristin[1]				Tricaprin[2]				Tripalmitolein[1]			
	Control		Irradiated		Control		Irradiated		Control		Irradiated	
	v	v/w	v	v/w	v	v/w	v	v/w	v	v/w	v	v/w
.10	28.4	10.8[3]	28.3	10.6	25.6	9.4	24.2	8.9	26.6	10.2	27.2	10.1
.15	33.9	8.5	32.6	8.2	29.6	7.3	29.1	7.2	33.0	8.3	31.0	7.8
.20	35.7	6.8	34.8	6.5	35.1	6.5	34.2	6.3	35.6	6.9	32.6	6.2
.30	40.8	5.1	39.8	5.0	40.8	5.0	39.2	4.8	41.6	5.2	39.6	5.0
.50	45.2	3.4	42.2	3.2	46.5	3.4	44.1	3.2	47.6	3.5	46.4	3.4

Control: Nonirradiated triglyceride. Irradiated: Triglyceride irradiated to 5.0 Mrad.

v = velocity during the first minute (per cent of syringe capacity).

w = weight of substrate in emulsion aliquot (grams).

Trimyristin and tricaprin: 0.2 ml of 0.2% steapsin suspension.

Tripalmitolein: 0.1 ml of 0.2% steapsin suspension.

[1]Titrated in 20-ml volume.

[2]Titrated in 2-ml volume.

[3]$v/w \times 10^{-3}$.

[4]Emulsion volume (ml).

deviations from linearity when weight of emulsified substrate is substituted for substrate concentration. The irradiated triglycerides tested do not inhibit lipase activity.

It is planned to study irradiated polyunsaturated triglycerides in more detail in regard to their emulsification, oxidation and degradation as they relate to lipase hydrolysis. With the availability of a radiation source, dose-hydrolysis rates will also be investigated.

STUDY NO. 4 — Vitamin B_6 metabolism; microbiological assays

PROBLEM:

Only limited information is available as to cellular distribution of vitamin B_6, its metabolites, and the body pool size and turn-over rate of this nutrient. With the availability of ^{14}C-labeled pyridoxine, such studies were feasible. The objectives of the laboratory rat studies were as follows:

1. Determine both the tissue (organs) and cellular distribution of vitamin B_6 and its metabolites.

2. Estimate the tissue and urinary turn-over rate of ^{14}C-labeled pyridoxine and its metabolites.

3. Identify the major metabolites of ^{14}C-labeled pyridoxine.

4. Investigate interrelationships of vitamin B_6 (with use of labeled pyridoxine) with vitamin B_6 artagonists (deoxypyridoxine, INH, etc.) and other dietary nutrients with respect to objectives in 1, 2 and 3.

The techniques and information obtained from the animal investigations will be applied to studies on the evaluation of the human requirement for vitamin B_6. In addition to these studies, supporting microbiological assays are conducted for folic acid, vitamin B_{12}, riboflavin, thiamine, niacin and vitamin B_6.

RESULTS AND DISCUSSION OF THE RESULTS:

A group of rats was placed on a vitamin B_6-free diet until their weight gain was essentially zero. The rats were then injected daily with 50 µg of vitamin B_6 (25 µg of pyridoxine hydrochloride and 25 µg of 3-hydroxy-4,5-dihydroxymethyl-2-methylpyridine-4,5-$^{14}C_2$-hydrochloride; specific activity,

30.8 μc/mg) for 30 days. The urines were collected daily. At this time, four rats were sacrificed in order to determine a ^{14}C tissue and celluiar distribution pattern. The remaining rats were then injected daily with 50 μg of cold pyridoxine hydrochloride, and the decrease in urinary ^{14}C excretion was determined on daily urine collections. Thirty days later, four more fats were sacrificed again to determine a ^{14}C tissue and cellular distribution pattern.

Upon the completion of this phase of the study, additional animals will be investigated to provide the information necessary to attain the objectives stated.

A considerable number of vitamin assays (folic acid, vitamin B_{12}, thiamine, riboflavin, vitamin B_6) were conducted in support of studies by other investigators or agencies.

CONCLUSIONS:

Investigations were initiated, using ^{14}C-labeled pyridoxine, to study the cellular and organ distribution, turn-over rate and metabolites of vitamin B_6. Since this study is only partially completed, no conclusions are warranted at this state of progress. Microbiological assay services have been provided other investigators and agencies as requested or required.

PUBLICATIONS:

None

STUDY NO. 5 — Chemistry and biochemistry of ascorbic acid

PROBLEM:

The objective of this work is to find out more about the chemistry and biochemistry of ascorbic acid. The essential biological function(s) of this much studied vitamin are unknown; much of its chemistry and most of its metabolism are yet to be worked out.

The following areas have been under active investigation during the past year:

1. Chemistry of the ascorbic and isoascorbic acids and their oxidation products.

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2. Synthesis of l.-ascorbic-6-^{14}C acid.

3. Distribution of radioactivity from ^{14}C- and ^{3}H-labeled ascorbate in rats.

4. Identification of urinary excretion products from ascorbic acid in the guinea pig.

5. Cellular and molecular distribution of label from ascorbate in guinea pigs.

The investigations represent a cooperative effort with the University of Colorado by Dr. B. M. Tolbert and his group, and the Chemistry Division of the USAMRNL.

RESULTS AND DISCUSSION OF THE RESULTS:

1. Chemistry of the Ascorbic and Isoascorbic Acids and Their Oxidation Products.

a. NMR Spectra of Ascorbates and Dehydroascorbates: This study was made to determine the structure by modern techniques of the various ascorbic acid isomers and their oxidation products. Computer analysis on an IBM 7044 by J. V. Mengenhauser, of the NMR spectra, showed ABC_2 spectra for L-xyloascorbic acid and D-araboascorbic acid, and an ABCD spectrum for L-xylodehydroascorbic acid. The calculated coupling constants and chemical shifts for these compounds have been determined.

The equivalence of the C-6 hydrogens in both L-xylo- and D-arabo-ascorbic acid rules out the possibility of intramolecular hydrogen bonding. The non-equivalence of the C-6 protons of the dehydroascorbic acid may be due to restricted rotation around the bond between carbons five and six. It is postulated that this restricted rotation is caused by a hemiacetal bond between the six hydroxyl and the three keto group. This molecule probably exists in water in a hydrated form.

b. Study of the Oxidation of D-isoascorbic Acid: In dehydroascorbic acid, the configuration of the C-5 and C-6 carbons are D and L, and are thus trans on ring closure. As observed, ring closure would be easy with a minimum conformational strain. In isoascorbic acid (D-araboascorbic acid), the C-5 and C-6 configurations are D-D, and conformational hindrance would occur on ring closure.

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NMR spectra of the oxidation products of D-araboascorbic acid indicate that the main product is not a dehydroascorbic acid. Work is now in progress to identify and characterize this compound.

2. Synthesis of L-Ascorbic-6-^{14}C Acid. This project has been reinitiated by E. M. Bell at the University of Colorado, who will use the procedures worked out by R. Bevill of the USAMRNL. However, the preparation will begin with glucose-1-^{14}C rather than sorbose-1-^{14}C since the glucose is a cheaper source. Glucose-1-^{14}C can be quantitatively reduced to sorbose using $NaBH_4$. A low level synthesis is underway, starting with glucose-1-^{14}C.

The first biological experiment planned for this material will be a serial study of the fate of ascorbate-4-^{3}H-6-^{14}C in the rat. If the $^{14}C/^{3}H$ ratio remains constant in the various organs following injection of a large single dose of the double-labeled material, we will have clear evidence that metabolism of ascorbate or its derivatives in vivo does not involve cleavage of the molecule between the C-4 and C-6 carbons.

3. Distribution of Radioactivity from ^{14}C- and ^{3}H-labeled Ascorbate in Rats. A group of rats was injected with 13.2 μc of ascorbic-1-^{14}C acid and 36.5 μc of ascorbic-4-^{3}H acid in 1 ml of solution containing 31.5 μM ascorbic acid. They were sacrificed at intervals of from 1 to 60 days. At 60 days, the radioactivity levels were no longer high enough to continue the experiment. The rats were dissected and portions of the various tissues were taken for carbon and tritium counting. It is apparent that a decrease in $^{14}C/^{3}H$ ratio would indicate a retention of a degraded ascorbate residue. The ^{14}C and equivalent ^{3}H ratio would correspond to a C-6 metabolite of ascorbate, or ascorbate itself. The retention of an excess of ^{3}H over the ^{14}C would be postulated as indicative of the incorporation of a C-4 fragment into the rat.

The initial data on both labels show a wide distribution of C-6 entities in all tissues. Highest concentrations in dpm/mg tissue were found, in order of decreasing concentrations, in the adrenals, pituitary, spleen, small intestines, liver and in the cerebellum, hypothalamus and large intestine. These last three were very comparable.

By the end of 20 days, the carbon-14 levels, corresponding to a C-6 entity, had fallen to very low values in all tissues except for the adrenals, gonads, cerebrum, cerebellum and the spleen, in order of decreasing amounts of C-6 entity. On the other hand, excess tritium, corresponding to a C-4 metabolite, was still significant in most tissues and highest in the pituitary, spinal cord and the hypothalamus.

The eye and the heart represented an interesting special case. While the initial concentration of C-6 entity was low, the carbon-14 was rapidly lost, and a long-term retention of excess tritium was observed.

These data clearly show long retention of a degraded metabolite of ascorbic acid. We suggest, therefore, that the tritium-labeled fragment of ascorbate with a long turn-over time is a bound form of L-threose or L-threonic acid, and that one of the biochemical roles of ascorbic acid is to function as a donor of this 4-carbon entity.

4. Identification of Urinary Excretion Products from Ascorbic Acid in the Guinea Pig. Two guinea pigs were injected daily with 10 μc ascorbic-4-^{3}H acid and 5 μc ascorbic-1-^{14}C acid in 3 mg total ascorbic acid content over a 14-day period. The urine was collected and thin-layer chromatographic analyses of the labeled excreted products were performed.

Preliminary data show that the majority of the labeled material excreted was as organic compounds which were not ascorbic acid or any of its common derivatives (analogous to the findings in the Iowa City human vitamin C experiment). Further, there appear to be four or five compounds formed as metabolites of labeled ascorbate in the guinea pig. All but one of these compounds are double-labeled and have approximately the same C/H ratio as the injected ascorbate, and are therefore probably six carbon compounds, possibly conjugated. One compound in which the carbon-1 label has been lost could be threonic acid.

5. Cellular and Molecular Distribution of Label from Ascorbate in Guinea Pigs. The guinea pigs used in Section 4 of this report were sacrificed and selected tissues were assayed for ^{14}C and ^{3}H. These tissues were then homogenized and the homogenate separated by centrifugation into four fractions: the membrane fraction, the mitochondrial fraction, the ribosomal fraction and the supernatant. Each fraction was assayed for ^{14}C and ^{3}H using the double spiking procedure and computer analysis of the data. Interesting aspects of the results of this study are:

a. The high concentrations of ascorbate in the brain are most remarkable. This result confirms the results shown in Section 3 of this report (Distribution of Radioactivity from ^{14}C- and ^{3}H-labeled Ascorbate in Rats). There is no great difference in radioactivity in the various parts of the brain.

b. Most of the activity in the gonad is in the cell plasma. Since the gonads retain activity for a long time, it seems likely that the ascorbate may be strongly bound to a soluble protein.

c. The radioactivity in the microsomal fraction of the various brain tissues is very high, indicating a bound form of ascorbate in these particles.

d. There is a very high excess concentration of ^{3}H in the liver supernatant. This suggests that cleavage of ascorbate, C_1-C_2 or C_2-C_3, may occur in the liver and the fragment retained for some time.

e. In most cases, the $^{14}C/^{3}H$ ratio was remarkably close to the value for the injected ascorbate, indicating that most of the radioactivity probably represents ascorbate or ascorbate metabolites containing all six carbons.

f. The summations of per cent "bound ascorbate" were much higher in all three brain tissues than they were in the liver and gonads.

These data and results further implicate an interesting and significant relationship between vitamin C and neurological processes.

CONCLUSIONS:

1. NMR spectra of xyloascorbic acid, isoascorbic (araboascorbic) acid, dehydroxyloascorbic acid and dehydroisoascorbic acid have been obtained.

2. The synthesis of L-ascorbic-6-^{14}C acid from glucose-1-^{14}C is being performed.

3. The tissue distribution pattern in the rat of radioactivity from administered ^{14}C- and ^{3}H-labeled ascorbate has been obtained, and the data indicate that there are several 6-carbon metabolites, as well as a possible 4-carbon metabolite, present.

4. The urinary metabolites of guinea pigs administered ^{14}C- and ^{3}H-labeled ascorbate have been studied and found not to be ascorbic acid but rather derivatives of it. There appear to be 4 or 5 compounds formed as metabolites of labeled ascorbate. All but one of these compounds are double-labeled and have approximately the same C/H ratio as that of the injected ascorbate and are therefore probably six-carbon compounds, possibly conjugated. The one compound which had lost carbon-14 radioactivity but retained tritium activity might be threonic acid.

5. The ^{14}C and ^{3}H activity levels of membrane, mitochondrial, ribosomal and supernatant fractions of selected tissues of guinea pigs who had been labeled with ^{14}C- and ^{3}H-ascorbic acid have been measured.

WORK UNIT NO. 074 - BODY OF REPORT

PUBLICATIONS:

1. Tolbert, B. M., E. M. Baker and J. C. Saari. Ascorbic acid metabolism in man. Fed. Proc., 2: 218, 1966 (abstract and presentation).

2. Bell, Ellen M. "Preparation and NMR Studies of Labeled Ascorbic Acid." M.S. Thesis, Department of Chemistry, University of Colorado, August 1966.

3. Bell, E. M., E. M. Baker and B. M. Tolbert. Synthesis of L-ascorbic-4-^{3}H acid. J. Labeled Compounds, II: 148, 1966.

4. Tolbert, B. M., A. W. Chen, E. M. Bell and E. M. Baker. Metabolism of L-ascorbic-4-^{3}H acid in man. Am. J. Clin. Nutr., 20: 250, 1967.

5. Baker, E. M., J. C. Saari and B. M. Tolbert. Ascorbic acid metabolism in man. Am. J. Clin. Nutr., 19: 371, 1966.

6. Tolbert, B. M., S. C. March, D. B. Karr, W. Scharf and E. M. Baker. Ascorbate function: donor of a four-carbon moiety? Fed. Proc., 26: 854, 1967 (abstract).

7. Bell, E. M., J. V. Mengenhauser, B. M. Tolbert and H. E. Sauberlich. NMR study of ascorbic and dehydroascorbic acid. Fed. Proc., 26: 854, 1967 (abstract).

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6337	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 07 66)	U / U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 00 076

10b. PRIOR NUMBER/CODE: None

11. TITLE: (U) Analytical Biochemistry (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry; 003500 Clinical medicine	07 66	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. NUMBER / b. TYPE / c. DATE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA	PRIOR FY 67	1	160
		CURRENT FY 68	1	94

19. GOV'T LAB/INSTALLATION/ACTIVITY
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Sauberlich, H. E.
TEL: 303 366-5311 X24214 TYPE: DA

21. TECHNOLOGY UTILIZATION: Medicine and Nutrition

22. COORDINATION: None

23. KEYWORDS: Analytical Biochemistry; Instrumentation; Automated Analyses; Methodology; Analytical Chemistry.

24. (U) Technical Objective: To develop and adapt new concepts in analytical biochemistry to biochemical procedures and research; provide analytical support to research programs within the Division and cooperatively with projects in other Divisions or cooperating agencies.

25. (U) Approach: Depending upon requirements and the availability of personnel, biochemical procedures will be developed or adapted through direct innovation or revision of existing methods. Whenever feasible, electronic controllers and mechanical devices will be incorporated to provide automated or semiautomated analytical procedures yielding maximum efficiency. A primary effort will be to provide analytical support to those task elements requiring unique equipment or specific methodologies. A continuing program will be maintained to provide for improved automation analysis in clinical chemistry, particularly routine analyses required in volume.

26. (U) Progress: In support of the above objectives, a significant degree of automation and personnel training have been accomplished. Fluorescent procedures for magnesium, calcium and serum glutamic oxalacetic transaminase, the enzymic assay of lactic dehydrogenase, the urinary analysis of creatinine, and the blood serum determination of total fatty acids have been automated and are capable of input to laboratory data acquisition systems now under procurement.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A025601A822 Military Internal Medicine

TASK NO. 01

WORK UNIT NO. 076 Analytical Biochemistry

The following investigations have been conducted under this work unit:

STUDY NO. 1 Analytical support and services

STUDY NO. 2 Development of analytical biochemistry procedures

1. Three phases of study have been pursued under this work unit -- analytical support, procedure development and method automation. Accomplishment in the latter two phases increases the capability for analytical support in areas of automated methodologies and specific techniques.

2. Enzymic assays have been automated for lactic acid, and a complete system is under development for the enzymes of the glycolytic pathway. The advantages of fluorometry have been applied to procedures for magnesium, calcium and serum glutamic oxaloacetic transaminase. Total fatty acids are now determined by an automated extraction-reaction method.

3. A comprehensive evaluation of data acquisition systems for analytical instrumentation showed two specific types of value. Procurement has been initiated for one unit, a digital print-out system based on maximum signal produced by an automated, time-sequenced analyzer. The second system of merit provides for on-line computation of concentration from a totally-integrated signal, derived from a variety of analytical instruments.

BODY OF REPORT

WORK UNIT NO. 076 Analytical Biochemistry

STUDY NO. 1 Analytical support and services

PROBLEM:

The requirements of the various task elements for analytical support and service demand the optimal use of personnel and equipment. To satisfy these demands, it is essential to investigate any mechanical, chemical, or electronic advances, capable of increasing the rate, decreasing the time, or improving the accuracy of analytical procedures.

RESULTS AND DISCUSSION OF THE RESULTS:

1. The use of automatic dilution devices, both manual- and electric-operated, has resulted in increased accuracy of dilutions, decreased man-hour requirements and reduced costs by eliminating expendable glassware.

2. Application of an electronic digital integrator to the output of an atomic absorption spectrometer has served a two-fold purpose — more precise quantitation of trace element analysis and elimination of the effects of sample viscosity. Specific volumes are aspirated in a total consumption burner and the entire transducer signal is integrated.

3. In those automated procedures wherein samples are analyzed on a time-sequence basis, reliable concentration values may be obtained by measurement of peak height (maximum millivolt signal) rather than total integration of individual sample peak areas. An eight-channel digitizer, capable of automatically calibrating its response and then detecting, digitizing and printing the maximum peak heights from simultaneously operating, synchronized, or non-synchronized instruments has been purchased. Time spent in transposing sample data, in plotting concentration curves or equations, in interpolating strip chart peaks, in calculating concentrations and in sorting and tabulating data will now be available for additional analyses and methods development.

CONCLUSIONS:

Applications of new electro-mechanical devices to problem areas in analytical biochemistry make it possible not only to increase productivity but also to improve accuracy and reproducibility.

WORK UNIT NO. 076 - BODY OF REPORT

RECOMMENDATIONS:

The advent of electronic data-gathering and analysis, as well as automated instrumentation, makes it imperative that the analytical biochemist keep current in today's technology, either by self-training or by special instruction. For the service laboratory to function effectively, full use must be made of systems programming.

STUDY NO. 2 — Development of analytical biochemistry procedures

PROBLEM:

The demand for innovation in analytical biochemistry is in direct proportion to advances in biochemistry itself. As new concepts arise, the need to evaluate them analytically becomes essential. To provide for precise data acquisition, it is vital that development be conducted on specific methodologies.

RESULTS AND DISCUSSION OF THE RESULTS:

1. Utilizing auto-analyzer techniques in conjunction with fluorometers with flow-through cells, procedures have been established for magnesium and calcium at the sub-microgram level, and for serum glutamic oxaloacetic transaminase at the clinical level.

2. The inadequacies of the titrimetric procedure for total fatty acids in lipid extracts have been overcome by conversion to an automated colorimetric method. The procedure is based on the solubility of copper soaps in chloroform with subsequent complexing of the copper with diethyldithiocarbamate and measurement of the extinction at 440 millimicrons.

3. An automated enzymic method for determining whole blood lactic acid levels has been established. The procedure is based on the conversion of lactate to pyruvate by the enzyme, lactic dehydrogenase. Concentrations as low as 30 micrograms/ml can be accurately determined with a reproducibility of ±0.6 micrograms.

WORK UNIT NO. 076 - BODY OF REPORT

CONCLUSIONS:

Automation of analytical biochemistry procedures provides the means for precise and reliable acquisition of data to the investigator. Development of such methods offers a solution to the analytical demands of large-scale studies and to the time requirements of the project leader.

RECOMMENDATIONS:

Methods development should be a key requisite in the program of the analytical biochemistry service group to provide for optimum use of the investigator and full utilization of the laboratory capabilities.

PUBLICATIONS:

1. Levine, R. A., R. S. Harding and G. Briggs. The effects of prolonged gluten and gliadin feeding on normal subjects. New Eng. J. Med., 274: 1109, 1966.

2. Levine, R. A., R. S. Harding, G. J. Isaac, E. T. Bongiovanni and C. L. Miller. Fasting plasma amino acids and nitrogen retention in man following prolonged supplementation with wheat gluten or egg whites. Am. J. Clin. Nutr., 20: 404, 1967.

3. Harding, R. S., G. A. Leveille, E. M. Baker, Z. Z. Ziporin and H. E. Sauberlich. Biochemical procedures -- volume I. USAMRNL Report No. 304, 23 January 1967.

4. Friedemann, Theodore E., Norman F. Witt, Bonnie W. Neighbors and Charles W. Weber. Determination of available carbohydrates in plant and animal foods. J. Nutrition, 91: 1-40, 1967.

GPO 770801

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6324	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (30 06 66)	U / U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
62156011 3A025601A822 00 077	None

11. TITLE:
(U) Nutritional Physiology (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
016200 Stress Physiology 002300 Biochemistry 005900 Environ Biology	10 64	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	2	35
		CURRENT FY 68	2	85

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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TEL. 202 OXford 6 5472	TEL. 303 366-5311 X22119 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Nutrition	None

23. KEYWORDS
Adaptation; Physiological; Nutrition; Metabolism; Environmental Stress

24. (U) Technical Objective: The purpose of these investigations is to study the phenomenon of the often-observed simultaneous metabolic adjustments of animals and humans to multiple stresses and their qualitative and quantitative effects upon nutritional adaptations and requirements.

25. (U) Approach: The problems will be approached through the study of: 1) simultaneous stresses in animals and, if possible, in humans; 2) responses common to two or more stresses; 3) time sequences of the onset of the responses 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) radiochemical studies; 5) determination of metabolic pathways; and 6) clinical observations.

26. (U) Progress: Hepatic lipogenesis from glucose was markedly decreased in rats fed a diet containing orotic acid. In contrast, ingestion of orotic acid enhanced lipogenesis in white adipose tissue. Lipogenesis in this tissue was not inhibited by cold stress. Activities of the urea cycle enzymes were increased in ground squirrels within two hours after arousal from hibernation. Oxidation of amino acids was also increased during this arousal from hibernation.

Publications: Fed. Proc. 26:336, 1967; Amer. J. Physiol. 213(1): July 1967. Comp. Biochem. & Physiol. 22: July 1967. Biochem. & Biophys. Acta, 1967 (In press). Canad. J. Physiol. & Pharmacol. 1967 (Submitted).

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) CFY + 1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A025601A822 Military Internal Medicine

TASK NO. 01

WORK UNIT NO. 077 Nutritional Physiology

The following investigations have been conducted under this work unit:

STUDY NO. 1: Orotic Acid and In Vitro Lipogenesis of Hepatic and Adipose Tissue from Cold-Stressed Rats

STUDY NO. 2: Metabolic Changes During Arousal From Deep Hypothermia in the Ground Squirrel

STUDY NO. 3: In Vitro Incorporation of Lysine and Methionine Into Livers of Fasted and Fasted-Refed Rats

Ingestion of orotic acid at either 25° or 6° markedly stimulates lipogenesis from glucose in white adipose tissue. By contrast, hepatic lipogenesis is decreased in orotic acid-fed rats. Arousal from hypothermia is associated with increased activities of the urea cycle enzymes and an increased oxidation of amino acids. During this period, protein biosynthesis is depressed, while gluconeogenic capacity, as indicated by an increased rate of CO_2 fixation, is significantly elevated. There is an increased turnover of protein during fasting.

BODY OF REPORT

WORK UNIT NO. 077 — Nutritional Physiology

STUDY NO. 1 — Orotic Acid and In Vitro Lipogenesis of Hepatic and Adipose Tissue From Cold-Stressed Rats

PROBLEM:

Orotic acid produces fatty livers in rats when fed as one percent of the diet. Fatty infiltration appears within three days and reaches a maximum level of approximately 25 percent of the wet weight in two weeks. The accumulated fat contains high levels of triglycerides and cholesterol, while the concentration of phospholipids remains relatively constant. Rats ingesting orotic acid also incorporate more tritium from H_3O into fatty acids of extrahepatic tissues than the corresponding controls. Accordingly, in vitro lipogenesis from glucose was studied in liver and white adipose tissue of rats ingesting orotic acid. In view of the fact that a mild cold stress depresses lipogenesis from glucose, the study was extended to include tissues from a group of animals fed orotic acid at 6° C.

Male Holtzman albino rats weighing 160-180 grams were exposed to 6° C while the corresponding controls were kept at 25° C. The animals were housed in individual wire cages with diets and water available ad libitum. The basal diet contained 18% casein, 75% sucrose, 3% corn oil and 4% USP Salt Mixture No. XIV, and a complete vitamin mixture. One percent of orotic acid was added to the basal diet at the expense of sucrose. Both the feeding period and cold exposure were of ten days' duration. At the end of this period, the animals were sacrificed and epididymal fat pads and livers were removed and placed in cold saline. In vitro lipogenesis from glucose-U-C^{14} in liver or fat pad slices was then studied using standard biochemical techniques.

RESULTS AND DISCUSSION:

The data indicate that ingestion of orotic acid at either 25° C or 6° C resulted in a markedly increased oxidation of glucose to CO_2, and in a markedly enhanced incorporation of glucose carbons into the following lipid fractions of white adipose: fatty acids, glyceride-glycerol, and the nonsaponifiable lipids. In contrast, incorporation of glucose carbon into glycogen was significantly reduced at 25° C, while no statistical difference was found at 6° C. Lipogenesis in this tissue was not inhibited by cold stress. By contrast, the capacity of liver for lipogenesis was decreased in animals fed the orotic acid diet. The environmental temperature had no effect on this phenomenon. Cold stress did not prevent hepatic lipid infiltration produced by orotic acid.

Nutritional Physiology (Continued)

STUDY NO. 2 Metabolic Changes During Arousal From Deep Hypothermia in the Ground Squirrel

PROBLEM:

The thirteen-lined ground squirrel withstands deep hypothermia (body temperature 4 - 5° C) for periods of up to four months. The physiological and biochemical phenomena which are operative during the process of arousal (return to a normal body temperature of 37° C) are of interest in relation to the adaptive mechanisms which function during and after hypothermia.

Accordingly, the following in vitro studies were conducted using hepatic tissues from animals in deep hypothermia and after a two-hour arousal period: (1) Activities of the urea cycle enzyme; (2) Oxidation of several amino acids; (3) Protein biosynthesis; (4) Activities of several NADPH-generating enzymes; (5) CO_2 fixation; (6) Lipogenesis from a number of precursors.

RESULTS AND DISCUSSION:

The results indicate that the activities of the urea cycle enzymes were increased within two hours after arousal. Oxidation of amino acids was also increased during the period. By contrast, incorporation of labeled amino acids into liver proteins was decreased after the arousal. Lipogenesis during arousal was depressed, however, CO_2 incorporation into glucose was accelerated. Activities of some NADPH-generating enzyme systems were slightly increased during the arousal period.

STUDY NO. 3 In Vitro Incorporation of Lysine and Methionine Into Livers of Fasted and Fasted-Refed Rats

PROBLEM:

Refeeding following a period of prolonged fasting has been shown to induce fatty livers which may be of sufficient severity to produce, or be associated with, degenerative changes. Previous data from in vivo experiments indicate that specific activities in the liver proteins of refed rats were slightly decreased when compared with either control or fasting animals. Accordingly, in vitro protein biosynthesis was studied in fasted and fasted-refed rats to determine whether a decrease in specific activities of liver proteins in refed rats is associated with hepatic microsomal fractions.

A group of rats weighing from 150-160 grams was subjected to five days' fasting. The control group was fed ad libitum, a diet

consisting of 15% crude casein, 78% sucrose, 3% corn oil, 4% mineral mixture and a complete vitamin supplement. Water was available to both groups at all times. At the end of the fasting period, the animals from each treatment group were sacrificed, the livers were quickly removed and chilled. Microsomal preparations were then incubated with either lysine-U-C^{14} or methionine-methyl-C^{14}, and incorporation of either amino acid into the microsomal proteins determined.

RESULTS AND DISCUSSION:

The data demonstrate that during fasting there was an increased incorporation of methionine or lysine into microsomal protein fractions. Upon refeeding, specific activities were decreased and were equal to that found in the ad libitum fed controls.

SUMMARY AND CONCLUSIONS:

Ingestion of orotic acid stimulates lipogenesis from glucose in white adipose tissue of rats maintained at either 25° or 6° C. Cold stress decreased lipogenesis in tissue of the animals fed the control diet. By contrast, cold stress did not inhibit lipogenesis in tissue of the animals fed orotic acid. An increased turnover of all the lipid components under these conditions could explain an accelerated incorporation of glucose carbon into the lipid metabolites. In view of the fact that orotic acid depresses hepatic lipogenesis, the increased lipogenesis in white adipose tissue, therefore, may serve as a compensatory mechanism for fatty acid synthesis.

Marked metabolic alterations in hepatic tissues of ground squirrels after a two-hour arousal from hypothermia are suggestive of an adaptation and reorganization of metabolic processes for existence at normal body temperature. Increased activities of the urea cycle enzymes, together with an increased oxidation of amino acids and a decreased biosynthesis of proteins, indicate that body proteins are utilized for energy purposes during the arousal. An increased rate of CO_2 fixation during arousal from hypothermia further indicates that gluconeogenesis may be of importance in the arousal process.

The increased incorporation of amino acids into liver proteins and the increased oxidation of amino acids to CO_2 during fasting suggest an increased turnover of protein during the period of starvation. Hepatic lipid infiltration following refeeding after fasting does not affect the capacity of microsomal fraction to incorporate amino acids into proteins.

PUBLICATIONS:

1. Klain, G. J. and R. F. Burlington. Gluconeogenesis during hibernation and arousal from hibernation. Fed. Proc. 26: 336, 1967.

2. Klain, G. J. and R. F. Burlington. Effect of cold on glucose metabolism of fasted and fasted-refed rats. Am. J. Physiol. 213:(1) July, 1967.

3. Burlington R. F. and G. J. Klain. Effect of hypoxia on gluconeogenesis in the albino rat and thirteen-lined ground squirrel. Comp. Biochem. Physiol. 20: 275, 1967.

4. Burlington, R. F. and G. J. Klain. Gluconeogenesis during arousal from hibernation. Comp. Biochem. Physiol. 22: July 1967.

5. Klain, G. J. and B. K. Whitten. The effect of orotic acid and cold stress on lipogenesis in white adipose tissue. Biochem. and Biosphys. Acta 1967 (In press).

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6343	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (10 04 67)	U RPT U WRK	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER CODE
62156011 3A025601A822 00 078	None

11. TITLE:
(U) Metabolic Response of Man to Nutrition or Disease (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
00350 Clinical Medicine	07 66	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	19 RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	a. DATE b. NUMBER NA c. TYPE d. AMOUNT	PRIOR FY 67	0	27
		CURRENT FY 68	0	33

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20 PERFORMING ORGANIZATION
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INVESTIGATORS PRINCIPAL ASSOCIATE Herman, R. H., LTC
TEL 303 366-5311 X10221 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Medical Problems in General	None

23. KEYWORDS
Fever; Periodic fever; Porphyria, Polymorphic

24. (U) Technical Objective: It is desired to determine under a variety of conditions how the metabolic functioning of man is altered. In a variety of states of nutrition, metabolic pathways are altered. In disease people stop eating and they are in a relatively starved condition. Thus, gluconeogenesis and ketosis occur. There is loss of muscle mass, body fat, minerals, water, and vitamins. This causes alterations in red cell, liver, muscle, and subcutaneous adipose tissue metabolism.

25. (U) Approach: A variety of disease states will be investigated. These include porphyria, the effect of a variety of derivatives from wheat on small bowel functioning in normal man, pulmonary hypertrophic osteoarthropathy, vitamin deficient states, malabsorptive diseases, and Klinefelter's syndrome.

26. (U) Progress: We have investigated a number of patients for porphyria and have been able to exclude the disease in a number of patients. Studies in this particular disease are limited by the availability of patients. We are now studying the effect of various fractions of gliadin on the absorption of fat by normal man. Studies are under way to determine the nature of thiamine deficiency in acutely ill patients and the role this plays in precipitating hemolytic states in glucose-6-phosphate dehydrogenase sufficiency. We currently have under study a series in rats in whom we are attempting to induce the lesions of pulmonary hypertrophic osteoarthropathy by injecting serum and urine obtained from a patient with such a condition secondary to a bronchogenic carcinoma.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		BR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A025601A822	Military Internal Medicine
TASK NO.	00	
WORK UNIT NO.	078	Metabolic Response of Man to Nutrition or Disease

Metabolic changes of profound nature occur in man when the nutritional state is altered or when disease states occur. We have investigated such metabolic changes in certain patients and in certain experimental conditions. We have tested certain fractions of gliadin which are toxic in patients with celiac disease in normal human subjects. The preliminary data so far indicates that as expected these gliadin fractions are non-toxic for normal man. We have tested serum and urine from a patient with hypertrophic pulmonary osteoarthropathy in rats to determine if we can detect the presence of any substance which causes similar lesions. No visible x-ray lesions were produced. Microscopic section of the injection sites is pending. We have investigated certain patients with selected metabolic disorders and have studied metabolic derangements in these patients. We now have available for study patients with hypopituitarism and diabetes insipidus.

BODY OF REPORT

WORK UNIT NO. 078 Metabolic Response of Man to Nutrition or Disease

DESCRIPTION:

During drastic changes in the nutrition of man profound alterations in metabolic processes occur. During disease states increased or decreased consumption of calories occur. In hypermetabolic states there may be increased food consumption competing with increased utilization. During many disease states starvation occurs with not only a negative nitrogen balance with wasting of tissue but a loss of electrolytes and vitamins which complicates an already difficult situation in many cases. In certain of the endocrine disorders abnormal utilization of nutrients, including minerals, vitamins, and water occurs. By virtue of our access to certain patients with selected metabolic disorders we are able to study these metabolic changes. Such disease states include porphyria, both hepatic and erythropoietic, klinefelter's syndrome, hypopituitarism, diabetes insipidus, vitamin deficiency, malabsorption, pulmonary hypertrophic osteoarthropathy, and so on.

PROGRESS:

In conjuction with Prof. O. D. Kowlessar of Jefferson Medical College in Philadelphia, Pennsylvania we have studied the effect of various gliadin fractions on the absorption of fat in normal human subjects. These fractions have been shown by Prof. Kowlessar to produce malabsorption in patients with celiac disease. However, the effect on normal man has not been tested heretofore. It would be predicted on the basis of studies to date that such fractions of gliadin would not be toxic in normal subjects with no evidence of celiac disease or other malabsortive disorder. Such studies have now been completed and the preliminary data so far show that as expected no change in fecal fat occurred indicating that the fractions toxic for patients with celiac disease have no effect on normal human subjects.

We have investigated the possibility of producing lesions in rats suggestive of hypertrophic pulmonary osteoarthropathy. Blood and urine was obtained from a patient with hypertrophic pulmonary osteoarthropathy secondary to a carcinoma of the lung. Blood and urine specimens were obtained before and after surgery. After the surgical removal of the neoplastic lesion the hypertrophic pulmonary osteoarthropathy regressed. Rats were then injected with the urine and serum obtained from this patient before and after surgery. Over a period of several weeks no x-ray changes could be seen in the limbs injected with the serum and urine. The animals were killed at fixed intervals and the limbs removed and fixed. The limbs injected with the serum and urine and limbs injected with saline for control purposes are now undergoing sectioning and will be examined microscopically to determine if any lesion at all was induced in these animals. Obviously, this study was carried out with a great number of assumptions any one of which may be incorrect. This may be the wrong species of animal, doses of serum and urine may be too small,

no substance may be present in urine or serum that are responsible for the condition, the substance, if present, may be unstable, and so on. Nevertheless, it is necessary to begin somewhere in any investigation and this seemed to be a reasonable approach, as a first approximation. The results of this study will now permit us to devise further studies in a more precise fashion.

Other patients that have been studied include three patients investigated for the possibility of porphyria, one patient with hyperparathyroidism, two patients investigated for hypopituitarism, one patient studied with diabetes insipidus, and one patient investigated for hyperaldosteronism. The result of these studies have excluded the possibility of porphyria and hyperaldosteronism. The diagnoses of hyperparathyroidism, diatebes insipidus, and hypopituitarism in one patient were proven. Thus, we now have access to several patients with proven metabolic disorders which can be studied as the needs of our research projects demand.

SUMMARY:

We have shown that fractions of gliadin which are toxic for patients with celiac disease have no effect on normal individuals. We have been unable to reproduce the lesions of pulmonary hypertrophic osteoarthropathy in rats using the serum and urine from a patient with this condition though certain of the data is still pending. We have investigated certain patients with selected endocrine and metabolic disorders and have acquired a small group of patients now with metabolic disorders who can be studied whenever our research programs require studies.

PUBLICATIONS:

None.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6347	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (09 01 67)	RPT U WRK U	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b PRIOR NUMBER/CODE
62156011 3A025601A827 00 070	61145011 3A014501B71R 05 080

11. TITLE:
(U) High Altitude Bioenergetics (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
016200 Stress Physiology 00590 Environmental Biology 012900 Physiology	07 66	NA	OTHER IDA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	1	28
		CURRENT FY 68	1	25

19. GOV'T LAB/INSTALLATION/ACTIVITY
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Krzywicki, H.J., Johnson, H.L.
TEL 303 366 5311 X25222 TYPE DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Mountaineering; Mining	None

23. KEYWORDS Hypoxia; Stress; Performance decrement; Work; Balance-metabolic; Blood gas analysis; Psychological tests; Spirometry

24. (U) Tech Objective: Locate and quantitate the human performance decrements to be expected in military operations at 10-18,000 feet and to measure the extent of and rates of acclimatization.

25. (U) Approach: A. Measure and compare symptoms; food, nitrogen, mineral, and water balance. B. Measure pulmonary, cardiovascular, and metabolic changes during rest, mild, moderate, and exhausting work and recovery in 16 sea level soldiers before, during, and after four weeks at altitude. C. Measure physiology of other mammals in chamber and altitude environments in multiple experiments.

26. (U) Progress: Eight Laboratory Reports Nos. 289, 290, 291, 292, 293, 294, 295, and 300 and two journal articles, Fed. Proc. 25:1380, 1966 and J. Appl. Physiol. 21:1732, 1966 have been published on humans at altitude. A new study has been proposed to study the problem of negative nitrogen balance at altitude on protein intakes c 0.9 gm/kg of body weight. A new study was completed on the effects of a high carbohydrate diet as it relates to mountain sickness symptoms. Body composition, energy, water, nitrogen, and electrolyte balances are also being evaluated.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY+1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A025601A827	Military Environmental Medicine
TASK NO.	01	
WORK UNIT NO.	070	High Altitude Bioenergetics

A broad program investigating physiology and behavior of humans at high terrestrial altitude is in progress. We plan to locate and quantitate the performance decrements to be expected in soldiers in military operations at 10, 000 to 18, 000 feet; to measure the extent of and the rate of acclimatization; to investigate the physiology, biochemistry, and pharmacology of the affected organ systems; and to ascertain how to minimize the decrements by personnel selection, physical conditioning, environmental exposure, nutrition, drugs, or other variables. Animal species are also being studied at high altitude. Acute mountain sickness and its prevention or treatment is one of the most serious military problems to be investigated.

BODY OF REPORT

WORK UNIT NO. 070 High Altitude Bioenergetics

PROBLEM:

Our objective is to locate and quantitate the performance decrements to be expected in soldiers in military operations at 10, 000 to 18, 000 feet; to measure the extent of and the rate of acclimatization; to investigate the physiology, biochemistry, and pharmacology of the organ systems causing the decrements; and to ascertain how to minimize the decrements by selection, conditioning, previous environmental exposure, nutrition, drugs, or other variables. We plan to measure and correlate personality, pulmonary, cardiovascular, metabolic, and biochemical parameters at rest, various levels of work and recovery in healthy human populations at both low and high terrestrial altitudes or with the use of altitude chambers or gas mixtures for short-term studies. We plan to participate in field studies with multiple performance measurements of actual military tasks and to study the effects of grade walking and load carrying. The physiology and biochemistry of several aspects of acclimatization will be investigated in the organ systems of several animal species at actual or simulated altitude with appropriate techniques. The effects of high carbohydrate diets and mineral supplementation as related to mountain sickness symptoms and to water, nitrogen, and electrolyte balances will be investigated.

RESULTS AND DISCUSSION OF THE RESULTS:

During the past year a series of four papers were completed and published on various phases of high altitude (three scientific journal articles and one Laboratory Report No. 300).

Human Performance at 3475 Meters. J. Appl. Physiol. 27:1732, 1966.

Maximal work capacity ($\dot{V}_{O_2}$) on the bicycle ergometer was decreased in three groups of men, one group acclimated to sea level, and two groups acclimated to 1610 meters. At 3475 meters, maximal $\dot{V}_{O_2}$ in milliliters per kilogram body weight per minute was reduced by 17% for the sea level group, and by 10% for the group from 1610 meters. Although there was a difference of approximately 7% in $\dot{V}_{O_2}$ between sea level and 1610 meters, there was no measurable beneficial effects of acclimatization at 1610

meters in improving maximal work at 3475 meters. Maximal work capacity and maximal $\dot{V}_{O_2}$ did not improve over a 20 day period at altitude. $\dot{V}_E$ STPD was decreased, and $\dot{V}_E$ BTPS increased on arrival at altitude with a gradual increase in both during prolonged exposure. Pulse rates at rest, and moderate exercise, were consistently high at high altitudes, whereas the maximal pulse rates gradually declined. Oxygen consumption at the basal, sitting rest, and moderate exercise states was not markedly changed by altitude. The physiological cause for the cessation of maximal work at altitude remains obscure. Under the conditions of this study, (a) the 1610 meter elevation did not seem to be beneficial in improving the maximal work at 3475 meters, (b) a 20 day acclimatization period at 3475 meters did not result in a superior submaximal or maximal work performance on return to sea level, and (c) individuals can adequately perform submaximal work even after the initial high altitude exposure.

Human Performance at 3475 and 4300 Meters. Fed. Proc. 25:1380, 1966.

Oxygen requirements and work performance were evaluated at 1610, 3475, and 4300 meter elevations. Maximal oxygen consumption liters per minute, STPD, was decreased with an increase in altitude. The maximal performance, $\dot{V}_{O_2}$ (milliliters per kilogram body weight per minute), averaged 40.5 ml at sea level, 37.0 at 1610 meters, 33.0 at 3475 meters, and 32.1 ml/kg body weight at the 4300 meter elevation. Basal metabolic rates, sitting rest, and submaximal work (liters per minute oxygen) were practically unchanged at all altitudes, even though the pulse rates were increased. One group showed a significant increase in BMR at 4300 meters during the first week of exposure. Pulse rates were decreased during maximal work at 3475 and 4300 meter altitudes. On the other hand, the pulse rates during sitting rest and submaximal work were increased with an increase in high altitude. The decrease in maximal oxygen pulse at high altitudes and the significant increase in oxygen equivalent at the 3475 and 4300 meter elevation reflect the penalty incurred due to decreased barometric pressure at these altitudes.

In this study there seemed to be no great beneficial effects of ascending to altitude either gradually or abruptly or

between the groups that exercised and those who did not exercise, although the physical well-being (reduced "mountain sickness" symptoms) of the men who ascended to altitude gradually was greatly improved over the men who ascended to altitude abruptly.

Alterations in Body Composition. J. Appl. Physiol. 21:1741, 1966.

Collaborative study with Physiology Division, USAMRNL, which will be reported by them.

Respiratory Function at High Altitude.

Respiratory function of two groups of normal young adults was studied at sea level and at an altitude of 4300 meters for 28 days. In this study, physical conditioning in one-half of the subjects proved to be beneficial since it resulted in an additional increase in MBC during high altitude exposure. This suggests that physical conditioning may be a factor in attaining a superior physical condition at altitude. All of the groups exposed to altitude showed significantly increased MBC, with increasing altitude, suggesting a decreased work of breathing the rarified air.

Maximal breath holding time in seconds, for all groups at altitude were significantly decreased, and again the physically conditioned group had significantly higher values than the non-exercisers during altitude exposure. These decreases may be related to the oxygen tension of the inspired air and the decreased gas volume in the lungs. It is suggested that the great changes one observes at altitude in MBC and maximal breath holding time could be used as an index of adaptation to high altitude. There is a marked improvement in these parameters with physical conditioning.

Resting and maximal work $\dot{V}_E$ (BTPS) were significantly increased at the 4300 meter altitude. This, in conjunction with the increased MBC values at altitude, strongly suggests that ventilation during maximal work is not limited by the strength of the respiratory muscles, but is regulated through the central nervous system.

Energy Metabolism and High Carbohydrate Supplementation as Related to Physical Endurance.

This study was recently completed on sixteen sea level soldiers brought abruptly to high altitude. The group was divided into two groups with eight men receiving a normal diet and eight receiving a high carbohydrate diet. Some of the immediate problems that were investigated were: (a) to measure the energy cost of the various military activities at high altitudes; (b) the decrement in work endurance at high altitude; (c) the efficiency of moderate work at altitude; (d) the effects of grade walking and load carrying on the treadmill; (e) psychological evaluation of performance; (f) body composition changes including body fat by displacement, body water using D_2O, plasma, blood and red cell volume using Evans blue dye; (g) water, nitrogen, and electrolyte balances; and (h) the influence of carbohydrate diets as related to the clinical symptoms and to endurance at high altitude. Information is being compiled and analyzed for a future report.

Calorie, Nitrogen, and Water Requirements (4300 Meters).

Balance studies were conducted on three groups of young healthy adults between the ages of 18 - 24 years. After control studies, Group I was taken to 4300 meters gradually, Group II was taken to 4300 meters abruptly and Group III remained at sea level during the entire study. One-half of each group were physically conditioned. No significant differences were observed in food intake, nitrogen, and fluid balances between (a) the groups that were taken to altitude gradually or abruptly or (b) between the groups that were physically conditioned and those who did not exercise. As a result, both groups were combined for comparative purposes.

Three factors were prominent during high altitude exposure to 4300 meters that included: (a) a decrease in food intake that in all probability is due to anorexia caused by the clinical symptoms; (b) although the protein intake was 60 gm/day, a negative nitrogen balance was observed which may be due to decreased protein synthesis at high altitude; and (c) a negative fluid balance, due to involuntary dehydration and other undetermined factors.

These and other related problems require considerable investigation in the near future.

CONCLUSIONS:

In maximal work, the oxygen uptake significantly decreases with an increase in altitude but during rest and submaximal work, oxygen uptake is practically unchanged.

Maximal breathing capacity is significantly increased with an increase in altitude and suggests decreased work of breathing the rarified air. Maximal breath holding time is significantly decreased with an increased altitude. Both of these parameters showed marked improvement with physical conditioning.

Information is now being compiled and evaluated on the effects of a high carbohydrate diet as related to mountain sickness symptoms.

Three factors were prominent during high altitude exposure to 4300 meters that included: (a) a decrease in food intake that in all probability is due to anorexia caused by the clinical symptoms; (b) a negative nitrogen balance which may be due to decreased protein synthesis or decreased protein utilization at high altitude; and (c) a negative fluid balance, due to involuntary dehydration and other undetermined factors.

These and other related problems require considerable investigation in the near future.

RECOMMENDATIONS:

1. Continue to investigate the energy cost of various military activities at high altitude.

2. Evaluate the effects of a high carbohydrate diet at high altitude.

3. Evaluate the use of mineral supplementation as a means of reducing the mountain sickness symptoms.

4. Further investigate the reasons for the negative nitrogen balances observed at high altitude on intakes of 60 gms of protein/day.

5. Evaluate body compartment changes at high altitude.

High Altitude Bioenergetics (Cont'd)

PUBLICATIONS:

1. Consolazio, C. F., L. O. Matoush, and R. A. Nelson: Energy metabolism in maximum and submaximum performance at high altitudes. Fed. Proc. 25:1380, 1966.

2. Consolazio, C. F., R. A. Nelson, L. O. Matoush, and J. E. Hansen: Energy metabolism at high altitudes (3475 m). J. Appl. Physiol. 21:1732, 1966.

3. Surks, M. I., K. S. K. Chinn, and L. O. Matoush: Alterations in body composition in men after acute exposure to high altitudes. J. Appl. Physiol. 21:1741, 1966.

4. Consolazio, C. F., H. L. Johnson, L. O. Matoush, R. A. Nelson, and G. J. Isaac: Respiratory function in normal adults at 3475 and 4300 meters. USAMRNL Report #300, January 1967.

5. Consolazio, C. F., L. O. Matoush, and H. L. Johnson: The calorie, nitrogen, and water requirements at high altitude. USAMRNL Report (in press).

GPO 778064

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6348	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 07 66)	U_{RPT} U_{WRK}	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
62156011 3A025601A827 00 071	None

11. TITLE:
(U) Cardiovascular and pulmonary Responses at High Altitude (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
00590 Environmental Biology; 012900 Physiology	07 66	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE / b. NUMBER / c. TYPE / d. AMOUNT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER NA	PRIOR FY 67	2	50
		CURRENT FY 68	2	20

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21. TECHNOLOGY UTILIZATION Medical problems in populations at high altitude

22. COORDINATION
None

23. KEYWORDS Environmental Stress; Physiological Adaptation; Acclimatization; Cardiovascularaand Pulmonary Function; High Altitude

24. (U) Technical Objective: The prime purpose of this work unit is to investigate the responses of the cardiovascular and pulmonary systems to hypoxia. Particular attention will be given to humans during actual altitude exposure. The studies will emphasize the interrelationships of cardiovascular and pulmonary function with the function of other body systems. The initial decrements in function, as well as the amelioration of these decrements through acclimatization or other procedures will be investigated.

25. (U) Approach: For the most part, conventional techniques and procedures will be utilized to assess cardiovascular and pulmonary function. In most instances, these measurements on humans will be a part of a multi-disciplinary investigation conducted during actual altitude, e.g., on Pikes Peak. Some of the other measurements such as work capacity, metabolic rate, renal function, temperature regulation, etc., will be influenced, but not directly controlled by the cardiovascular and pulmonary systems. Thus, the factors influencing and/or controlling cardiopulmonary function at high altitude, as well as the interrelationships of various body functions will be of prime interest.

26. (U) Progress: The influence of varying arterial PO_2, PCO_2 and pH levels on hemodynamic and pulmonary function was studied in nine subjects at Brooke Hospital and subsequently during a 16-day exposure on Pikes Peak. In these studies, the arterial CO_2 level was altered by having the subjects breathe gas mixtures containing CO_2 concentration up to 7%. The data obtained in this experiment are currently being analyzed. This has been delayed somewhat due to the departure of the principal investigator.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CPY + 1	

DD FORM 1 AUG 66 1498 (Items 1 to 36 identical to NASA Form 1121) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A025601A827 Military Environmental Medicine

TASK NO. 01

WORK UNIT NO. 071 Cardiovascular and Pulmonary Responses at High Altitude

During the past year, one study "Effects of High Altitude Exposure on Cardiopulmonary Responses to Incremental Changes in Arterial PCO_2" has been conducted under this work unit.

The purpose of this study was to delineate any altitude-induced alterations in the control or modification of cardiovascular and pulmonary functions by changing levels of arterial carbon dioxide. Volunteer subjects respired graded levels of CO_2 from 1 to 7% initially at low altitude (Brooke Army Hospital) and subsequently during exposure for one or two weeks at high altitude (Pikes Peak).

Many of the data gathered during this experiment are still being analyzed. However, we do know that the set-point of the respiratory center to arterial PCO_2 is rapidly shifted to the left during the first two days of exposure. However, the sensitivity of the center to incremental increases and decreases in CO_2 is not altered by high altitude exposure. The efficiency of the respiratory control system is maintained at sea level values throughout the acute stage of altitude exposure.

BODY OF REPORT

WORK UNIT NO. 071 — Cardiovascular and Pulmonary Responses at High Altitude

STUDY NO. 1 — Effects of High Altitude Exposure on Cardiopulmonary Responses to Incremental Changes in Arterial PCO_2

PROBLEM:

Past studies at this laboratory and elsewhere have shown high altitude exposure causes marked alterations in cardiopulmonary function. Included among these alterations are transient increases in cardiac output and a sustained increase in ventilation. Although these alterations and others are essential to high altitude acclimatization, information pertinent to the underlying mechanisms which control cardiopulmonary function at high altitude is very limited. The purpose of the present study was to assess the responsiveness of the cardiovascular and pulmonary systems to graded increases and decreases in arterial PCO_2. Toward the end, a group of nine soldier volunteers were studied initially at low altitude at Brooke Army Hospital and subsequently after two and five days' exposure to 14,000 feet on Pikes Peak. At each location, the subjects first respired gas mixtures containing CO_2, the CO_2 concentration being increased 7.5 mm Hg. every three minutes until the respiratory minute volume was tripled (about 45 - 52 mm Hg. CO_2 in the inspired mixture). Then, the CO_2 content of the respired mixture was reduced in 7.5 mm Hg. increments, again at three-minute intervals, until the respired gas mixture contained no CO_2 and the ventilation was normal. Physiological measurements were made prior to breathing the gas mixtures, at the end of the three-minute periods on each incremental increase or decrease in respired CO_2 and finally when the subjects were again breathing gas mixtures containing no CO_2.

RESULTS AND DISCUSSION:

At both low and high altitude hysteretic loops were derived by plotting changes in ventilation as a function of changes in arterial PCO_2. The position of these loops indicated the ventilatory set-point, their slopes indicated the sensitivity of the respiratory center to CO_2, and their areas represented the efficiency of the respiratory control system. The findings are summarized as follows: (1) There is an initial rapid shift in set-point which occurs during the first two days of high altitude exposure and is not further changed after five days' exposure; (2) Respiratory center sensitivity to CO_2 is not altered, at least during the acute stages of high altitude exposure, and (3) The

efficiency of the respiratory control system is maintained at sea level values during the first five days at high altitude.

Cardiovascular measurements on each mixture of CO_2 included: arterial pH, PCO_2 and PO_2; cardiac output; heart rate; stroke volume; arterial and venous pressure; total peripheral resistance; and heart work. At present these data are still being analyzed, this analysis being somewhat delayed due to the departure of the responsible scientist from the laboratory.

RECOMMENDATIONS:

This type of research seems to be a most fruitful approach to understanding the mechanisms underlying cardiovascular and pulmonary function at high altitude. Future work on the responsiveness of these systems to alterations in PO_2 and various humoral agents would seem desirable.

PUBLICATIONS:

1. J. A. Vogel, J. E. Hansen and J. P. Hannon. Hemodynamic alterations in humans and animals during chronic high altitude exposure. Proceedings Ann. Army Res. Conf., West Point, New York, 1966.

2. Vogel, J. A. and J. E. Hansen. Cardiovascular function during exercise at high altitude. Proc. International Symposium on The Effects of Altitude on Physical Performance, pp. 47-51, Albuquerque, New Mexico, 1966.

3. Hannon, J. P., J. L. Shields and C. W. Harris. High altitude acclimatization in women. Proc. International Symposium on The Effects of Altitude on Physical Performance, pp. 37-44, Albuquerque, New Mexico, 1966.

GPO 778864

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6349	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 07 66)	U RPT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE
62156011 3A025601A827 00 072

10b. PRIOR NUMBER/CODE
61145011 3A014501B71R 05 080

11. TITLE:
(U) Metabolic Effects of Altitude (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
016200 Stress Physiology	07 66	NA	OTHER DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C.In-House	a. DATE b. NUMBER NA c. TYPE d. AMOUNT	PRIOR FY 67	3	60
		CURRENT FY 68	3	65

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21. TECHNOLOGY UTILIZATION
Environmental Factors Affecting Life and Health

22. COORDINATION
None

23. KEYWORDS
Altitude; Adaptation; Metabolic; Biochemistry; Stress; Physiology

24. (U) Tech Objective: Research under this work unit will be directed toward describing the various metabolic defects produced in human volunteers by direct or indirect exposure to hypoxia, especially at high terrestrial altitudes. It will also be concerned with describing the pattern and extent of metabolic adaptations associated with hypoxic exposure, both acute and chronic. Attention will be given to the basic physiological mechanisms which underlie the defects and adaptations which are observed.

25. (U) Approach: Human volunteers will be subjected to actual and simulated high altitude environments and various physiological, radioisotope and biochemical measures will be applied to describe the metabolic defects associated with hypoxia. Generally, these studies will be limited to the more gross aspects of metabolic function such as the changes in nutritional state, energy, metabolism, blood and urine chemistry, etc.

26. (U) Progress: Nine human subjects exposed to 14,100 feet (Pikes Peak) for two weeks had markedly decreased serum levels of essential amino acids. The level of the non-essential amino acids increased slightly. In contrast, the serum levels of water, total protein, taurine, creatinine, urea, uric acid, glutamic acid, phospholipids and inorganic P were significantly increased. These subjects also showed a marked reduction in plasma and extracellular fluid space but an increase in intracellular fluid space during altitude exposure. High altitude exposure was also associated with a normal or slightly elevated body water content, normal body K^{40} and a marked reduction in body weight and fat content.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) CFY + 1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORMS 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A025601A827	Biomedical Investigations
TASK NO.	00	Military Environmental Medicine
WORK UNIT NO.	072	Metabolic Effects of Altitude

Two high altitude studies were conducted under this work unit during the past year:

STUDY NO. 1: Effects of High Altitude on Body Fluid Compartments

STUDY NO. 2: Effects of High Altitude on Serum and Plasma Metabolites

Several groups of human volunteers participated in these investigations. Control, low altitude measurements were made at Brooke Hospital, after which the subjects were flown to Pikes Peak, Colorado where sequential measurements were made for periods of exposure lasting up to two weeks. High altitude exposure is associated with a marked and sustained decrease in extracellular space, but an increase in intracellular space. Body water content decreases slightly during the first day or so of exposure, but increases to exceed control levels after two weeks. Altitude exposure is associated with a marked loss of body fat. High altitude exposure produced no alterations in the serum levels of various nucleotides or enzymes. However, it does cause a decrease in the serum levels of certain essential amino acids. Some nonessential amino acids increase as do the total concentrations of nitrogenous metabolites.

BODY OF REPORT

WORK UNIT 072 — Metabolic Effects of Altitude

STUDY NO. 1 — Effects of High Altitude on Body Fluid Compartments

PROBLEM:

Humans, as well as other species, exhibit a marked reduction in plasma volume when exposed to high altitude. At 14,000 feet this reduction is apparent after a few hours and becomes more pronounced over the next week or ten days, where it remains for months and perhaps indefinitely. Studies of high altitude acclimatization in women showed this reduction was not due to dehydration, but to an actual loss of plasma from the circulation, probably as a result of an elevated rate of lymph formation. These observations raised questions concerning the effects of high altitude on other body fluid compartments. Consequently, the plasma, extracellular and intracellular volumes of nine soldiers were measured initially at low altitude and subsequently over a two-week period at Pikes Peak. Other measurements included total body water, lean body mass, body fat and body K^{40} content.

RESULTS AND DISCUSSION:

Not only plasma volume but also extracellular volume were markedly reduced during the first week of exposure. Thereafter, extracellular volume recovered slightly. Intracellular space increased in proportion to the decrease in extracellular space, i.e., about three liters in both instances. Total body water content showed an initial, slight decrease followed by a return to normal levels after one week and a slight increase above normal values after two weeks' exposure on the Peak. Lean body mass and body K^{40} content were not significantly altered by high altitude exposure. Body fat content, on the other hand, was markedly reduced.

CONCLUSIONS:

There is a large shift of body fluid from the extracellular to the intracellular space. The cause of this is not due to dehydration; it is due to a loss of body fat.

RECOMMENDATIONS:

New studies should be initiated to determine the actual anatomical sites of these fluid shifts and whether they are associated with certain hormonal changes, particularly aldosterone, A.D.H. and catecholamines.

Information on the relationships, if any, of these fluid changes to altitude sickness and performance decrements should be investigated.

STUDY NO. 2: Effects of High Altitude on Serum and Plasma Metabolites

PROBLEM:

Exposure to high altitude is accompanied by marked alterations in intermediary metabolism. Since the early 1900's, investigators have reported negative nitrogen balances in humans and animals exposed to high altitudes of 10,000 - 18,000 feet. Body composition studies in humans and rats at 14,000 feet have indicated that there is a shift in muscle protein to nonmuscle protein. A decreased incorporation of labeled amino acids into livers of rats at 14,100 feet has also been reported. In addition, plasma albumin degradation was increased markedly for 24 to 48 hours within the first three days in humans exposed to high altitude.

In view of these observations, changes in concentration of serum metabolites were studied in humans exposed to 14,100 feet for varying periods of time up to two weeks. In Group A, blood samples were drawn at sea level and on Days 1, 3 and 5 at 14,100 feet. In Group B, blood samples were drawn on Days 1, 3, 7 and 14 at 14,100 feet.

RESULTS AND DISCUSSION:

Compared to sea level concentrations, no changes in the following metabolites or enzyme activities were observed in Group A: NAD, NADH, NADP, NADPH, ATP, ADP, AMP, glutamic-oxalacetic and glutamic-pyruvic transaminase, lactic, malic and β-hydroxybutyric dehydrogenase.

In Group B, concentrations of the following metabolites decreased at high altitude: lysine, threonine, valine, total lipids and ornithine. Concentrations of the following metabolites were increased: plasma protein, glutamic acid, alanine, taurine, urea, α-aminobutyric acid and phospholipids. In general, concentrations of total nitrogenous metabolites increased, total non-essential amino acids increased slightly and total essential amino acids decreased. None of these changes can be explained by a change in serum water content, which increased about one percent. Measurements of various serum lipid concentrations remain to be completed.

CONCLUSIONS:

There seems to be little evidence that any major organ, e.g., liver, is subjected to damaging hypoxia during exposure to 14,000 feet. Such damage would probably be reflected in an increased serum level of nucleotides and enzymes. The reduction in certain essential amino acids along with the increase in total nitrogenous metabolites would support the concept that the early stages of altitude exposure are very similar to conditions of moderate starvation.

GPO 778084

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6350	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 67	D. Change (01 07 66)	U RPT U WRK	NA	NL	A.WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
62156011 3A025601A827 00 073	61145011 3A014501B71R 05 080

11. TITLE:
(U) Physiological and Psychological Aspects of Performance at Altitude (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
013400 Psychology; 012600 Pharmacology; 012900 Physiology	07 66	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	a. DATE b. NUMBER NA c. TYPE d. AMOUNT	PRIOR FY 67	1	55
		CURRENT FY 68	1	39

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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Mountaineering; Medicine	None

23. KEYWORDS Acute altitude sickness; Acetazolamide; Preconditioning; Hypoxia - Hypocapnia Voluntary Apnea; Fluid balance

24. (U) Technical Objective: To study various aspects of symptomatology in individuals exposed to 10,000-18,000 foot altitudes. Efforts will be directed towards: 1) Obtaining quantitative estimates of symptom severity; 2) Establishing causal relationships between symptomatology and physiological and biochemical alterations; 3) Investigating prophylactic and therapeutic measures to prevent or ameliorate altitude sickness; and 4) Elucidating criteria for prediction of individual susceptibility to altitude sickness.

25. (U) Approach: Develope statistically valid estimates of symptom severity by subject self-rating of individual symptoms. Computerized item analysis will be used to refine self-rating questionnaire. Overall sickness will be evaluated by paired comparison method with previous illnesses and by physician interview. Experimentally induced improvements in symptomatology will be correlated with any observed reductions in physiological and/or biochemical alterations. Efficacy of promising medications, diet and preconditioning will be evaluated with double-blind techniques where feasible. Various physiological and psychological parameters will be examined as criteria for prediction of altitude sickness susceptibility.

26. (U) Progress: Symptomatology as a function of time at altitude has been established. The General High Altitude Questionnaire is presently being modified by item analysis. New techniques of subjective evaluation are being developed. Acetazolamide was shown to be slightly advantageous in reducing certain symptoms of altitude sickness. Preconditioning to hypoxia or hypocapnia alone has been shown to be somewhat beneficial. Voluntary apnea at sea level shown promise as a predictor of natural acclimatization to altitude. Shifts of fluid balance at altitude are currently being correlated with symptom incidence. A study comparing the efficacy of codeine and phenformin in alleviating altitude sickness has been designed and is awaiting approval.

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
CFY + 1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) REPLACES DD FORM 613 & 613C WHICH ARE OBSOLETE.

ABSTRACT

PROJECT NO.	3A025601A827	Military Environmental Medicine
TASK NO.	01	
WORK UNIT NO.	073	Physiological and Psychological Aspects of Performance at Altitude

The following investigations have been conducted under this work unit:

STUDY NO. 1: Evaluation of Acetazolamide in the Treatment of Acute Mountain Sickness

STUDY NO. 2: Evaluation of Hypoxia and Hypocapnia Separately as Causal Factors in Acute Mountain Sickness

The present work unit is designed to evaluate physiological and psychological impairment at high altitude and to attempt to evaluate certain promising medications relative to their ability to prevent or ameliorate the symptoms of acute mountain sickness. Acetazolamide, a carbonic anhydrase inhibitor, was evaluated in a double-blinded placebo situation. Previously it was found that acetazolamide afforded a slight but significant protection against certain symptoms commonly reported in low altitude subjects upon initial altitude exposure. Although acetazolamide administration caused improvements in altitude-induced changes of PO_2 and pH, while contributing to hypocapnia in human subjects, the results would not warrant recommendation that acetazolamide be adopted as an adequately successful therapeutic for symptoms of acute altitude sickness.

Additional information was collected on human subjects pre-acclimatized to hypocapnia or hypoxia by appropriate gas mixtures for one week prior to ascent to Pikes Peak (14, 110 feet). Results indicate that breathing hypoxic gas mixtures containing 5% CO_2 and 14% oxygen or forced hyperventilation on sea level air (causing hypocapnia and no hypoxia) were slightly and equally effective in reducing high altitude symptomatology. Periods of pre-acclimatization with these breathing treatments were carried out for nine hours a day for five days prior to ascent to Pikes Peak. From this we would conclude that symptoms of acute mountain sickness are not solely attributable to either hypoxia or hypocapnia alone, but must be related to an interaction between each alteration.

BODY OF REPORT

WORK UNIT NO. 073 — Physiological and Psychological Aspects of Performance at Altitude

STUDY NO. 1 — Evaluation of Acetazolamide in the Treatment of Acute Mountain Sickness

PROBLEM:

The present study was designed to evaluate acetazolamide, a carbonic anhydrase inhibitor, as a therapeutic agent for the syndrome of acute mountain sickness. Reports from studies at simulated altitude had shown acetazolamide partially prevented an increase in arterial pH and decrease in PO_2 of subjects at high altitudes compared to placebo controls. Incidence of symptoms in these subjects were also reported to be considerably reduced. This study was initiated to confirm these impressions at actual altitude using a 26-symptom questionnaire with subject self-rating of each symptom on a scale of five. Acetazolamide was given in four 250 mg. doses one day prior to ascent and two 250 mg. doses the first two days on Pikes Peak. Sixteen subjects were studied.

RESULTS:

Fourteen of the 26 symptoms were improved significantly with acetazolamide; however, improvement was only slight or not at all on the more debilitating symptoms such as: headache, nausea, insomnia, anorexia, somnolence. When compared to a previous study using codeine, acetazolamide is inferior as an agent to combat acute mountain sickness both in terms of total symptomatology and most debilitating symptoms.

The effect of acetazolamide on arterial blood gases and pH during maximum symptom severity was as follows (means of groups of eight): (1) PO_2 - placebo 52 mm Hg., acetazolamide 62 mm Hg.; (2) PCO_2 - placebo 30 mm Hg., acetazolamide 24 mm Hg.; (3) pH - placebo 7.43, acetazolamide 7.37.

CONCLUSIONS:

Acetazolamide is not the drug of choice in combating the subjective symptomatology of acute mountain sickness.

RECOMMENDATIONS:

On the basis of this experience, we would recommend: (1) Continued evaluation of promising drugs as agents for therapy and prevention of severe debilitation due to acute mountain sickness

and (2) Investigation of acetazolamide as a prophylactic agent followed by another drug at altitude (codeine would seem the choice at present).

STUDY NO. 2: Evaluation of Hypoxia and Hypocapnia Separately as Causal Factors in Acute Mountain Sickness

PROBLEM:

This study was designed to determine whether preconditioning to hypoxia or hypocapnia alone prior to actual altitude exposure could influence the severity of symptomatology and course of natural altitude acclimatization. This was attempted by exposing eight human volunteers for nine hours per day over a five day period at sea level to gas mixtures containing 5% CO_2 and 14% O_2 to produce hypoxia without hypocapnia. Arterial blood samples showed average PO_2 in the range of 60 mm Hg. with average PCO_2 of 42 mm Hg. A second group of eight subjects breathed ambient air with forced hyperventilation by Bennet respirators for an equal preconditioning period. Arterial blood gas analysis showed hypocapnia (average PCO_2 - 16 mm Hg.) without hypoxia (average PO_2 - 120 mm Hg.

RESULTS:

The results of this study indicate that preconditioning to hypocapnia (forced hyperventilation) or hypoxia (5% CO_2, 14% O_2) nine hours/day for five days just prior to ascent to Pikes Peak were slightly and equally effective in reducing high altitude symptomatology. The degree of protection observed was somewhat less than we have seen with codeine. All of the statistical evaluation has not been completed as yet, but it would appear that certain symptoms (headache, nausea, insomnia) are reduced by this type of preconditioning. When data are completely reduced, more definitive statements about the role of hypocapnia vs. hypoxia in the detrimental effects of acute high altitude exposure may be possible.

PUBLICATIONS:

1. Evans, W. O. and Witt, N. F. The interaction of high altitude and psychotropic drug action. Psychopharmacol. (Berl.), 10: 184-188, 1966.

2. Evans, W. O. The measurement of the subjective symptomatology of acute high altitude sickness. Psychol. Rep., 19: 815-820, 1966.

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11. TITLE:
(U) Microbial Flora of Human Subjects: Possible Effects of Altitude and/or Drugs (06)

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910100 Microbiology 016200 Stress Physiology

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19. GOV'T LAB/INSTALLATION/ACTIVITY
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21. TECHNOLOGY UTILIZATION: Mining, agriculture, aeronautics

22. COORDINATION: None

23. KEYWORDS: Altitude, blood cell count, microbiology, skin, feces, temperature, humidity

24. (U) Technical Objective: Man's physiological and psychological reaction to a defined environment and altitude has been the subject of numerous studies. The effect of altitude on the indigenous microbiota of man, however, has not been investigated in depth. In this study, three parameters interacting with man and his indigenous microflora was to be investigated (1) Environment, which included altitude, temperature, and relative
25. humidity; (2) drugs used to suppress or reduce symptoms of altitude exposure; (3) idiopathic or altitude - induced illness distinguished from infectious diseases.
25.(U) Approach: To study the effect of environment and/or specific drugs on the indigenous microflora, the following approach was used; swabs from forehead and back were cultured for the isolation and enumeration of micrococci, yeasts and general aerobic and anaerobic flora; throat cultures the normal flora, group A streptococci and Mycoplasma stool cultures to detect any changes from the normal flora. Venous blood for hematology and electrophoretic patterns to determine evidence of significant antibody changes air sampling of the subject's environment. All subjects were selected from volunteers at low altitude training centers where baselines were established. The subjects were
26. moved to high altitudes and the sampling procedures repeated.
26.(U) Progress: Two groups of 16 each were studied. Baselines were established at Ft. Sam Houston, Texas. Altitude samples were obtained from the subjects after transfer to Pikes Peak, Colo. No qualitative nor quantitative changes in the microbial flora were noted. Hematologic changes, specifically platelettes, were seen. A complete report presenting the data and conclusions is in preparation.

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ABSTRACT

PROJECT NO.	3A025601A827	Military Environmental Medicine
TASK NO.	00	
WORK UNIT NO.	074	Microbial Flora of Human Subjects: Possible Effects of Altitude and/or Drugs

The purpose of this project was to study the effect of environment and/or specific drugs on the indigenous microflora of man. Three parameters interacting with man and his microflora was investigated. These parameters were:

(1) environment; i.e. altitude, temperature, and relative humidity;

(2) drugs used to suppress or reduce symptoms of altitude exposure;

(3) infectious diseases to be distinguished from altitude induced illness.

Two groups of 16 each were studied. Baselines were established at Ft. Sam Houston, Texas. Sampling to determine the effect of altitude was obtained after transfer to Pikes Peak, Colorado. No qualitative or quantitative change in the microbial flora were noted. Hematologic changes, however, were observed. A complete report presenting the data is in preparation.

BODY OF REPORT

WORK UNIT NO. 074

Microbial Flora of Human Subjects: Possible Effects of Altitude and/or Drugs

PROBLEM:

Man's physiological and psychological reaction to a defined environment, including altitude, has been the subject of numerous studies. The effect of environment and the indigenous microbiota of man, however, has not been the subject of in depth investigations. In this study, three parameters interacting with man and his indigenous microflora were investigated for evaluation:

(1) environment, which included altitude, temperature, and relative humidity;

(2) the effect of drugs used to suppress or reduce symptoms of altitude exposure;

(3) idiopathic or altitude-induced illness distinguished from infectious diseases. The project was initiated during this reporting period with three surveys being conducted, two at sea level at Ft. Sam Houston, Texas and one at high altitude on Pikes Peak, Colorado. The sampling procedure used during these surveys was to obtain swabs from the forehead and back and culture for the presence and enumeration of micrococci, yeasts and fungi and general aerobic and anaerobic bacterial flora. Throat swabs were obtained for the presence of group A streptococci and Mycoplasma sp., and stool specimens were cultured to detect any quantitative or qualitative changes from the normal flora. Venous blood was obtained for hematology and electrophoretic patterns to determine any significant antibody changes; air sampling of the general area and of the subject's environment was accomplished.

RESULTS AND DISCUSSION OF THE RESULTS:

Two groups of 16 subjects each were studied. Considerable data of a broad baseline character were derived. No qualitative or quantitative changes in the microbial flora from specimens cultured from the skin or feces or evidence of antibody changes were seen. Hematologic changes, specifically, increased percentages of reticulocytes, were observed. Preliminary studies to establish methods indicated that we might expect to find significant differences in the skin microflora of individuals from different geographic areas. This was not confirmed by our surveys. The probability exists that the duration of exposure was not sufficient to alter the microbial flora.

CONCLUSIONS:

None.

RECOMMENDATIONS:

Future studies will be concerned with the sampling of subjects living and working at various altitudes and states of relative humidity.

PUBLICATIONS:

None. A complete report presenting the data collected during the surveys in in preparation.

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13. ABSTRACT

Basic Research in Support of Military Medicine: Studies of Performance Physiology, Molecular Biochemistry, Studies in Lipids, Nutrition and Metabolism. Mineral Metabolism, and Haemopoietic Metabolism.

Research in Biomedical Sciences: Experimental Surgery, Maintenance of Animals, Nutritional and Metabolic Adaptations and Interrelationships, Work Performance and Body Composition, Muscle Metabolism, Microbial Metabolism, High Altitude Bioenergetics, Cardiovascular and Pulmonary Responses, Microbial Flora and Myocardium of animals have been studied utilizing humans, dogs, and small laboratory animals using various techniques.

In-House Laboratory Independent Research: Research has been conducted under the following work units during the past year: Symbiosis and Intestinal Flora, Means for Measurement of Work Decrement in the Rat, Body Temperature Control by Adrenal Steroids, Examination of Mature German Shepherd Dogs Fed Rice Base Diets, Coronary Blood Flow Studies and Natriuretic Property of 16-Alpha-Hydroxyprogesterone.

Biomedical Investigation: These studies include: Tuberculosis Research, Computer Classification of Pulmonary Disability, Computer Instrument Linkages, Effects of IN[illegible] on Animal Histology, Intravenous Fat Emulsion, Human Nutrition, Nutritional Studies of Military Populations, Nutritional Physiology, Analytical Biochemistry, Nutrients and Response of Man to Nutrition or Disease.

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