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AUTHORITY

ST-A USAMRNL LTR, 21 JUN 1971

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REPORTS CONTROL SYMBOL MEDDH-288

ANNUAL RESEARCH PROGRESS REPORT

30 JUNE - 1965

USARMY 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 1964 - 30 June 1965

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

3A014501A71F - Military Internal Medicine

01 - Internal Medicine
Sub-Tasks 21 through 31

02 - Metabolism & Nutrition
Sub-Tasks 11 through 25

04 - Pharmacology of the Combat Soldier
Sub-Task 11

3A014501A71N - Military Environmental Medicine

01 - Environmental Medicine
Sub-Task 70

3A014501B71P - Basic Research in Supply of Military Medicine

03 - Biochemistry
Sub-Tasks 01 through 04

09 - Physiology
Sub-Task 06

5016 6.11.30.01.1 - In-House Laboratory Independent Research

3A013001A91C - In-House Laboratory Independent Research

01 - In-House Laboratory Independent Research
Sub-Tasks 58 through 60
66 through 70
99

SUMMARY

Military Internal Medicine: Microbiological Research in Tuberculosis, Experimental Surgery, Computer Classification, Histopathology of Laboratory Animals, Intravenous Fat Emulsions, Studies of Nutritional Status of Both Civilian and Military Populations, as well as studies of Radiated Foods, together with Amino Acids and Proteins, Carbohydrate Studies and Fatigue and Exercise Physiology studies during the FY year.

Military Environmental Medicine: Studies of the interrelationships of hypoxia, diet and temperature on work performance, cardiopulmonary physiology, nutritional status and organ and body metabolism. These studies utilize humans, dogs and small laboratory mammals with various techniques applied.

Basic Research in Support of Military Medicine: Studies of Lipids and Related Compounds, Mineral Metabolism, Carbohydrates, Nutritional Biochemistry of Chemotherapeutics and the Functional Aspects of Body Composition have been intensely studied during the period and the findings in each field have been reported.

In-House Laboratory Independent Research: Total of nine sub-tasks, seven initiated during FY 1965, will be incorporated into the mission program in FY 66 or 67. Several papers which report the findings of investigators working on In-House projects have been published in scientific journals.

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
		DA OA 6300	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	RPT U WRK U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 65

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 01 21

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		18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER: c. TYPE: NA	a. DATE: d. AMOUNT:	PRIOR FY 65	2	75
			CURRENT FY 66	3	83

19. GOV'T LAB/INSTALLATION/ACTIVITY
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INVESTIGATORS PRINCIPAL: Morse, W. C., LtCol
ASSOCIATE: Rothlauf, M.V: Tull, A.H.
TEL: 303 366 5311 X25223 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
Hospitals; Sanitariums	None

23. KEYWORDS: Mycobacterium; Drug Resistance; Microbial; Bacteriological Technics; Environment Virulence; Metabolism; Microbial; Biological Assay; Histological Technics; Chemotherapy

24. (U) Tech Objective Investigations are directed towards improving procedures for rapid isolation, identification, and drug susceptibility patterns of mycobacteria isolated from patients and to improve patient tolerance of antimycobacterial drugs by altering dosage regimens based on serum concentrations determined by biological and chemical assays, and in obtaining histo-pathological data from a new in situ culture method which may suggest duration and amount of drugs required for better chemotherapy.

25. (U) Approach Modifications of current procedures in specimen preparation, medium composition, incubation environment, drug susceptibility methodology and the application of biochemical aids used in identification of mycobacteria are constantly in progress. The rate of bacterial conversion in humans on anti-tuberculosis drug therapy is correlated with drug dosage, time of drug administration and serum drug concentrations. The histopathological and in situ culture findings of surgical specimens from tubercular patients are correlated with the previous drug regimens employed.

26. (U) Progress: (Oct 64-Jun 65) Results of multiple studies on the improvement of isolation, identification and drug susceptibility methodology were summarized in USAMRNL Report #283. Continuous studies for improvement of techniques are in progress. High peak serum concentrations of anti-tubercle drugs provided in a single daily dose have been shown to correlate well with bacterial conversions of the sputum to negative. The results of histopathological and in situ cultural experiments are extensive and will be shortly presented for publication.

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) OVER

ABSTRACT

MICROBIOLOGICAL RESEARCH IN TUBERCULOSIS

a. Improved methodology for isolating and identifying mycobacteria are presented in USAMRNL Report #283, November, 1964.

b. Biological or chemical assays of antimycobacterial drug in patients' serum are performed to determine if levels obtained inhibit the specific mycobacterial species causing the disease, but are not at toxic levels.

c. The study of mental aberrations attributed to increased blood ammonia induced by isoniazid (INH) administration is delayed awaiting the development of a precise method for blood ammonia.

d. Analyses of p-amino salicylic acid (PAS) concentrations continue. These varied studies investigate blood levels versus dosage; compare different PAS preparations, levels of PAS achieved when the drug is taken in the fasting state, with food and before sleep, and the clinical efficacy of once-a-day versus twice-a-day treatment regimens.

e. Studies on the comparative pathogenicity for guinea pigs of drug-resistant strains of M. tuberculosis are being pursued.

f. Culture of surgical specimens from tuberculosis patients using a new slide culture technique demonstrates no advantage over the use of conventional methods.

g. Reports that INH induces lung tumors in mice led to a joint study with members of the Pathology Division, USAMRNL, and will be reported in detail by that division.

h. A new method for testing disinfectants against M. tuberculosis is being evaluated.

BODY OF REPORT

MICROBIOLOGICAL RESEARCH IN TUBERCULOSIS

Description: Improvement of Laboratory Techniques

a. Mycobacteriology procedures were revised and simplified to achieve isolation, identification and drug-resistance data after two to three weeks' incubation. Studies included examination of digestion and decontamination procedures, requirements for CO_2 for primary isolation of mycobacteria from clinical material, the effects on growth of water of different mineral content and the study of different chemical tests as aids to identification of different mycobacterial species.

Progress:

a. The laboratory procedures employed by this unit for isolation and identification of mycobacteria continue to be modified to include new methods and techniques which will simplify the problems of distinguishing the "unclassified mycobacteria" from M. tuberculosis strains and from the nonpathogenic mycobacteria. A new mucolytic agent, n-acetyl-l-cysteine (AC), has been studied as an aid in digesting sputum for subsequent decontamination by sodium hydroxide (NaOH). The conventional digestion-decontamination with trisodium phosphate, in use in this unit for many years, requires 48 hours of treatment and results in loss of approximately 90% of viable mycobacteria. The use of AC and 3% NaOH reduces the time of alkali exposure to 30 minutes, a factor which significantly reduces the loss of mycobacteria from the clinical specimens. The AC-NaOH procedure is now used exclusively in this unit.

Results of studies on the effects of increased CO_2 environment during incubation of cultures of mycobacteria, at primary isolation from clinical specimens, demonstrates that 2%-5% CO_2 stimulates all strains, is required by some, particularly certain drug-resistant mutants, and is inhibitory to none. It has been found that the requirement for increased CO_2 tensions is probably of fairly short duration; i.e., the first four to six days of incubation.

Results of comparative studies show that the source of water for use in the preparation of media and reagents is important. Demineralized, glass-distilled water is best. Tap or distilled water, when used to prepare the semi-synthetic oleic acid-albumin-agar medium (7H10-OA) used in this unit, demonstrates an inhibitory effect on growth of many strains of mycobacteria.

An extensive study of the "Niacin Test", which measures the production of niacin by the human strain tubercle bacillus (M. tuberculosis) shows that this test is the best single test for distinguishing M. tuberculosis from all other nonchromogenic mycobacteria.

With the results of these and other studies now completed and summarized, a revised edition of this unit's published procedures was completed in November, 1964. This methods manual, "Mycobacteriology Laboratory Methods, Report #283, USAMRNL", is available to requestors.

MICROBIOLOGICAL RESEARCH IN TUBERCULOSIS

Summary and Conclusions:

a. Tuberculosis laboratory procedures continue to be revised and simplified, with the aim of achieving complete isolation, identification, and drug susceptibility results in two weeks. These attempts have included studies on digestion of sputum decontamination of specimens with sodium hydroxide, n-acetyl-l-cysteine as compared to trisodium phosphate, the requirements of CO_2 for primary isolation of tubercle bacilli and the effects of different water sources for reagent and media preparation. Also evaluated was the niacin test. A revision of published procedures was completed for distribution as of 1 November 1964.

Description: Determination of Antituberculosis Drug Levels

b. Biologic and chemical assays of antituberculosis drugs in serum and body fluids of tuberculous patients receiving assigned dosages are performed to determine which concentrations achieved inhibit the mycobacterial species determined to be the causative agent of the disease. Differences in metabolic inactivation of the antituberculosis drugs by individuals make necessary continual surveillance of serum concentrations on all patients administered these agents.

Progress:

b. Biologic or chemical assays of antituberculosis drugs in serum of tuberculous patients continue to be an important phase in their management during chemotherapy. In addition to the knowledge that all of the antituberculosis drugs exhibit toxic side effects and that rates of metabolism of these drugs vary from patient to patient, it is important, particularly with respect to oral drugs, to study the effect on serum levels of the administration of the drugs with food. Results of preliminary studies show that serum levels obtained when INH and PAS were taken with meals or prior to bedtime were significantly reduced as compared to those levels obtained when the same subject was tested in a fasting state. The importance of these observations rests on the accumulating evidence which clearly suggests that peak drug levels, rather than extended lower levels, can be related to chemotherapeutic efficacy. Drugs taken with food or at night prior to sleep do not produce optimal or required peak levels.

Summary and Conclusions:

b. Assays for concentrations or antituberculosis drugs in serum help to monitor chemotherapy by providing information that sufficient drug is present to inhibit tubercle bacilli but not to induce toxicity.

Description: Relation of Isoniazid to Blood Ammonia Levels

c. Investigations being conducted are directed towards correlating serum INH concentrations, blood ammonia levels and the results of psychometric analyses with the toxicity found in many tuberculosis patients

receiving INH. The study is also directed towards determining the effect, if any, of the administration of the amino acids, glutamic acid and arginine on the reduction of blood ammonia concentrations and on toxicity.

Progress:

c. Recent reports in the literature have suggested that blood ammonia levels in subjects ingesting INH do not rise as was previously stated. (See Annual Report, Chemistry Division, USAMRNL, 1963-1964.) Further study on this project is halted until blood ammonia methodology can be thoroughly investigated and a reliable and valid method can be developed.

Summary and Conclusions:

c. Further progress is temporarily suspended pending development of a more precise and specific blood ammonia method.

Description: Efficacy of Various Antituberculosis Drug Regimens

d. Blood concentrations achieved and clinical effectiveness of a new form of p-amino salicylic acid (PAS-C) continue to be investigated. Areas of study include the effect on serum levels and clinical effectiveness of the administration of PAS-C with food and the efficacy of once-a-day versus twice-a-day administration.

Progress:

d. Several years' experience with PAS-C (PAS-Ascorbic), a highly purified form of p-amino salicylic acid, has shown that six grams per day is sufficient to achieve successful chemotherapy. Once-a-day drug (QD) administration was shown to be equal if not superior to the same total dosage given in two divided dosages (BID). The superiority of QD drug administration is probably based on the highest levels obtained with the larger dose and that the blood level-depressing effect of PAS-C taken with food is considerably reduced. A paper comparing q.i.d. versus b.i.d. drug administration has been prepared jointly with the staff of the Pulmonary Disease Service, FGH, and accepted for publication by the journal, "Diseases of the Chest".

Summary and Conclusions:

d. Results of extensive studies on PAS-C have led to the adoption of this form of PAS by FGH. A paper on the efficacy of treatment regimens has been accepted for publication.

Description: Mycobacterial Drug Resistance Versus Virulence

e. Other studies are aimed at determining if differences in mycobacteria pathogenicity and virulence in the guinea pig can be attributed to the type of drug-resistant mutant of tubercle bacilli, and particularly if differences found

can be correlated with the in vitro sequential order of selection of the drug-resistant mutant, primarily those selected resistant to INH, PAS and Streptomycin (Sm). Earlier reported work, (Amer. Rev. Tuberc. 69:464, 1954 and Proc. Soc. Exper. Biol. Med., 89:468, 1955), showed that mutants of tubercle bacilli resistant to INH and deficient in catalase activity were highly attenuated for guinea pigs: Attenuation was directly related to quantitated loss of catalase activity. The pathogenicity (or attenuation) of mutants of tubercle bacilli resistant to INH and one or more other drugs (Sm, PAS) and the sequential order to which they became resistant has not been determined.

Progress:

e. To date, 32 drug-resistant strains of M. tuberculosis have been inoculated by aerosol cloud techniques into six guinea pigs each. The challenge suspension was standardized in terms of numbers of fiable cells. Catalase activity on the standardized inoculum of each strain was determined by respirometry. Three challenged animals were sacrificed at three weeks and three at six weeks. Portions of the lung at post were cultured to recover the challenge strain to determine change, if any, in the resistance pattern, and other portions were submitted for pathologic examination. Thirty more strains are scheduled for study.

Twenty-seven of the 62 strains were selected resistant by in vitro techniques, i.e., large inocula of drug-susceptible tubercle bacilli were plated on media containing inhibitory concentrations of the drug or drugs. Spontaneously occurring drug-resistant mutants in the susceptible population were thus "selected" out from the predominantly drug-susceptible inoculum. One drug-resistant mutant exists with approximately every 1×10^8 drug-susceptible cell. The remainder was recovered from patients. Final correlation will be made in terms of animal pathogenicity, catalase activity and drug-resistance pattern.

Summary and Conclusions:

e. This project is now well under way and should be completed within the fiscal year 1966.

Description: Cultural and Histological Appraisal of Chemotherapy Effectiveness

f. A study, conducted in collaboration with the Pathology Service, Fitzsimons General Hospital, is designed to provide information which may favorable influence the duration of drug treatment required to bring about a complete chemotherapy cure of tuberculosis. The bacteriologic findings on tissue treated immediately after surgical removal, and cultured by a new technique, are being correlated with histologic changes.

Progress:

f. A total of 125 surgical and two autopsy specimens have been cultured for mycobacteria using the coverglass technique. Culture results of specimens processed, additional to those previously reported, do not substantiate the

impression that the technique has an advantage over conventional methods used to culture mycobacteria from surgical specimens. The promising early results shown with the addition of yeast RNA (0.05 Y/ml) to the 7H10 OA culture medium was not borne out by the additional specimens so treated.

Related to the above study, but of general application as well, the fluorescent staining technique has been evaluated for detection of mycobacteria in tissue sections. It was found to be a more satisfactory method for finding mycobacteria and more rapidly than the carbol-fuchsin acid-fast techniques.

Summary and Conclusions:

f. Early results obtained from the application of this new method of recovering viable tubercle bacilli from tissues indicated an increased rate of positives over results of conventional methods. More results contradict the earlier results. RNA addition to the medium did not influence an increased rate of positive cultures. A publication is being prepared.

Description: Carcinogenicity of Isoniazid

g. A study of a reported relationship of the administration of INH to an increased incidence of lung tumors in Strong A and Balb C strains of white mice seeks to evaluate these questionable observations.

Progress:

g. Progress on this collaborative study conducted with the Pathology Division, USAMRNL, has now reached the phase of histologic examination. Details of current status will be found in the Pathology Division's report.

Summary and Conclusions:

g. Preliminary results of this project will be reported by the Pathology Division, USAMRNL.

Description: Effectiveness of Germicides on Mycobacteria

h. A method has been developed which measures the efficacy of germicides against tubercle bacilli contained in sputum.

Progress:

h. This method uses sputum films or microscopic slides prepared from sputum from patients excreting tubercle bacilli. The films are air dried, exposed by immersion to varied concentrations of the "germicide" being tested, washed to remove the germicidal agent (by dilution or neutralization and then immersed and incubated in a liquid culture.)

MICROBIOLOGICAL RESEARCH IN TUBERCULOSIS

Summary and Conclusions:

b. This technique, reported in the previous USAMFNL Annual Report, continues to be used in evaluation studies of newer germicides. Some modifications of the methodology have been made and further testing of disinfecting compounds will be conducted during the coming year. Many "germicides" labelled as "tuberculocidal" which have been tested by this new method have failed to kill tubercle bacilli under the realistic test conditions.

The methodology employed, and an evaluation of this new successful test procedure for measuring the activity of disinfectants against tubercle bacilli in sputum is being prepared for publication.

List of Publications:

b. Comparison of Fluorometric and Microbiologic Procedures for Measuring Isoniazid in Serum. J. H. Peters, W. C. Morse, and L. H. Schmidt The American Review of Respiratory Diseases, 91:225, 1965 (February).

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		None	None

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 10 64	B. COMPLETED	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501A71F 01 022

10b. PRIOR NUMBER/CODE: None

11. TITLE: (U) Microbiological Research in Mycotic Infections

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
010100 Microbiology	---	NA	OTHER 1 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 65	---	---
		CURRENT FY 66	---	---

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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INVESTIGATORS PRINCIPAL: Morse, Warren C. Lt.Col.Sproat,E.
ASSOCIATE: Weiser, Orman L.
TEL: 303 366 5311 X 25223 TYPE: DA

21. TECHNOLOGY UTILIZATION: ---

22. COORDINATION: None

23. KEYWORDS: Histoplasma; Coccidioides; Fluorescent antibody; Complement-fixation; Immuno-diffusion; Skin tests; Anti-fungal agents; Chemotherapy of mycoses.

24. (U) Skin testing of persons with positive Histoplasmin skin reactions can cause an increase in complement-fixing titer to Histoplasma antigens. Valuable support for detecting and following the course of pulmonary mycoses can be supplied to a pulmonary disease center by culture and serological facilities. A microcomplement-fixation technique for detecting Coccidioides antibodies correlates well with the standard technique and saves time and reagents. With Histoplasma antigens, however, the correlation is not satisfactory to date. A fluorescent antibody technique for detecting Histoplasma in smears would be useful and is being investigated. An immuno-diffusion serological test for coccidioides antibodies also is under study. Infection of mice with yeast-phase Histoplasma was not successful. Infection of such mice with simultaneously injected M. tuberculosis appears to enhance the tuberculosis infection. Yeast-phase Histoplasma, unlike Cryptococcus neoformans, grows well at human fever temperatures.

As previously reported, culture work associated with pulmonary mycosis was discontinued and suspect cases of pulmonary mycosis are being evaluated by serologic methods. This work unit is in direct support of the Pulmonary Disease Service and therefore is being transferred to Miscellaneous Microbiological Support in Clinical Research.

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
NA	

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

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

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to NASA Form 1122) OVER

ABSTRACT

MICROBIOLOGICAL RESEARCH IN MYCOTIC INFECTIONS

a. A high-titered rabbit antiserum has been produced for use in detecting the organism in sputum.

b. More versatile serologic testing procedures for mycotic diseases are available to authorized requesting agencies. Emphasis has been placed upon development of new, improved testing procedures.

BODY OF REPORT

MICROBIOLOGICAL RESEARCH IN MYCOTIC INFECTIONS

Description: Histoplasma in Sputum

a. Detection of Histoplasma capsulatum in sputum preparations.

Progress:

a. The fluorescein-labelled antibody against Histoplasma capsulatum was tested against culture films and tissue sections positive for the yeast phase of Histoplasma capsulatum. These tests showed that the antibody had been successfully tagged. The serum was turned over to the FGH Microbiology Laboratory for further evaluation.

Summary and Conclusions:

a. A final report will be submitted by personnel of the Pathology Service, FGH, when sufficient data are obtained through results of extensive trial with the fluorescein-tagged serum.

Description: Fungal Serologic Methodology

b. Serologic tests using fungal antigens to detect and quantitate specific humoral antibody in pulmonary mycoses.

Progress:

b. Utilization of Ouchterlony gel-diffusion proved a valuable adjunct to the complement fixation test in screening sera of patients with suspected coccidioidomycosis. On two occasions precipitating antibody confirmed clinical symptoms of the disease before C-F antibody became demonstrable. Subsequent C-F tests of these patients' sera elicited positive results, attesting good specificity of the method. The chief objection of immunodiffusion is the 72 hour incubation interval for reactivity to become visually evident. Materials and equipment have been ordered to investigate the applicability of a new method which substitutes cellulose acetate paper for the gel medium and promises reliable results in only 24 hours. This method also holds the added attraction of reactant economy concomitant with microbiology methods.

The 10,088 fungal serology procedures performed during this period indicate a continued increase of requests for this type of testing as an aid in differential diagnosis. Correlative testing of patients' sera utilizing a new fluorometer technique versus C-F methods will begin in September with a view toward ultimate replacement of the C-F test. The advantage of the fluorometric technique is its rapidity of obtaining quantitative results, its simplicity, and its cost-saving potential.

Tentative plans to investigate a method of testing fractionated humoral antibody with discrete fungal antigen constituents are contemplated in an attempt to eliminate the problem of cross-reactivity in C-F testing and

to give more significance to titer evaluations. Short supply of fungal antigens and the paucity of early acute mycotic disease sera may be limiting factors in this investigation.

Summary and Conclusions:

b. Immuno-diffusion has proved its worth as an integral part of a well balanced fungal serology program. Criticality of supply of fungal antigens and problems of cross-reactivity emphasize the need for new testing methods to supplant those in use. Investigation and analysis of new techniques will be conducted toward achieving this goal. Final report.

Further studies in serologic methodology will be carried under Project No. 3A014501A71F, Task No. 01 - Internal Medicine, Sub-task No. 23.

List of Publications:

None.

RESEARCH AND TECHNOLOGY RESUME		3. GOVT ACCESSION	4. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6301	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SEC	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 066

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 01 23

11. TITLE:
(U) Miscellaneous Microbiological Support in Clinical Research (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 a. DATE: b. NUMBER: c. TYPE: NA d. AMOUNT:	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House		PRIOR FY 65	2	32
		CURRENT FY 66	2	35

19. GOV'T LAB/INSTALLATION/ACTIVITY
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INVESTIGATORS PRINCIPAL: Blair, E.B., LtCol
ASSOCIATE: Morse, W.C., LtCol; Weiser, O.L.
TEL: 303 366 5311 X25223 TYPE: DA

21. TECHNOLOGY UTILIZATION: Hospitals; Sanitariums

22. COORDINATION: None

23. KEYWORDS: Mycoplasma pneumoniae; adenovirus; Influenza viruses; Staphylococcal phages; culture media; Lidwell phage applicator; epidemiology

24. (U) Tech Objective To determine the incidence of upper respiratory pathogens in hospital patients and normal control groups. To facilitate the diagnosis and therapy of non-TB bacterial and viral respiratory diseases; to evaluate and attempt to improve methodology for isolation and identification of these microorganisms. To simplify staphylococcal phage typing techniques.

25. (U) Approach Material for testing is obtained from hospital patients and periodic surveys of a normal Army population. Testing for serum antibodies to Mycoplasma pneumoniae, adeno and influenza viruses, in addition to attempts to isolate these agents are in progress. Incidence of upper respiratory bacterial pathogens and staphylococcal carriers among these populations are being determined. Tests of replicability of the Lidwell apparatus and optimal times for maximum demonstration of phage plaques are in progress.

(U) Progress: (Oct 64-Jun 65) All studies are in progress and conclusive results are not yet available.

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
CDCG 1412 a	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands) FY	36.

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to NASA Form 1122) OVER

ABSTRACT

MISCELLANEOUS MICROBIOLOGICAL SUPPORT IN CLINICAL RESEARCH

On studies of urine from pyelonephritis patients as well as urine from normal individuals, it has been found that a Gram's stain and determination of the catalase activity of urine may be used for the detection of significant bacteriuria. In a Mycoplasma study, C-F antibodies and positive cultures have been obtained from material collected from newborn infants. Micro-serologic techniques are being used in Mycoplasma antigen studies in attempts to increase the specificity.

A seven months' survey for upper respiratory bacterial pathogens among normal hospital personnel yielded few group A streptococci or meningococci. Nasal and throat carriage of staphylococci in this group was 37% to 40% and 25%, respectively. Procedures using the Lidwell phage applicator were not sufficiently reproducible in replicates used for determining inactivation rates of phage on stamped agar plates stored at 4°C and used for one year.

BODY OF REPORT

MISCELLANEOUS MICROBIOLOGICAL SUPPORT IN CLINICAL RESEARCH

Description: Catalase Activity of Urine

a. Microbiological methods for determining significant bacteriuria.

Progress:

a. Details and result of the methods used in a pyelonephritis study will be presented in a paper which is nearly ready for publication. A total of 702 urines were studied by bacteriologic methods, and 500 urines evaluated for catalase activity. In no instance was the catalase test negative where significant bacteriuria was present. The incidence of false positives was present in less than 1% of the urines studied. It is suggested that the catalase test be used for preliminary screening in support of the diagnosis of bacteriuria and for selection of urines for quantitative bacteriologic study.

Summary and Conclusions:

a. From the results of the pyelonephritis study it is evident that two simple and rapid procedures, the Gram's stain and catalase activity of the urine, may be used for screening and preliminary diagnosis of bacteriuria. In no instance was either of these tests negative when significant bacteriuria was present. The methods used and the results have been submitted for publication.

Description: Upper Respiratory Viruses

b. A study of respiratory viruses associated with diseases of the chest, both chronic and acute.

Progress:

b. Laboratory support in studies of respiratory viruses, Mycoplasma sp. and Colorado tick fever was continued. A survey, conducted in cooperation with the 249th General Hospital, was finished. Throat washings and sera obtained during this survey are being studied bacteriologically and serologically. The results will be evaluated and possibly published.

Summary and Conclusions:

b. No unusual incidences of respiratory disease associated with Adenoviruses or Influenza viruses were seen in this area during the period of this report.

Description: Mycoplasma in Respiratory Diseases

c. A study of the role of Mycoplasma sp. in respiratory diseases.

MISCELLANEOUS MICROBIOLOGICAL SUPPORT IN CLINICAL RESEARCH

Progress:

c. A study of Mycoplasma sp. in newborn will be completed and is being prepared for publication.

Summary and Conclusions:

c. In our continuing study of Mycoplasma sp. and their incidence in healthy as well as diseased individuals, we have uncovered one rather unexpected finding in that a significant number of cord bloods do have detectable antibody to Mycoplasma pneumoniae and that Mycoplasma sp. are being cultured from material obtained from some of the newborn. The methods used and the results are being prepared for publication.

Description: Fungal Serology

d. A study of antigen-antibody interaction using microtechniques.

Progress:

d. Therapy for coccidioidomycosis infections is generally initiated on the basis of pre-established serologic titer values; however, on evaluation, the quantitative micro-CF technique did not possess the reproducibility required to form a valid basis for therapy. The micro double-diffusion test of Crowle was utilized to determine efficacy and specificity of our Mycoplasma antigen preparation. No additional precipitin bands were observed when compared with the macro gel-diffusion method. Possibly this indicates a comparative lack of complexity of the Mycoplasma antigen molecule or reflects its amenability to diffusion dynamics.

Summary and Conclusions:

d. The microtechniques used to evaluate our Mycoplasma antigens indicated a lack of complexity of the antigens and will be expanded further in the study of these antigens in an attempt to obtain increased specificity.

Description: Upper Respiratory Bacterial Flora

e. A survey of the upper respiratory bacterial flora among normal army hospital personnel.

Progress:

e. Details of methods used in surveying for upper respiratory bacterial pathogens in an army population were given in ARTR 36707, October 1964. Nasal and throat swabs from personnel of the 249th General Hospital were collected and examined monthly from October 1964 through April 1965. The specimens were inoculated to 5% sheep blood agar, chocolate agar, salt milk agar, mannitol salt agar and salt mannitol plasma agar. These specimens were remarkable for their almost complete absence of group A

streptococci and meningococci. Based on receipt of specimens from dispensary or hospital sources, this nonrecruit population experienced very little upper respiratory disease. Staphylococcal carriage among members of this group is discussed in par. f. A final report covering this aspect of the study is in preparation.

Summary and Conclusions:

e. Examination of a series of six monthly throat and nasal cultures on personnel of the 249th General Hospital revealed a remarkable lack of group A streptococci and meningococci.

Description: Staphylococcal Carrier Surveys

f. Nasal and throat carriage of Staphylococcus aureus among normal hospital personnel.

Progress:

f. The incidence of coagulase-positive staphylococcal carriers among this group was found to be in the range 36% to 45% during the seven-month testing period. Carriers could be classified as (a) permanent - staphylococci present at all times although individuals might change strains; (b) intermittent - staphylococci absent on one or more occasions; and (c) persistently negative.

Recovery of staphylococci on salt milk agar from a small area of skin over the mandibular area of the face was much lower (8.6%) than from the nose (36.4%). Phage typing showed 7.3% of the skin isolates to be identical with the staphylococci found in the anterior nares.

Throat carriage of staphylococci was observed in about 24% of the personnel examined. Comparison of throat types with isolates from the nose revealed that the majority carried the same type in the nose and throat (11.9%) while different phage types were present simultaneously in the nose and throat of 6.8% of this group. Staphylococci were present in the throat, but not the nose of 5.1% of the personnel and present only in the nose of 25.4%. Total carriage of staphylococci in this group was 49.1%. There is a wide variation in incidence of throat carriage (4% - 64%) reported in the literature.

Summary and Conclusions:

f. The incidence of staphylococcal carriers, nasal and throat, among these personnel was 36% to 45% and 24%, respectively. Carriage in both nose and throat was classed as permanent, intermittent and negative. Staphylococci isolated from the face were, in most cases, the same as carried in the nose. A final report on this phage of the study is being prepared.

MISCELLANEOUS MICROBIOLOGICAL SUPPORT IN CLINICAL RESEARCH

Description: Staphylococcal Surveys

g. Surveillance for Staphylococcus aureus in the hospital environment.

Progress:

g. This laboratory continues to support the Fitzsimons General Hospital infectious disease committee by phage typing staphylococcal isolates from the hospital environment. Pertinent results from staphylococcal surveys on personnel of the 249th General Hospital and advanced medical technician classes were given to interested supervisors, inasmuch as many of these personnel perform on-the-job training in the hospital. There was no unusual increase in hospital-acquired staphylococcal disease during the reporting year. Studies are in progress on laundering procedures and the adequacy of soaps for disinfection of woolen blankets.

Summary and Conclusions:

g. Phage typing of all staphylococci isolated from patients and staff of Fitzsimons General Hospital indicates no unusual increase in hospital-acquired cross-infection. Studies on disinfection and laundering of woolen blankets are in progress.

Description: Phage Typing of Staphylococci

h. Simplification of bacteriophage methodology.

(1) A study to determine the replicability plaque counts using the Lidwell phage apparatus.

(2) A study to determine the time required for maximum demonstration of plaque-forming units (PFU) of the 22 standard typing phages.

(3) A study to determine the effects of storage on lyophylized and liquid phage suspensions.

Progress:

h. (1) The Tarr-Lidwell phage applicator (Monthly Bull. Pub. Health Lab. Svc. (British) 18:49, 1959) has greatly simplified this aspect of bacteriophage typing. However, lagging acceptance of this technique lies in the lack of evidence showing adequate reproducibility of lytic reactions on replicate plates. Experiments in this laboratory, wherein a phage suspension containing 5-25 PFU per loopful were placed on 20 replicate sterile agar plates and reacted with the homologous propagating strain. An analysis of counts obtained from these plates showed the replicate plaque counts produced by the Lidwell apparatus to be well within established standards. However, it is necessary to more precisely define the variation between loops within plates and between plates in order to evaluate the decrease in lytic activity

on agar plates which had been stamped with phage suspensions and stored for varying times at 4°C. Preliminary tests have shown no significant variation between loops within plates; however, in certain instances, there has been significant ($p = .01 - .001$) variation between loops between plates. Each step of the procedure is being reviewed in an attempt to reduce this variation.

(2) Preliminary studies involving plaque counts indicate that incubation times up to five days at the standard 30°C incubation temperature are required for maximal appearance of PFU from certain of the standard typing phages. This effect on replication studies, described in par. h. (1), is obvious and therefore must be evaluated.

(3) Studies confirm the reported longevity of liquid phage suspensions held at 4°C as well as lyophilized preparations of these phages. The original titering dilutions of phages propagated in June - July 1964 and held in the refrigerator are still in routine use. No drop in titer was observed during this period in 13 of 22 phages. Eight phages decreased less than one log in titer, while one phage decreased one log. Lyophilization results in initial destruction of 50% - 60% of phage, but little or no subsequent loss has been observed. These observations will be continued.

Summary and Conclusions:

h. (1) Analysis of counts from replicate plating of staphylococcal phage suspensions shows no significant variation between counts between loops within plates, but in certain instances significant differences were noted between loops between plates. Attempts will be made to reduce this variation.

(2) Certain typing phages require up to five days for maximal appearance of PFU. This effect on variation between replicates is being investigated.

(3) Studies on high titer (RTD > 1000) liquid phage suspensions show that a single set of dilutions held at 4°C can be used for at least one year. Lyophilized phage remained at a constant titer during the same period. These observations will be continued indefinitely.

List of Publications:

None.

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RESEARCH AND TECHNOLOGY RESUME

2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
	DA OA 6318	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 65	A. NEW	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 02 055

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 01 24

11. TITLE: (U) Experimental Surgery (06)

12. SCIENTIFIC OR TECH. AREA: 012900 Physiology; 005900 Environ Biology; 016200 Stress Phys

13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
08 54	NA	OTHER / DA

16. PROCURE. METHOD: C. In-House

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

18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	0	24
CURRENT FY 66	1	26

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ASSOCIATE: Hansen, J.E., LtCol
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21. TECHNOLOGY UTILIZATION: Human Job Performance

22. COORDINATION: None

23. KEYWORDS: Surgical Techniques and Equipment: Aortic Valve, Mitral Valve; Wound Healing; Teaching; Environment; Physiological Functions

24. (U) Tech Objective: The technical objectives are to provide surgical techniques, equipment and personnel to support research studies in the laboratory proper; to provide same for the surgical residency program of FGH, and to conduct basic and applied experimental surgical investigations. Techniques of cardiovascular catheterization, electromagnetic flowmeter implantation and surgically induced partial heart block are the principal areas of interest in support of research in the laboratory proper. Residents and staff personnel from the Departments of Thoracic Surgery, General Surgery, Opthalmology, and Ob-Gyn have used the facility, equipment, and personnel during the reporting period. Training has included mitral/aortic valve placement, wound healing, vascular surgery, intraocular trauma and repair, and specialized surgical technique teaching.

(U) Approach: Basic procedures being undertaken are preliminary pilot studies with preanesthetic and anesthetic agents. Intravascular thermocouple placement for recording internal body temperatures at various positions within the body are in development stage. It is anticipated that present work will serve to develop techniques in previously mentioned areas of interest and enhance the capabilities of surgical residents from Fitzsimons General Hospital.

(U) Progress: (Oct 64-Jun 65) Surgical procedures supporting other research projects continue with effort made to improve techniques at no expense to the supported project. Interest in conducting experimental animal surgery by FGH residents and staff is increasing. Pilot studies using analgesics, tranquilizers, and barbiturates as preanesthetic and anesthetic agents to establish range effect of each prior to additional stresses have started. Preliminary intravascular implants to test temperature response in body core have begun. Results thus far give indications that some techniques may have merit and will increase the sophistication of the testing methodology.

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) OVER

ABSTRACT

PROSTHETICS AND EXPERIMENTAL SURGERY (FY 1965)
EXPERIMENTAL SURGERY (FY 1966)

Under this subtask surgical support is given to investigators from this laboratory and from Fitzsimons General Hospital in the development of techniques or prosthetics and the attainment of proficiency in their use. In the past, the long-term efficacy of nylon, dacron, orlon and teflon thoracic aorta prosthetics and the surgical feasibility of lessening vascular repair time, using short pronged stainless steel rings was demonstrated. General surgeons have received training in cardiovascular and thoracic surgical techniques in dogs. Present work includes the implanting of catheters and thermocouples in dogs plus evaluating various surgical techniques and equipment prior to use in humans.

BODY OF REPORT

PROSTHETICS AND EXPERIMENTAL SURGERY (FY 1965)
EXPERIMENTAL SURGERY (FY 1966)

Description: A Study of Various Plastic Materials in Arterial Grafting

To assist in the development of prosthetic, physiological and surgical techniques, and equipment in laboratory animals prior to their use in other laboratory animals or humans.

Progress:

In past years the long-term efficacy in dogs of dacron, nylon, orlon and teflon thoracic aorta prosthetics was demonstrated. The surgical feasibility of insertion of short pronged stainless steel rings by surgeons not trained in vascular surgery and the tolerance of the rings without significant subsequent thrombosis was shown. Recently we have, in dogs, worked on the development of techniques and equipment to allow the long-term use of arterial and venous catheters in humans with simultaneous sampling and temperature recording of the blood. Electromagnetic flowmeters were inserted in several locations on the thoracic aorta of dogs to evaluate the tolerance by the animals and the accuracy and reliability of the meters. An ophthalmological freezing instrument which may allow less traumatic and easier removal of the lens is being evaluated. Several combinations of preanesthetic drugs were tested in an attempt to find a formula which would lessen post anesthetic recovery excitement. Fitzsimons General Hospital surgical residents received training in thoracic and cardiovascular techniques and implanted various suture materials in animals to observe and evaluate tissue reactions.

Summary and Conclusions:

Worthwhile experience was gained and techniques were improved.

Publications:

None

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RESEARCH AND TECHNOLOGY RESUME

2.	3. GOVT ACCESSION	4. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6319	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY WORK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 6145011 3A014501B71R 02 056

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 01 25

11. TITLE: (U) Lung Structure, Function, Pathophysiology (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002600 Biology 012900 Physiology	06 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)
		PRIOR FY 65	1	16
		CURRENT FY 66	1	18

19. GOV'T LAB/INSTALLATION/ACTIVITY
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ASSOCIATE: Stelter,G.P.Maj; Fairchild,D.G.,Capt
TEL: 303 366 5311 X26112 TYPE: DA

21. TECHNOLOGY UTILIZATION: Pulmonary Research Laboratory

22. COORDINATION: None

23. KEYWORDS: Lung; Anatomy; Respiratory System; Alveolus; Bronchi

24. (U) Tech Objective: Pulmonary structure and function are intimately interrelated. Physiologic studies are hampered by lack of visualization of the airways and vasculature while the usual anatomic and pathologic studies do not allow a three-dimensional comprehension of structure.

25 (U) Approach: By reconstructing a small area of normal pulmonary parenchyma better visualization of the airways and vasculature can be made. These observations can be applied physiologically and clinically. The major technical problem has been in obtaining adequate serial sections of normal lung tissue.

26 (U) Progress: (Oct 64-Jun 65) A highly magnified reconstruction of normal dog lung has been thoroughly studied. Marked complexity of the terminal airways was observed. The distal airway has a "polychotomous" branching pattern, as opposed to the generally dichotomous pattern seen in the larger airways. A distal airway is defined as a polyhedral conduit of air whose edges consist of epithelial covered bundles of connective tissue, muscle fibers and vessels; whose vertices are intersections of these bundles, and whose facets are defined by curving planes of gas, limited by the edges of the facet. Each airway has a single inlet facet and two to fourteen outlet facets. The total area of the outlet facets invariably exceeds that of the base or inlet facet. Alveoli are defined as airway terminations not further subdivided by septs. Alveoli vary markedly in shape and size supporting the concept of arrested alveolar development due to adaptation to available space. Destruction of the bifurcating supporting elements of the distal airways appears to be of considerable significance in the pathogenesis of emphysema. Acrylic is being considered to reconstruct human pulmonary tissue to provide better definitions of the pulmonary vasculature.

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) CPY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) OVER

ABSTRACT

LUNG STRUCTURE, FUNCTION, PATHOPHYSIOLOGY

The intimate relationship of pulmonary structure and function has been repeatedly emphasized. Numerous investigators have stressed the lack of adequate 3-dimensional visualization of the most distal airways, which represent the true respiratory zone of the lung. A fiberboard model of one cubic millimeter of normal dog lung was built at a magnification of 530X. We have found that alveolar ducts, alveolar saccules and atria are all similar and may properly be redefined as "distal airways". Contrary to present diagrams and opinions, the length of the average distal airway is no greater than its mean diameter. The branching pattern of this airway is "polychotomous" and dichotomous. Alveoli are of various sizes and shapes and arise in no regular manner from the distal airway. The vascular and lymphatic portions of this area of the lung could not be adequately evaluated due to the opaque material used in the reconstruction. Colonel Vorder Brugge expressed interest in this project, especially in regard to the relationship of anatomic features to pathologic processes involving the lung. We are attempting to obtain adequate normal human lung tissue in order to make an acrylic reconstruction to overcome the visual difficulties encountered on the present model and to more fully evaluate the course and relationships of the terminal pulmonary vessels and lymphatics in relation to the distal airways and alveoli. Two manuscripts will be submitted for publication by the end of August 1965.

In the dog, the distribution of pulmonary emboli is influenced by density of emboli, position of the dog and apparently the anatomy of the pulmonary vascular tree but not by sites of introduction used (femoral and jugular veins). No further studies have been done on this subject.

No progress was made in developing a chronic lung window.

BODY OF REPORT

LUNG STRUCTURE, FUNCTION, PATHOPHYSIOLOGY

Description:

Pulmonary structure and function are as intimately interrelated as are the airways and vasculature. Physiologic studies are hampered by lack of visualization of the pulmonary tissue in a functional state. Anatomic and pathologic studies often fail to show these structures as they exist in life. Three-dimensional models of normal and perhaps abnormal portions of animal and human lung will be made.

Wagner and Filley at the University of Colorado have photographed the surface of normal dog lung in the closed chest animal. This superb technique will be extended to a chronic preparation.

Pulmonary embolization is a common clinical problem and is inconsistently followed by recognizable infarction. Factors influencing distribution, pharmacologic, neurologic, and ventilatory changes following embolization may be studied in the dog.

Progress:

After several preliminary trials, a normal dog's lung was removed at thoracotomy after ligation of the vasculature; the lung was fixed with formol-Zenkers solution intrabronchially, mounted in paraffin, sectioned serially at 7μ thickness, stained, projected, and drawn on 200 sections of virgin Kraft paperboard; and the resultant sections were cut and assembled in layers giving a model of approximately one cubic millimeter magnified 530 times. The model has been labelled, colored, and intensively studied. Two papers are in preparation.

Significant pleural reaction occurred to all substances implanted in the thorax. This project is not being pursued.

Dogs have been embolized by the jugular and femoral veins in six positions relative to gravity. Emboli, spherical in shape and of varying densities, were used and the site of impaction recorded after sacrifice.

Summary and Conclusions:

1. Study of the model has led us to redefinitions. Alveolar ducts, alveolar saccules and atria are all similar and may be properly called distal airways. A distal airway is a polyhedral conduit of gas without major change in axial direction, whose edges consist of epithelial covered bundles of connective tissue, smooth muscle or vessels, whose vertices are intersections of these bundles and whose facets are defined by planes of gas limited by the edges. Alveoli, which are not further subdivided by septa, are the lateral or distal termination of airways. Alveolar orifices are

generally ovoid and are partially or completely defined by circumferential septal tissue. Alveoli vary markedly in size and shape. The branching pattern of the distal airways is polychotomous and not dichotomous. There is little or no change in the mean orifice diameter of succeeding distal airway generations. This geometric pattern, among other advantages, gives minimal travelling distance from trachea to maximal diffusing surface and minimizes the obstructing potential of intraluminal liquid or solid substances. The surface area of the model was found to very closely approximate the surface area calculated by the mean chord length formula of Weibel.

2. The "chronic" lung window does not seem attainable at this time.

3. In the dog, the distribution of pulmonary emboli is influenced by the density of the emboli, the position of the dog, and apparently the anatomy of the pulmonary vascular tree but not by the sites of introduction used (femoral and jugular veins).

List of Publications:

1. Model displayed at the 7th Annual Aspen Conference on Pulmonary Diseases, Aspen, Colorado, 10-13 June 1964.

2. Three papers reporting the above work are in various stages of preparation.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6302	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / ENTIRE	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 067

10b. PRIOR NUMBER/CODE: 61125011 3A012501A803 01 26

11. TITLE: (U) Computer Classification of Pulmonary Disability (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
003500 Clinical Medicine 002400 Bioengineering, 009800 Med & Hosp Equip	03 61	NA	OTHER 1 DA

16. PROCURE. METHOD	17. CONTRACT/GRANT		18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	b. NUMBER:	c. DATE:	PRIOR FY 65	1	17
	c. TYPE: NA	d. AMOUNT:	CURRENT FY 66	1	19

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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INVESTIGATORS PRINCIPAL: Syner, J.C., Lt.Col.
ASSOCIATE: Ruffner, A.B.
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) Tech Objective: To design, program and test-run a computer control system for processing medical information in clinical practice and experimental data in medical research. In its fully debugged form, this integrated bio-medical information processing system will function within the hospital, clinic and laboratory environments on a production basis.

25. (U) Approach: Biomedical data enters the system from various clinics and laboratories within the hospital complex. The information flows into an integrated programming network which executes all commands for storage, retrieval and analysis (Mathematical and logical). The completion of the reasoning foundations essential to decision making is made within the computer programming.

26. (U) Progress: (Oct 64-Jun 65)
With return of principal investigator in March 1965, data collection and systems programming to effect production time functions in hospital and laboratory environments is being accelerated.

27. COMMUNICATIONS SECURITY	29.	30. OSD CODE	30. BUDGET CODE
☐ COMSEC OR COMSEC RELATED ☒ NOT RELATED		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATION
CDOG 1412a	

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) OVER

ABSTRACT

COMPUTER CLASSIFICATION OF PULMONARY DISABILITY

A medical logic system is being programmed for a general purpose digital computer. The primary components of the system are the specialized information and the reasoning foundations practiced in patient care. The system encompasses all aspects of information collection, storage, retrieval and analysis utilized in medical decision making. The class of problem programmed for solution is the classification of disability resulting from pulmonary diseases.

BODY OF REPORT

COMPUTER CLASSIFICATION OF PULMONARY DISABILITY

Description:

To solve the problem under consideration, the system demanded that programming be developed which could derive an estimate of the current and predictable state of health. This required that provision be made for deriving a diagnosis and prognosis, evaluating the role of treatment, i.e., drugs, surgical, environmental controls, and assessing, principally by statistical methods, the relative weight to be attributed to the components of information, individually and in sequenced combinations.

The classification of disability resulting from pulmonary disease is a member of a class of medical problems. A digital computer system for any one member of the class (i.e., gastro-intestinal, cardio-vascular, etc.) could serve as a model for the techniques, procedures, and hardware required to implement information processing for total patient care.

Progress:

The plan adopted to solve the problem under consideration calls for two basic efforts:

1. Conceptualize and write the logic of the computer system.

2. Phase the programming into production runs at Fitzsimons General Hospital.

Conceptualizing and writing computer programming for the medical logic system has been the principal requirement to date. Significant advances have been made since the previous report in the development of systems programming.

1. A directory of medical information organized by subject into master files has been established. The information content of these files represents the medical knowledge and experience considered essential for solving the problem at hand. A system for placing, under computer control, the information collected from hospital clinics and laboratories is under continued refinement. Current information is continuously added to the files and obsolete information deleted.

2. File maintenance procedures to rearrange, edit, sort, provide quality control, add, delete and combine messages in master files, are more fully automated under improved computer controls. In the file maintenance system, quality control of information is being further developed through the use of logical and statistical techniques to question every item in all messages, and to reject doubtful items for re-evaluation at the data source.

3. The directory of medical terms is now in an improved state of readiness to perform essential translations from machine language encoding to English language to the medical terms and phrases required in stating the disability classification in traditional medical language. The directory is being used as a coding/encoding interface for all storage and retrieval problems involving non-numeric information.

4. A system for the storage and retrieval of literature abstracts pertinent to the problem under consideration is being programmed. Retrieval will be made for authors, titles, subjects, keywords and experimental observations and statistical results. This provides a capability to pool and integrate the experience of other clinicians and investigators with the results of practice at Fitzsimons General Hospital. The system is designed around two ideas: first, it is to serve as an automatic retrieval system to print out literature abstracts about particular subjects in the area of disability upon request from the user; and second, it is to chain the information in this literature into the medical logic system to assist in deriving a disability classification of the individual being evaluated.

5. Three additional statistical methods have been programmed for the analysis system:

a. "T" test.

b. Generalized analysis of variance computation.

c. Tau non-parametric correlation coefficient.

Phasing the systems programming into production runs at Fitzsimons General Hospital has been accomplished to a limited degree since the previous report. Progress to be noted in this area is as follows:

1. A clinical information system which processes symptoms, past and present illnesses and functional classification on patients has been written. This system operates entirely under computer controls. The user can make his interrogation by a simple message stating the individual's name and the question under consideration.

Summary and Conclusions:

Progress in programming the medical logic system and phasing this into production runs at Fitzsimons General Hospital has been delayed by the requirements encountered in learning the technical and logical characteristics of the computer and the overseas assignment of the principal investigator. Gaining technical proficiency in use of the computer has been very demanding and has required a prolonged period of time. With problems of technique now at a minimum the rate of progress on the classification and systems problems is greatly increased. This is reflected in an increased capability of translating generalized conceptual systems into the required programming instructions necessary to productively chain the various segments of the system in an entirely automated manner.

Publications:

None.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6303	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 65	A. NEW	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 068

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 01 27

11. TITLE: (U) Computer Instrument Linkages (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry 002400 Bioengineering, 009800 Med & Hosp Equip	12 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	NA	PRIOR FY 65	0	27
		CURRENT FY 66	1	30

19. GOV'T LAB/INSTALLATION/ACTIVITY
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TEL: 202 Oxford 6 5472

20. PERFORMING ORGANIZATION
NAME: USA Medical Research & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240
INVESTIGATORS PRINCIPAL: Ruffner, A.B.
ASSOCIATE:
TEL: 303 366 5311 x25130 TYPE: DA

21. TECHNOLOGY UTILIZATION: Biological Laboratory Instrumentation

22. COORDINATION: None

23. KEYWORDS: Computer; Instrument; Linkage; Programmer; Analog; Digital; Conversion; Bio-Medical; Curve Analysis

24. (U) Tech Objective To design, program, test run, and implement in feasible areas, the data storage, retrieval and analysis of the punched tape digital output of an analog/digital conversion system attached to various laboratory instruments.

25. (U) Approach
The current method of data analysis of stripchart recordings is broken down into logical components available to computer programming -- programming to be accomplished by joint effort of computer personnel and instrument specialists.

26. (U) Progress: (Oct 64-Jun 65)
Analog/digital conversion system DAR approved -- Specifications have been sent to manufacturers.
Flow charting and programming completed but not tested for Nitralyzer

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) OVER

ABSTRACT

COMPUTER INSTRUMENT LINKAGES

In analog to digital conversion systems wherein the volume or complexity of the analysis of the output data is dependent on a digital computer, computer programming is considered to be the single greatest drawback to successful automatic systems. The problem of this study is to program logic systems that handle the digital output of a variety of analog instruments utilized routinely in research studies performed at U. S. Army Medical Research and Nutrition Laboratory and Fitzsimons General Hospital.

BODY OF REPORT

COMPUTER INSTRUMENT LINKAGES (FY 1965)

Description:

A study is being made of the programming and systems planning necessary to store, retrieve, and analyse the punched tape digital output of an analog to digital conversion system attached to various laboratory instruments. An initial conversion system capable of operation at a wide range of voltage inputs and with a large number of input channels has been requested. Programming will be a joint function of the Computer Division and the personnel most familiar with the data sources.

Progress:

1. An analog to digital conversion system "Data Automation Requirement" has been approved.

2. Programming has been completed for initial storing of "N" channels of input data from paper tape to the record file of the computer system.

3. Flow charting and programming completed for nitralyzer.

4. Programming for output analysis of energy expenditure data has been completed in the Bioenergetics Division.

Summary and Conclusions:

The electronics for an analog to digital conversion system have been ordered.

The Bioenergetics Division will have initial installation of the conversion system, as a pilot study, because of the considerable programming and system planning already accomplished.

It is anticipated that as programming is developed for the output of other equipment in the laboratory, the portable system will be moved to the equipment sites for test running and program debugging.

Publications:

None.

Previous page was blank, therefore not filmed.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION: DA OA 6304	REPORT CONTROL SYMBOL: CSCRD 103	
4. DATE OF RESUME: 01 07 65	5. KIND OF RESUME: A. NEW	6. SECURITY: U (RPT) U (WRK)	7. REGRADING: NA	8. RELEASE LIMITATION: NL
9. LEVEL OF RESUME: A. WORK UNIT				

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 069

10b. PRIOR NUMBER/CODE: None

11. TITLE: (U) Wound Healing (06)

12. SCIENTIFIC OR TECH. AREA: 013800 Radiation Shielding and Protection 0141 00 Radiobiology

13. START DATE: 04 63

14. CRIT. COMPL. DATE: NA

15. FUNDING AGENCY: OTHER DA

16. PROCURE. METHOD: C. In-House

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

18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	1	35
CURRENT FY 66	1	40

19. GOV'T LAB/INSTALLATION/ACTIVITY

NAME: HEADQUARTERS

ADDRESS: US ARMY MED RSCH & DEVELOPMENT COMD Washington, D. C. 20315

RESP. INDIV. Rosenberger, Lt. Col., A. E.

TEL: 202 OXford 6 5472

20. PERFORMING ORGANIZATION

NAME: USA Medical Rsch & Nutr Lab

ADDRESS: Fitzsimons General Hospital Denver, Colorado 80240

INVESTIGATORS: PRINCIPAL: Kinnamon, K. E., Capt. ASSOCIATE:

TEL: 303 366 5311 X 26111 TYPE: DA

21. TECHNOLOGY UTILIZATION: Health Physics Application Surgical Proced Following Radiation Exposure

22. COORDINATION: None

23. KEYWORDS: Wound Healing; Radiation Injury; Trauma; Methionine; Collagen, Metabolism; Radiation Protection; Radiation, nonthermal; Radiation, protective agents

24. (U) Tech Objective: Injury from ionizing radiation produced by nuclear devices is an incompletely understood phenomenon. The combination of traumatic injury and radiation injury would be evident in many patients following a nuclear explosion. There is need to better define the types of interaction which exist between radiation injury and mechanical trauma and how these interactions influence healing.

25. (U) Approach: Using rats as experimental subjects, various effects of ionizing radiation upon healing traumatic wounds, or vice versa, are being studied. Doses of radiation ranging from 200 rad to 1300 rad have been used in these experiments. Chemical analyses, radioassay techniques and histopathological studies as well as gross observations have been used to determine what effects one type of injury may have on the other.

26. (U) Progress: (Oct 64-Jun 65) Eight hundred rad whole body radiation received by rats has not been shown to affect wound regenerate content of total methionine or total cystine. Neither does the acute exposure alter methionine-S^{35}, cystine-S^{35}, total sulfur-S^{35}, nor total sulfur-^{35} minus sulfate-S^{35} regenerate levels after tracer injections of methionine-S^{35}. The irradiation did, however, cause a reduction in wound collagen content. In another experiment, a study was conducted to determine the effect of prior traumatic injury upon rats receiving 1300 rad ionizing radiation. Our investigation failed to confirm a previous report that critically timed prior traumatic injury will afford substantial protection to radiation injury. The effects of dietary methionine and/or zinc are also being studied to ascertain their influence upon wound healing with and without total body irradiation (800 rad). There is evidence both of these dietary additives play a cardinal role in wound healing. Too, whole body radiation is known to markedly influence the metabolism of both these nutrients. The data from this last study is presently being evaluated.

27. [illegible]	28.	29. [illegible] CODE: AR	30. [illegible] CODE: 1
31. [illegible]: CDOG 1412 a		32. [illegible]: NA	
33. [illegible]	34. [illegible]		
35. [illegible]			

DD Form 1498

ABSTRACT

WOUND HEALING

The effects of 800 rads of total body irradiation on the distribution and excretion of intraperitoneally injected radiozinc have been studied in rats fed diets containing elevated levels of methionine and/or zinc. Alterations in zinc metabolism have been observed. Radiozinc retention in regenerating wound tissue of irradiated rats of the methionine plus zinc group was significantly elevated but no alterations in the rate of wound healing, as determined by collagen formation, were seen. In other studies, standard skin wounds administered to albino rats at various times prior to 1300 rads of whole body irradiation were found to impart no protection.

BODY OF REPORT

WOUND HEALING

Description:

At another laboratory, the addition of both methionine and zinc to standard rat diets has been shown to promote the healing of excised wounds in non-irradiated rats. Neither methionine alone nor zinc alone as feed additives produced consistent changes in the healing rate. More recently, incised wounds have been shown to preferentially accumulate zinc-65 during the acute healing stage. In this laboratory an effort has been made to determine the influence of 800 rads of total-body ionizing radiation upon the excretion and distribution of zinc-65 in regenerating wound tissue and other selected major tissues of rats maintained on rations with increased levels of methionine and/or zinc.

The search for a chemical substance which affords protection against ionizing radiation has been a diligent one since the late 1940's. However, those chemical agents shown to possess radioprotective properties, besides affording only limited protection, have been found to possess certain undesirable characteristics. The chemical protectants discovered so far, almost invariably, require dosages uncomfortably near toxically lethal levels. Recently, there has been a report from another laboratory describing a method of protection that is more effective than any reported so far. The report claims that by subjecting test rats to a standard traumatic skin wound on the seventh, eighth, or ninth day prior to a lethal dose of radiation, an LD 100/10 (approximately 1200 to 1500 rads) can be reduced to an $LD_{35-45/10}$. Presumably the physiological responses to injury and healing stimulates production of sufficient quantities of certain agents which afford considerable protection from total body irradiation. Studies have been carried out in this laboratory in an attempt to more clearly define the nature of this "time after trauma - radiation protection" response.

Progress:

Eight hundred rads of total-body ionizing radiation to rats have been found to affect the metabolism of intraperitoneally injected $Zn^{65}Cl_2$. Regenerating wound tissue of rats fed a basal control ration, a ration containing 20 milligrams of methionine per gram of diet, or a ration containing 0.133 milligrams of zinc per gram of diet, responded in general to a single radiation exposure in a manner similar to other tissues throughout the body. Regenerate levels of radiozinc in regenerating tissue of irradiated rats fed both methionine and zinc at the above levels were higher on the 6th day following exposure than those found in any dietary group whether irradiated or non-irradiated. No significant differences in wound collagen levels were found. Excretion of radiozinc was significantly reduced temporarily by the acute radiation exposure. Recovery of zinc excretory function occurred on the 3rd to 5th day following exposure. Urine zinc-65 excretion did not appear to recover as rapidly in irradiated animals fed diets high in methionine.

WOUND HEALING

Investigation conducted in this laboratory has failed to confirm a report from another laboratory that critically timed prior traumatic injury will afford substantial protection to radiation injury. Male adult Holtzman rats were exposed to 1300 rads of whole body irradiation on days 1, 3, 5, 7, 8, 9, 11, 13 and 15 following wounding. Although mortality was used as an end point, ante mortem observations and necropsy findings were also evaluated.

Summary and Conclusions:

Eight hundred rads of total-body ionizing radiation to rats significantly affects zinc metabolism. Zinc levels of regenerating wound tissue are much higher in rats fed diets high in methionine and zinc; however, healing, as determined by wound collagen formation, is not accelerated. Critically timed prior traumatic injury has not been found to confer any degree of protection to rats exposed to 1300 rads of total-body irradiation.

List of Publications:

1. Kinnamon, Kenneth E. Radiation and Wound Healing: Influence of Dietary Methionine and Zinc upon Zn^{65} Distribution and Excretion in the Rat. Manuscript being edited.

2. Kinnamon, Kenneth E. and Fairchild, David G. Effects of Traumatic Injury on Response to Subsequent Total Body Irradiation in Albino Rats. Intended as a USAMRNL Report.

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 RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 02 057

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 01 29

11. TITLE:
(U) Histopathology and Clinical Pathology of Laboratory Animals (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 a. DATE: b. NUMBER: c. TYPE:	d. AMOUNT:	18. RESOURCES EST.	c. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House	NA		PRIOR FY 65	3	52
			CURRENT FY 66	3	57

19. GOV'T LAB/INSTALLATION/ACTIVITY
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TEL: 202 OXford 6 5472

20. PERFORMING ORGANIZATION
NAME: USA Medical Research & Nutrition Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

INVESTIGATORS PRINCIPAL: Fairchild, D. G., Jones, L. D.
ASSOCIATE: Hageman, D. R., Ackerman, L. J.
TEL: 303 366 5311 X23230 TYPE: DA

21. TECHNOLOGY UTILIZATION
Basic Life Sciences

22. COORDINATION

23. KEYWORDS: Histopathology; experimental animals; histology; clinical pathology; staining technics

24. (U) Purpose: To gain knowledge of the diseases of laboratory animals and to apply this knowledge to a more accurate interpretation of experimental data obtained in the use of experimental animals. Technical methods are continously being reviewed to meet new demands as they arise.

(U) Approach: Routine and special histopathologic and histochemical technics are used in conjunction with transmitted, polarized, ultraviolet, phase and electron microscopy to elucidate the cause, pathogenesis and possible significance of lesions encountered in histopathologic examinations.

(U) Progress: Oct 64-Jun 65: Approximately 1800 cases, of which 1100 are necropsies, have been accessioned in this period resulting in 1800 bags of wet tissue, 8000 paraffin blocks of tissue, 1600 H&E stained slides, 2000 special stained slides, and 2500 feet of 35 mm film strip which was utilized in the production of serial sections of tissue. When rare or unusual lesions of histopathologic significance are encountered, they are submitted for publication in the open literature. Normal study sets of microscopic slides with written descriptions are being prepared on all experimental animals used in this laboratory. To date rabbit and mouse sets have been assembled. Dog, chicken, and guinea pig study sets are in the final stages of preparation. Extensive clinico-pathologic examinations are performed on a routine basis on dogs and rabbits to study the overall health of these animals. The animal care branch has been responsible for the procurement, rearing and maintenance of a relatively disease free colony of about 5000 population which includes mice, guinea pigs, rats, dogs, rabbit, quail and chickens

1. Fairchild, et al. Multiple Neoplasia in a Dog. JAVMA 145, 991, 15 Dec 64. 2. King, et al. Bilateral Hermaphroditism in a Dog. JAVMA 145, 999-1001, 15 Nov 64. 3. Castleberry, et al. Mucoid Impaction of the Canine Bronchi. JAVMA 146, 607, 15 Mar 65. 4. Jones, L.D. Avian Cerebellar Teratoma. Avian Diseases, 8, 580, Nov. 64

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) OVER

BODY OF REPORT

HISTOPATHOLOGY OF LABORATORY ANIMALS (FY 1965)
HISTOPATHOLOGY AND CLINICAL PATHOLOGY OF LABORATORY ANIMALS (FY 1966)

Description:

This sub-task provides the pathologist the opportunity to gain additional experience and insight into the spontaneous and experimental diseases of laboratory animals. This enables him to render a more accurate interpretation of experimental results obtained from those research projects of the Laboratory which employ laboratory animals as experimental models.

Progress:

A program has been developed and is in use whereby all anatomic and pathologic data is being placed into an RCA 301 computer system for data retrieval and data analysis.

All further projects encompassing the special techniques of histochemistry and/or microautoradiography will be reported under this task instead of Subtask No. 30 - Histochemical Methods in Biological Research which was terminated in September 1964.

Consultations are routinely solicited from this division concerning the problem of spontaneous experimental animal diseases and the effect of these diseases on the interpretation of experimental data.

The Animal Service Branch has been responsible for the procurement, maintenance, disposal and coordination of laboratory facilities so that approximately 6,000 animals of various species were supplied to investigators during FY 65. A healthy animal colony is maintained under supervisory controls of a staff Veterinary Officer. All animals are observed at least once daily and extensive clinical pathology is performed weekly, or more often if deemed necessary, on rabbits and dogs to keep constant control of the overall health of the entire colony.

In FY 65 approximately 1,900 accessions were entered, 1,400 of which were necropsied and 500 were surgical specimens. The Histopathology Laboratories Branch processed approximately 2,000 bags each of wet and embedded tissues, 15,000 blocks of embedded tissues, 46,600 routinely processed stained and unstained microscopic slides, 16,000 feet of serial sections on film strips, and 7,770 specially stained histochemical or autoradiographic slides.

Under this task, officers have prepared normal histology study sets on dog, rat, mouse, guinea pig, chicken and rabbit. These sets are used as a reference tissue library.

The specimens are constantly examined and reviewed to increase the knowledge of spontaneity of diseases in the laboratory animals routinely used at this installation. When material is felt to be of sufficient interest to be of value

to the scientific community, manuscripts are prepared for publication.

The manuscripts which were prepared from this study during FY 65 are summarized below:

1. A Method for Mounting Cestodes and Nematodes for Microscopic Examination. This method utilizes EPON 812® epoxy resin as a mountant. This results in a permanent and durable media for the preservation of parasite specimens. The resultant transparent preparation does not distort or discolor the specimen upon ageing.

2. An Improved Method for Accelerated Giemsa Stain. The standard procedure has been modified so that tissue sections fixed in most fixatives (except those that contain mercurial compounds) can be deparaffinized, stained, and coverslipped in a total time of twelve minutes, instead of the usual 1-1/2 hours. Modifications were made in staining solutions and in the temperature at which staining was performed.

3. The Use of the Electronic Computer in Retrieval of Veterinary Pathologic Data. A practical and usable program of data storage and retrieval was devised for an RCA 301 computer system. Anatomicopathologic entities, genetic records, and identification codes were the types of information stored in the computer. Data retrieval programs have been effective in processing the data quickly and accurately.

4. Avian Cerebellar Teratoma. Teratomas are rarely found in the chicken and when present occur most frequently in the ovary and testicle. Both di- and tridermic forms have been reported. No reports have been found of primary location at other sites in the chicken. This manuscript describes the first reported case of an avian cerebellar teratoma in a chicken.

5. Malignant Mastocytoma with Leukemic Manifestations in a Dog. Surgery was performed three times in an attempt to remove a malignant mast cell tumor of the skin. During clinical observation of three months, the neoplasm metastasized to superficial and visceral lymph nodes and most of the internal visceral organs. The WBC count prior to death was 27,800 and the differential was:

	Percent	#/mm^3	Normal #/mm^3
Band	16	4,448	0-540
Neutrophil	58	16,124	3,600-13,860
Lymphocyte	2	556	720-5,400
Monocyte	3.7	1,028	180-1,800
Basophil	11	3,058	0
Eosinophil	0.4	111	120-1,800
Unclassified	7	1,946	

Gross necropsy findings included recurrence at the primary site and widespread metastatic neoplasm in most organs including bone marrow. Microscopic findings largely substantiated gross findings, and microscopic evidence of neoplastic cells were found in all tissues examined.

The training of personnel of this and other installations or institutions has been increasing over previous years. In FY 65, sixteen military and seventeen civilian medical or histology technicians were trained in techniques of histopathology by staff personnel of this division.

Summary and Conclusions:

This sub-task is a general area of research which is conducted to gain insight into the diseases of experimental animals and to evaluate the significance of the lesions which occur in test and control animals. Materials and methods employed are both routine and specialized, encompassing areas of tissue histochemistry, autoradiography and electron microscopy.

Manuscripts are published on significant findings.

List of Presentations and Publications:

Presentations:

1. Ackerman, L. J. Principles of Histopathologic Techniques. Medical Laboratory Specialists MOS Training Course (MOS 931), USAMRNL, 20 Oct 64.

2. Fairchild, D. G., Knappenberger, T. E., Ackerman, L. J. and Houston, C. J. Histopathologic Methods in Research. Presented as workshop at 6th Annual Postgraduate Course in Medical Technology, University of Colorado School of Medicine, 18-19 Mar 65.

Publications:

1. King, N. W. and Garvin, C. H. Bilateral hermaphroditism in a dog. J.A.V.M.A., 145:997, 1965.

2. Houston, C. J. A method for mounting cestodes and nematodes for microscopic study. Submitted for publication.

3. Houston, C. J. An improved method for accelerated Geimsa stain. Submitted for publication Am. J. Stain. Tech.

4. Castleberry, M. W., Ferrell, J. F., Jones, L. D. and Garvin, C. H. Mucoid impaction of the canine bronchi. J.A.V.M.A., 146:607, 1965.

5. Castleberry, M. W., Jenkins, E. D. and Thompson, S. W. The use of the electronic computer in retrieval of veterinary pathologic data. Accepted for publication in Am. J. Vet. Res.

6. Jones, L. D. Avian cerebellar teratoma. Avian Diseases, 8:580, 1964.

7. Fairchild, D. G., Ferrell, J. F., Davis, C. L. and Garvin, C. H. Multiple neoplasm in a dog. J.A.V.M.A., 145:991, 1964.

8. Fairchild, D. G. and Hageman, D. R. Malignant mastocytoma with leukemic manifestations in a dog. In manuscript.

* Shell Chemical

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION: None	REPORT CONTROL SYMBOL: None

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY PRJ	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 10 64	B. COMPLETED	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501A71F 01 030

10b. PRIOR NUMBER/CODE: None

11. TITLE: (U) Histochemical Methods in Biological Research

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

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

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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Denver, Colorado 80240
INVESTIGATORS PRINCIPAL: Castleberry, M. W. Lt. Col.
ASSOCIATE: Knappenberger, T. E. Capt.
TEL: 303 366 5311 X 23230 TYPE: DA

21. TECHNOLOGY UTILIZATION: --

22. COORDINATION: None

23. KEYWORDS: Histochemistry; Autoradiography; Laboratory Animal Diseases; Fluorescence Microscopy; Electron Microscopy

24. (U) To conduct studies in the development and application of histochemical methods to the study of diseases of laboratory animals and in support of other projects in which histochemical demonstration of tissue components is necessary.

25. Histochemistry is a technique of pathology which allows the demonstration of many tissue components not ordinarily demonstrated in routine tissue specimens. Histochemical techniques for the demonstration of proteins, carbohydrates, lipids, and enzymes have been applied to the study of viral inclusion bodies, sheep rumenal pigment and intravenous fat pigment. Microautoradiography and enzyme histochemistry have been used in support of studies dealing with collagen metabolism in experimental lathyrism, bone growth in tissue cultures, and cartilage growth in rickets. A capability in electron microscopy now permits the study and application of this tool in bio-research.

26. Studies have been completed and published on viral inclusion bodies, sheep rumenal pigment, and portions of the intravenous fat program. The work unit was closed out and work continued under Histopathology and Clinical Pathology of Laboratory Animals.

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
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 1172) OVER

ABSTRACT

HISTOCHEMICAL METHODS IN BIOLOGICAL RESEARCH

Since all major studies started in FY 64 have been completed and reported except the publication of "Selected Histochemical and Histopathological Methods", Thompson, S. W., Lt. Col., VC, Charles C. Thomas, Springfield, Illinois, which is scheduled for publication this calendar year, all further studies will be conducted and reported under Project 3A14501A71F, Task 01, Sub-task 29, Histopathology and Clinical Pathology of Laboratory Animals.

BODY OF REPORT

HISTOCHEMICAL METHODS IN BIOLOGICAL RESEARCH

Description:

All studies initiated under this task have been completed and reported. Further studies involving the development and/or use of histochemical methods in biological research at this laboratory will be reported under Project 3A014501A71F, Task 01, Sub-task 29.

Progress:

N/A

Summary and Conclusions:

N/A

List of Publications:

Thompson, S. W. Selected Histochemical and Histopathological Methods. Charles C. Thomas, Springfield, Illinois. In Press.

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RESEARCH AND TECHNOLOGY RESUME

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 65	A. NEW	U (RPT) U (RMK)	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 070

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 01 31

11. TITLE: (U) The Effects of INH on Murine Pulmonary Histology (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CAT. 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)
	a. NUMBER:	b. DATE:		PRIOR FY 65	2	37
C. In-House	c. TYPE: NA		d. AMOUNT:	CURRENT FY 66	2	41

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20. PERFORMING ORGANIZATION
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Denver, Colorado 80240
INVESTIGATORS PRINCIPAL: Fairchild, D.G.
ASSOCIATE: Jones, L. D., Knappenberger, T. E.
TEL: 303 366 5311 X 23230 TYPE: DA

21. TECHNOLOGY UTILIZATION: Tuberculosis therapy

22. COORDINATION: None

23. KEYWORDS: Lung; mice; oncology; isonized; tuberculosis; pathology; carcinogenicity

24. (U) To ascertain the effect of isoniazid (INH) on marine pulmonary histology, principally to investigate the potential pulmonary carcinogenicity of isoniazid in mice.

(U) Approach: Published reports of studies indicate that INH is a pulmonary carcinogen when administered to mice orally or parenterally. Previous studies have used limited numbers of animals and were not carefully controlled. A controlled experiment using a large number of animals was undertaken. Saline or INH is administered to Cb and Strong A mice at varied dosages by varied routes. Thorough histopathological examination, including serial sectioning of lungs, was performed to evaluate the incidence of pulmonary neoplasia in these mice.

(U) Progress: (Oct 64-Jun65) Phase I has been completed. Strong A mice received parenteral injections 0.2ml 1% INH while control animals received 0.2ml of normal saline every other day for 18 weeks. Twelve weeks after the last injection all animals were necropsied and a thorough histopathologic examination was performed. Results: a significant increase (P .05) of pulmonary neoplasms was seen only in female mice receiving INH. The incidence of pulmonary neoplasia in control males and females, and males injected with INH was not significantly different. Difficulty was encountered, due to biological variation, when Cb and Strong A mice were injected with 0.2 ml 1% INH. This level drug was toxic to the test mice, 69 of 218 died before completion of this phase. Phase II is partially completed. Cb and Strong A mice were injected with 0.2ml of 0.2% INH every other day for 18 weeks with a 12 week post injection period. All animals will be necropsied by 19 Apr 65 and histologic examination and evaluation of results will be completed by 31 May 65.

Future plans include oral administration of INH and subsequent examination for increase of pulmonary neoplasia, and a study to ascertain why only the female mice that received INH have a significant increase in pulmonary neoplasia when compared with male and female control, and male animals that received INH.

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++	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) OVER

ABSTRACT

THE EFFECTS OF ISONICOTINIC ACID HYDRAZIDE (INH) ON MURINE PULMONARY HISTOLOGY

This study was designed to critically evaluate the production, pathogenesis, and incidence of pulmonary neoplasia reported to have been produced in mice by isonicotinic acid hydrazide (INH) when given orally or parenterally for long periods. Two strains of mice, both sexes, and in sufficient numbers to provide statistical significance, have been employed. Results of the first tests substantiate the early reports of other investigators only in the female. Strain differences to INH drug sensitivity were demonstrated. Drug sensitivity to INH was not offset by pyridoxine, but instead pyridoxine appeared to potentiate the sensitivity or toxicity. Standard mouse food with added INH was very poorly accepted thus creating an unfavorable experiment for comparison with studies using the parenteral route of administration.

BODY OF REPORT

THE EFFECTS OF ISONICOTINIC ACID HYDRAZIDE (INH) ON MURINE PULMONARY HISTOLOGY

Description:

One of the drugs of choice in the treatment of tuberculosis in man is isonicotinic acid hydrazide (INH). 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 peril. Like other antituberculosis drugs it must be employed continuously throughout long periods of time. It has been recognized as a Vitamin B_6 antagonist and as such it is a neurotoxic drug capable of producing peripheral neuritis, convulsive seizures, and toxic psychoses. INH has also been implicated in the production of hepatocellular alterations, dysfunction, and jaundice.

Recent 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 reported only relatively very 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 is designed to more critically evaluate the production, pathogenesis, and incidence of pulmonary neoplasia reported to have been produced by the administration of INH. In comparison with those reports in the literature this study is to be enlarged to include both sexes and larger numbers of experimental animals for the purpose of securing significant statistical analyses. Two strains of mice are included for strain susceptibility determination. 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 parts: I - Breeding of the required number of mice; II - Administration of INH to test animals and physiological saline solution (PSS) to test control animals; and III - Evaluation of results. There are six basic foundation phases in Part II:

Phase 1.	A.	to Strong-A mice: 0.2 ml. 1% INH sol.	Subcutaneous
	B.	to Strong-A mice: 0.2 ml. PSS	"
Phase 2.	A.	to BalbC mice: 0.2 ml. 1% INH sol.	"
	B.	to BalbC mice: 0.2 ml. PSS	"
Phase 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	"

	D.	to Strong-A: 0.2 ml. 0.2% INH sol.	Subcutaneous
Phase 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	"
Phase 5.	A.	to Strong-A: (in food) INH	Orally
	B.	to Strong-A: (in food) NO INH	
Phase 6.	A.	to BalbC: (in food) INH	Orally
	B.	to BalbC: (in food) NO INH	

Progress:

Part I. Enlargement of the mouse breeding colonies as the preparatory function has been accomplished and is now in continuous operation furnishing experimental animals in increments as required. The mice are about seven weeks of age (20-25 grams) when put on experiment.

Part II.

Phase 1. This phase has been completed. In this phase the effect of INH for strain Strong-A mice was studied. These mice were given 0.2 ml. of a 1% INH solution subcutaneously every other day for 18 weeks. For controls an equal number of mice received the same amount of PSS. Following a 12-week observation period, the survivors were sacrificed, a complete necropsy was performed, and a histopathological study made of all major organs. Particular attention was paid to the lungs. Lungs without gross evidence of a neoplasm were completely serially sectioned at a thickness of 10 microns, mounted on film strips and stained with hematoxylin and eosin for histopathological study. The mouse lung tumor incidence is shown below:

Mouse Lung Tumors: Strong-A Mice

		INH		
Sex	Neg	Pos	% Incidence	Total
Male	52	39	42.85	91
Female	50	67	57.26	117
Total	102	106	50.96	208
		PSS		
Male	66	36	35.29	102
Female	61	45	42.45	106
Total	127	81	38.94	208

Statistical analysis of these data disclosed that the incidence of lung tumors was significantly greater in the females receiving INH than the controls ($P<.05$); and that the incidence in the females receiving INH was significantly greater ($P<.05$) than the males on the same regimen. There was no significant change in incidence in males receiving INH.

Phase 2. This phase duplicated the first phase except BalbC strain mice were used. A great deal of difficulty resulted. This strain of mice exhibited a very low tolerance to the INH resulting in hyperesthesia, convulsive seizures, shock, and death. In an attempt to offset these untoward effects various forms of pyridoxine supplementations were attempted. This was in keeping with current practices employed with human patients to counteract the development of neurotoxic symptoms produced by INH. Instead of obtaining the anticipated alleviation of ill effects, the symptoms and deaths continued. Despite numerous deaths this phase was completed as scheduled. Results are now being evaluated.

Phase 3. D. (Added) This portion of this phase duplicated the first phase employing Strong-A mice, but were given 0.2 ml. of a 0.2% INH solution in the same manner. The same number of mice for control received an equal amount of PSS. This part of the phase has been completed and results are under study.

Phase 5. Some difficulty, delay, and unsatisfactory results have been experienced so far. The mice in this experiment have been given two grams of INH incorporated into each kilogram of their diet. Their weight performance has been very poor. This is consistent with similar results noted in other investigators' reports. This problem is presently being investigated with special consideration directed toward various metabolic and nutritional aspects.

Summary and Conclusions:

Limited studies by several investigators indicate a significantly high degree of pulmonary carcinogenicity in mice attributable to isonicotinic acid hydrazide (INH) when administered orally or parenterally for long periods. This finding has been substantiated only in female mice by the present work which also demonstrates stain differences in drug sensitivity. The use of pyridoxine not only failed to offset, but instead appeared to potentiate the toxic symptoms produced by the drug.

Standard mouse food with INH added was very poorly accepted thus creating an unfavorable experiment for comparison studies with the parenteral route of administration.

List of Publications: (None)

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RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA 0A 6306	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 65	A. NEW	&U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 071

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 02 11

11. TITLE: (U) Intravenous Fat Emulsions (06)

12. SCIENTIFIC OR TECH. AREA: 003500 Clinical Medicine
002300 Biochemistry, 012600 Pharmacology

13. START DATE: 05 53

14. CRIT. COMPL. DATE: NA

15. FUNDING AGENCY: OTHER | DA

16. PROCURE. METHOD: C. In-House

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

18. RESOURCES EST.

	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	2	33
CURRENT FY 66	2	36

19. GOV'T LAB/INSTALLATION/ACTIVITY
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ASSOCIATE: Jones, L. D. DVM
TEL: 303 3665311 X 21108 TYPE: DA

21. TECHNOLOGY UTILIZATION: Clinical Medicine, Pharmaceutical Industry

22. COORDINATION: None

23. KEYWORDS: Fat Emulsions; Intravenous Fat; Lipids; Oils; Fatty Acids; Balance-Metabolic; Toxicology; Lipid Metabolism; Parenteral Feedings; Phosphatide; Pathology; Electron Microscopy

24. (U) Tech Objective To produce a non-toxic intravenous preparation which will provide a relatively high caloric intake per ml. of solution for patients unable to assimilate sufficient nourishment.

(U) Approach The high caloric density and the absence of osmotic effect of fat emulsions offer a number of theoretical advantages for intravenous alimentation 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.

(U) Progress A symposium containing twenty-six papers devoted to basic and applied studies of intravenous fat emulsions has been compiled, edited and published. See Am. J. Clin. Nutrition, Vol 16, pages 1 thru 223, 1965. Toxicity testing of newly developed experimental high caloric, high osmotic, carbohydrate-alcohol solutions (Extracal) was evaluated but was found to be clinically inferior to available fat emulsions. This Laboratory continues to perform histopathological and electron microscopic examinations of tissues from animals utilized in various parenteral nutrition studies performed at other institutions. Studies have failed to establish any relationship between vitamin E administration and the development of intravenous fat pigment deposition after multiple infusions of fat emulsions.

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) OVER

ABSTRACT

INTRAVENOUS FAT EMULSIONS - INTRAVENOUS NUTRIMENT PROGRAM

Continuing studies were conducted in animals and humans to assess the acute and chronic toxicity of intravenous fat emulsions and combinations of emulsion components. These studies were conducted in association with other laboratories and investigators including: (1) Vanderbilt University, Nashville, Tennessee (2) Louisiana State University, New Orleans, Louisiana; (3) Applied Science Laboratories, Inc., State College, Pennsylvania; (4) U. S. Department of Agriculture, Southern Utilization Research and Development Division, New Orleans, Louisiana; (5) Mount Sinai Hospital, New York City, New York; (6) Vitrum AB, Stockholm, Sweden and (7) Karolinska Institute, Stockholm, Sweden.

An active testing program was conducted including examination of commercial proprietary preparations and four experimental fat emulsions. The examinations included the administration of the preparation to dogs, rabbits and rats. A study of the effects included clinical response, reactions at the sites of injection, clinical pathology, hepatic function, gross lesions, histopathology, and electron microscopy of selected tissues.

Some of the conclusions made from the results of these investigations and other activities include: (1) Intravenous fat pigment deposits were produced by all fat emulsions studied. (2) When decolorized, glandless cottonseed oil was used in the preparation of fat emulsions containing highly purified egg lecithin as the emulsifier, the emulsions possessed better physical characteristics than when purified cottonseed oil was used. (3) Dietary supplementation with choline did not decrease the magnitude of ultrastructural changes in hepatocytes following intravenous infusions of emulsions. (4) Toxicity testing of a newly developed, experimental high caloric, high osmotic, carbohydrate-alcohol solution (Extracal) revealed the solution to be clinically inferior to available fat emulsions. (5) Studies have failed to establish any relationship between intravenous fat pigment deposition and the pigmentation seen with vitamin E deprivation. Dogs receiving vitamin E enriched Intralipid did not have hepatic lipoid micro-granulomas on histopathological examination as is normally seen following multiple infusions of fat emulsions. (6) A symposium containing twenty-six papers devoted to basic and applied studies of intravenous fat emulsions was compiled, edited and published in the American J. of Clinical Nutrition, January 1965 (pages 1 thru 223).

BODY OF REPORT

INTRAVENOUS FAT EMULSIONS - INTRAVENOUS NUTRIMENT PROGRAM

Description:

A basic problem in the treatment of battle casualties, burn patients, radiological casualties and patients with certain surgical or medical disease states is the maintenance of adequate nutrition to support the therapeutic measures and natural healing processes in progress. The use of intravenous fat emulsions would provide the caloric needs and protein sparing effect necessary in patients incapable of ingesting the required nutrients. The development of a stable, nontoxic fat emulsion has been the goal of the Surgeon General's Intravenous Nutriment Program. To support this program, this laboratory has conducted a multifaceted research program to determine the clinical, the histopathological, and the cellular ultrastructural toxicity of intravenous fat emulsions and those aspects of lipid transport or metabolism which influence or are influenced by fat emulsion infusions.

Major areas in which work was accomplished during the past year include: 1. Animal toxicity testing of experimental emulsions and of emulsion components; 2. Histopathological studies of tissues from animals involved in the toxicity testing program. This is a supportive function provided to the investigators of the Intravenous Fat Toxicity Testing Group and it has been utilized by investigators at (a) Vanderbilt University, Nashville, Tennessee, (b) Louisiana State University, New Orleans, Louisiana, (c) Karolinska Institute, Stockholm, Sweden, (d) Vitrum, AB, Stockholm, Sweden, and (e) this laboratory; 3. Physical and chemical studies to identify the previously described intravenous fat pigment; 4. Metabolic studies utilizing the isolated perfused rat liver to determine the cellular uptake, metabolism and toxicity of fat emulsions; 5. Studies to determine the relationship of choline deficiency or enrichment to the hepatic cellular ultrastructural changes noted following prolonged intravenous fat emulsion infusions; and 6. Monitorship of the Surgeon General's Intravenous Nutriment Program.

Progress:

Toxicity Testing: Animal toxicity testing of experimental emulsions and emulsion components has been performed at this laboratory utilizing rats and rabbits. Test emulsions were infused intravenously daily, with 15 ml/kg body weight for five days each week for three weeks. An appropriate pre-infusion adaptation and observation period was observed. During the infusion and post-infusion weeks laboratory tests and extensive observations of the clinical

status were made. Detailed necropsy, generally performed in the second post-infusion week, plus histopathological examination was made on all animals. Utilizing this procedure, the following solutions have been tested.

1. Experimental Emulsion X64107: Prepared by Riker Laboratories, Inc., Northridge, Calif. 20% soybean oil, 1.2% phospholipids, and 2.25% glycerol in distilled H_2O.

2. SR 4734-176: Prepared by W.S. Singleton of USDA, Southern Utilization Research and Development Division, New Orleans, Louisiana (SUR&DD). 2.5% lecithin from freeze dried egg yolk, 2.6% glycerol and distilled water.

3. SR 4734-181: Prepared by W.S. Singleton, SUR&DD. 20% cottonseed oil, 1.25% egg-lecithin (freeze dried); and 2.6% glycerol in distilled water.

4. SR 194-10*: Prepared by W.S. Singleton, SUR&DD. 10% decolorized cottonseed oil, 0.6% pure egg lecithin, and 2.0% glycerol in distilled water.

5. Extracal: An experimental product of Cutter Laboratories, Berkeley, California, containing 15% glycerol, 7.5% glucose, 7.5% fructose, 5% anhydrous ethanol and 0.016% sodium bisulphite in distilled H_2O.

*Emulsion administered at rate of 30 ml/kg body weight.

The results of the tests utilized to evaluate SR 194-10 have not been analyzed and are unavailable for presentation. The results of the testing of the other solutions are summarized below. Riker X64107, Extracal and SR 181 produced minimal decreases in the hematocrits and hemoglobins of rabbits to which they were administered. Riker X64107 produced a more marked decrease in the red blood cell count than did SR 181. SR 176 did not influence the hematological findings in the rats or rabbits to which administered. Mild to moderate thermogenic responses were noted in the rabbits after infusion with Riker X64107, SR 181 and SR 176, but not following saline infusions. An average gain in weight was observed in rats and rabbits during the infusion periods with all the test materials except in rats receiving Extracal. However, the average weight gain was not as great as that observed in control animals receiving normal saline infusions. In rabbits, Extracal produced perivascular hemorrhage, inflammation and alopecia about the sites of the ear veins used for infusion. Thrombophlebitis was moderate to marked. Rats receiving

Extracal exhibited violent reactions including reddness of the ears, dyspnea, epistaxis, weakness, apparent fatigue, shock and death. Four of six rats died with death occurring after each of the following infusions: first, fifth, seventh, and tenth. The infusions were terminated following the 12th infusion due to the poor tolerance of the animals to the material.

HISTOPATHOLOGY:

Histopathological examination of tissues from animals receiving fat emulsions disclosed the intravenous fat pigment and lipoid microgranulomas in the organs in which the reticuleondothelial system significantly contributed to the organs' composition. Lipoid microemboli were found in the lungs of one rabbit after infusions with Riker X64107. This was interpreted as a result of the obvious physical instability of the emulsion. It was of marked interest to note that in the livers and spleens of the rats and rabbits which received SR 176 - a solution which contained no neutral fat - lipoid microgranulomas were noted which contained pigment having some of the characteristics of intravenous fat pigment. Vasculitis, thrombosis, bile ductular hyperplasia and mild portal cirrhosis were found in the tissues from rabbits which received Extracal.

The findings of the histopathological examination of the tissues submitted by L. S. U. and Vanderbilt are not included but will be reported in the contract reports from these two institutions.

Tissues received from Karolinska Institute and Vitrum, AB of Stockholm, Sweden were from animals which had been utilized in a series of interesting studies. Multiple tissues from seven dogs which had received daily infusions of Intralipid at 9 grams/kg body weight for 28 consecutive days disclosed: lipoid microgranulomas and intravenous fat pigment in hepatic and splenic tissue but the number of microgranulomas and the extent of pigment deposition was not as marked as generally seen following cottonseed oil emulsions. Fatty metamorphosis of the spleen and liver was noted in tissues from some of the animals, and pulmonary lipoid microemboli were found in tissues of two animals. Tissues from three dogs which had been maintained for ten weeks on total parenteral feedings, including 6 grams of fat/kg/day thru use of Intralipid, were examined and the following histopathological findings were noted: lipoid microgranulomas and intravenous fat pigment deposition in the spleens and livers of all animals; I. V. fat pigment deposition was present in lung tissues of two animals; lipoid microgranulomas were noted in the lung tissue of one animal; fat microemboli were noted in the pulmonary tissues of one animal; hemosiderosis was a common finding in the pulmonary, hepatic and

splenic tissues; focal hyperplasia of the R. E. system was noted in the splenic tissue of two animals; and renal lesions, though present, could not be attributed to the infused fat emulsion. Multiple infusions of Intralipid containing twice (2. 4%) the normal phospholipid content produced the usual hemosiderosis, I. V. fat pigment deposition, and lipoid microgranulomas. In the pulmonary and renal tissues of one animal recieving this emulsion multiple intravascular microscopic thrombi of undetermined origin were found. The most remarkable findings in the hepatic tissue from animals which received multiple infusions of Intralipid enriched with four times the normal amount of alpha tocopherol was the presence of moderate fatty metamorphosis, the absence of lipoid microgramulomas but the usual expected presence of I. V. fat pigment.

OTHER STUDIES:

Further studies utilizing the isolated perfused rat liver as a technique for evaluating the hepatic uptake and utilization of synthetic fat emulsions confirm the previous observation that the endoportal administration of an emulsion produced a significant reduction of hepatic blood flow. Electron microscopy demonstrated that there was direct assimilation of the emulsion by the hepatocytes. Previous work at this Laboratory suggested that choline dietary enrichment would partially prevent the electron microscopically observed vacuolation and dilatation of the rough endoplasmic reticulum of hepatocytes of animals receiving multiple infusions of Intralipid. Repeat studies failed to confirm this observation. In recent studies aimed at identifying the chemical nature of intravenous fat pigment, gradual clearance of the pigment from rabbit spleens was noted as the time subsequent to the infusions lengthened. This observation is contrary to previous observations in canines receiving multiple infusions of Liporaul. Carefully controlled tests are currently in progress to evaluate this observation.

MONITOR FUNCTIONS:

Monitorship functions of the Surgeon General's Intravenous Nutriment Program were curtailed due to a marked increase in the monitor's responsibilities in other areas and the marked reduction in suitable emulsions for testing. Considerable effort was expended in procurement of suitable material for evaluation. Close collaboration with the staff at Riker Laboratories, Inc., initially promised a possible nontoxic emulsion. Technical problems encountered in the commercial production of Intralipid resulted in that company's decision in June 1965 to withdraw from manufacture of the emulsion. Initial human clinical testing has begun on material produced by Riker after it had been clinically screened by investigators collaborating with Riker Laboratories, Incorporated. Results of such

testing by members of the Surgeon General's Intravenous Nutriment Program are too preliminary for comment. The final withdrawal of the Upjohn Company in June 1965 from manufacture of Lipomul, I. V. has further limited the source of commercial material.

Site visits made and conferences attended included: (1) Attendance at the 1st World Fat Congress - including functioning as co-chairman of one of three sessions of the congress devoted to Parenteral Nutrition, Hamburg, Germany, 12-18 October 1965; (2) Visits to Vitrum, AB, Stockholm, Sweden, October 1964; (3) Visit to the School of Public Health, Karolinska Institute, Stockholm, Sweden to view and discuss ongoing parenteral nutrition research; (4) Visits with two Swedish investigators active in research in the field of nutrition, October 1964; (5) Participated in the organization meeting of the International Society for Parenteral Nutrition, Hamburg, Germany, October 1965; (6) Site visits with Dr. H.C. Meng, Vanderbilt University, Nashville, Tenn. and discussion of the status of the Intravenous Nutriment Program with interested investigators and members of the Surgeon General's Advisory Committee on Nutrition in Nashville, Tennessee, May 1965; (7) Organized and participated in a meeting of the investigators of The Surgeon General's Intravenous Nutriment Program at USAMRNL, March 1965.

During FY 65 the monitor shared editorial responsibilities with Dr. John F. Mueller, Professor of Medicine and Physician-in-Chief, The Brooklyn-Cumberland Medical Center, Brooklyn, New York in completing a published "Symposium on Intravenous Fat Emulsions". This Symposium which consisted of reports of research performed by various investigators of the Surgeon General's Intravenous Nutritional Program, was published in the January 1965 issue of The American Journal of Clinical Nutrition. The symposium was two hundred twenty-six pages in length and included twenty-six papers.

SUMMARY AND CONCLUSIONS:

Production of experimental fat emulsions, chemical evaluation, animal toxicity testing and histopathological examinations of tissues from animals receiving various commercial or experimental intravenous fat emulsions by members of Toxicity Testing Group of the Surgeon General's I. V. Nutriment Program was continued as outlined in the Annual Progress Report, Sub-task 11, 1 July 1963 - 30 June 1964. The following conclusions have been made:

1. Use of a glandless cottonseed oil homogenized with a chromatographically homogeneous egg yolk lecithin produces an emulsion which has good physical characteristics including stability. The percent lecithin needed as the emulsifier to obtain this result is less than previously used.

2. Extracal, a high caloric intravenous solution, composed of glycerol, glucose, fructose and alcohol, prepared by Cutter Laboratories, was not well tolerated in rats and produced thrombophlebitis and injection site inflammatory reaction in rabbits.

3. Deposition of intravenous fat pigment and lipoid microgranulomas developed in animals receiving all of the emulsions tested. Multiple infusions of a solution composed of 2.5% egg lecithin and 2.6% glycerol resulted in deposition of a pigment which had some of the characteristics of intravenous fat pigment despite the lack of neutral fat in the emulsion.

4. Dogs were maintained on complete parenteral nutrition for 10 weeks without any apparent clinical deleterious effect. Intravenous fat pigment deposition, formation of lipoid microgranulomas and hemosiderosis in the splenic and hepatic tissues of these animals was quite marked. Tissues from other dogs which had received multiple infusions of tocopherol enriched Intralipid did not demonstrate hepatic lipoid microgranulomas but did have moderate fatty metamorphosis and deposition of intravenous fat pigment.

5. Electron microscopy examination of hepatocytes from animals previously infused with fat emulsions disclosed pigment deposition, mitochondrial swelling and vacuolization of the rough endoplasmic reticulum (rER). The vacuolization of the rER resembled that seen in acute choline deficiency. Repeated studies demonstrated that choline enrichment of diets fed to animals receiving multiple infusions of Intralipid did not prevent, completely or partially, the dilatation and vacuolization of the rER.

6. The lack of suitable experimental or commercial test emulsions has seriously curtailed the work of the Surgeon General's Intravenous Nutriment Program.

LIST OF PUBLICATIONS:

1. Mueller, J. F. and Canham, J. E.: Preface to Symposium on Intravenous Fat Emulsions. Am. J. Clin. Nutrition 16: 1, 1965.

2. Meng, H. C., Kuyama, T., Thompson, S. W., II and Ferrell, J. F.: Toxicity Testing of Fat Emulsions. I. Tolerance Study of Long-Term Intravenous Administration of Intralipid in Rats. Am. J. Clin. Nutrition 16: 29, 1965.

3. Sasaki, H., Schaffner, F., Thompson, S.W., II and Hunt, R.D.: Toxicity Testing of Fat Emulsions. II. Ultrastructual Changes in the Liver Following Administration of a New Intravenous Fat Emulsion (Intralipid). Am. J. Clin. Nutrition 16: 37, 1965.

4. Thompson, S.W., Jones, L.D., Ferrell, J.F., Hunt, R.D., Meng, H.C., Kuyama, T., Sasaki, H., Schaffner, F., Singleton, W.S. and Cohn, I.: Testing of Fat Emulsions for Toxicity. III. Toxicity Studies with New Fat Emulsions and Emulsion Components. Am. J. Clin. Nutrition 16: 43, 1965.

5. Jones, L.D., Castleberry, M.W., Canham, J.E. and King, N.W.: Toxicity Testing of Fat Emulsions for Intravenous Administration. Am. J. Clin. Nutrition 16: 62, 1965.

6. King, N.W., Jones, L.D., Sasaki, H. and Schaffner, F.: The Effects of Choline on Hepatic Ultrastructual Changes Associated with the Intravenous Administration of Fat. Am. J. Clin. Nutrition 16:88, 1965.

7. Canham, J.E., Jones, L.D., King, N.W. and Levine, R.A.: Metabolic and Toxicity Studies of Intravenously Administered Fat Emulsions (abstract). Abstracts of Papers - 1st World Fat Congress. Pub., Aschendarffsche Buchdruckerei, Munster Westf, 1964.

8. Levine, R.A. and Canham, J.E.: Hemodynamic Alterations Produced by Artifical Fat Emulsions Perfused Through the Isolated Rat Liver, in "Symposium on Parenteral Nutrition", Verlagsschriftleiter Pallas-Verlaz, Munich, 1965.

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION DA OA 6307	REPORT CONTROL SYMBOL CSCRD 103
4. DATE OF RESUME 01 07 65	5. KIND OF RESUME A. NEW	6. SECURITY U RPT U WRK	7. REGRADING NA	8. RELEASE LIMITATION NL	9. LEVEL OF RESUME A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 072

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 02 12

11. TITLE: (U) Studies of Nutritional Status of Populations (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	NA	PRIOR FY 65	1	10
		CURRENT FY 66	1	11

19. GOV'T LAB/INSTALLATION/ACTIVITY
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INVESTIGATORS PRINCIPAL: Sauberlich H. E.
ASSOCIATE: Consolazio, C.F.;Canham, LtCol,J.E
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21. TECHNOLOGY UTILIZATION: Medicine, agriculture

22. COORDINATION:

23. KEYWORDS: Nutrition, medicine, nutrition disorders, survey, nutrition surveys, vitamins, deficiency diseases, protein, protein deficiency, minerals, calorie

24. (U) Tech 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.

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 studied are suggested.

26. (U) Progress: (Oct 64-Jun 65)

During the reporting period, the final report of the ICNND nutrition survey of Malaya was completed and various members of the Malaysian government provided with copies. Consulting services in the areas of nutrition were provided the government of Burma and members of the SEATO laboratory in Bangkok, Thailand. Several members of the USAMRNL assisted the Institute of Nutrition of Central America and Panama during February and March in association with the nutrition survey of Guatemala.. One biochemist participated in the ICNND nutrition survey of Nigeria.

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) OVER

ABSTRACT

STUDIES OF NUTRITIONAL STATUS OF POPULATIONS

This laboratory has continued to assist the Interdepartmental Committee on Nutrition for National Development (ICNND) and other agencies of the National Institutes of Health through the participation of personnel in nutrition surveys or other nutrition projects. Personnel have also assisted in analysis of food, blood and urine samples collected in nutrition surveys or projects, in briefing sessions on nutrition problems or surveys, in preparation of nutrition reports and as consultants.

BODY OF REPORT

STUDIES OF NUTRITIONAL STATUS OF POPULATIONS

Description:

Assistance is provided in studies on the nutritional status of populations, either domestic or foreign, for the primary purpose of appraising and improving the nutritional status of the population concerned. A major assistance is provided by participation of personnel of this laboratory 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 studied are suggested.

Progress:

During the reporting period, the final report of the ICNND nutrition survey of Malaya was completed and various members of the Malaysian government provided with copies. A member of USAMRNL consulted with Malaysian government officials concerning the results of the survey. Assistance and consulting services in the area of nutrition were provided nutritionists and members of the government in Indonesia and Burma and members of the SEATO laboratory in Bangkok, Thailand and of the NAMRU-2 laboratory in Taipei, Taiwan. Two members of the USAMRNL assisted the Institute of Nutrition of Central America and Panama during February and March in association with the nutrition survey of Guatemala. One biochemist participated in the ICNND nutrition survey of Nigeria held in January to March period. Currently, two officers from USAMRNL are participating in the nutrition survey being conducted in Paraguay.

During the past year, analytical chemistry assistance has been provided nutritionists in the SEATO laboratory in Bangkok and INCAP laboratory in Guatemala. Two members of USAMRNL participated in a workshop on clinical and experimental studies on urolithiasis (particularly with respect to the bladder stone problem of northeast Thailand) held at the Naval Medical Research Institute in Bethesda, Maryland.

One foreign officer from Thailand received training at USAMRNL during the past nine months in the field of nutrition and in various biochemical and dietary techniques employed in the evaluation of nutritional status.

Summary and Conclusions:

During the past year, personnel participated in the nutrition surveys conducted in Nigeria, Guatemala and Paraguay. Additional assistance and guidance were provided investigators and officials in Indonesia, Malaysia, Burma and Taiwan.

STUDIES OF NUTRITIONAL STATUS OF POPULATIONS

List of Publications:

1. Federation of Malaya Nutrition Survey. A report of the ICNND, September 1964.

2. Assistance was provided in the preparation of the new "Manual for Nutrition Surveys," ICNND publication, December 1964.

3. Northeast Brazil Nutrition Survey, March-May 1963. A report of the ICNND, May 1965.

4. Fort Belknap Indian Reservation Nutrition Survey, August-October 1961. A report of the ICNND and the Division of Indian Health, U. S. Public Health Service, December 1964.

5. Blackfeet Indian Reservation Nutrition Survey, August-September 1961. A report of the ICNND and the Division of Indian Health, U.S. Public Health Service, December 1964.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		None	None

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 05 65	B. COMPLETED	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501A71F 02 013

10b. PRIOR NUMBER/CODE: None

11. TITLE: (U) Nutritional Potentialities of Algae

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

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

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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ASSOCIATE: Sauberlich, Howerde E.
TEL: 303 366 5311 X2214 TYPE: DA

21. TECHNOLOGY UTILIZATION
-

22. COORDINATION

23. KEYWORDS: Algae; digestibility; protein; lipid; amino acids

24. (U) The nutritional potential of algae has been studied from an analytical standpoint and by means of animal and human feeding experiments. Analytically, algae has been found to have a high nutrient content. However, many difficulties have been encountered in feeding studies. The cell wall is not readily digested and algae is not very palatable. The protein of algae is rather poor because of its low content of the sulfur-containing amino acids. Because of the highly unsaturated nature of the algal lipids, an increased requirement for vitamin E may exist.

Because of inability to obtain sufficient quantities of algae and lack of equipment to produce adequate amounts of algae at this laboratory, the project was terminated.

25.

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
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) OVER

ABSTRACT

NUTRITIONAL POTENTIALITIES OF ALGAE

Studies on the nutritional potentialities of algae have been discontinued because of the inability to obtain sufficient quantities of algae for further feeding experiments. Reports resulting from algae studies conducted thus far at this laboratory are listed. In general, the algae studied were adequate in vitamins, but were deficient in several amino acids, including histidine, glycine and methionine. Poor digestibility of the algae further lowered the biological value of the protein. Human subjects could tolerate small quantities of algae in the diet, but further processing would be necessary if algae is to be of use as a major food source.

BODY OF REPORT

NUTRITIONAL POTENTIALITIES OF ALGAE

Description:

Studies conducted under this project were directed toward investigating the nutritional potentialities of algae and towards ways of improving its nutritional qualities. Three approaches to the problem were taken, 1) analytical studies of algae, 2) animal feeding experiments and 3) human feeding studies.

Progress:

Analytical data were obtained pertaining to the vitamin, protein, amino acid, nitrogen, ash, mineral, lipid, fatty acids and caloric content of several species of algae. The algae analyzed were available in sufficient quantity to permit feeding studies. Part of the analytical findings were published in J. Nutrition 75: 7, 1961. Additional analytical findings will appear in a U. S. Army Medical Research and Nutrition Laboratory report. Marked differences in the vitamin, protein and lipid contents were noted between species of algae. In general, the algae were low in histidine, glycine and sulfur amino acid contents.

Studies were performed on the protein quality and amino acid deficiencies of three algae samples with the use of growing rats and chicks. The animals who were fed diets in which the protein was supplied by 1) a mixture of Scenedesmus obliquus and Chlorella pyrenoidosa or 2) Spongiococcum excentricum grew less well and had lower protein efficiency ratios than their respective controls receiving soybean oil meal or casein protein. Amino acid supplementation studies revealed all the algae fed to be deficient in methionine. Moreover, the mixed algae was deficient in glycine for the chick and the algae C. pyrenoidosa was deficient in histidine for the growing rat (J. Nutrition 76: 423, 1962). Poor digestibility of algae and the resulting lowered availability of the algae protein appeared to contribute to the inferior growth-supporting qualities of algae.

Algae was fed to young adult male volunteers in a short feeding study (J. Nutrition 75: 7, 1961). Small amounts of algae (25 g/day) were nearly acceptable when properly added to the diet, but amounts above 100 g/day were very poorly tolerated because of gastrointestinal symptoms along with other symptoms such as nausea, vomiting, malaise and headache. It was concluded that algae could be tolerated as a food supplement, but further processing would be necessary if algae is to be useful as a major food source.

Summary and Conclusions:

Because of the inability to obtain sufficient quantities of algae and the lack of equipment to produce adequate amounts of algae at this laboratory, it

was considered necessary to terminate this study.

Although algae have potential value as a food material, it possesses various nutritional inadequacies, particularly with respect to acceptability, digestibility and protein quality.

List of Publications:

Studies at this laboratory on the nutritional potentialities of algae have resulted in the following reports and publications:

1. Powell, R. C., E. M. Nevels and M. E. McDowell. Algae feeing in humans. J. Nutrition 75: 7, 1961.

2. Leveille, G. A., H. E. Sauberlich and J. A. Edelbrock. The influence of enzyme supplementation on the digestibility of algae. U. S. Army Med. Rsch. and Nutrition Lab. Rpt. No. 259, 20 June 1961.

3. Leveille, G. A., H. E. Sauberlich and M. E. McDowell. The nutrient content of various algae and the amino acid adequacy for growth of rats and chicks. Report AMRL-TDR-62-116 of the Symposium-Workshop on Biologistics for Space Systems, Dayton, Ohio, 6570th Aerosopace Medical Research Labs., p. 405, October 1962.

4. Leveille, G. A., H. E. Sauberlich and J. W. Shockley. Protein value and the amino acid deficiencies of various algae for growth of rats and chicks. J. Nutrition 76: 423, 1962.

5. McDowell, M. E. and G. A. Leveille, Feeding experiments with algae. Fed. Proc. 22: 1431, 1963.

6. _____ Algae as a food source for space. Proc. Conf. on Space Nutrition and Related Waste Problems, NASA-Space Science Board, National Acad. Sci., Tampa, Florida, 27-30 April 1964.

RESEARCH AND TECHNOLOGY RESUME			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 65	A. NEW	U U	NA	NL	A. Work Unit

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 073

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 02 15

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	NA	PRIOR FY 65	1	59
		CURRENT FY 66	2	65

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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Denver, Colorado 80240

INVESTIGATORS PRINCIPAL/ASSOCIATE: Consolazio, C. F., Canham, J. E.
Matoush, L. O., Sauberlich, H. E.
TEL: 303 3665311 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), environment

(U)Tech Objective: Due to changing needs of the military, there is a constant need to evaluate the capability of current and newly developed rations to provide adequate nutrition. Evaluations of nutritional status, work performance, body composition and capacity of the soldier is essential to insure that the effective military performance is not impaired by improper nutrition. Such improvement could limit the capability of the Army's cadre at a time when instant readiness is mandatory. Previous studies, though helpful, do not provide the necessary answers.

(U)Approach: Two studies have been designed to help answer some of the above problems. The first study evaluated the nutritional adequacy and acceptability of a variety of high caloric density rations, under nonresupply conditions. The second will include 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. An initial, extensive survey was conducted at Fort Carson, Colorado. A subsequent study was designed to evaluate the energy requirements and food adequacy of the Ranger Battalion at Fort Benning, Georgia.

(U)Progress: (Oct 64-Jun65) The first study (Fort Bragg) is complete except for the daily water flux data. High calorie density rations supplying 2000-2600 Calories/day appear to be acceptable for nonresupply patrol activities, if sufficient variety in food items is provided. The caloric intake was adequate for maintaining physical efficiency after a 10 day test period. Analysis of the collected samples and data of the Fort Carson and Fort Benning Studies is in progress.

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 24 identical to NASA Form 1122) OVER

ABSTRACT

MILITARY NUTRITION SURVEYS AND RATIONS (FY 1965)
APPLIED NUTRITION STUDIES OF MILITARY POPULATIONS (FY 1966)

a. Nutrition Surveys: 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 situations. Current, newly developed and experimental rations are to be evaluated for nutritional adequacy.

b. Ration Studies: Due to changing needs of the military there is a constant need to evaluate the capability of current and newly developed rations to provide adequate nutrition. Evaluations of nutritional status, work performance and body composition of the soldier is essential to insure that the effective military performance is not impaired by improper nutrition. Such improvement could limit the capability of the Army's cadre at a time when instant readiness is mandatory. Previous studies, though helpful, do not provide the necessary answers.

Three studies were designed to help answer some of the above problems. Two nutrition surveys were conducted to ascertain the soldier's dietary intake, the clinical and biochemical status of the men, the body composition and the work capacity. An initial study was conducted at Fort Carson, Colorado and the second nutrition survey was designed to evaluate the energy requirements and food adequacy of the Ranger Battalion at Fort Benning, Georgia. The third study was designed to evaluate the nutritional adequacy and acceptability of a variety of high caloric density rations, under nonresupply conditions, on Special Forces troops at Fort Bragg, North Carolina.

BODY OF REPORT

MILITARY NUTRITION SURVEYS AND RATIONS (FY 1965)
APPLIED NUTRITION STUDIES OF MILITARY POPULATIONS (FY 1966)

Description

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 is essential to insure that the effective military performance of the soldier, during his duty career, is 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.

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, habits and nutritional status.

Progress

Camp Study - Bioenergetics Division: The second Army Post Nutrition Survey was conducted at Fort Benning, Georgia during the second quarter of FY 1965 on a Ranger class, during their three week training period (44th Company, 4th Student Battalion, Student Brigade). The food consumption from the mess hall alone averaged 4129 and 4021 Calories/man /day for the analyzed and calculated values, respectively. When one includes the 272 Calories consumed from sources outside the mess hall (candy bars, etc.), the total consumption from all sources averaged 4400 and 4293 Calories for the same analyzed (food composites) and calculated values.

The troops gained body weight during the first phase of the study (Days 1-8), which was due to the long hours of lectures, demonstrations, etc., in preparation for the field phase. In the 8 day field phase, the body weight loss was more than 1 kg even though the food intake was increased to 4563 Calories/day. The total weight loss for the complete 18 days averaged -1.15 kg or a -70 gm/man/day.

The hemoglobin and hematocrit values were not significantly changed during the complete training phase. Plasma proteins were decreased by 0.5 gm% during the training period ($P < 0.001$). The decrease in the plasma proteins could be indicative of retention of body water, however, other factors could be responsible for the decrease in plasma proteins. Nevertheless, it is felt that it can be unequivocally stated that no dehydration was present at the time this study was concluded and that the weight loss observed was not due to loss of body water. Mean body waist circumference decreased by 2.8 cm during the course of the training period. This is probably an indication of the loss of body fat but could be indicative of increased tonus of the abdominal musculature secondary to the physical training.

Based upon the data given above, an attempt has been made to more clearly estimate the average daily caloric intake necessary to supply the energy requirements of the men undergoing the Ranger Training Cycle. The caloric equivalent of the mean body weight loss amounted to 442 Calories. If this value is added to the known mean caloric intake of 4293 (calculated) or 4400 (analyzed) Calories, values of 4735 Calories/man/day or 4842 Calories/man/day, is obtained. Therefore it can be seen that the energy expenditure improved by the conditions of training exceeded the caloric intake.

Ration Study - Bioenergetics, Physiology Divisions: The combat patrol ration study was conducted at Fort Bragg, North Carolina (Pisgah National Forest) during July-September 1964, utilizing Special Forces troops.

The primary purpose of the ration study at Fort Bragg, North Carolina was to evaluate the nutritional adequacy and acceptability of 7 incomplete or complete "rations" designed for nonresupply patrol type situations of 10 days' duration. One of the rations, the US Meal Combat Individual Ration (M-T) was studied at two caloric intake levels. The rations are as follows:

MILITARY NUTRITION SURVEYS AND RATIONS (FY 1965)
APPLIED NUTRITION STUDIES OF MILITARY POPULATIONS (FY 1966)

		Quantity Offered Daily
1.	High Caloric Density No. 1 (Commercial)	
	Dry Powder (454 gm) 1 pound	1800
	Wafers, 18 @ 25 Calories each	450
	Sugar, cream, tea and coffee	138
	Total Cal/Day	2388
2.	High Caloric Density No. 2 (Aid Station, US)	
	Dry Powder (454 gm) 1 pound	2213
	Sugar, cream, tea and coffee	138
	Total Cal/Day	2351
3.	High Caloric Density No. 3, Food Bars	
	6 Coated Bars @ 192 Calories each	1142
	5 Uncoated Bars @ 162 Calories each	800
	Sugar, cream, tea and coffee	168
	Total Cal/Day	2110
4.	High Caloric Density Ration No. 4, Bandoleer Food Packet Combat	
	3 Units Daily @ 740 Calories each, Cal/Day	2220
5.	US Food Packet Survival	
	3 Units Daily @ 829 Calories each, Cal/Day	2487
6.	Packet Subsistence, Long Range Patrol (Quick Serve)	
	2 Units Daily @ 1075 Calories each, Cal/Day	2148
7.	US Army Meal Combat, Individual (M-T)	
	Served at 2/3 Daily Level (2 units), Cal/Day	2310
8.	US Army Meal Combat, Individual (M-T)	
	3 Units Daily @ 1155 Calories each, Cal/Day	3465

Special Forces Center, Fort Bragg, North Carolina assigned 60 troops as test subjects. These men were divided into 12 teams of 5 men each. The experimental design consisted of two 10 day patrols, with a 4 day rehabilitation phase between each field patrol. Each ration was tested by three different teams for a 10 day period.

Information gathered included body weight changes, a bicycle maximum performance test, a fasting blood for RBC transketolase, hemoglobin and hematocrit, and a fasting timed urine sample for measuring water flux after a test dose of D_2O. These studies were done prior to going into the field and at the end of each phase (Day 11). In addition, the men performed a contest march on the morning of Day 12.

The daily food consumption averaged 1649 Calories/man/day for High Density No. 1, 1953 for High Density No. 2, 125' for High Density No. 3, 1727 for High Density No. 4, 2260 for the US All Purpose Survival Ration, 1899 for the Packet, Subsistence, Long Range Patrol, 2352 for the Meal Combat Individual 2/3 level, and 3064 Calories/man/day for the Meal Combat Individual, Complete.

The two liquid rations, High Density No. 1 and 2, and High Density No. 4 (Bandoleer) were quite acceptable. The food bars, High Density No. 3 were not acceptable, even though the men in the second patrol phase consumed the maximum number issued. The "All Purpose Survival Ration" was the greatest disappointment of all. The acceptability was poor, with the cheese and imitation meat bars being quite unacceptable.

The "Long Range Patrol, Dehydrated" ration was highly acceptable even though it required water. During the first few days of the patrol the men felt that the two meals were too much, but after Day 4, most of them felt that they could have easily consumed a third ration. This ration was easily reconstituted (15-20 minutes), was lightweight, but bulky.

High caloric density rations supplying 2000 - 2600 Calories/day appear to be acceptable for nonresupply patrol activities, if sufficient variety in food items and adequate water is provided. The caloric intake was adequate for maintaining physical efficiency after a 10 day test period.

Summary and Conclusions

Nutrition Survey, Fort Benning, Georgia: AR 40-564 prescribes the minimal nutrient intake/man/day of a physically active individual living in a temperate environment and subsisting on a garrison or field type ration to be 3600 Calories/day. The daily food consumption over the entire training period, using the chemical analyses of the food composites, averaged 4400 Calories/day, or an increase of 22.2% over the daily prescribed intakes.

When one utilizes the caloric equivalent of the body weight change (-70 gm/man/day or 442 Calories) the energy requirements averaged 4842 Calories/man/day. This was equivalent to an increase of 34.5% over the prescribed daily minimal intakes.

This fairly high requirement was not unexpected since the men worked long hours, especially during the field training phase and were under continuous physical and mental stress.

Ration Study - Fort Bragg, North Carolina: Seven high density type rations were each studied three times by men on 10 day simulated combat patrols. These men were issued food at approximately 2/3 the daily ration caloric level and were expected to lose at least 5 pounds in 10 days. Under the conditions of the field test there seemed to be no great difference in any of the "rations" in maintaining maximum efficiency and performance during the 10 day patrols. It was clearly shown that a highly motivated individual can subsist on any fairly reasonable ration for a 10 day period, including the liquid type rations issued in this test.

One problem encountered was voluntary dehydration of one-third of the men who completed the test in Phase I. This significantly decreased the maximum performance of these men at the end of the test period.

Publications

1. Consolazio, C. Frank: The Fort Bragg Ration Test. Army Research Office, Life Sciences Division, Arlington, Virginia, 21 September 1964 (Presentation).

2. Van Reen, R., Minard, D., Dalser, A. R., Raica, N. Jr. and Nelson, R. A.: Nutrition of Recruits During a Summer Habitability Study. J. Am. Diet. Assoc. 45: 117, 1964.

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RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6309	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 074

10b. PRIOR NUMBER/CODE: 61145011 1K025601A033 02 11

11. TITLE:
(U) Nutritional Studies of Irradiated Food (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry; 01400 Radio & Radiation Chem; 006500 Food Management	06 60	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 65	2	74
			CURRENT FY 66	1	30

19. GOV'T LAB/INSTALLATION/ACTIVITY
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ASSOCIATE: Sauberlich, H. E.
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21. TECHNOLOGY UTILIZATION
Food preservation and food technology

22. COORDINATION
None

23. KEYWORDS: Food irradiation; food preservation; nutrition; food analysis; food; food-processing industry; radiation; radiation biochemistry; irradiate

24. (U) Tech Objective:

The objectives are 1) to establish the wholesomeness and nutritional adequacy of foods sterilized with ionizing radiation and 2) to determine whether or not nutrients are affected by dose-rate.

25. (U) Approach:
Although previous long-term feeding studies have established the wholesomeness of gamma ray (Co-60 or spent fuel rods) sterilized (6 Mrads) foods, certain nutritional problems have not been clarified. Analysis is being made to compare nutrient changes in foods irradiated at a low dose rate (4.5×10^6 rad/hour) and at a high dose rate (av. 10^9 rad/hour). The rate of lipase hydrolysis on irradiated fats and pure triglycerides is also being studied to determine whether or not lipase inhibitors are produced by irradiation.

26. (U) Progress: (Oct 64-Jun 65)
No differences attributable to the dose rate of irradiation were found in thiamine, fatty acids, amino acids or other parameters in five foods (ham, pork, beef, chicken and bacon) irradiated to about 5×10^6 rads with Co-60, 24 or 11 Mev electrons. These data cannot be evaluated critically because temperature and total dose of the electron irradiated foods was not controlled. Instrumentation and methodology for the lipase-irradiated fat studies have been completed, but no data are available at this time.

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 1132) OVER

ABSTRACT

NUTRITIONAL STUDIES OF IRRADIATED FOOD

a. Technical assistance and liaison between TSGO and TSGO irradiated food contractors has continued. Progress reports have been duplicated and distributed. Meetings were attended with participation.

b. Preliminary data obtained in cooperation with U. S. Army Materiel Food Radiation Laboratories, Natick, Massachusetts, have not shown any nutrient changes in five meat items which would suggest changes attributable to the rate of irradiation to 5 megarads.

c. Studies on the hydrolysis rate of lipase on irradiated pure fats have been initiated.

d. Orally administered maintenance levels of vitamin K (2-4 µg/day) increases the utilization of carotene as measured by liver vitamin A stores in the vitamin K deficient rat.

BODY OF REPORT

NUTRITIONAL STUDIES OF IRRADIATED FOOD

Description:

Monitorship of TSGO irradiated food program is being continued.

Irradiation destruction of meat nutrients is being investigated to determine whether or not high dose-rate irradiation (electrons) are more or less destructive than gamma radiation from a high intensity Co-60 source.

Studies have been initiated to investigate in vitro hydrolysis of pure and natural fats by several lipase preparations.

Carotene utilization in vitamin K deficiency is being studied.

Progress:

Technical assistance and liaison between TSGO and TSGO irradiated food contractors is being continued. Reports have been duplicated and distributed to interested investigators and agencies. Meetings were attended with participation.

In the dose-rate nutrient destruction studies, there were no consistent differences in the five foods: chicken, ham, pork, beef or bacon irradiated with Co-60, 11 or 24 Mev electrons to 5 megarads which could be attributed to dose-rate. Data on frozen stored and 3-month room temperature stored foods included amino acids, thiamine, fatty acids, peroxide numbers, TBA color development, saponification numbers and proximate analysis. Because of temperature differences during irradiation and the variable dose delivered by the electron irradiation, these studies are being repeated under more closely controlled irradiation conditions.

To be included in this study will be buffered aqueous solutions of pure micronutrients which have in the past shown high radiation sensitivity. Foods will be packaged in the recently developed laminated plastic containers instead of in cans. The data obtained from this study will be useful to future food irradiation programs if, as anticipated, electron sources will be extensively used for food irradiation. These studies are a cooperative effort between U. S. Army Materiel Command Food Radiation Laboratories, USAMRNL and TSGO.

The in vitro lipase studies with irradiated pure fats have just been initiated. Preliminary instrumentation and emulsification procedures have been explored. There are no data to report at this time.

Nutritional Studies of Irradiated Food (Cont'd.)

A vitamin K-carotene interrelationship has been found in rats to support previous reports of a vitamin K-vitamin A interrelationship. More carotene is utilized as measured by liver vitamin A stores when vitamin K (Menadione) is in direct contact with carotene. Comparable results were obtained when both vitamins were incorporated into the diet or administered simultaneously in an oil solution. This beneficial effect of vitamin K was not observed when the vitamin K was administered intramuscularly as Menadione bisulfite at doses comparable to the physiological levels in the diet.

Because of the high carotene to vitamin K ratio (10-20:1), it is not very likely that the effect observed is entirely due to the possible antioxidant properities of vitamin K, particularly since the diet is adequately supplied with tocopherol. Further studies are not anticipated at this time because of the difficulties encountered in obtaining and maintaining the rat in a vitamin K deficiency state without adequate prevention of coprophagy. Future studies are planned in germfree animals. More desirable, however, would be in vitro studies of the influence of vitamin K on the carotene to vitamin A conversion if an active carotenase enzyme system could be found.

Summary and Conclusions:

The irradiation of five meat items with high dose-rates with 11 or 24 Mev electrons from a linear accelerator did not appear to be any more or less destructive to nutrients than comparable doses (5 Mrad) delivered by a high intensity Co-60 source.

Orally administered physiological levels of vitamin K to vitamin K-deficient rats increased carotene utilization as measured by vitamin A liver stores. Comparable doses of intramuscularly administered vitamin K did not increase carotene utilization.

List of Publications:

1. Raica, N., Jr., M. E. McDowell and H. E. Sauberlich. Wholesomeness of foods preserved by cold-sterilization and induced radioactivity. Proc. 8th QM Irradiated Food Contractors' Meeting, Oct. 1963, Natick, Mass. U. S. Dept. of Commerce, Office of Technical Services, PG 166166, Wash., D. C., 1964.

2. _____ Present status of the wholesomeness program. Ibid.

3. Raica, Nicholas, Jr. and H. E. Sauberlich. Current status of the U. S. Army Food Irradiation Program. J. Colo. Wyo. Acad. Sci. Proc. 5:25, 1964.

4. Raica, Nicholas, Jr. Data on wholesomeness studies - A progress report. Proc., International Conf. on Radiation Preservation of Foods. Sept. 1964, Boston, Mass. (in press).

5. Raica, Nicholas, Jr. Data on wholesomeness studies. A progress report. Food Irradiation 5, A2-A7, 1965.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		None	None

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 05 65	B. COMPLETED	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61145011 3A014501A71F 02 017	None

11. TITLE:
(U) Histopathology of Mice Eating Irradiated Foods

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002600 Biology	--	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	--	--
		CURRENT FY	--	--

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ASSOCIATE: Jenkins, E. D. Jones, L. D.
TEL: 303 366 5311 X 23230 TYPE: DA

21. TECHNOLOGY UTILIZATION	22. COORDINATION
--	None

23. KEYWORDS: Mice; Irradiated Food; Cardiac Disease; Carcinogenicity; Pathology, Growth Rates; Genetic Effects; Computer Programming

24. (U) The object of this experiment was to determine the possible harmful effects of feeding a diet of 100% irradiated foods composed of pork, chicken, milk, carrots, and potatoes. Particular attention was directed to a heart lesion which affected the left auricle and had previously been incriminated as being caused by an irradiated food diet. During the three and one-half years of the experiment, 4,300 mice repre-
25. senting different strains and generations of animals had been utilized for the feeding studies. Each animal received close clinical observation and after sacrifice or death, each animal was thoroughly examined for gross and microscopic lesions. All lesions were recorded and analyzed for incidence rates and distribution observed within experimental populations. No correlation between lesions and the feeding of irradiated foods could be found in this study. Under the conditions of the protocol used in this study, the mice fed irradiated foods appeared to be as healthy as those which were fed the control diet.

26. All gross and histopathological findings observed during the study have been incorporated into a computerized system which was developed to provide an efficient memory bank for future evaluations of laboratory animal diseases. Future studies pertaining to these findings will be reported under the work unit "Histopathology and Clinical Pathology of Laboratory Animals".

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
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) OVER

ABSTRACT

HISTOPATHOLOGY OF MICE EATING IRRADIATED FOODS

The basic project has been completed and a report submitted as USAMRNL Report No. 279. Irradiated foods were not incriminated in producing lesions or ill effects in a controlled study of some 4,000 mice fed a lifetime diet of food preserved by ionizing radiation.

BODY OF REPORT

HISTOPATHOLOGY OF MICE EATING IRRADIATED FOODS

Description:

This project was designed to evaluate the possible harmful effects in all organ systems of mice fed a composite 100% irradiated diet.

Progress:

Although all work has been completed, a manuscript is in preparation which will be submitted for publication in the open literature.

Summary and Conclusions:

Basically, no lesions were found which could be attributed to the feeding of irradiated foods. A detailed report of the experimental design and findings can be found in USAMRNL Report No. 279.

Feasibility studies have been completed on the usage of electronic data processing procedures to implement the analysis of the information generated in this project. A program has been written and is workable. Analysis will begin as soon as all data from this project has been entered into the computer. Furture reporting of this phase of the project will be reported in Sub-task 29, Histopathology and Clinical Pathology of Laboratory Animals.

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RESEARCH AND TECHNOLOGY RESUME

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 65	A. NEW	U U	NA	NL	A. WORK UNIT

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

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 02 22

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

12. SCIENTIFIC OR TECH. AREA: 002300 Biochemistry; 002600 Biology; 012900 Physiology

13. START DATE: 05 65

14. CRIT. COMPL. DATE: NA

15. FUNDING AGENCY: OTHER | DA

16. PROCURE. METHOD: C. In-House

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

18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	2	57
CURRENT FY 66	2	63

19. GOV'T LAB/INSTALLATION/ACTIVITY
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21. TECHNOLOGY UTILIZATION
Nutritient utilization and requirements

22. COORDINATION
None

23. KEYWORDS: Periodicity of eating, meal-eating, nibbling, adaptation, enzymes, lipogenesis, fatty acid

24. (U) Tech Objective:
To determine the mechanisms and significance of biochemical adaptations in the mammal exposed varied patterns and levels of macronutrient and micronutrient intakes.

25. (U) Approach:
To induce adaptive changes, primarily by dietary means, and to elucidate the biochemical basis for such changes.

(U) Progress: (Oct 64-Jun 65)

2d. Limiting an animal's access to food to two hours/day (meal-eating) results in a 10-50 fold increase in lipogenesis as compared to the control nibbling animal.

2. The enhanced lipogenesis is partly due to an increase in the activity of Key enzymes, namely, glucose-6-phosphate dehydrogenase and NADP-malic dehydrogenase.

3. The major source of cytoplasmic acetyl-CoA appears to be citrate via the citrate cleavage reaction.

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 6 1	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) OVER

ABSTRACT

NUTRITIONAL AND METABOLIC ADAPTATIONS

Kidney cortex of rats or hamsters exposed to a mild cold stress, and/or fed low-carbohydrate diets was found to have an increased capacity for glucose formation from a number of non-carbohydrate precursors. This is apparently an adaptive reaction, adjusting the metabolism to the needs of the organism.

Lipogenesis from glucose is significantly increased in the liver from animals which are fasted and refed a high carbohydrate diet. Incorporation of C^{14} glucose into triglycerides and total lipids is drastically increased in liver from fasted-refed rats studied *in vivo*. Similar results were obtained when lipogenesis was measured in liver and adipose tissue *in vitro*. A marked decrease in lipogenesis from glucose occurred when animals were fasted for four days. Mild cold stress has a lipotropic effect in fasting and fasting-refed animals.

BODY OF REPORT

NUTRITIONAL AND METABOLIC ADAPTATIONS (FY 1965)

Description:

The objective of this sub-task is to investigate the capacity of mammals, including humans, to adapt to, and utilize for prolonged periods of time, diets of abnormal or unusual composition. It includes studies of the adverse physiological effects of such diets, the rate and extent of adaptation to different nutritional and environmental conditions, the influence of other factors (e.g. drugs, hormones) on nutritional adaptations, and the fundamental physiological and biochemical mechanisms which underlie and sustain metabolic adaptation processes.

Progress:

Renal gluconeogenesis in cold-stressed animals. Young growing rats fed either a high-protein, a high-sugar or a high-fat diet, were exposed to 5 $\pm$ 1°C for varying periods of time. The control groups were maintained on similar diets at 25 $\pm$ 2°C. Five animals from each treatment group were sacrificed at weekly intervals and the rate of glucose formation in slices of kidney cortex suspended in a saline medium was measured from the following substrates: alanine, arginine, aspartic and, glutamic acid, glycerol, glycine, ketoglutarate, lactate, proline, propionate, pyruvate and valine.

At the end of the first week, the cold-stressed animals fed the high-protein diet had an increased capacity for gluconeogenesis from all the above substrates, when compared with the corresponding controls at 25°C. For contrast, the animals fed the high-sugar diet at 5°C used only glycerol for glucose formation. The cold stressed animals fed the high fat diet had an increased capacity for gluconeogenesis mainly from glycerol, followed by pyruvate and ketoglutarate.

For similar experiments the rate of glucose formation in kidney slices was compared in cold-exposed adult rats and hamsters. The animals were fed a commercial laboratory diet (Purina Chow) and were exposed to 5 ± 1°C for two weeks. After cold exposure, the rate of glucose production was markedly increased from the following substrates: pyruvate, lactate, ketoglutarate, glycerol, aspartate and glutamate. Although an increased capacity for gluconeogenesis was apparent in both species following cold exposure, the greatest increases occurred in hamster kidney.

Glucose metabolism in cold-exposed, fasted and refed rats.

Adult rats maintained on a high-carbohydrate diet were exposed to 5±1°C and subjected to fasting and refeeding with groups of 3 - 5 individual rats selected for study at four days of fasting and at three days of refeeding following four days' fasting. Corresponding treatment groups were maintained at 25 ± 2°C. In in vivo experiments a "tolerance-type" dose of glucose containing 5μc of glucose-u-C^{14} was injected intraperitoneally and animals were sacrificed four hours after the injection. The data indicate a drastically increased incorporation of C^{14} into triglycerides of liver in fasted-refed animals kept at room temperature. Total lipids were also markedly increased in the livers of these animals. For contrast, incorporation of C^{14} into triglycerides and the level of total fat in the cold-stressed and/or fasted rats was markedly decreased. Specific activities of liver glycogen was increased approximately ten-fold in fasting rats at 25°C and about seven-fold in those maintained at 5°C. Specific activities of cholesterol was decreased in fasting animals compared with either the nonfasting or the refed animals. The environmental temperature had no effect on the incorporation of C^{14} into liver cholesterol.

The metabolism of glucose in liver and white adipose tissue was also studied using in vitro techniques. The conversion of uniformly labeled C^{14} glucose to CO_2, glycogen, fatty acids, glyceride-glycerol and nonsaponifiable fats was measured in tissue from cold-exposed, fasted and fasted-refed animals. In adipose tissue from fasted rats, significantly less glucose carbon was incorporated into CO_2, glycogen and fatty acids as compared to control animals. Cold exposure and fasting lead to further decreases in glucose metabolism in vitro. A significant increase in glucose conversion to CO_2 and fatty acids was noted in adipose tissue from fasted rats which had been refed. A marked decrease in lipogenesis and CO_2 production grom glucose occurred in liver tissue from fasted animals kept at 5° or 25° ± 1°C. Refeeding fasted animals elicited a significant increase in lipogenesis in liver from animals kept at room temperature but not in cold-exposed animals. Glycogenesis as measured in vitro, in liver tissue was not notably affected by fasting, cold stress or refeeding after fasting.

Summary and Conclusions:

The capacity of renal tissue to synthesize glucose from non-carbohydrate precursors is significantly affected by low environmental temperature or changes in diet. An increased capability for gluconeogenesis in cold and/or when the diet is low in carbohydrate represents an adaptation to environmental as well as to nutritional abnormalities.

NUTRITIONAL AND METABOLIC ADAPTATIONS

Significant changes in liver and adipose tissue glucose metabolism occur when animals are maintained on a high carbohydrate diet and exposed to cold, fasted or refed after fasting. Refeeding fasted rats results in super normal lipogenesis from glucose in the liver and adipose tissue. This phenomenon is partially alleviated by mild cold stress. Lipogenesis is markedly decreased in liver and adipose tissue from fasted animals whereas the incorporation of glucose carbon into glycogen in the liver is significantly increased. Therefore, metabolism of glucose in fasted and fasted-refed rats at 5 or 25 ± 1°C is markedly different from pathways observed in non-fasted normal rats.

List of Publications:

1. Klain, George J. Renal gluconeogenesis in cold-stressed rats. Fed. Proc. 24:148, 1965 (Abstract). Manuscript in preparation.

2. Burlington, Roy F. Renal gluconeogenesis in cold-exposed rats and golden hamsters. Journal of Colorado-Wyoming Academy of Science 6:__, 1965 (Abstract - in Press).

3. Klain, George J. The phenylalanine requirement of cold-exposed rats. Journal of Colorado-Wyoming Academy of Science 6:__, 1965 (Abstract - in Press).

4. Klain, George J. and Roy F. Burlington. Glucose metabolism in cold-exposed fasted and refed rats. Manuscript in preparation.

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RESEARCH AND TECHNOLOGY RESUME

3. GOVT ACCESSION	4. AGENCY ACCESSION	REPORT CONTROL SYMBOL
	DA OA 6324	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 077

10b. PRIOR NUMBER/CODE: 61125011 3A012501A803 02 19

11. TITLE:
(U) Nutritional Physiology (06)

12. SCIENTIFIC OR TECH. AREA: 016200 Stress Physiology
002300 Biochemistry 005900 Environ Biology

13. START DATE: 10 64

14. CRIT. COMPL. DATE: NA

15. FUNDING AGENCY: OTHER | DA

16. PROCURE. METHOD: C. In-House

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

18. RESOURCES EST.

	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	1	33
CURRENT FY 66	2	36

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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INVESTIGATORS PRINCIPAL: Dr. George Klain
ASSOCIATE: Dr. Roy Burlington
TEL: 303 366 5311 X22119 TYPE: DA

21. TECHNOLOGY UTILIZATION
Nutrition

22. COORDINATION
None

23. KEYWORDS
Adaptation; Physiological: Nutrition: Metabolism: Environmental Stress

24. (U) Tech 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.

28. (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 metabolities; 4) radiochemical studies; 5) determination of metabolic pathways; and 6) clinical observations.

36. (U)Progress: (Oct 64-Jun 65) The rate of glucose formation from non-carbohydrates is markedly increased in cold-stressed rats fed a high-protein or a high-fat diet, when compared with the corresponding animals kept at room temperature. In contrast, gluconeogenesis was not significantly affected by cold stress when the rats were fed a high-carbohydrate diet. The rate of "supernormal lipogenesis" was markedly decreased in cold stressed rats that had been refed following starvation. Lipid deposition was particularly diminished in the heart. In adipose tissue, cold and/or starvation elicits a marked decrease in lipogenesis from glucose in vitro, whereas, an increase in lipogenesis is noted in tissues from starved, refed animals. Report 1: Renal gluconeogenesis in cold-stressed rats. Fed. Proc. 24: 148, 1965.

27. COMMUNICATIONS SECURITY	28.	29. OSC CODE	30. BUDGET CODE
a. COMSEC OR COMSEC RELATED ☐ b. NOT RELATED ☒		BR	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 36 identical to NASA Form 1122) OVER

ABSTRACT

ENVIRONMENTAL NUTRITION (FY 1965)
NUTRITIONAL PHYSIOLOGY (FY 1966)

Extremes of environmental conditions including cold, dry heat, moist heat or altitude have been reported or demonstrated to effect the nutritional needs of acclimatized or unacclimatized humans. The minimal and optimal macro- and micro-nutrient requirements to satisfy these altered needs have only been partially established. Studies have been completed or are in progress to better elucidate and quantitate the altered nutritional needs imposed by environmental stress. Repeated experiments have been conducted on men performing work and sedentary activities in hot environments to determine the magnitude of the loss of nutrients through sweating and the best methods for measuring sweat losses. Previous reports have established that sufficient amounts of calcium and nitrogen are lost via sweat to produce negative balances. To insure that the arm-forearm method of sweat collections utilized in the previous studies accurately reflected total body sweat composition, a study was performed on 12 healthy males exposed in the environmental chamber to 38.5°C and 30% relative humidity. After an acclimatization period forearm-arm and total body sweat was collected over 3 different time periods. Nitrogen determined from total body sweat ranged from 86 to 93% of that derived by extrapolation from the arm sweat. The mean total body sweat loss of nitrogen averaged 2.22 and 3.75 gm for 12 and 24 hour collection periods. A study on the trace mineral losses of men exposed to hot environments was completed. Very little progress on the relationship of relative humidity on energy expenditure and heat acclimatization was shown during the past year.

BODY OF REPORT

ENVIRONMENTAL NUTRITION (FY 1965)
NUTRITIONAL PHYSIOLOGY (FY 1966)

Description:

Extremes of environmental conditions including cold, moist heat, dry heat or altitude affect the nutritional needs of the acclimatized or unacclimatized human. The minimal macro- and micro-nutrient requirements to satisfy these altered needs have not been clearly elucidated. Due to the geographic range of current military commitments and the necessity for preparedness for possible future commitments, it is imperative that the nutritional requirements imposed by environmental extremes be established. Published information by this Laboratory on the "Energy Requirements of Men Living Under Extreme Environmental Conditions" have been incorporated in the new NRC's "Dietary Allowances" 1964. The energy requirements are now increased by 5% in a hot environment (40°C) and are practically unchanged in a cold environment.

The loss of essential nutrients through perspiration from men working in hot environments has been demonstrated. Studies to determine the sifnificance of these sweat losses have been evaluated. Increased environmental heat has been shown to increase the energy requirements for men performing a given amount of work but little is known of the affect that variation in the relative humidity has upon energy requirements. The affect of relative humidity upon energy expenditure and heat acclimatization has been studied as has the magnitude and composition of respiratory water loss under varying environmental conditions.

Progress:

Sweat Studies - Arm-Forearm versus Total Body (Bioenergetics Division): Human males were maintained in an environmental chamber at 38.5°C and 30% humidity for 7, 12 and 24 hour periods. The composition of the sweat collected from the forearm and arm was compared to the composition of total body sweat. Considerable individual variation in the concentration of nitrogen in the upper extremity was found. Nitrogen determined from total body sweat ranged from 86 to 93% of that derived from the arm sweat extrapolation. These values are in better agreement if one takes into account the respiratory water loss (approximately 7.5%) which has been included in the calculation of the total arm sweat values. The mean total body sweat loss of nitrogen averaged 1.90, 2.22 and 3.75 gm for 7, 12 and 24-hour collection periods, respectively. A final report was completed.

Trace Mineral Sweat Losses - Bioenergetics Division: Three healthy males, receiving a constant dietary intake were exposed 7.5 hours/day in the environmental chamber at 37.7°C and 50% relative humidity for 16 days. The subjects performed 30 minutes of moderate physical activity on the bicycle ergometer with the remainder of the daily exposure period spent in sedentary type activities. A final report was completed.

Effects Produced by Variations of Relative Humidity at Constant Temperature - Bioenergetics and Metabolic Divisions: Two studies have been conducted to determine the effect of variation of relative humidity at fixed environmental temperature on energy requirements and heat acclimatization. In the first study, 7 normal young men performing constant levels of physical activity were exposed daily, for 8 hours/day, at 75 and 100°F with set periods of relative humidity of 10, 40 and 70% at both temperature levels. Metabolic rates were determined daily at rest and during two activity levels on the bicycle ergometer. Other measurements included sweat rates, respiratory water loss, chemical composition of collected expired water, urinary steroids, and serum lipid levels. The subjects, though previously acclimatized to 100°F and 40% relative humidity, were not acclimatized to 100°F when the relative humidity was increased to 70%.

Eleven healthy young males were utilized in the second study. After a brief training period the subjects were exposed daily (6 hrs/day) in the environmental chamber to 100°F temperature at fixed periods of relative humidity of 30, 70 and 92-95%. Metabolic rates were determined at rest and during a fixed level of physical activity on the bicycle ergometer. Sweat and expired respiratory water were collected and determined. Urinary steroid determinations and complete nitrogen, calcium, sodium, potassium and water balance studies were performed. During both studies hyperventilation with syncope occurred in some subjects acclimatized to 100°F at 30 and 40% relative humidity when exposed to the higher levels of relative humidity. A downward shift of blood CO_2 content and maximum binding capacity with an alkaline shift of the pH was found after the subjects were exposed to 100°F and 93% relative humidity. Accumulation, computation and analysis of data from these two studies are only partially complete at this time, due to the pressing high altitude and ration studies.

Summary and Conclusions:

1. Comparison of the excretions of nitrogen between arm and total body sweat showed fairly good agreement. Nitrogen determined from the total body sweat ranged from 86 - 93% of that derived by extrapolation from the arm sweat. These values are in better agreement if one takes into account the approximate 7.5% respiratory water loss, which has been included in the

calculations of the total arm sweat value. Correlation between the excretion in arm and total body sweat is excellent if one eliminates all arm sweat samples less than 16 gm weight. On the basis of these comparisons the nitrogen and mineral losses in sweat reported in our previous paper, still remain high.

These findings again reaffirm the presence of additional nitrogen, calcium and iodine losses in sweat that have been ignored in many balance studies. Past calcium and nitrogen balance studies conducted on active individuals in a warm or hot environment where equilibrium was apparently attained should be reevaluated, with consideration for these sweat losses. These sweat losses are probably of little practical significance in studies of fairly inactive subjects living in an air-conditioned ward or in a temperate environment.

It is recommended that in future studies related to sweat collection, the total body sweat procedure should be utilized, wherever possible.

2. Trace mineral balances, using losses in the sweat were negative for copper, molybdenum and nickel. Sweat losses during a 7.5 hour collection period averaged 1.0 to 1.9 mg of copper, 0.30 to 0.37 mg of selenium, 0.86 to 1.10 mg of strontium, 2.16 to 2.41 mg of zinc and 0.06 to 0.12 mg for manganese. Sweat losses decreased with acclimatization.

3. Two studies to evaluate the influence of changes in relative humidity at fixed temperature upon energy expenditure have been completed. Computation and analysis of the data are still in progress.

4. Men acclimatized to 100°F at 30% or 40% relative humidity were found not to be acclimatized to the same temperature while performing similar activities when the relative humidity was increased to 70% or 92%.

List of Publications:

1. Consolazio, C. F., Matoush, L. O., Nelson, R. A. and Isaac, G. J.: Comparison of Calcium and Iodine Excretion in Arm and Total Body Sweat. USAMRNL Report No. 282, August 1964.

2. Consolazio, C. F., Nelson, R. A., Matoush, L. O., Hughes, R. C. and Urone, P.: The Trace Mineral Losses in Sweat. USAMRNL Report No. 284, August 1964.

3. Consolazio, C. F. and Shapiro, R.: Environmental Physiology and Psychology in Arid Climates. Arid Zone Research XXIV. Proceedings of the Lucknow, India Symposium, UNESCO, 1964.

4. Consolazio, C. F.: Calorie Requirements of Long Flights. Conf. on Nutrition in Space and Related Waste Problems. NASA Publication SP70, 1965.

5. Consolazio, C. F., Nelson, R. A., Matoush, L. O. and Canham, J. E.: Comparisons of Nitrogen, Calcium and Iodine Excretion in Arm and Total Body Sweat. Fed. Proc., Atlantic City, N. J., April 1965, Vol. 24, Page 312 (Abstract & Presentation).

6. Matoush, L. O., Consolazio, C. F., Nelson, R. A. and Canham, J. E.: Sweat Losses in Relation to Trace Mineral Balance. Fed. Proc., Atlantic City, N. J., April 1965, Vol. 24, Page 312 (Abstract and Presentation).

7. Consolazio, C. F.: NRC Recommended Dietary Allowances, Revised 1964, Wyoming Dietetic Assoc., Casper, Wyoming, 24 April 1965 (Presentation).

8. Consolazio, C. F.: Nitrogen Losses in Sweat. NAS, NRC Gordon Conference, New London, New Hampshire, August 1964 (Presentation).

9. Consolazio, C. F.: a) Caloric Requirements; b) Nutrient Losses in Sweat; c) Operational Rations; and d) Selected ICNND Studies. Nutrition Review for the Therapeutic and Research Dietitian, WRAIR, Walter Reed Army Medical Center, Washington, D. C., August 1964 (Presentation).

10. Consolazio, C. F.: Nutritional Variation in World Populations and Performance Potential. NY Academy of Science, Conference on "The Biology of Human Variation", New York, N. Y., February 1965 (In Press).

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6322	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U U (CITY) (AGY)	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 02 059

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 02 20

11. TITLE: (U) Amino Acids & Proteins (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry	03 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: c. TYPE: NA d. AMOUNT:	PRIOR FY 65	3	94
		CURRENT FY 66	3	103

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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INVESTIGATORS PRINCIPAL: Leveille, G. A.
ASSOCIATE: Sauberlich, H.E.; Levine, R. A.
TEL: 303 366 5311 X 24214 TYPE: DA

21. TECHNOLOGY UTILIZATION: Medicine

22. COORDINATION: None

23. KEYWORDS: Protein, protein deficiency, protein metabolism, protein metabolism disorders; amino acids

24. (U) Tech Objective:
To study protein and amino acid nutrition and determine what factors alter protein and amino acid requirements, and the mechanisms by which such alterations are affected.

25. (U) Approach:
Experiments are designed with the use of animals and human subjects in which factors that may influence protein or amino acid requirements are examined. Malabsorption syndromes and imbalance conditions have been studied in the early phases of the experiments

26. (U) Progress: (Oct 64-Jun 65)
During the reporting period, additional studies were conducted on the influence of wheat gluten diet on absorption of various nutrients and on plasma amino acids. Plasma amino acids were studied in samples of human subjects from Brazil and Ft. Carson, Colorado. The influence of PAS and INH (isonicotinic acid hydrazide) on plasma amino acids in humans is currently under study.

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) OVER

ABSTRACT

AMINO ACIDS AND PROTEINS

a. Possible harmful effects of prolonged gluten administration were investigated in 37 studies performed on a metabolic ward in 12 healthy, young adult volunteers, 13 convalescent patients and 3 patients with asymptomatic steatorrhea. After a 2-week control period, either a 100 grams of gluten or egg whites were added to the diet for at least 8 weeks. Five of 10 patients receiving gluten supplementation developed significant abnormalities including steatorrhea, lowered serum carotene, vitamin A and cholesterol levels and abdominal distention. No histologic abnormalities developed. No deleterious effects on gastrointestinal absorption or morphology were evident in any of the other subjects.

b. Factors considered related to the intolerance of some individuals to gluten included age and the presence of an associated illness. It is postulated that gluten may compete with fat for the pathways of fat absorption by altering sterol and bile acid metabolism.

BODY OF REPORT

AMINO ACIDS AND PROTEINS

Description:

Studies in this area of protein and amino acid nutrition have been designed to determine what factors alter protein and amino acid requirements and the mechanisms by which such alterations are effected. The effects of prolonged gluten administration have been investigated in a series of studies to determine if gluten tolerance is present in persons without non-tropical sprue and to assess the feasibility of excessive gluten administration as a practical screening test in persons with asymptomatic steatorrhea and apparent normal gastrointestinal absorption.

Progress:

Thirty-seven studies were performed on 28 male subjects in a metabolic ward. Thirteen convalescent patients and 12 healthy young adult male volunteers were used in this study and initially were shown not to have malabsorption by a variety of screening tests, including jejunal mucosal biopsies. Three convalescent patients with asymptomatic steatorrhea were also housed in the metabolic ward and concomitantly investigated with gluten supplementation. All patients were ambulatory and compensated regarding their disease status. Four gluten-free diets and a fifth high gluten diet, each consisting of 4-7 menus of equivalent nutrient composition were rotated daily through the study. After a 2-week control period, either 100 grams of gluten or egg whites were added to the daily diet for at least 8 weeks. The diets and supplements, by analysis, supplied 115-125 grams of fat, 172-215 grams of protein, 440-590 grams of carbohydrate and 3475-4249 Calories per day.

The mucosa of the proximal jejunum was biopsied serially between 3 and 6 times in all subjects. Continuous 5-day (group 1) or 7-day (group II-VII) pooled stool collections were analyzed for total fat and nitrogen. Measurement of a 5-hour urinary collection of D-xylose after a 25 grams oral dose was performed weekly in all patients. Every 3rd or 4th day throughout the study, fasting blood samples were analyzed for serum lipids (cholesterol, lipid phosphorus, total glycerides and plasma non-esterified fatty acids), total serum proteins and albumin, glucose, prothrombin time, serum calcium, vitamin A, carotene, hematocrit, leukocyte count and differential smear. Completeness of urine collections was checked by creatinine determinations. The data were evaluated statistically by the paired t test method using a mean of the last value for each weekly period.

Group 1 consisted of 5 compensated tubercular patients on chemotherapy, one patient with Laennec's cirrhosis and one with viral hepatitis; group II consisted of 2 compensated tuberculosis patients, one subject with a diagnosis of alcoholism, one with Laennec's cirrhosis and one with chlorpromazine

hepatitis. Both groups received a 100 grams daily gluten supplement. Groups III and V each had 4 subjects on egg white supplement. Group III were compensated tubercular patients; group V were healthy subjects. Group IV, 5 healthy subjects, received a gluten-gliadin supplement. The two healthy subjects in group VI received a 200 grams supplement of dextrose. The 9 healthy men in group VII received a 150 grams supplement of cooked gluten.

Summary and Conclusions:

Except for group 1, there were no significant changes in fecal nitrogen, body weight, total glycerides, lipid phosphorus, plasma esterified fatty acids, total serum proteins and albumin, serum calcium, blood glucose, glucose tolerance tests, prothrombin time, hematocrit, leukocyte count, differential blood smear or radiographical studies of the gastrointestinal tract. The jejunal mucosa in all subjects was normal with dissecting and light microscopy. Microbiologic studies of the proximal jejunal aspirate demonstrated essentially sterile juice, although colony counts ranged from 0 to < 10 microorganisms/ml. The most consistently abnormal findings were in group 1. Levels of vitamin A, carotene and cholesterol and body weight showed significant changes. Four patients had persistent steatorrhea between 4 and 8 weeks. In the remaining 3 others, fecal fat increased from control values in all subjects, but remained within the normal range.

Intolerance to gluten was postulated to be related to age and the presence of an associated illness. Gluten may compete with fat for the pathways of fat absorption by altering sterol and bile acid metabolism. As a screening test for gluten intolerance, a 100 grams gluten "cocktail" was found to be as practical and well tolerated as smaller doses previously used.

List of Publications:

Levine, Robert A., Gordon W. Briggs and Richard S. Harding. Effects of prolonged gluten administration in humans without malabsorption. I. Clinical, biochemical and morphologic studies. (Submitted for publication).

RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			DA OA 6323	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U RPT U DOC	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71R 02 060

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 02 21

11. TITLE:
(U) Carbohydrates & Related Compounds (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300, Biochemistry; 000700 Agricultural Chemistry	01 64	NA	OTHER I DA

16. PROCURE. METHOD	17. CONTRACT/GRANT		18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
	a. DATE:				
	b. NUMBER:				
C. In-House	c. TYPE: NA	d. AMOUNT:	PRIOR FY 65	1	27
			CURRENT FY 66	2	30

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21. TECHNOLOGY UTILIZATION
Nutrition

22. COORDINATION
None

23. KEYWORDS
Cellulose, carbohydrates, carbohydrate metabolism, cotton cellulose

24. (U) Tech Objective
1. To establish the nutritional utilization of cellulose in the non-ruminant.

2. To prepare a cell-free system for the biosynthesis of cellulose.

25. (U) Approach: To accomplish objective 1, the following tasks will be performed: (a) Uniformly labeled C^{14} cellulose will be grown and isolated from cotton plants and the product analytically defined; (b) Labeled cellulose will be fed to subjects to determine if the substance can be metabolically utilized; (c) Control experiments will be performed with germfree animals to determine the role intestinal microflora play in such utilization. To accomplish objective 2, the following experiments will be performed: (a) C^{14}-labeled sugar nucleotides (UDPG, GDPG, CDPG, ADPG, TDPG) will be synthesized and characterized; (b) Cell-free extracts of cotton seedlings and mature cottonplants will be prepared; (c) After incubation of the sugar nucleotides and the cell-free extracts, the product (if any) will be characterized with regard to structure and radioactive sugar incorporation; (d) Attempts will be made to purify the enzyme(s) responsible for cellulose biosynthesis.

26. (U) Progress: (Oct 64-Jun 65)
Objective 1: (a) Radioactive C^{14} cotton cellulose has been prepared and characterized in preparation for feeding experiments; (b) Germfree animal equipment has been set up and is operative.

Objective 2: Work to be done during the summer of 1965.

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

31. MISSION OBJECTIVE	32. PARTICIPATION
NA	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) OVER

ABSTRACT

CARBOHYDRATES AND RELATED COMPOUNDS

Studies conducted within this sub-task have been related to the metabolism and utilization of cellulose by man and experimental animals. In one study, normal adult males were fed a 2500 Calorie diet composed of normal foodstuffs to which, after a suitable control period, 174-200 grams of cellulose was added. Over the 5-week period of supplementation, the average per cent degradation of the cellulose during passage through the gastrointestinal tract was 22.4%. Protein digestibility was adversely influenced during 3 of the 5 weeks of supplementation. However, these studies did not establish utilization of the degradative products in the human. To better study the metabolism of cellulose and its possible utilization in the human and laboratory animal, ^{14}C-labeled cotton was produced by the injection of labeled glucose in the stems of immature bolls followed by harvesting of the mature cotton bolls. Radioactive carbon fibers were further purified to yield labeled alpha cellulose. The digestibility of this material is under study with human subjects and rats.

BODY OF REPORT

CARBOHYDRATES AND RELATED COMPOUNDS

Description:

The increase in world population has caused investigators to explore the potential usability of various natural organic substances as possible foodstuffs. Cellulose, a polysaccharide component of the crude fiber of plants, is known to be unaffected by mammalian enzymatic digestion, but is digested by certain celluloytic microorganisms. Results of previous studies at this laboratory have indicated a high rate of digestibility of cellulose in the rat. Cellulose digestibility studies in man dating back almost 100 years have disclosed considerable variation with digestibility ranging from 0 to 79%. However, the amount of cellulose ingested in the above studies was generally quite small. In a previous study conducted in this laboratory, normal adult males were maintained on a synthetic diet to which a uniform microcrystalline pure cellulose was added at the level of 10, 20 and 30%. The digestibility of this cellulose ranged from 5.8 to 49.2% with a mean of 23.9%. Further studies have been conducted under this sub-task to determine the metabolism and utilization of carbohydrates, particularly cellulose and the more unusual pentoses and hexoses by both man and experimental animals.

Progress:

Human gastrointestinal degradation of a microcrystalline cellulose

Eleven adult males were fed a 2500 Calorie diet composed of natural foods. After a 4-week control period, 174 or 200 grams of a microcrystalline cellulose was added to the daily diet of 6 of the subjects by including the cellulose in the food during preparation. This supplement was maintained during the next 5 weeks of the study. A moderately vigorous exercise program was maintained throughout the study with the result that, on the caloric intake fed, there was a progressive loss of weight. Urinary excretion of riboflavin, thiamine and niacin were unchanged. While the digestibility of fat was not influenced, the addition of cellulose did adversely and significantly influence the digestibility of protein during 3 of the 5 weeks of the supplementation. Fecal analysis of cellulose disclosed that the average per cent of degradation of cellulose was 22.4% with a range of 10.5 to 29.4%. Although it was impossible to establish whether the degradative products of cellulose entered into the metabolism of the subjects receiving the supplement, analysis of the curves depicting weekly decrease in body weight suggested that possibly calories were being obtained from this source. However, utilization of the degradative products appeared definable only by the performance of studies utilizing carbon-14 labeled cellulose.

Studies utilizing carbon-14 labeled cellulose

During this past year, a qualified investigator was made available to initiate studies in this area. Previously, pure carbon-14 labeled cellulose was not available

for refined human or animal studies. During the current year, such material was produced. Cotton plants were grown and ^{14}C-glucose was injected into the stems of immature bolls. Procedures for this technique were successfully developed. The cotton fibers were then harvested when the bolls matured. The fibers were further purified by extraction methods to provide pure alpha cellulose. The material was adequately labeled for human and animal metabolism studies.

Thus far, only one digestion study has been conducted using the labeled material. A normal human recently received a quantity of the cotton fibers and the expired CO_2 was monitored for radioactivity for the following 72 hours. Only trace amounts of activity were observed. No activity was noted in the voided urine during this period. In future studies, the cellulose will be modified in various ways (physical, particles, partial degradation etc.) in order to study further its possible utilization. Similarly, rat experiments will be conducted in order to validate earlier observations made at this laboratory with studies conducted on other ^{14}C-labeled cellulytic materials.

Summary and Conclusions:

1. Two balance studies utilizing normal adult males have been performed to measure the extent of degradation of cellulose during passage through the human gastrointestinal tract. The average per cent of degradation (23.9% and 22.4%) was similar in both studies though the basic diets were quite dissimilar. Urinary thiamine and niacin excretion were influenced in the first study, but not in the second. While protein digestibility was unchanged when cellulose was added to a synthetic diet, it was significantly influenced when the cellulose was added to a diet composed of normal foods.

2. Pure ^{14}C-labeled alpha cellulose was produced by labeling immature cotton bolls with ^{14}C-glucose. The digestibility of the labeled cellulose is being studied with the human and the rat.

List of Publications:

Canham, J. E., R. S. Harding, C. F. Consolazio and N. F. Witt. Gastrointestinal degradation of cellulose in the human. Fed. Proc. 24: 1018, 1965 (abstract).

RESEARCH AND TECHNOLOGY RESUME

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U (RPT) U (ABS)	NA		A. WORK UNIT

3. GOVT ACCESSION	4. AGENCY ACCESSION	REPORT CONTROL SYMBOL
	DA OA 6310	CSCRD 103

10a. CURRENT NUMBER/CODE: 62156011 3A025601A822 01 075

10b. PRIOR NUMBER/CODE: 61145011 3A014501A71F 02 18

11. TITLE: (U) Nutritional & Metabolic Aspects of Micronutrients (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry; 003500 Clinical Medicine; 008500 Isotopes	01 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. NUMBER: b. TYPE: NA c. DATE: d. AMOUNT:	PRIOR FY 65	2	64
		CURRENT FY 66	3	70

19. GOV'T LAB/INSTALLATION/ACTIVITY
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INVESTIGATORS PRINCIPAL ASSOCIATES: Sauberlich, H. E.; Baker, Lt.Col. E.M.; Canham, Lt.Col. J.E.
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21. TECHNOLOGY UTILIZATION
Nutrition, clinical medicine, biochemistry

22. COORDINATION
None

23. KEYWORDS
Vitamin B_6, pyridoxine, pyridoxine metabolism, vitamins, micronutrients

24. (U) Tech Objective

To investigate the nutritional requirements of the micronutrients, particularly the vitamins, and the factors and interrelationships that may alter these requirements and to study the metabolic aspects of the nutrients.

25. (U) Approach: The initial studies will be concerned with the interrelationship between vitamin B_6 and vitamin C that has been shown to exist in previous studies carried out in this laboratory. This will be accomplished by labeling human volunteers with L-ascorbic-1-C^{14} acid to determine vitamin C pool size and utilization. Following this, the subjects will be given a pyridoxine (B_6) supplement of 300 mg/day for a 30-day period. At the end of the B_6 supplementation period, the subjects will again be labeled with the C^{14} ascorbate to determine if there has been any change in their vitamin C pool and utilization.

26. (U) Progress: (Oct 64-Jun 65)

The C^{14} labeling has been completed and the C^{14} labeled oxalic acid and C^{14} labeled ascorbic acid excreted in the urine have been isolated and are being counted. Complete results will be obtained as soon as the specific activity of the urinary ascorbate samples are obtained.

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 64 1498 (Items 1 to 36 identical to NASA Form 1152) OVER

ABSTRACT

VITAMINS (FY 1965)
NUTRITIONAL AND METABOLIC ASPECTS OF MICRONUTRIENTS (FY 1966)

Approval for the use of tracer amounts of labeled vitamins in human subjects was received from the Atomic Energy Commission. As a result, several previously planned studies on the metabolism and requirement of vitamins in humans were initiated. In one study, the influence of high intakes of vitamin B_6 on vitamin C requirement was investigated. Preliminary evaluation indicates that there was an increase in the utilization of vitamin C. In another study, ^{14}C-labeled pyridoxine was employed in a human subject in a study of pool size, metabolism and turnover of this vitamin. The turnover rate was observed to be approximately 15 days. The metabolism of the administered pyridoxine was rather complex and is under further study.

BODY OF REPORT

VITAMINS (FY 1965)
NUTRITIONAL AND METABOLIC ASPECTS OF MICRONUTRIENTS (FY 1966)

A. Effect of high intake of pyridoxine on vitamin C in man

Description:

In previous studies at this laboratory, a close relationship between vitamin C and vitamin B_6 appeared to exist. During B_6 deficiency, plasma ascorbic acid levels fell rapidly from control levels. Though supplementation with minimal amounts of pyridoxine hydrochloride produced a rise in serum vitamin C, the level did not return to normal until adequate B_6 supplementation was given. The observed oxaluria with B_6 deficiency, as reported by Farber and others could be due to impaired protein metabolism, but altered vitamin C metabolism may be a factor in producing oxaluria. In studies carried out in this laboratory, we have never observed increased oxalate excretion in B_6-deficient subjects. However, in every case when subjects were given a 10-gram DL-tryptophan load, increased oxalate excretion did occur.

In two subsequent studies at this laboratory, normal adults receiving normal diets have been supplemented with high levels of pyridoxine hydrochloride. In both studies, the whole blood ascorbic acid values rose to remarkably high levels during the period of pyridoxine hydrochloride supplementation and remained elevated until two weeks after cessation of supplementation. In a group on a low-protein intake, the urinary excretion of vitamin C followed the pattern of whole blood ascorbate, but the group on a high-protein intake showed an elevated urinary ascorbate pattern only after cessation of B_6 supplementation. Urinary oxalate excretion increased during pyridoxine supplementation and readjusted more slowly towards normal levels after cessation of the B_6 supplementation. There appears to be a definite interrelationship between vitamin B_6 and vitamin C, but the relationship needs defining.

In the current study, an attempt was made to determine if there is any change in vitamin C pool size and utilization following a 30-day period of pyridoxine hydrochloride supplementation (300 mg/day) by the use of L-ascorbic-1-^{14}C acid to label the body pool.

Progress:

The experiment has been completed; however, no definite statement can be made concerning any changes in vitamin C pool size or utilization until the DNPH derivatives are counted to determine the ^{14}C specific activity of the ascorbate. The samples are being presently counted by Dr. Tolbert at the University of Colorado. The urinary oxalate has been isolated and counted to determine the level of ^{14}C activity. From the

oxalate ^{14}C values, it appears that we can say that there was a definite increase in the vitamin C utilization in the subjects who received the 300 mg of vitamin B_6/day for 30 days. This is with the assumption that oxalate excretion is an accurate reflection of ascorbate metabolism.

Summary and Conclusions:

Two human volunteers were given L-ascorbic-1-^{14}C acid to label the body pool so that the vitamin C pool size and utilization could be measured. They were then given a pyridoxine (B_6) supplement of 300 mg/day for a 30-day period. At the end of the supplementation period, the subjects were again labeled with the ^{14}C ascorbate to determine their vitamin C pool size and utilization. The ^{14}C ascorbate specific activities have not as yet been obtained; however, from the ^{14}C urinary oxalate values, it appears as though there was a definite increase in the vitamin C utilization.

List of Publications:

None at present.

B. Metabolism of ^{14}C-labeled pyridoxine (vitamin B_6) by man

Description:

^{14}C-labeled pyridoxine was made available to USAMRNL in order to study the requirement of this vitamin and its metabolic pathways in man.

Progress:

Thus far, only a single subject has received the labeled pyridoxine and its utilization and metabolism followed. Oral administration was followed by over 97% absorption with no conversion to carbon dioxide as noted by monitoring of the radioactivity of the expired air. Within approximately 14-15 days, 50% of the radioactivity had appeared in the urine. The radioactive components appearing in the urine are numerous in number. Separation, identification and measurement of the compounds are under extensive study employing column, paper and thin layer chromatography.

Summary and Conclusions:

^{14}C-labeled pyridoxine was administered to a human subject and the utilization and metabolism studied in order to provide information on the requirement for this vitamin. The study is still in progress.

Vitamins (FY 1965)
Nutritional and Metabolic Aspects of Micronutrients (FY 1966) (Cont'd.)

List of Publications:

1. Sauberlich, H. E. Human requirement for vitamin B_6. Vitamins and Hormones 22: 807, 1964.

2. Ziporin, Z. Z., W. T. Nunes, R. C. Powell, P. P. Waring and H. E. Sauberlich. Excretion of thiamine and its metabolites in the urine of young adult males receiving restricted intakes of the vitamin. J. Nutrition 85: 287, 1965.

3. _____ Thiamine requirement in the adult human as measured by urinary excretion of thiamine metabolites. J. Nutrition 85: 297, 1965.

4. Sauberlich, H. E. Determination of the vitamin B_6 group. The Vitamins, IV. Academic Press, New York (in press).

Previous page was blank, therefore not filmed.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		None	None

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	6. SECURITY PAR	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 05 65	B. COMPLETED	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501A71F 02 023

10b. PRIOR NUMBER/CODE: None

11. TITLE:
(U) Analytical Biochemistry in Nutrition

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry	-	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	-	-
		CURRENT FY	-	-

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21. TECHNOLOGY UTILIZATION
-

22. COORDINATION

23. KEYWORDS
Analytical biochemistry; instrumentation; automated analyses; methodology

24. (U) To utilize new concepts in analytical chemistry and to facilitate their adaptation to biochemical procedures, methodology is developed by direct innovation or by a revision of existing procedures. Use of electronic controllers, mechanical devices and new chemical intermediates provides a means of automating and controlling biochemical methods with greater accuracy and reliability. Support is given to those task elements requiring unique equipment or specific methodologies. A program for automating analyses in clinical chemistry, in determining total nitrogen and amino acids and in fluorometry and spectrophotometry was instituted. Collaborative studies are conducted to determine both normal and abnormal values in biological fluids, tissues and foods as indices of nutritional adequacy.

26. Primary objective to the sub-task has been accomplished, i.e., the adaptation of new biochemical procedures and equipment to accomplish the overall updating of the techniques employed in nutritional research studies. All future improvements of methodology in analytical biochemistry will be reported under the specific project for which developed.

27. COMMUNICATIONS SECURITY	28.	29. GEO CODE	30. SUBJECT CODE
☐ a. CONFID ☐ b. SECRET		AR	1

31. MISSION OBJECTIVE	32. PARTICIPATING
NA	NA

33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT

35. EST. FUNDS (In thousands)	36.
FY:	

DD FORM 1498 (Items 1 to 36 identical to DASA Form 1130)

ABSTRACT

ANALYTICAL BIOCHEMISTRY IN NUTRITION

An enzymatic method for determining whole blood lactic acid, based on the conversion of lactate to pyruvate by lactic dehydrogenase, has been automated to provide a sampling rate of 70 per hour. Standards in the range of 30-300 ugm/ml were reproducible with a standard deviation of ± 0.011 OD units. Duplicate samples were reproducible within ± 0.6 ugm/ml. The dilution and sampling of specimens for analysis by atomic absorption spectroscopy was automated to give a recorded analysis at the rate of 120 per hour.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		None	None

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / WORK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 05 65	B. COMPLETED	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE
61145011 3A014501A71F 02 023

10b. PRIOR NUMBER/CODE
None

11. TITLE:
(U) Analytical Biochemistry in Nutrition

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry	-	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	-	-
		CURRENT FY	-	-

19. GOV'T LAB/INSTALLATION/ACTIVITY
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RESP. INDIV. Rosenberger, E.A. Lt. Col.
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20. PERFORMING ORGANIZATION
NAME: USA Med Rsch & Nutr Lab
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Denver, Colorado 80240

INVESTIGATORS PRINCIPAL: Harding, Richard S.
ASSOCIATE: Leveille, Gilbert A.
TEL: 303 366 5311 X 24214 TYPE: DA

21. TECHNOLOGY UTILIZATION
-

22. COORDINATION

23. KEYWORDS
Analytical biochemistry; instrumentation; automated analyses; methodology

24. (U) To utilize new concepts in analytical chemistry and to facilitate their adaptation to biochemical procedures, methodology is developed by direct innovation or by a revision of existing procedures. Use of electronic controllers, mechanical devices and new chemical intermediates provides a means of automating and controlling biochemical methods with greater accuracy and reliability. Support is given to those task elements requiring unique equipment or specific methodologies. A program for automating analyses in clinical chemistry, in determining total nitrogen and amino acids and in fluorometry and spectrophotometry was instituted. Collaborative studies are conducted to determine both normal and abnormal values in biological fluids, tissues and foods as indices of nutritional adequacy.

26. Primary objective to the sub-task has been accomplished, i.e., the adaptation of new biochemical procedures and equipment to accomplish the overall updating of the techniques employed in nutritional research studies. All future improvements of methodology in analytical biochemistry will be reported under the specific project for which developed.

27. COMMUNICATIONS SECURITY	28.	29. DOD CODE	30. BUDGET CODE
☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED		AR	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) OVER

ABSTRACT

ANALYTICAL BIOCHEMISTRY IN NUTRITION

An enzymatic method for determining whole blood lactic acid, based on the conversion of lactate to pyruvate by lactic dehydrogenase, has been automated to provide a sampling rate of 70 per hour. Standards in the range of 30-300 ugm/ml were reproducible with a standard deviation of ± 0.011 OD units. Duplicate samples were reproducible within ± 0.6 ugm/ml. The dilution and sampling of specimens for analysis by atomic absorption spectroscopy was automated to give a recorded analysis at the rate of 120 per hour.

BODY OF REPORT

ANALYTICAL BIOCHEMISTRY IN NUTRITION

Description:

To utilize new concepts in analytical chemistry and to facilitate their adaptation to biochemical procedures, methodology is developed by direct innovation or by a revision of existing procedures. Use of electronic controllers, mechanical devices and new chemical intermediates provides a means of automating and controlling biochemical methods with greater accuracy and reliability. Support is given to those task elements requiring unique equipment or specific methodologies. Collaborative studies are conducted to determine both normal and abnormal values in biological fluids, tissues and foods as indices of nutritional adequacy.

Progress:

An automated enzymatic method for determining whole blood lactic acid levels has been developed. The method is based upon the conversion of lactate to pyruvate by the enzyme, lactic dehydrogenase (Lactate + NAD $\overset{LDH}{\rightleftharpoons}$ pyruvate + $NADH_2$). Lactic acid concentrations as low as 30 µgm/ml can be accurately determined. An automatic sampler, a variable speed proportioning pump, a time-delay coil, an ultraviolet spectrophotometer with a flow-through cuvette and a potentiometric strip chart recorder are employed.

Protein-free filtrates (previously prepared manually with the use of equal volumes of blood and 6.0% $HClO_4$) are automatically aspirated and mixed with glycine-hydrazine buffer (pH 9.0), LDH (0.45 mgm protein/ml in 2.2 M $(NH_4)_2SO_4$), NAD in H_2O (2.8 µmole/ml). A reaction time of approximately 20 minutes is obtained by the time-delay coil immersed in a water bath at 26°C. The OD of the reduced coenzyme ($NADH_2$) is monitored at 340 mµ and continuously recorded. Standards in the range of 30-300 ugm/ml were reproducible with a standard deviation of ± 0.011 OD units. Reproducibility of unknown samples determined in duplicate was within limits of ± 0.6 µgm/ml.

A controlled sampling device has been connected in line with a proportioning pump equipped with a plastic tubing manifold to provide automatic sampling and dilution of specimens for introduction into the burner assembly of an atomic absorption spectrometer. Samples may be analyzed and recorded at the rate of 120 per hour.

Collaborative research projects:

1. U. S. Navy Medical Research Laboratory: Analysis of nutrient value of diet composites for metabolic studies.

2. Fitzsimons General Hospital: Analysis of amino acid levels of urine and plasma of infants and adolescent children afflicted with mental and/or physical deficiencies resulting from phenylketonuria and other amino-acidurias is continuing with attempts to define abnormal values.

3. ICNND - Brazil nutrition survey: Analyses of amino acid content of food composites and blood plasma of military and civilian groups were performed to determine correlation with accepted normals.

Summary and Conclusions:

The automation of analytical procedures for blood lactic acid and atomic absorption spectroscopy increases the precision and reliability of the method as well as the rate of analysis. Because the majority of the work done under this subtask has been in support of research performed under other subtasks, it is being terminated. Future methodological innovations or improvements will be reported under the appropriate subtasks.

Publications:

Please see: Subtask #21 - "Carbohydrates and Related Compounds"; Subtask # 20 - "Amino Acids and Proteins"; Subtask # 12 - "Studies in Nutritional Status of Populations"; Subtask # 70 - "High Altitude Studies"; and Subtask # 02 - "Mineral Metabolism".

RESEARCH AND TECHNOLOGY RESUME

4. DATE OF RESUME: 01 05 65	5. KIND OF RESUME: 3. COMPLETED	6. SECURITY: U U	7. REGRADING: NA	8. RELEASE LIMITATION: NL
2. AGENCY ACCESSION: None	REPORT CONTROL SYMBOL: CSCRD 103			
9. LEVEL OF RESUME: A. WORK UNIT				

10a. CURRENT NUMBER/CODE: 61145011 3A014501A71F 02 24

10b. PRIOR NUMBER/CODE: None

11. TITLE: (U) Human Studies in Vitamin B_6 Metabolism

12. SCIENTIFIC OR TECH. AREA: 006500 Food Management; 002300 Biochemistry

13. START DATE: 1957

14. CRIT. COMPL. DATE: NA

16. FUNDING AGENCY: OTHER | DA

16. PROCURE. METHOD: C.In-House

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

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

18. GOV'T LAB/INSTALLATION/ACTIVITY NAME:
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TEL: 202 OXford 6 5472

20. PERFORMING ORGANIZATION
NAME: USA Med Rsch & Nutr Lab
ADDRESS: Fitzsimons General Hospital
Denver, Colorado 80240

INVESTIGATORS PRINCIPAL: Canham, John E.
ASSOCIATE: Sauberlich, Howerde E.
Baker, Eugene M., Raica, Nicholas, Jr

TEL: 303 366 5311 X21108 TYPE: DA

21. TECHNOLOGY UTILIZATION: Evaluate adequacy of diets

22. COORDINATION: None

23. KEYWORDS: Vitamin B_6; Pyridoxine; Xanthurenic Acid; Tryptophane Metabolism; Transaminase Activity; Deficiency States; Vitamins; Vitamin Requirements; Vitamin B_6 Metabolism

24. (U) Tech Objective: The objectives are to determine the adult human requirement of vitamin B_6; the clinical and biochemical manifestation of vitamin B_6 deficiency and toxicity; the normal excretory pattern of vitamin B_6 and its metabolites; and the relationship of vitamin B_6 to other dietary micro and macronutrients.

25. (U) Approach: Normal adult humans have been fed a variety of diets of fixed composition and with known vitamin B_6 intake. Clinical observations to determine the effects of vitamin B_6 deficiency or high vitamin supplementation have been made. Biochemical tests to determine the effect of deficiency or high level supplementation of vitamin B_6 on metabolic processes known to be or suspected to be dependent upon an adequate vitamin B_6 intake were performed.

26. (U) Progress: During this report period emphasis was placed on evaluating means of assessing an individual's vitamin B_6 nutritional status. The urinary excretion of xanthurenic acid following a tryptophane load of the various forms and at different levels of tryptophane revealed that a 5 gram L or a 10 gram D, L-tryptophane load produced significant equal results while, under similar conditions, a 2 gram L or a 5 gram D-tryptophane load did not produce significant change in xanthurenic acid excretion. In acute vitamin B_6 deficiency whole blood glutamic - pyruvic transaminase activity and its *in vitro* pyridoxal-5-phosphate stimulation appeared to be good indicators of the status of vitamin B_6 intake.

27. COMMUNICATIONS SECURITY: ☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED	28.	29. OSD CODE: AR	30. BUDGET CODE: 1
31. MISSION OBJECTIVE: NA		32. PARTICIPATION: 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) OVER

ABSTRACT

HUMAN STUDIES IN VITAMIN B_6 METABOLISM

Studies to better define the metabolic consequences of vitamin B_6 deprivation and the adult human requirement of the vitamin have continued. Emphasis was placed upon clarification of the sensitivity and validity of tests utilized to evaluate the vitamin B_6 status of an individual.

In a study conducted to ascertain which form and dosage level of tryptophane was most suitable for evaluation of an individual's vitamin B_6 status as measured by net xanthurenic acid excretion after tryptophane loading it was determined that: net xanthurenic acid excretion following either a 10 gram D, L or L-tryptophane load was equal; no significant increase in xanthurenic acid excretion was observed under the similar conditions when a 2 gram L-tryptophane or a 5 gram D-tryptophane load was administered; a 5 gram D-tryptophane load did not interfere with the determination of xanthurenic acid; and xanthurenic acid excretion in vitamin B_6 deficient individuals was not related to total body weight but was related to a measurement of lean body mass.

Extensive study of the influence of multiple levels of vitamin B_6 intake upon various measurements of transaminase activity (plasma, WBC, RBC, alcohol GPT or GOT) has revealed the following: whole blood glutamic-pyruvic transaminase activity is a reasonably good indicator of vitamin B_6 status if in vitro pyridoxal-5-phosphate stimulation values are also obtained; and red blood cell glutamic oxalacetic transaminase activity tends to reflect vitamin B_6 nutriture better than plasma transaminase activity, it is not responsive to minor variations in vitamin B_6 intake (less than 2 milligrams/day).

To provide better administrative control and due to a predictable decreased activity under this subtask, this subtask is to be phased out. Future studies, within the same research area, will be funded and reported under the work unit entitled "Nutritional and Metabolic Aspects of Micronutrients".

BODY OF REPORT

HUMAN STUDIES IN VITAMIN B_6 METABOLISM

Description:

The human need for vitamin B_6 was established with recognition of vitamin B_6 deficiency states in infants. Definition of the dietary requirements for this vitamin in infants rapidly followed but the adult requirements have not been clearly established. Animal studies elsewhere and human studies at this Laboratory indicated that certain of the Army rations were deficient in vitamin B_6 - dependent upon the ration ingredients and the conditions under which the rations were stored. These studies emphasized the need for clearer definition of the adult human vitamin B_6 requirements. A series of studies have been conducted at this Laboratory to determine: the adult requirements of the vitamin in man and to evaluate the clinical and biochemical manifestations of B_6 deficiency; the normal excretory pattern of vitamin B_6 and its metabolites; the relationship of B_6 to other micro or macronutrients; and the clinical and biochemical manifestations of B_6 toxicity. A weak link in the evaluation of vitamin B_6 requirements has been the difficulty in evaluating the vitamin B_6 status of a given individual due to the multiple factors which influence the biochemical or microbiological procedures normally used in the measurement of the vitamin B_6 status of an individual. The problem is further complicated by the lack of uniformity in testing procedures amongst the various investigators involved in this field. Therefore, during this report period, emphasis has been placed upon the various techniques utilized to assess the vitamin B_6 status.

Progress:

The metabolism of tryptophan is markedly influenced by the presence of adequate vitamin B_6 and hence, the loading of an individual with a given dose of tryptophan with subsequent measurement of the urinary excretion of xanthurenic acid and other metabolites of tryptophan has been frequently used as a measurement of the vitamin B_6 status. A study was conducted to determine which form of tryptophan, i.e. D, L, or DL-tryptophan and dosage level most suitable for use in humans. Thirteen normal adult males were placed upon a control hospital diet for a suitable control period followed by a liquid formula diet deficient in vitamin B_6 and succeeded by a diet containing natural foods but sub-optimal amounts of vitamin B_6. During the period of study repeated loadings with 10 grams

DL, 5 grams D, 5 grams L or 2 grams L-tryptophan were made. The study revealed that the net increase in xanthurenic acid excretion following either a 10 gram DL or a 5 gram L-tryptophan load in vitamin B_6 deficient subjects was equal. There was no significant increase in xanthurenic acid excretion in the same subjects following a 2 gram L-tryptophan load or a 5 gram D-tryptophan load. The 5 gram D-tryptophan load did not interfere with the xanthurenic acid determination. The increased xanthurenic acid excretion following a 10 gram DL or the 5 gram L-tryptophan load was not related to body weight but was related to a measure of lean body mass as determined by whole body counting for a measurement of the total potassium content. No increased oxalate excretion was observed in the B_6 deficient subjects although a slight increase in oxalate excretion followed loading with 10 grams DL or 5 grams of L-tryptophan.

Study B - Transaminase Activity- Dr. Nicholos Raica, Jr.

Preliminary data reported previously on blood transaminase activity in vitamin B_6 deficient human subjects has been verified. Whole blood glutamic-pyruvic transaminase (GPT) activity seems to be a reasonably good indicator of vitamin B_6 nutriture, particularly if in vitro pyridoxyl-5-phosphate stimulation values are also obtained. Although red blood cell glutamic-oxalacetic transaminase (GOT) tends to reflect vitamin B_6 nutriture better than plasma transaminase activity, it is not as responsive to small amounts of pyridoxine (less than 2 milligrams/day) supplements. When the subjects were ingesting less than the recommended intake of vitamin C during the vitamin B_6 deficiency period, red blood cells GOT activity was not restored towards normal values when adequate vitamin B_6 was included in the diet. Whole blood GPT activity was not affected by vitamin C supplementation. It should be emphasized that these are acute studies in which subjects served as their own controls. Whether or not similar results would be obtained with subjects having a chronic vitamin B_6 deficiency it is difficult to speculate from the data obtained.

Study C - Sub-marginal Vitamin B_6 Intake

A study is currently in progress to determine the effect of the chronic ingestion of sub-marginal vitamin B_6 intakes will have upon the human particularly as manifested by the rate of development and in the magnitude of biochemical and clinical abnormalities. The data is too preliminary to report at this time.

Human Studies in Vitamin B_6 Metabolism (cont'd)

Due to the predictable decreased activity under this subtask during the forseeable future, this subtask is to be discontinued and future studies performed under this subtask will be reported under this subtask will be reported under the subtask entitled "Nutritional and Metabolic Aspects of Micronutrients".

Summary:

Studies to better define the metabolic consequences of vitamin B_6 deprivation and the adult human requirement of the vitamin have continued. Emphasis was placed upon clarification of the sensitivity and validity of tests utilized to evaluate the vitamin B_6 status of an individual.

In a study conducted to acertain which form and dosage level of tryptophane was most suitable for evaluation of an individual's vitamin B_6 status as measured by net xanthurenic acid excretion after tryptophane loading it was determined that: net xanthurenic acid excretion following either a 10 gram D, L or L-tryptophane load was equal; no significant increase in xanthurenic acid excretion was observed under the similar conditions when a 2 gram L-tryptophane or a 5 gram D-tryptophane load was administered, a 5 gram D-tryptophane load did not interfere with the determination of xanthurenic acid; and xanthurenic acid excretion in vitamin B_6 deficient individuals was not related to total body weight but was related to a measurement of lean body mass.

Extensive study of the influence of multiple levels of vitamin B_6 intake upon measurements of transaminase activity (plasma, WBC, RBC, alcohol blood GPT or GOT) has revealed the following: whole blood glutamic-pyruvic transaminase activity is a reasonably good indicator of vitamin B_6 status if in vitro pyridoxal-5-phosphate stimulation values are also obtained; and red blood cell glutamic oxalacetic transaminase activity tends to reflect vitamin B_6 nutriture better than plasma transaminase activity, it is not responsive to minor variations in vitamin B_6 intake (less than 2 milligrams/day).

Human Studies in Vitamin B_6 Metabolism (cont'd)

List of Publications:

1. Baker, E. M., Canham, J. E., Nunes, W. T., Sauberlich, H. E. and McDowell, M. E. Vitamin B_6 requirements for the adult male. Am. J. Clin. Nutrition 15: 59, 1964.

2. Raica, N., Jr. and Sauberlich, H. E. Blood cell transaminase activity in human vitamin B6 deficiency. Am. J. Clin. Nutrition 15: 67, 1964.

3. Baker, E. M. and Canham, J. E. Xanthurenic acid excretion after loading with various forms of tryptophane in the evaluation of vitamin B_6 status (abstract). Fed. Proc. 24: 624, 1965.

4. Canham, J. E., Nunes, W. T, and Eberlin, E. W. Electro-encephalographic and central nervous system manifestations of B6 deficiency and induced B6 dependency in normal human adults (abstracts). Nutrition Proc. of the 6th Int. Congress. Edinburg 1963. C. F. Mills and R. Passmore, Eds., Pub. - E. &S. Livingstone, Ltd., London, 1964, p 537.

5. Sauberlich, H. E., Baker, E. M., Canham, J. E. and Raica, N., Jr. Vitamin B6 requirement of the human (abstract). Nutrition Proc. of the 6th Int. Congress. Edinburg 1963. C. F. Mills and R. Passmore, Eds., Pub. - E. & S. Livingstone, Ltd., London, 1964, p 538.

6. Canham, J. E. and Sauberlich, H. E. Vitamin B_6. Chap. 15, Sect. I. Handbook of Nutrition. A. B. Eisenstein, Ed., Pub. - Am. Med. Assoc., Council on Foods and Nutrition, (in press).

7. Harding, R. S., Canham, J. E. and Sauberlich, H. E. The free amino acids in the plasma and urine of human subjects on vitamin B6 deficient diet. Technicon Symposium, Automation in Analytical Chemistry, 8-10 Sept. 65, N. Y., N. Y.

8. Moorman, J. A., and Harding, R. S. Automated ultraviolet spectrophotometric determination of lactic acid by enzymatic assay. Proc. 4th National Meeting, Society for Applied Spectroscopy, Denver, Colo. 30 Aug - 3 Sept 65.

RESEARCH AND TECHNOLOGY RESUME

1.	3. GOVT ACCESSION	5. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		None	None

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 10 64	B. COMPLETED	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: (U) 61145011 3A014501A71F 02 025

10b. PRIOR NUMBER/CODE: None

11. TITLE: Periodicity of Eating

12. SCIENTIFIC OR TECH. AREA: 002300 Biochemistry; 002600 Biology; 012900 Physiology

13. START DATE: -

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	-	-
CURRENT FY	-	-

19. GOV'T LAB/INSTALLATION/ACTIVITY
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INVESTIGATORS PRINCIPAL: Leveille, Gilbert A.
ASSOCIATE: Hanson, Richard W. Capt MSC
TEL: 303 366 5311 X 24214 TYPE: DA

21. TECHNOLOGY UTILIZATION: -

22. COORDINATION: None

23. KEYWORDS: Metabolism; carbohydrates; body fats; lipids; glucose tolerance; rats; chickens; food rhythm (physiology); body composition

24. (U) It has been shown that intermediary metabolism in certain animals may be influenced by the rate of caloric ingestion (nibbling or gorging). Previous studies have indicated that the nibbling rat has a lower respiratory quotient, lower blood lipids and better protein utilization than the rat force fed or trained to eat the same daily caloric load in one daily meal. The gorged rat has increased lipogenesis, increased ratio of body fat, alteration in adrenal secretion and altered thyroid
25. function.

The enhanced lipogenesis induced by meal-feeding (gorging) was related to an increase in the activities of glucose-6-phosphate dehydrogenase and NADP-malic dehydrogenase. These enzymes are more active in adipose tissue from meal-fed than in tissue from ad libitum-fed (nibbler) rats. The activity in adipose tissue of isocitric dehydrogenase, 6-phosphogluconic dehydrogenase and NAD-malic dehydrogenase did not increase significantly in response to meal-feeding. No increase in lipogenesis or enzyme activity could be demonstrated in adipose tissue from rats fed a high fat
26. diet. Lipase activity of adipose tissue was increased by meal-feeding a normal diet and decreased by meal-feeding a high fat diet and enhanced in rats meal-fed a high carbohydrate diet. Diaphragm or slices of liver from high fat-fed rats oxidized palmitate-1-^{14}C more rapidly than did tissue from ad libitum-fed animals.

Data were obtained which are consistent with the concept that citrate is a major source of acetyl-CoA for extramitochondrial lipogenesis. The oxaloacetate formed from the cleavage of citrate is converted to malate and then to pyruvate with a concomitant transhydrogenation of NADH to NADP by the coupling of cytoplasmic NAD-malic dehydrogenase and NADP-malic dehydrogenase.

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

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

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1332) OVER

ABSTRACT

PERIODICITY OF EATING

a. The effects of meal-eating (single 2-hour meal/day) on carbohydrate and lipid metabolism were studied in rats. Food consumption was greater in ad libitum-fed rats (nibblers); however, the rate of gain was similar for both groups following an initial weight loss for the meal-eaters. Isolated adipose tissue from meal-eaters converted more glucose to CO_2, fatty acids, non-saponifiable lipids and glycogen than tissue from nibbling animals. Acetate incorporation into fatty acids and cholesterol also was higher in adipose tissue from meal-eaters. A higher level of hexose monophosphate shunt activity in adipose tissue was indicated for meal-eating rats.

b. Re-feeding for up to 2 hours following a 22-hour fast resulted in increased lipogenesis in adipose tissue from meal-eaters. Total oxidized pyridine nucleotide coenzyme (NAD-NADP) levels and the rate of glucose oxidation to CO_2 in rat epididymal fat pads were found to decrease during the first 30 minutes of re-feeding in meal-eaters. Changes noted in metabolic pattern induced by re-feeding were apparently not due to de novo enzyme synthesis.

c. The enhanced lipogenesis induced by meal-feeding (gorging) was related to an increase in the activities of glucose-6-phosphate dehydrogenase and NADP-malic dehydrogenase. These enzymes are more active in adipose tissue from meal-fed than in tissue from ad libitum-fed (nibblers) rats. The activity in adipose tissue of isocitric dehydrogenase, 6-phosphogluconic dehydrogenase and NAD-malic dehydrogenase did not increase significantly in response to meal-feeding. No increase in lipogenesis or enzyme activity could be demonstrated in adipose tissue from rats fed a high fat diet. Lipase activity of adipose tissue was increased by meal-feeding a normal diet and decreased by meal-feeding a high fat diet and enhanced in rats meal-fed a high carbohydrate diet. Diaphragm or slices of liver from high fat-fed rats oxidized palmitate-1-^{14}C more rapidly than did tissue from ad libitum-fed animals.

d. Data were obtained which are consistent with the concept that citrate is a major source of acetyl-CoA for extramitochondrial lipogenesis. The oxaloacetate formed from the cleavage of citrate is converted to malate and then to pyruvate with a concomitant transhydrogenation of NADH to NADP by the coupling of cytoplasmic NAD-malic dehydrogenase and NADP-malic dehydrogenase.

BODY OF REPORT

PERIODICITY OF EATING

Description:

Previous studies have demonstrated distinct metabolic alterations in animals fed one or two daily meals as compared to those fed ad libitum. The studies described were designed to elucidate the mechanisms involved in the meal-eating response and to ultimately clarify the possible relationship of these alterations to food utilization, the problems of obesity and the degenerative diseases of military importance.

Progress:

Study A

Body weight and food consumption of meal-eating and nibbling rats were determined over a 16-week period. The body weight and food consumption of meal-eating animals were lower than that of nibblers. The meal-eating rats lost weight during the first week and thereafter the body weight curves were virtually parallel for both groups. The feed efficiency (grams gain/grams feed consumed) for the last 15 weeks, omitting the first week as an adjustment period, was higher for the meal-eating rats. This group had a feed efficiency of 0.111 ± 0.004 (mean for 22 rats ± S.E.) as compared to 0.086 ± 0.004 (mean for 24 rats) for the nibbling rats. This difference was highly significant ($P < 0.001$), demonstrating that the meal-eating rat, although weighing less than the nibbling animal, was more efficient in utilizing its food.

Data were obtained suggesting an adaptive increase in the anabolic capacity of tissues from the meal-eating rat. Adipose tissue from meal-eaters converted more glucose to CO_2, fatty acids, non-saponifiable lipids and glycogen than similar tissue preparations from ad libitum-fed animals. The incorporation of acetate into cholesterol by liver tissue and into fatty acids by adipose tissue was also higher ($P < 0.01$) in the meal-eating rat. These data demonstrate the marked adaptive alteration induced by changes in dietary pattern, most notably the increased synthesis of lipid and glycogen in adipose tissue. However, this increase in synthetic capacity was not reflected in a changed oxidative rate in muscle and liver.

The influence of time of re-feeding on the oxidation of glucose to CO_2 and the incorporation of acetate into fatty acid by adipose tissue were studied. In meal-eating rats, changes in nucleotide levels (NAD and NADP) and CO_2 production from glucose paralleled each other, while an inverse relationship existed between glucose oxidation and acetate incorporation into fatty acids. Glucose oxidation to CO_2 decreased during the first 30 minutes and was essentially constant thereafter for meal-eating animals.

Periodicity of Eating (Cont'd.)

The level remained unchanged for nibbling rats over the 2-hour period. Similar changes were observed in nucleotide levels of adipose tissue from rats of each group after 30 minutes of re-feeding. Acetate incorporation into fatty acids by adipose tissue from meal-eaters increased throughout the 2-hour period of re-feeding, while the rate of incorporation into tissue from nibbling animals was lower initially and remained unchanged.

It seemed possible that the difference observed between meal-eating and nibbling animals caused by re-feeding might have been influenced by the greater food consumption of the meal-eating animals during the 2-hour re-feeding period. However, intravenous administration of glucose resulted in increased ($P < 0.05$) acetate incorporation into fatty acid with time in meal-eating rats only. These results support the contention that the re-feeding response observed in the meal-eating rat is a metabolic adaptive change rather than a reflection of the amount of food consumed.

The involvement of de novo enzyme synthesis in response to re-feeding was tested. After 2 hours of re-feeding, the incorporation of acetate into fatty acids and non-saponifiable lipid was unaltered ($P > 0.05$) by the administration of an inhibitor of protein synthesis, puromycin, in either meal-eating or nibbling rats.

The rate of $^{14}CO_2$ formation from glucose-1-^{14}C and glucose-6-^{14}C and the response to insulin by isolated adipose tissue from meal-eating and nibbling animals was studied. A higher C-1/C-6 ratio was observed for the meal-eating rats, both in the presence and absence of insulin in the medium, suggesting increased hexose monophosphate shunt activity.

The data presented illustrate the adaptive changes induced in the rat by meal-feeding. These changes are most striking in adipose tissue where the pattern of glucose utilization is markedly altered. The metabolism of this tissue is geared to the synthesis, storage and release of lipid. Glucose serves both as a source of acetyl-CoA for lipogenesis and of glyceride-glycerol. Because of the inability of the fat cell to use free glycerol, the α-glycerophosphate required for triglyceride synthesis must be derived from glucose. These functions take on a special importance in the meal-eating rat because the long periods between meals demand an efficient conversion of dietary carbohydrate and fat into storage lipid. The efficiency of the adaptive response can be seen by comparing the rate of glucose oxidation to CO_2 and conversion to fatty acid, non-saponifiable lipid and glycogen by adipose tissue from meal-eating and nibbling rats. There is a sharp increase in each of these parameters attributable to meal-feeding.

The results of the C-1/C-6 ratio experiments further demonstrate the efficiency of the adaptive response. These data show a greater $^{14}CO_2$ production by adipose tissue

from glucose-1-^{14}C in meal-fed rats with a C-1/C-6 ratio of 9.03 as compared to a ratio of 3.87 for nibbling animals. These data add qualitative support to the concept that an increased functioning of the HMP shunt in adipose tissue is an important part of the adaptive response to meal-feeding.

The data obtained illustrate two distinct aspects of the adaptation induced by meal-feeding. The meal-eating regime apparently patterns the metabolic machinery of the fat cell, resulting in increased lipogenesis from glucose and acetate. Another aspect is the further enhancement of the rate of lipid synthesis noted in re-fed, meal-eating animals. This response is observed in rats given carbohydrate alone. The inverse relationship between the rate of CO_2 production from glucose and fatty acid synthesis from acetate suggests that the increased availability of metabolic intermediates (i.e., acetyl-CoA, NADPH) stimulate lipogenesis. The patterning induced by meal-eating may explain why substrate stimulation of fatty acid synthesis with time of re-feeding is noted in adipose tissue of meal-eating, but not of nibbling rats.

Study B

The adaptive response to meal-feeding appeared to be the result of the relatively large inflow of carbohydrate over the short, 2-hour daily feeding period. An increased activity of enzymes in pathways supporting lipogenesis in adipose tissue was therefore anticipated. The extent to which the specific substrate influenced the alteration of enzyme activity was also tested by feeding diets high in fat or high in carbohydrate. Glucose-U-^{14}C utilization and lipogenesis from acetate-^{14}C by adipose tissue were increased markedly as a result of meal-feeding a diet high in carbohydrate, whereas these processes were depressed sharply by the feeding of high fat diets. Of the five adipose tissue enzymes studied (glucose-6-phosphate dehydrogenase, NAD and NADP-malic dehydrogenases, isocitric dehydrogenase and 6-phosphogluconic dehydrogenase), only glucose-6-phosphate dehydrogenase and NADP-malic dehydrogenase (malic enzyme) were increased significantly by meal-feeding a high carbohydrate diet. Enzyme activity was depressed by feeding the high fat diet and no differences were noted between ad libitum and meal-fed rats. The activity of isocitric dehydrogenase was not affected by meal-feeding either of the diets. NAD-malic dehydrogenase activity was not increased significantly by meal-feeding, but the level of activity was far higher than that of other enzymes studied.

The increased activity of glucose-6-phosphate dehydrogenase could stimulate lipogenesis by increasing the level of NADPH for reductive synthesis of fatty acids. However, calculations show that NADPH generation via the hexose monophosphate pathway cannot totally support lipogenesis. The concerted action of NAD-malic dehydrogenase and malic enzyme could supply additional reduced NADP. The adaptability

of malic enzyme, coupled with the extremely high levels of NAD-malic dehydrogenase in adipose tissue, strongly supports the suggestion that transhydrogenation of NADH to NADP via the two malic dehydrogenase systems is an important source of NADPH.

The relative amounts of glucose-U-^{14}C incorporated into CO_2, glyceride-glycerol, fatty acid, non-saponifiable lipid and glycogen were increased markedly by meal-feeing a high carbohydrate diet. Meal-feeding the high fat diet did not stimulate the conversion of glucose to any of these intermediates, while severely depressing the total glucose utilized. These findings agree with the enzyme data showing a depression of enzyme activity in tissues of meal eaters consuming the high fat diet.

The effects of meal-feeding and dietary composition on the pattern of glucose utilization by adipose tissue are of interest. The percentage of total glucose incorporated into fatty acids was greater in high carbohydrate meal-fed rats, but lower in nibbling rats, while the percentage converted to glyceride-glycerol was greater in the nibblers. In the high fat-fed animals, there was a greater percentage conversion of glucose to glyceride-glycerol than noted in the high carbohydrate-fed rats. This is interpreted to mean that the small amounts of glucose available in the high fat-fed diet are shunted into the production of α-glycerophosphate required for triglyceride formation. In animals consuming a carbohydrate-free diet, the rate of α-glycerophosphate synthesis may limit triglyceride formation since, in adipose tissue, the glycerol formed from lipolysis is not re-utilized.

The differences in glucose conversion to α-glycerophosphate induced by meal-feeding and variations in dietary composition are closely reflected in the uptake of palmitate-1-^{14}C by adipose tissue. Uptake was greater in high carbohydrate-fed meal eaters and was depressed significantly by feeding the high fat diet. We are presently measuring the activity of α-glycerophosphate dehydrogenase in adipose tissue to determine if changes in fatty acid uptake and/or α-glycerophosphate formation from glucose are related to the activity of this enzyme.

The oxidation of palmitate-1-^{14}C by diaphragm was not changed by meal-feeding, while liver slices from meal-fed rats oxidized significantly greater amounts of the fatty acid. Oxidation of palmitate-1-^{14}C was also enhanced by ingestion of the high fat diet. In study A, we were unable to demonstrate any statistically significant difference in the ability of diaphragm or liver to oxidize fatty acids as a consequence of meal-feeding; however, the differences noted were in the same direction as observed in this study. These findings are in line with reports demonstrating that muscle has the capacity to oxidize large quantities of fatty acids. The increase in fatty acid oxidation by liver slices of high fat-fed rats may indicate an adaptation of one or more enzymes involved in the utilization of this substrate.

Periodicity of Eating (Cont'd.)

The lipase activities studied include the enzyme active at pH 8.5 and released from adipose tissue when incubated with heparin. This enzyme is generally considered to be lipoprotein lipase. The pH 6.8 active lipase is similar to the epinephrine sensitive enzyme. Although controversy exists concerning the significance of these enzymes, lipoprotein lipase is generally thought to hydrolyze glycerides prior to their uptake into adipose tissue, while the pH 6.8 enzyme is active in FFA release from adipose tissue. An increase in the pH 6.8 enzyme activity in the adipose tissue of meal-fed rats was anticipated since FFA release would provide the principal energy source during the period between meals. In animals meal-fed a high fat diet, and consequently depositing large quantities of preformed lipid, the activity of lipoprotein lipase might also be enhanced. Meal-eating did increase the activity of both lipases and the release of lipoprotein lipase by heparin. However, feeding a high fat diet depressed the activity of both the pH 8.5 and 6.8 enzymes. The significance of these data is not clear, particularly the lowered activity observed as a consequence of meal-feeding. However, lipoprotein lipase may not be essential for the uptake of preformed lipid or this enzyme may be present in excess and therefore may not be adaptive. Further study is necessary to elucidate the meaning of these results.

There has been considerable interest in the role of mitochondrial permeability as a factor in the control of lipogenesis. The synthesis of fatty acid from carbohydrate involves the extramitochondrial formation of pyruvate which would then be converted to acetyl-CoA by oxidative decarboxylation within the mitochondria. Since fatty acid synthesis is thought to occur in the cell cytoplasm, acetyl-CoA must be transferred out of the mitochondria and acetyl-CoA diffusion out of the mitochondria is two slow to meet the demands for rapid lipogenesis. Other possible mechanisms for circumventing the problem of mitochondrial impermeability would involve the cleavage of mitochondrial acetyl-CoA to acetate and CoASH and the diffusion of the acetate into the cytoplasm to be reactivated to acetyl-CoA by an acetate activating enzyme.

There is considerable experimental evidence for a third pathway involving the intramitochondrial formation of citrate. The citrate would leave the mitochondria and be cleaved to acetyl-CoA and oxaloacetate by citrate cleavage enzyme. Recently, Young et al. (Biochem. 3: 1687, 1964) have presented evidence suggesting a conversion of the oxaloacetate, formed by citrate cleavage, to pyruvate. This would couple the NAD and NADP-malic dehydrogenases extramitochondrially, with a resultant increase in cytoplasmic NADPH. The pyruvate formed would then enter the mitochondria for decarboxylation to acetyl-CoA. This pathway would require cytoplasmic NAD-malic dehydrogenase and malic enzyme as well as citrate cleavage enzyme. The levels of activity of NAD-malic dehydrogenase and malic enzyme were found to be much higher in the cytoplasmic fraction of adipose tissue cells. Meal-feeding a high carbohydrate diet greatly enhanced the levels of citrate cleavage enzyme. In addition, the greatest activity of NAD-malic dehydrogenase occurred in the soluble fraction of the fat cell. Malic enzyme has been demonstrated to be an extramitochondrial enzyme.

Periodicity of Eating (Cont'd.)

The present studies demonstrate citrate incorporation into fatty acid by isolated epididymal fat pads and a reduction of this incorporation by the addition of unlabeled acetate. The synthesis of fatty acid from α-ketoglutarate via a "reversal" of the Krebs cycle to citrate has been demonstrated. In this study, the problem of the pathway of lipogenesis was approached by using specifically labeled glutamate-^{14}C and aspartate-^{14}C. The former compound would enter the Krebs cycle as α-ketoglutarate and the latter as oxaloacetate.

Values for the incorporation of C-1, C-2 and C-5 of glutamate-^{14}C into fatty acids by adipose tissue demonstrate a reversal of the glutamate derived α-ketoglutarate to citrate and, subsequently, to acetyl-CoA for fatty acid synthesis. This pathway is significantly more active in adipose tissue from meal-eating than from nibbling animals. The pattern of lipogenesis from C-3 and C-4 of aspartate-^{14}C also fits the pathway proposed. Aspartate-4-^{14}C is oxidized to $^{14}CO_2$, but none of the activity is incorporated into lipid. The higher rate of incorporation of aspartate-3-^{14}C into fatty acids as compared to the C-2 or C-5 of glutamate-^{14}C suggests that the intramitochondrially formed oxaloacetate, and presumably citrate, is a much more significant precursor of cytoplasmic acetyl-CoA than is the citrate formed from a "reversal" of β-ketoglutarate through the Krebs cycle.

This suggestion was more fully studied by measuring lipogenesis from glutamate labeled in the C-2, C-5 or C-3 plus C-4 positions. The C-2 and C-5 of glutamate-^{14}C would only be incorporated into fatty acid by a "reversal" of α-ketoglutarate, while glutamate-3,4-^{14}C would be incorporated by both forward and backward reactions. Therefore, the difference between the incorporation of C-2 and C-4 and the C-3 plus C-4 of glutamate would be a measure of the forward activity of the Krebs cycle. The results of such an experiment indicate a greater activity of the forward reaction of α-ketoglutarate, accounting for almost 80% of the total glutamate-^{14}C found in fatty acids. The magnitude of both pathways was increased by meal-feeding.

The incorporation of glutamate carbon into lipid from the forward reaction was assumed to be mediated through citrate cleavage, although our data on this point are equivocal. However, the citrate cleavage enzyme is both active and adaptive in adipose tissue and citrate-1,5-^{14}C and glutamate-2-^{14}C and 5-^{14}C are incorporated into fatty acid. This is strong evidence favoring lipogenesis from oxaloacetate via citrate formation and citrate cleavage. These data further underline the importance of citrate and citrate cleavage enzyme in the supply of acetyl-CoA for lipid synthesis.

The labeling pattern observed in this study indicates that during periods of rapid lipogenesis, i.e., meal-feeding, substrates are shunted into lipid synthesis at the expense of the oxidative pathways. This is well demonstrated by the inverse relationship noted between the relative amounts of ^{14}C found in $^{14}CO_2$ and fatty acids.

Periodicity of Eating (Cont'd.)

A consideration of enzyme activities, cellular distribution of NAD and NADP-malic dehydrogenases and citrate cleavage enzyme, as well as the data on the incorporation of labeled substrates into lipid, strongly supports the proposed pathway of lipogenesis. In such a pathway, the role of citrate in the transfer of mitochondrially formed acetyl-CoA and the role of NAD and NADP-malic dehydrogenase are of prime importance. Such a pathway is self-priming in that citrate can stimulate acetyl-CoA carboxylase activity which is thought to be the rate limiting enzymatic step in fatty acid formation. Thus, the precursor, citrate, stimulates lipogenesis by affecting a rate limiting enzyme and the by-product of the cleavage of citrate is metabolized in a manner which provides essential reduced coenzymes.

Several enzymes at key points in the metabolic pathways of lipid metabolism may also respond to meal-feeding. We have preliminary evidence to indicate that liver β-hydroxybutyrate dehydrogenase activity is increased in meal-eating rats and suggestive evidence that α-glycerophosphate dehydrogenase and acetyl-CoA carboxylase activity may also be affected.

Summary and Conclusions:

Meal-eating (feeding a single 2-hour meal/day) results in enhanced lipogenesis in adipose tissue and liver of rats. The data obtained suggest that the increased rate of lipogenesis is related, at least partly, to an increase in the activities of glucose-6-phosphate dehydrogenase and NADP-malic dehydrogenase.

Data have been obtained supporting a pathway of lipogenesis in which pyruvate is oxidized to acetyl-CoA which condenses with oxaloacetate to form citrate within the mitochondria. The citrate then diffuses to the cytoplasm where, under the action of the citrate-cleavage enzyme, citrate is broken down to acetyl-CoA and oxaloacetate. The acetyl-CoA formed serves as a substrate for cytoplasmic fatty acid synthesis, while the oxaloacetate is reduced to malate by NAD-malic dehydrogenase which is then oxidatively decarboxylated to pyruvate by malic enzyme. The conversion of oxaloacetate to pyruvate occurs extramitochondrially and as a result of this conversion there is a transhydrogenation from NADH to NADP. The formed NADPH can be used to support cytoplasmic reductive synthesis.

Data were also obtained demonstrating that α-ketoglutarate can be "reversed" to citrate cytoplasmically; however, the citrate so formed only supplies ~ 20% of the total glutamate carbon incorporated into lipid. These data suggest that greater amounts of cytoplasmic citrate are formed from α-ketoglutarate via the "forward" reaction than via the "reverse" reaction.

Periodicity of Eating (Cont'd.)

Further studies to delineate the metabolic effects of the rate of food ingestion will be performed. However, to improve administrative procedures, this sub-task is to be terminated and the studies will be performed under the sub-task entitled "Nutritional and Metabolic Adaptations."

List of Publications:

1. Leveille, G. A. and R. W. Hanson. Metabolic studies in "meal eating" and "nibbling" rats and chicks. J. Colo. Wyo. Acad. Sci. 5: 23, 1964 (abstract).

2. _____ Influence of a high fat diet on the adaptations induced in rats by a single daily meal. Fed. Proc. 24: 438, 1965 (abstract).

3. _____ Influence of periodicity of eating in the chicken. Am. J. Physiol. (in press).

4. _____ Adaptive changes in enzyme activity and metabolic pathways in adipose tissue from meal-fed rats. J. Lipid Res. (in press).

5. _____ Influence of periodicity of eating on lipid and carbohydrate metabolism in the rat. Can. J. Physiol. and Pharmacol. (in press).

6. _____ Enzymatic adaptations in adipose tissue induced by meal-feeding. J. Colo. Wyo. Acad. Sci. (abstract)(in press).

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 RPT	6. SECURITY WRK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71P 07 030

10b. PRIOR NUMBER/CODE: None

11. TITLE:
(U) Performance Physiology (06)

12. SCIENTIFIC OR TECH. AREA: 005900 Environmental Bio
012900 Physiology, 016200 Stress Phy

13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
06 63	NA	OTHER | DA

16. PROCURE. METHOD: C. In-House

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

18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	2	50
CURRENT FY 66	3	66

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INVESTIGATORS PRINCIPAL: Vogel, J.A.
ASSOCIATE: Hansen, J.E. LtCol, Hannon, J.P.
Consolazio, C.F.
TEL: 303 366 5311 X26112 TYPE: DA

21. TECHNOLOGY UTILIZATION: Human Job Performance

22. COORDINATION: None

23. KEYWORDS: Exercise, Exhaustion, Work, Fatigue, Performance, Antifatigue

24. (U) Tech Objective: For over a decade this laboratory has been interested in exercise physiology, particularly physical performance as related to environment. With an increased military interest in combat performance, a program of evaluating possible anti-fatigue agents was initiated. Drug screening has emphasized, however, the lack of basic understanding of the physiology and biochemistry of exercise, fatigue and exhaustion. The current objective is to develop a better understanding of the basic aspects of physical performance.

25. (U) Approach: Chronically instrumentized dogs are used to characterize the physiological and biochemical changes that occur with the onset of fatigue and exhaustion in treadmill exercise. Treadmill exercised rats will be employed to study the nature of long term exercise stress, particularly at the tissue and cell level. Specific problems of the physiology of exercise are to be studied with the catheterized human working on a bicycle ergometer. Environmental conditions, i.e. temperature, altitude and food deprivation will be studied as imposing factors on physical performance in man to help elucidate adaptive mechanisms related to fatigue and exhaustion.

26. (U) Progress: (Oct 64-Jun 65) Bicycle ergometer performance tests under high altitude conditions and as a part of nutrition field studies have been completed and are undergoing analysis. Further refinement in chronic catheterization and instrumentation has been achieved in dogs. A modified fishline acute catheterization technique for humans has been developed and improvements made in the respiratory gas analysis for bicycle work studies.

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
N/A	N/A

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) OVER

ABSTRACT

FATIGUE AND EXERCISE PHYSIOLOGY (FY 1965)
PERFORMANCE PHYSIOLOGY (FY 1966)

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 all ages of combat soldiers. Methodology developed includes the continuous measurement of heart rate, ventilation rate and volume, oxygen consumption and carbon dioxide output during stable or increasing and exhaustive workloads on the bicycle ergometer in humans; and 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. Work is as of yet incomplete on studies relating age and body composition to bicycle work performance. Data, including metabolic rates before, during and after exhaustive exercise, K^{40} counting, body fat estimation from water displacement and skinfold measurements are being made on laboratory personnel. Further improvements have been made in the development of a respiratory face mask for exercising humans as well as streamlining the respiratory analysis equipment and adapting its output for an RCA digital computer. Chronic instrumentation of the treadmill exercising dog is still proceeding. Major difference in the physiological and biochemical responses between man and dog may prevent many extrapolations between these two species.

BODY OF REPORT

FATIGUE AND EXERCISE PHYSIOLOGY (FY 1965)
PERFORMANCE PHYSIOLOGY (FY 1966)

Study No. 2 - Maximum and Submaximum Performance in Relation to Age and Body Composition. I. Laboratory Personnel

Authors: C. Frank Consolazio, Richard A. Nelson, LeRoy O. Matoush, Harry J. Krzywicki and John E. Canham.

Description:

The preliminary study was conducted on military and civilian personnel from the U.S. Army Medical Research and Nutrition Laboratory of all ages and was divided into two phases, a physical or anthropometric and physiological phase. Case histories were taken on each man prior to the physical examinations, which included blood pressure, nude body weights to the nearest 20 gm and heights to the nearest 0.2 centimeters. Skinfold measurements were taken at 4 different body sites.

Physiological measurements included a maximum and submaximum performance test on the bicycle ergometer. Metabolic 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 and K^{40} for lean body mass (includes K in bone as a fluid electrolyte) (using a total body counter). Urine samples were also collected for the measurement of creatinine excretion.

As a precaution EKG's were done on every man over 35 years of age.

Progress:

No progress in analysis and correlation of the accumulated data during the past year due to pressing high altitude and ration studies.

FATIGUE AND EXERCISE PHYSIOLOGY (FY 1965)
PERFORMANCE PHYSIOLOGY (FY 1966)

Study No. 3 - (Bioenergetics Division) Development and Application of a Continuous Direct Reading Instrument for Measuring Oxygen Consumption During Maximal and Submaximal Exercise

Authors: Richard A. Nelson, C. Frank Consolazio, LeRoy O. Matoush and Bert M. Tolbert.

Description:

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

Progress:

The continuous oxygen consumption analyzer has been in almost constant use during the preceding year on the high altitude studies. It has been shipped throughout the United States, many times both by air freight and by truck and has proved to be very reliable. Trouble developed in the infra-red carbon dioxide analyzer in the form of distorted output curve. This has pointed out the need for a regular detailed check of the output curves rather than only a "zero" and "span" check as has been done in the past.

The face mask for measuring energy metabolism during maximal performance, which was developed for the analyzer (The Monagham Company, 500 Alcott Street, Denver, Colorado 80204), has been improved with a different type of valve system. It has proven to be very dependable.

Plans:

Based on the experience with the present prototype model, a new instrument is being built which should be more transportable and easier to operate.

A parallel gas stream sampling system will be used rather than the present series type system. All the output signals will be recorded on a single 12 channel potentiometric recorder which will allow a more compact instrument. The components will be mounted in a desk height aluminum console for lighter weight and greater maneuverability.

FATIGUE AND EXERCISE PHYSIOLOGY (FY 1965)
PERFORMANCE PHYSIOLOGY (FY 1966)

Study No. 3 (Cont'd)

Present plans call for the digital conversion of the output signals to allow automatic data processing of the energy expenditure measurements. This will be accomplished by scanning (multiplexing) the signals, converting them in a digital volt meter and punching them on paper tape in a format for entering the RCA 301 computer. A program for handling mean signals has been written and is presently in use. The addition of a program for obtaining means from the continuous data will allow use of the data converting system for automatic handling of the data.

Summary and Conclusions:

The continuous respiratory gas analyzer has been used extensively with a great deal of reliability. Present plans call for the construction of a new updated instrument based on the experience gained with the present prototype model. Digital data conversion equipment will be added to the instrument to allow automatic data processing in the RCA 301 computer.

Publications:

In preparation.

FATIGUE AND EXERCISE PHYSIOLOGY (FY 1965)
PERFORMANCE PHYSIOLOGY (FY 1966)

Study No. 6 - Study and Treatment of Acute Physical Fatigue in the Treadmill Exercised Dog

Authors: James A. Vogel and John P. Hannon

Description:

In attempting to understand the basic physiological and biochemical factors underlying performance ability and fatigue, the treadmill exercising dog has been selected as an experimental animal situation to investigate this area at the intact system level. Emphasis has been placed upon instrumentation which will enable many factors to be recorded and analyzed simultaneously while the exercise is actually being performed with the least amount of interference to the animal.

The first phase of this work is to complete instrumentation development for the dog and to characterize, physiologically and biochemically, the nature of physical fatigue and exhaustion in the treadmill exercised dog. This will be followed by more detailed studies on particular aspects which appear to be key limiting factors in performance.

Progress:

A number of disadvantages in the use of the dog in the study of performance have come to light. Persistent alkalemia rather than acidemia with exercise occurs in the dogs as a result of effective carbon dioxide blow-off and delayed onset of anaerobic metabolism and lactic acid accumulation. The latter phenomena in itself is of major significance when contrasting performance between man and dog. The more labile, contractible spleen of the dog as compared to man may prove to have a considerable influence on cardiovascular responses in some cases.

Instrumentation-wise, collection of respiratory gases is definitely a major problem when employing treadmill exercising dogs. Progress is being made in this regard with a forced flow mask system. Masks are now being constructed which will avoid covering the eyes, a disadvantage of previous designs. Progress has been slow on developing techniques for chronic implantation of electromagnetic aortic flowmeters. Various coating materials are presently being tried to prevent tissue reaction to the electrical lead wires.

FATIGUE AND EXERCISE PHYSIOLOGY (FY 1965)
PERFORMANCE PHYSIOLOGY (FY 1966)

Study No. 6 (Cont'd)

Summary and Conclusions:

Instrumentation development to facilitate continuous physiological measurements during exhaustive exercise is till underway. Preliminary work has indicated that many significant differences exist between man and dog which necessitate that care be taken in attempting extrapolations between these species.

List of Publications:

None

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RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
			OA 6312	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 65	A. NEW	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 62503015 ARPA 5720
61145011 3A014501B71R 05 080

10b. PRIOR NUMBER/CODE: None

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

12. SCIENTIFIC OR TECH. AREA: 016200 Stress Physiol.
005900 Env. Biology, 013400 Phychology

13. START DATE: 08 63

14. CRIT. COMPL. DATE: NA

15. FUNDING AGENCY: OTHER | DA

16. PROCURE. METHOD: C. In-House

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

18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	6	281
CURRENT FY 66	8	375

19. GOV'T LAB/INSTALLATION/ACTIVITY
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ASSOCIATE: Consolazio, C.F., Hannon, J.P.
Evans, W.O., Capt
TEL: 303 366 5311 x26112 TYPE: DA

21. TECHNOLOGY UTILIZATION: Mountaineering; Mining

22. COORDINATION: None

23. KEYWORDS: Anoxia, 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 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 5,200 feet. B. Extensively measure pulmonary, cardiovascular and metabolic changes during rest, mild, moderate and exhausting work and recovery in sixteen sea level soldiers before, during and after four weeks at altitude, as affected by physical conditioning and abruptness of ascent; symptoms and psychometrics; food and water balance and other parameters. C. Measure similar findings in eight normal female students at sea level and during nine weeks at 14,100 feet. D. Compare double blind psychometrics and performance measures in volunteers exposed for 48 hours in chambers to high altitude and given placebo and one of two levels of codeine-amphetamine mixture. E. Measure physiology of other mammals in chamber and altitude environments in multiple experiments. F. Participate in field study with multiple performance measurements of actual military tasks.

(U) Progress: (Aug 63-Jun 65) Data Collection: (A) Feb - Apr 1964; (B) Aug - Nov 1964; (C) May - Aug 1965; (E) Jan 1964 on - Analyses still underway. Symptoms due to 11,400 and 14,100 feet severe for one - five days but strikingly lessened by intermediate altitude exposure. Maximum oxygen consumption decreased 10 - 15% from sea level without improvement in three - four weeks. Fed. Proc. 24:215, 1965.

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
N/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 36 identical to NASA Form 1122) OVER

ABSTRACT

HIGH ALTITUDE STUDIES

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 rate of acclimatization; to investigate the physiology, biochemistry and pharmacology of the affected organ systems; and to ascertain how to minimize the decrements by selection, conditioning, previous environmental exposure, nutrition, drugs, or other variables. Animal species are also being studied at high altitude. Acute mountain sickness is a more serious military problem than is the small reduction in maximal work capacity. Pistol firing and a measure of quick energy mobilization were also initially significantly altered at 14,100 feet.

BODY OF REPORT

HIGH ALTITUDE STUDIES (FY 1965)

Description:

The military necessity for physiologic and behavioral studies at high terrestrial altitude became apparent with the Chinese invasion of India. Several members of this laboratory, because of their natural interests, the geographical location of the laboratory at Colorado and the support of the Army Research Office and Medical Research and Development Command, began working on this problem in 1963. 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 rate of acclimatization; to investigate the physiology, biochemistry and pharmacology of the organs 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. 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.

Progress:

Data collection on a pilot study utilizing fourteen soldiers and six staff members as subjects was accomplished at sea level (USARIEM, Natick, Massachusetts), 5,200 feet (USAMRNL, Denver, Colorado) and 11,400 feet (Climax, Colorado) in February to April of 1964. Data collection on a major human study utilizing twenty-four sea level soldiers was accomplished at sea level, Denver, Climax sites and at 14,100 (Pike's Peak, Colorado) in August to November 1964. The studies include repeated measurements of many physiologic and behavioral parameters. Calculation of the raw data is nearly complete. Correlation and interpretation of the data continues. Definitive studies have been done on rats and to a lesser extent on dogs, measuring changes in biochemical or physiologic parameters often with the use of radioisotopes at 5,200 feet; 11,400 feet or 14,100 feet actual altitude or at simulated altitudes with gas mixtures. Two human studies have been planned for

the fall of 1965. One will study the effect of codeine and amphetemine on the symptomatology and performance of normal human volunteers at a chamber altitude of approximately 15,00 feet with placebo chamber altitude. The second study will measure the turnover of radioactively tagged thyroxine and albumin during actual exposure to 14,100 feet. The study of acclimatization over the nine-week period in normal female volunteers at 14,000 feet with particular emphasis on symptomatology, resting electrolyte changes, blood volumes and vectorcardiographic changes is in progress.

Summary and Conclusions:

1. Normal sea level soldiers brought abruptly from sea level to 14,100 feet become significantly ill within twenty-four hours. The illness is characterized primarily by severe generalized or localized headache, particularly severe in the morning hours, severe insomnia and nausea or vomiting. The symptoms generally subside within several days to one week. The symptoms are less severe in those that underwent a physical conditioning program prior to coming to altitude and continued this program after arrival. Normal sea level soldiers coming gradually to 14,000 feet over a two-week period did not have the above symptoms. Both groups had shortness of breath, palpitation, and dryness of the mucous membranes, persisting throughout the stay at high altitude. Acute high altitude pulmonary edema was not recognized in the sixteen subjects, but occurred in two of twenty staff members brought to 14,100 feet.

2. The oxygen uptake for a given amount of sub-maximal work is approximately equal at all altitudes measured. The total volume of air ventilated (BTPS) gradually increases over a two-week period at higher altitudes. There is an abrupt decline in ventilation on arrival at altitude followed by an increase over the next two-weeks period. Upon return to sea level, the immediate elevation of ventilation over pre-altitude exposure, expressed both BTPS and STPD, gradually subsides. The true oxygen or percent of oxygen extracted from the ventilated air changes reciprocally to the volume of air ventilated STPD.

3. In normal young men, maximal oxygen uptake and maximal work on the bicycle ergometer fall approximately fifteen to twenty percent with an abrupt or gradual change from sea level to 14,100 feet. Neither changes significantly for the following two weeks. Other aspects of performance of greater military significance may fall more or less and may recover in less than two weeks at altitude. For example, the accuracy of pistol fire fell and the speed of fire increased early at high altitude. Chinning, a measure of general strength was unaffected by altitude per se. Medicine

ball putting, a measurement of quick energy mobilization was significantly decreased in the first week of high altitude and returned to normal values within two weeks. The greatest decrease occurred in those subjects that went abruptly to altitude.

4. The partial pressure of oxygen in the trachea falls from approximately 150 mm. of mercury at sea level to 85 mm. of mercury at 14,100 feet. The most important single factor preventing an equiv. fall in oxygen saturation of blood and in maximal oxygen consumption is the shape of the oxyhemoglobin dissociation curve. On ascent to high altitude ventilation BTPS, hematocrit, pH, maximal stroke volume, and maximal cardiac output promptly rise, whereas the buffering capacity of the blood, arterial PCO_2 and minimally attainable mixed venous saturation promptly fall. After two weeks at high altitude there is a further rise in hematocrit, ventilation, arterial P_{O_2}, arterial saturation with the decline in maximum pulse, stroke volume, cardiac output and buffering capacity of the blood.

5. The electrocardiographic changes at high altitude include a transient negativity of the T waves in the right precordial leads, a transient decrease in QRS voltage, and a persistent and progressive shift posterior and rightwards of the QRS axis. These changes are not explainable on the basis of a change of heart position due to increased ventilation.

6. There is an increase in total serum protein, thyroxine-binding globulin, protein-bound iodine and serum free thyroxine which is maximal approximately one week after arrival at high altitude. The findings in humans imply an initial decrease in the plasma water and a marked diminution of peripheral thyroxine degradation or an increase in thyroid secretion of thyroxine.

7. On the basis of two administrations of ^{14}C labeled ascorbic acid to eleven individuals, it is concluded that the vitamin C requirement of young adult men is not increased by the stress of 14,100 feet altitude.

8. There is no striking change in the dietary preferences of soldiers at 11,400 or 14,100 feet, although anorexia and weight loss is evident at the latter altitude.

9. Rats were maintained in a hypoxic state, using gas mixtures or actual high altitude exposure, on a low or normal iodine diet and injected with $^{131}I^-$. Hypoxic rats on low iodine diets had reduced thyroid weights. Histological and autoradiograph examinations suggested a "resting gland". Thyroidal uptake of $^{131}I^-$, protein-bound iodine, protein-bound-131-iodine and a conversion ratio of di-131-iodotyrosine to thyroxine were reduced in the hypoxic animals.

HIGH ALTITUDE STUDIES (Cont'd)

10. Rats at high altitude injected intraperitoneally with ^{14}C glutamic acid and ^{14}C alinine expired 20% more tagged CO_2 and incorporated 18% less tagged CO_2 into liver proteins. Carbon 14 labeled lysine and methionine values were unchanged. The above results occurred after twenty-four hour exposure to hypoxia. Values were normal after five weeks of hypoxia.

11. Rats maintained at 11,400 feet on high-fat, high-carbohydrate, or high-protein diet gained weight less rapidly than their controls at 5,200 feet. At both altitudes the greatest weight gain occurred in the animals on a high-fat diet.

12. Dogs did not run as well on a treadmill at 11,400 feet as they did before or after 5,200 feet. Cardiac output was elevated both at rest and exercise initially at 11,400 feet with a gradual decline to their normal 5,200 feet values.

13. Fractional distribution of tissue blood flow was determined in thirty tissues of acutely and chronically exposed rats at 5,200 and 14,100 feet by the rubidium-86 dilution technique. High altitude was associated with differential increases in blood flow to the duodenum, jejunum, liver, myocardium, salivary gland, pancreas, adrenal and tail skin; differential decreases were observed in the colon, thyroid and foot skin.

Publications:

1. Abstracts Presented and Oral Presentations:

(a) Cardiovascular physiology of man during maximal work at high altitude. J. A. Vogel and J. E. Hansen, Colorado Academy of Science, April 1965.

(b) Cardiovascular Metabolic and Nutritional Studies in Animals at 5,200 feet and 11,400 feet Altitude. J. A. Vogel, J. P. Hannon and K. S. K. Chinn, Colorado-Wyoming Academy of Science, May 1964.

(c) Cardiovascular responses of man to maximal work at sea level and 14,110 feet. J. A. Vogel, J. E. Hansen and J. P. Hannon. Federation of the American Society for Experimental Biology, p. 215, April 1965.

(e) Human physiological and nutritional comparisons at sea level and altitudes. J. E. Hansen, C. F. Consolazio, R. A. Nelson and L. O. Matoush. Colorado-Wyoming Academy of Science, May 1964.

HIGH ALTITUDE STUDIES (Cont'd)

(f) Physiological changes in high altitude operations. J. E. Hansen and C. F. Consolazio. 114th Annual Convention of the AMA, 22 June 1965.

2. Abstracts only:

The alveolar-arterial oxygen gradient during high altitude exercise. J. E. Hansen, G. P. Stelter, J. A. Vogel, and C. F. Consolazio. Clinical Research, 13: 348, April 1965.

3. Abstracts Submitted:

(a) Hemodynamic alterations in humans and animals during chronic high altitude exposure. J. A. Vogel, J. P. Hannon and J. E. Hansen. Symposia on Environmental Physiology, Kyoto, Japan, September 1965.

(b) Circulatory and blood gas responses of exercising dogs at high altitude. J. A. Vogel and J. P. Hannon, American Physiological Society, Fall Meeting, August 1965.

(c) Factors influencing ECG changes at high altitude. C. W. Harris, 38th Scientific Seminar of the American Heart Association, 1965.

4. Papers Submitted:

(a) Electrocardiographic changes in man at rest and with exercise during exposure to high altitude. C. W. Harris and J. E. Hansen submitted to the American Heart Journal, May 1965.

(b) Problems of troop performance at high altitudes. W. O. Evans and J. E. Hansen. Accepted for publication in the magazine, Army.

5. Oral only:

(a) Problems resulting from unusual altitudes, heat and cold. J. E. Hansen and C. F. Consolazio. 17th Annual Symposium on Pulmonary Diseases, Fitzsimons General Hospital, 22 September 1964, moderator and panelist.

(b) Cardiovascular responses of man and dog to high altitude exposure. J. A. Vogel. Colorado Interschool Physiology Seminar, May 1965.

(c) Medical problems at high altitude. J. P. Hannon, C. W. Harris, G. P. Stelter and J. A. Vogel. Aurora and Adams County Medical Society, May 1965 - moderator and panelists.

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RESEARCH AND TECHNOLOGY RESUME	1.	2. GOVT ACCESSION	3. AGENCY ACCESSION DA OA 6313	REPORT CONTROL SYMBOL CSCRD 103
4. DATE OF RESUME 01 07 65	5. KIND OF RESUME A. NEW	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 3A014501B71P 01 055

10b. PRIOR NUMBER/CODE 61145011 3A014501B71P 03 01

11. TITLE:
(U) Lipids and Related Compounds (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochemistry; 002600 Biology; 012900 Physiology	07 60	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT a. DATE: b. NUMBER: c. TYPE: NA d. AMOUNT:	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. In-House		PRIOR FY 65	1	46
		CURRENT FY 66	1	52

19. GOV'T LAB/INSTALLATION/ACTIVITY
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21. TECHNOLOGY UTILIZATION
NA

22. COORDINATION

23. KEYWORDS: Lipids, bile acids, cholesterol, lipoproteins, phospholipids, fatty acids, triglycerides

24. (U) Tech Objective:

Develop techniques for the study of lipid metabolism. To delineate pathways of lipid metabolism which will enable a better understanding of their role in health and disease.

25. (U) Approach:
To study various parameters of lipid metabolism, including sterol metabolism, in experimental animals and ultimately to extend such studies to man.

(U) Progress: (Oct 64-June 65)
1. Dietary lithocholic acid, which had previously been demonstrated to induce a biliary hyperplasia, has been shown to increase cholesterol and bile acid synthesis and to increase the half-life of circulating cholesterol. Preliminary evidence suggests that entry of circulating lipids into liver cells is impaired by dietary lithocholic acid.

2. Dietary pectin has been shown to lower liver cholesterol levels in cholesterol-fed rats by inhibiting the absorption and, consequently, enterohepatic recirculation of bile acids.

27. COMMUNICATIONS SECURITY ☐ a. COMSEC OR COMSEC RELATED ☒ b. NOT RELATED	28.	29. OSD CODE BR	30. BUDGET CODE 1
31. MISSION OBJECTIVE NA		32. PARTICIPATION 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) OVER

ABSTRACT

LIPIDS AND RELATED COMPOUNDS

a. Investigations during this reporting period have been concerned with 1) the metabolic effects of dietary lithocholic acid, 2) the mechanism by which dietary pectin alters serum cholesterol levels, 3) the relationship of ketone bodies to lipid and carbohydrate metabolism in rat epididymal fat pads, 4) NAD biosynthesis by adipose tissue in vitro.

b. The elevated plasma lipid levels observed in chicks ingesting lithocholic acid appeared to be due to an enhanced hepatic lipogenesis. The normal homeostatic mechanisms controlling lipogenesis are seemingly not functioning. Preliminary evidence suggests that the entry of lipids into the hepatocyte is impaired and, consequently, normal feed-back control does not occur. The rate at which the biochemical and histological changes take place has also been studied. Biochemical changes are evident as early as 2-3 days following the initiation of dietary supplementation of lithocholic acid while clear histological changes are seen after 4-5 days.

c. Dietary pectin has been shown to depress plasma cholesterol levels of cholesterol-fed rats by depressing normal enterohepatic circulation of bile acids. Intestinal absorption of bile acids across a concentration gradient is depressed approximately 50% by pectin.

d. Studies of ketone body metabolism have shown an influence of ketone bodies on carbohydrate metabolism in adipose tissue. The conversion of β-hydroxybutyrate to acetoacetate appears to be an important point of control in the metabolism of ketone bodies. Data obtained suggest that NADPH generation is a factor determining the rate of lipogenesis from ketone bodies. Acetoacetate influences the utilization of glucose-U-^{14}C and triglyceride synthesis by adipose tissue in vitro.

e. Adipose tissue has been shown to synthesize NAD from nicotinamide-1-^{14}C or nicotinic acid-1-^{14}C plus ^{32}P. The ribose moiety of NAD is derived from glucose via the hexose monophosphate pathway. The level and rate of synthesis of NAD in rat adipose tissue are increased by re-feeding following a fast.

BODY OF REPORT

LIPIDS AND RELATED COMPOUNDS

Description:

Disease states involving lipid metabolism can in many cases be related to alterations or failure of homeostatic control mechanisms. Consequently, studies under this sub-task are designed to evaluate the dietary control of lipid metabolism as well as mechanisms involved in the control of this process.

Progress:

Dietary Lithocholic Acid and Lipid Metabolism

Studies previously reported demonstrated that the ingestion of diets containing 0.2% lithocholic acid by chicks resulted in elevated plasma lipid levels, increased size of the liver and a marked proliferation of bile ductules. The elevated plasma cholesterol levels resulted from a decrease in the rate of cholesterol turnover and an increase in hepatic synthesis. Investigations performed during the past year have shown a greater rate of glucose utilization by liver slices from lithocholic acid-fed as compared with normal chicks. The increase in glucose utilization appears to be via the hexose monophosphate pathway. This is supported by increased oxidation of glucose-1-^{14}C to $^{14}CO_2$ and elevated glucose-6-phosphate dehydrogenase activity in the liver of lithocholic acid-fed chicks.

Preliminary evidence also suggests that the plasma cholesterol of lithocholic acid-fed chicks does not readily enter the liver cell. Mice injected intravenously with serum from lithocholic acid-fed chicks show no inhibition of cholesterol synthesis from acetate-1-^{14}C while mice injected with serum from cholesterol-fed chicks do. The reasons for the lack of effect of cholesterol in the plasma of lithocholic acid-fed chicks are not clear and are presently under investigation.

Hypocholesterolemic Effect of Pectin

Dietary pectin has been shown to depress serum cholesterol in man and liver and serum cholesterol levels in cholesterol-fed rats. However, the mechanism by which pectin exerts this effect was obscure. Experiments conducted during this reporting period have shown that (1) the fecal excretion of bile acids is increased by pectin ingestion, (2) cholesterol absorption is decreased by dietary supplements of pectin; however, the decrease in absorption is not sufficient to explain the hypocholesterolemia observed, (3) feeding sulfa drugs does not influence the effects of pectin, and (4) in vitro absorption of bile acids is decreased approximately 50% by dietary pectin. These data are interpreted to indicate that dietary pectin acts by decreasing the enterohepatic circulation of bile acids and, consequently, increasing the rate of

catabolism of cholesterol to bile acids.

Ketone Body Metabolism by Adipose Tissue

The metabolism of ketone bodies by epididymal fat pads and its effect on the metabolism of carbohydrate and lipid in this tissue were studied.

Ketone bodies are oxidized to CO_2 and converted to fatty acid by adipose tissue. The points of control of ketone body metabolism were explored and data were obtained implicating the reaction converting β-hydroxybutyrate to acetoacetate. This reaction was shown to be influenced by nicotinamide in vitro, presumably by increasing the intracellular levels of NAD, and also by succinate. The synthesis of fatty acids from β-hydroxybutyrate was enhanced by glucose plus insulin and various citric acid cycle intermediates. An association between NADPH generation and lipogenesis from ketone bodies is indicated.

Ketone bodies affected the utilization of glucose by adipose tissue in vitro. Glucose-U-^{14}C conversion to $^{14}CO_2$, fatty acid-^{14}C and glycogen-^{14}C was increased, while α-glycerophosphate-^{14}C formation was decreased in the presence of acetoacetate. As a consequence of this reduction of α-glycerophosphate formation from glucose, there was a reduction in triglyceride synthesis from palmitate-1-^{14}C by adipose tissue. The increase in $^{14}CO_2$ output from glucose-^{14}C was attributable to an increased activity of the hexose monophosphate shunt.

NAD (Nicotinamide Adenine Dinucleotide) Biosynthesis by Adipose Tissue

Previous work from this laboratory on the effect of nicotinamide on NAD concentrations in adipose tissue suggested that this tissue synthesizes the coenzyme. The importance of pyridine nucleotide coenzymes in the control of metabolic processes, most notably lipogenesis by adipose tissue, is well established. However, there is no information concerning those factors which influence the synthesis of the coenzyme itself. This work was designed to elucidate those factors.

Work to date has indicated that NAD is synthesized by adipose tissue from nicotinamide-1-^{14}C or nicotinic-1-^{14}C acid plus ^{32}P. The presence of both ^{32}P and ^{14}C in the NAD molecule in a ratio of 2/1 is evidence for the de novo synthesis of NAD rather than an exchange of nicotinamide into preformed NAD via the enzyme NADase. Glucose is also incorporated into the ribose moiety of NAD through the hexose monophosphate pathway. Evidence for this was gained by measuring the radioactivity found in NAD after incubation of adipose tissue with glucose-1-^{14}C or glucose-6-^{14}C. Glucose-6-^{14}C was incorporated at a rate 50 times higher than glucose-1-^{14}C, a finding consistent with the formation of ribose-5-phosphate via the hexose monophosphate pathway. In "superlipogenesic"

rats (48-hour fast followed by 48-hour re-feeding), both the concentration of intracellular NAD and the newly synthesized NAD in epididymal fat pads was markedly increased when compared to fat tissue from normal animals. Work is continuing to further clarify the role of NAD biosynthesis as well as factors which control this synthesis in adipose tissue.

Summary:

The lipemia observed in lithocholic acid-fed chicks appears to be due to an enhanced lipogenesis. Preliminary evidence suggests that the cellular permeability of liver to lipids is impaired and, consequently, normal feedback control is altered.

The hypocholesterolemic effect of dietary pectin in cholesterol-fed rats appears to be the result primarily of an impaired enterohepatic circulation of bile acids and secondarily by depressing cholesterol absorption.

The conversion of β-hydroxybutyrate to acetoacetate appears to be an important point in the control of ketone body metabolism in adipose tissue. The rate of lipogenesis from ketone bodies is apparently determined by the rate of NADPH generation. The pattern of glucose utilization by adipose tissue is influenced by acetoacetate.

Adipose tissue has also been shown to be capable of synthesizing NAD from nicotinamide-1-^{14}C or nicotinic acid-1-^{14}C and 32 P.

Publications:

1. Goad, W. C. and G.A. Leveille. Lipid metabolism in lithocholic acid fed chicks. J. Colo. Wyo. Acad. Sci., 5: 17, 1964 (abstract).

2. Leveille, G. A. Relationship of nutrition to cholesterol metabolism and atherosclerosis. J. Colo.-Wyo. Acad. Sci., 5:20, 1964 (abstract).

3. Leveille, G. A. and H. E. Sauberlich. Plasma and liver lipids of mice as influenced by dietary protein and sulfur-containing amino acids. J. Nutrition, 84:10, 1964.

4. Leveille, G. A. and H. E. Sauberlich. Relative distribution of cholesterol in plasma and liver compartments of chicks fed different fatty acids. Proc. Soc. Exp. Biol. Med., 117: 653, 1964.

5. Hanson, R. W. The interrelationship of ketone body metabolism and glucose utilization by adipose tissue *in vitro*. Arch. Biochem. Biophys, 109: 98, 1965.

6. Hanson, R. W. Influence of insulin and ketone bodies on glucose metabolism by adipose tissue. J. Colo.-Wyo. Acad. Sci., 5: 23, 1964 (abstract).

7. Hanson, R. W., Z. Z. Ziporin and H. E. Sauberlich. Ketone body metabolism by adipose tissue in vitro. Fed. Proc. 24: 1718, 1965.

8. Hanson, R. W., and Z. Z. Ziporin. NAD biosynthesis by adipose tissue. J. Colo.-Wyo. Acad. Sci. (in press).

9. Goad, W. C. and G. A. Leveille. Metabolic effects of lithocholic acid. J. Colo. -Wyo. Acad. Sci. (in press).

10. Hanson, R. W. and Z. Z. Ziporin. Factors influencing the utilization of ketone bodies by adipose tissue in vitro. J. Lipid Res. (accepted for publication).

11. Leveille, G. A. and H. E. Sauberlich. Mechanism of the cholesterol depressing effect of pectin in the cholesterol-fed rat. J. Nutrition (to be submitted).

12. Leveille, G. A. and D. G. Fairchild. Lack of effect of dietary chenodeoxycholic acid on plasma and liver lipids and the ductular cell reaction in the chick. Poultry Sci. (to be submitted).

13. Leveille, G. A., N. W. King, H. E. Sauberlich and D. G. Fairchild. Induction and regression of biochemical and morphological changes induced by dietary lithocholic acid in chickens (in preparation).

14. Leveille, G. A., W. C. Goad and H. E. Sauberlich. Influence of dietary lithocholic acid on fatty acid synthesis, glucose utilization and cholesterol turnover in the chick (in preparation).

15. Ziporin, Z. Z. and R. W. Hanson. A chromatographic procedure for the separation and detection of pyridine nucleotides and related compounds from tissue extracts (in preparation).

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6314	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71P 01 056

10b. PRIOR NUMBER/CODE: 61145011 3A014501B71P 03 02

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

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002300 Biochem.; 003500 Clin. Med.	01 64	NA	OTHER | DA

16. PROCURE. METHOD	17. CONTRACT/GRANT			18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
	a. NUMBER:	a. TYPE:	d. AMOUNT:			
C. In-House		NA		PRIOR FY 65	2	51
				CURRENT FY 66	2	56

19. GOV'T LAB/INSTALLATION/ACTIVITY
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20. PERFORMING ORGANIZATION
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INVESTIGATORS PRINCIPAL: Ziporin, Z. Z.
ASSOCIATE: Kinnamon, Capt. K. E.
TEL: 303 366 5311 X 24214 TYPE: DA

21. TECHNOLOGY UTILIZATION: Clinical medicine, nutrition, orthopedic surgery, orthopedics, hematology

22. COORDINATION: None

23. KEYWORDS: Calcification, calcium, calcium metabolism disorders, calcium dietary, calcinosis, bone and bones, vitamin D, phosphorus metabolism disorders, copper, molybdenum, zinc, iron metabolism

24. (U) Tech Objective: Study of copper, molybdenum, zinc and iron for trace mineral interrelationship as they affect growth and blood elements. Study of calcium and phosphorus metabolism in relation to calcification of cartilage, especially as they are related to fracture healing and bone disorders such as osteoathritis and calcinoses.

(U) Approach: Trace mineral studies-Pregnant and non-pregnant female rats are fed high and low levels of minerals in various combinations. Tissue distribution in various organs and excretions are measured.
Calcium and phosphorus studies-Cartilages from rachitic and healing rachitic rats are assayed for enzyme activities. These studies are performed on extracts made from the epiphyseal and metaphyseal cartilages, and assayed for specific enzyme activities.

(U) Progress: (Oct 64-Jun 65) Trace mineral-Studies on the effects of inorganic sulfate on copper, molybdenum and zinc have been completed, and the effects of latter minerals on iron metabolism are now being conducted. Inorganic sulfate has no influence on the above trace minerals. Publication: Kinnamon, K.E. and G.E. Bunce; Effects of copper, molybdenum and zinc on Zinc-65 distribution and excretion in the rat (accepted by J. Nutrition).
Calcium and phosphorus studies-Cartilage extracts from rachitic and healing rickets have enzyme activities. Some enzymes of the triose phosphate metabolism and of Krebs cycle have been measured, Results are in a preliminary phase of analysis.

27. COMMUNICATIONS SECURITY	28.	29. DOD 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) OVER

ABSTRACT

MINERAL METABOLISM

a. The biochemical pathways involved in the calcification of rachitic cartilage are now being studied. Since literature reports have indicated the importance of anaerobic glycolysis in calcification, these enzyme activities in cartilage are under investigation. Preliminary results indicate that the calcifying and non-calcifying cartilage have enzyme activities which are not significantly different.

b. A study concerning the effects of dietary sulfate, alone and in combination with dietary copper, molybdenum and zinc in the pregnant rats with respect to distribution and excretion of administered Zn-65 and Mo-99 has been completed. Diets high in sulfate with both copper and molybdenum added did affect urine excretion of Mo-99. However, increased dietary sulfate alone or in combination with any one of these elements was not found to alter fetal or parent tissue retention, absorption or excretion of administered Mo-99 or Zn-65. The role of iron in some of these mineral interrelationships is presently being studied.

c. The use of magnesium oxide in the prevention of renal calculi in the patient prone to recurrent urinary calculi continues in a study of the long-term efficacy of its use. Results thus far have been encouraging and warrant continued study. The mechanism of action of the magnesium oxide is under investigation. Studies with animals and preliminary results with human subjects would indicate an interaction with magnesium, calcium and phosphorus.

BODY OF REPORT

MINERAL METABOLISM

Study A

Description:

Enzyme assay methods have been developed which can measure the activities of specific enzymes in the anaerobic glycolytic cycle as well as certain enzymes of the Krebs cycle. Application of these methods to calcifying and non-calcifying cartilage has revealed that these cartilages are not significantly different in terms of those enzymes studied.

Since these enzymes are active in specific portions of the glycolytic sequence, other metabolic patterns must be investigated. In the future, it is expected that metabolic intermediates in the glycolytic series will be isolated, identified and quantitated. Differences in the oxidative pathways will be determined by measuring $^{14}CO_2$ production from specified substrates.

Progress:

Previous experience in this laboratory has shown that weanling rats placed on a rachitogenic diet will manifest evidence of rickets in 21 days. When such animals are placed on the USP Line Test for 7 days, and are given a uniform dose of vitamin D, the cartilage in the radius of these animals will calcify with a uniform response as measured by degree of healing. Such animals provide an excellent tool for the investigation of calcification of cartilage. When vitamin D is administered, the processes which lead to calcification are set into motion, allowing a comparison of biochemical patterns in those given vitamin D with those not so treated. Thus, it may be said that a comparison is made between calcifying and non-calcifying cartilage.

Extracts of these cartilages have been tested for activities of the following enzymes: phosphoglyceromutase, enolase, pyruvic kinase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, NAD- and NADP-malic dehydrogenase and isocitric dehydrogenase. Preliminary results, which have not yet been evaluated statistically, would indicate that there is no significance difference in the activities of these enzymes from calcifying and non-calcifying cartilage.

Summary and Conclusions:

Triosephosphate enzyme activities of the glycolytic series as well as some enzymes of the Krebs cycle have been measured in calcifying and non-calcifying rachitic rat cartilage. These cartilage preparations do not appear to differ significantly in terms of the enzyme activities measured.

Mineral Metabolism (Cont'd.)

List of Publications:

1. Waring, P. and Z. Z. Ziporin. The separation of hexosephosphates and triosephosphates by thin layer chromatography. J. Chromatog. 15: 168, 1964.

2. Ziporin, Z. Z. and P. P.Waring. The relationship of Ca x P product in the serum and vitamin D to mineralization of rachitic rat cartilage. Fed. Proc. 24 (2): 566, 1965.

Study B

Description:

Studies dealing with the effect of sulfate on the copper-molybdenum-zinc interrelationship have been compiled. The role of iron in the copper-zinc interrelationship is not clear. The zinc-induced anemia observed in rats can be largely overcome by dietary supplements of copper, but completely overcome by supplements of copper plus iron. It has been suggested that the apparent antagonistic effect of zinc and copper is purely a reflection of an interference with iron metabolism by zinc. In any case, the mechanism of this relationship is obscure.

Progress:

Diets high in copper, molybdenum, zinc and sulfate have been fed to female albino rats. After being fed their respective rations for 4 or 5 weeks, each animal was bred. At 11-15 days gestation, tracer molybdenum-99 or zinc-65 was administered. Forty-eight and 96 hours later, respectively, blood was drawn, animals killed and fetal tissues taken, then assayed. Increased dietary sulfate alone or in combination was not found to significantly affect: Mo-99 or Zn-65 retention in fetal tissues or placental membranes (amnion, chorion, yolk sac, allantois and decidua); Mo-99 digestive tract absorption or excretion via urine or feces; Mo-99 blood levels; Zn-65 excretion via urine or feces; or volume of urine excreted. Diets high in sulfate with both copper and molybdenum affected urine excretion of radiomolybdenum. Animals fed high molybdenum rations showed a lower blood, fetal and placental structure retention and higher urine excretion and radiomolybdenum after 48 hours. This study has been completed.

In another study, diets with increased levels of copper, iron and zinc were fed to weanling albino rats in multiple combinations. After 5 weeks, each animal received a tracer dose of $^{59}Fe_3Cl$ by stomach tube. Ninety-six hours after ^{59}Fe administration, all animals were killed and the desired tissues taken, then assayed.

Mineral Metabolism (Cont'd.)

All analyses have been completed, but the data have not been completely evaluated. Preliminary indications are that the total iron content of the liver is markedly reduced by high zinc diets, yet recently introduced iron is not significantly influenced by the elevated dietary zinc. A study is presently under way to more clearly define this alteration in iron or zinc metabolism. Both ^{59}Fe and ^{55}Fe are being used in the latter study.

Summary and Conclusions:

Increased dietary levels of sulfate alone or in combination with diets high in copper, molybdenum or zinc do not affect zinc or molybdenum metabolism. Diets high in sulfate, copper and zinc do alter molybdenum metabolism. This alteration is apparently not due to a change in molybdenum absorption from the digestive tract.

List of Publications:

1. Kinnamon, Kenneth E. Copper, molybdenum and zinc interrelationships: The influence of inorganic sulfate upon distribution and excretion of Zn^{65} and Mo^{99} in pregnant rats. Manuscript being edited.

2. Kinnamon, Kenneth E. A study of the role of iron in the copper-zinc interrelationship in the rat. Manuscript in preparation.

Study C

Description and Progress:

Studies continue on the use of MgO in the treatment of human subjects with recurrent urinary calculi. The long-term efficacy of this treatment is under evaluation. Results thus far continue to be encouraging and warrant continued study. A number of subjects in various parts of the United States are under study through the cooperation of clinics and urologists. Urinary biochemical analyses are performed on samples provided prior to receipt of magnesium oxide and periodically following its use. A preliminary evaluation of the data indicate interactions between magnesium, calcium and phosphorus. These findings are similar to those observed in the rat where the effect of dietary phosphorus on magnesium and calcium balance was dependent on the plane of magnesium nutrition. The studies with the rat are currently in press and were reported in last year's progress report.

Summary and Conclusions:

The use of MgO in the treatment of human subjects with recurrent urinary calculi continues to be encouraging and is under further clinical trials and biochemical investigation.

Mineral Metabolism (Cont'd.)

List of Publications:

1. Moore, C. A. and G. E. Bunce. Reduction in frequency of renal calculus formation by oral magnesium administration. Invest. Urology 2: 7, 1964.

2. Bunce, G. E., H. E. Sauberlich, P. G. Reeves and T. S. Oba. Dietary phosphorus and magnesium deficiency in the rat. J. Nutrition (in press).

Presentation:

Sauberlich, H. E. Magnesium in nutrition, 8-9 June 1965. Workshop on clinical and experimental studies on urolithiasis held at Naval Medical Research Institute, Bethesda, Maryland.

RESEARCH AND TECHNOLOGY RESUME

3. GOVT ACCESSION	4. AGENCY ACCESSION	REPORT CONTROL SYMBOL
	DA OA 6315	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U U (RPT) (PRJ)	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71P 01 057

10A. PRIOR NUMBER/CODE: 61145011 3A014501B71P 03 03

11. TITLE: (U) Basic Studies of Carbohydrates (06)

12. SCIENTIFIC OR TECH. AREA: 002300 Biochemistry; 006500 Isotopes; 012100 Organic Chemistry

13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
01 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: c. TYPE: NA d. AMOUNT:	PRIOR FY 65	1	14
		CURRENT FY 66	1	15

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ASSOCIATE: Saari, 1/Lt. J.C.
TEL: 303 366 5311 X 24214 TYPE: DA

21. TECHNOLOGY UTILIZATION: Biochemistry, nutrition, clinical medicine

22. COORDINATION: None

23. KEYWORDS: Ascorbic acid, vitamin C, monodehydroascorbic acid, ascorbic acid dependent NADH oxidase, vitamins

24. (U) Tech Objective:
1. The biological role of ascorbic acid and its oxidative degradation products at the enzymic level is being studied.
2. The chemical characterization of ascorbic acid and its oxidative degradation products are being studied in relationship to their biological activity.

25. (U) Approach: 1. Suitable in vitro techniques will be performed with tissue slices and homogenates establishing: (a)The role of this enzyme system in hydroxylation reactions; (b)the role of this enzyme system in microsomal electron transport. Conventional enzymological techniques will be used in an attempt to purify the system and establish the number and nature of the components.
2. Ascorbic acid, monodehydroascorbic acid and the oxidative degradation products of ascorbic acid are being extensively studied by infrared, NTR, mass spectra and chromatographic procedures.
3. Uniformly labeled ^{14}C ascorbate and ascorbic-6-^{14}C acid will be synthesized this summer for use in further metabolic studies.

26. (U) Progress: (Oct 64-June 65): 1. The hog adrenal microsomal system has been prepared and the original observations of Standinger et al. have been confirmed.
2.(a)The NMR spectra for ascorbic acid are complex, ABCD and ABC systems;(b) mass spectra of ascorbate and D-araboascorbate show an intact molecule peak (at 176). Major splitting of the molecule occurs at the C-1 to C-2 bond and at the C-2 to C-3 bond to give CO_2 and a C_4 and a C_5 fragment. (c)Monodehydroascorbic acid has been prepared. Mass spectra shows a small dimeric peak confirming the previously postulated free radical dimer.

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 1112) OVER

ABSTRACT

BASIC STUDIES OF CARBOHYDRATES

Studies have continued on the chemistry of ascorbic acid (vitamin C) and its biological role. The NMR spectra for ascorbic acid are complex, ABCD and ABC systems; mass spectra of ascorbate and D-araboascorbate show an intact molecule peak at 176. Major splitting of the molecule occurs at the C-1 to C-2 bond and at the C-2 to C-3 bond to give CO_2 and a C_4 and a C_5 fragment. Monodehydroascorbic acid has been prepared. Mass spectra shows a small dimeric peak confirming the previously postulated free radical dimer. An enzyme system has been developed for the study of the biological role of ascorbic acid which is currently under investigation.

BODY OF REPORT

BASIC STUDIES OF CARBOHYDRATES

Description:

Several basic studies on carbohydrates are in progress or planned under this project.

1. Studies on the chemistry and the biological roles of ascorbic acid (in progress). These studies represent a joint effort with the University of Colorado (Dr. B. Tolbert) and USAMRNL.

2. Enzymatic investigations on the biosynthesis of cellulose in higher plants (in preliminary experimental stage).

3. Metabolism of 6-deoxyhexoses (planning phase).

Progress:

The following progress report is provided on the studies being conducted under item 1 above.

The immediate objectives of this study have been:

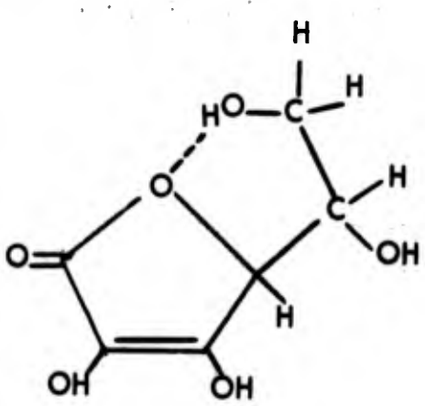
1. The characterization and study of the ascorbic acid and its oxidative degradation products, including the free radical intermediate, monodehydroascorbic acid.

2. The preparation of deuterium and tritium labeled ascorbate and isoascorbate (L-araboascorbic acid).

3. Biological role on the enzymic level of ascorbic acid and its oxidative degradation products.

Progress in characterizing ascorbic acid and its oxidative intermediates

Ascorbic acid (AsH_2) has been studied by NMR and mass spectrometry. NMR spectra of L-ascorbic acid show a non-equivalence for the two methylene protons on carbon-6, which give a complex ABCD (after Roberts) spectrum. This splitting could arise not only from the adjacent asymmetric carbon-5 atom, but also by hydrogen bonding of the 6-hydroxy hydrogen to the lactone ring oxygen. Pulman and Pulman (Quantum Biochemistry, published by Interscience, (1963) p. 707) have calculated a high negative charge for this oxygen, which would strengthen such a hydrogen bonded secondary ring:



The NMR spectrum of the 6-triphenylmethyl (trityl) ether of L-ascorbic acid supports the interpretation of a hydrogen bonded system obtained in L-ascorbic acid. Simply heating the sample of ascorbic acid has also given NMR changes which appear to be the result of breaking the hydrogen bonded system. All NMR spectrum were run in D_2O except the trityl ether which was run in chloroform. Ascorbic-4-d_1 acid is being prepared and its NMR spectra helps clarify the structure of this molecule as it shows a simpler splitting pattern (ABC). Both spectra are sufficiently complex that molecular angles must be calculated from the NMR data on the IBM 709 computer.

Mass spectra of ascorbic acid show an intact molecule peak (at 176). Major splitting of the molecule occurs at the C-1 to C-2 bond and at the C-2 to C-3 bond to give CO_2 and a C_4 and a C_5 fragment, plus a complex spectra of smaller particles.

Fragmentation patterns of L-ascorbic acid and its diastereoisomer, D-araboascorbic acid (isoascorbic), give identical patterns: each display a primary mode of decomposition through the loss of CO_2. Secondary modes involve the loss of oxalate with intensive rearrangement of 5 and 6 carbon fragments and further fragmentation of these rearrangement products. Overall interpretation implies that the stabilities of larger fragments of L-ascorbic acid are much less than those resulting from D-araboascorbic acid. This is based on abundance ratios obtained.

Monodehydroascorbate (AsH·) has been prepared. Its mass spectra shows a small dimeric peak confirming the previously postulated free radical dimer. We are now attempting to prepare larger quantities of this interesting compound by oxidation of ascorbate with dehydroascorbate (dAs).

Preparation of ascorbic acid oxidation products

Methods for the preparation of dehydroascorbic acid are being evaluated to establish an adequate method to obtain this compound in sufficient quantities to support our

biochemical studies. Bromine and iodine oxidation of L-ascorbic acid have both yielded good amounts of a reported methanol complex of L-ascorbate (m.p. 103-105). These oxidations were run in absolute methanol. The oxidation of L-ascorbic acid in aqueous solution by benzoquinone yielded the same compound (103-105°). The derivatives (phenylhydrazine and 2,4-dinitrophenylhydrazine) from the three oxidations were identical and gave very similar infra-red spectra. It is felt that this compound is not a methanol complex but rather a hydrate of dehydroascorbic acid. The infra-red spectrum does not indicate a methoxy grouping in the molecule but does show an intact lactone ring, primary and secondary hydroxyl groups, as well as appropriate C-C skeletal vibrations.

Continued work on the oxidation of ascorbic acid (Pecherer, J. Am. Chem. Soc. 74: 3827 (1951)) has now given dehydroascorbic acid in pure form and in sufficient yield. Infra-red spectra have supported the structure, i.e., triketo grouping and γ-lactone. The dehydroascorbic acid is now being studied by N.M.R. and will also be studied by E.S.R., both by itself and in relation to the comproportionation reaction with ascorbic acid. A mixture of AsH_2 and dAs gives a yellow color, which we hope can be confirmed as formation of AsH.

Progress in preparing labeled ascorbates

Low specific activity ascorbate-4-H^3 has been prepared by reflux of ascorbate in methanolic 1 N KOH solution. Thin film chromatography shows a pure compound. 17.6 grams of ascorbic acid were refluxed for 15 hours in 200 ml of a 50% aqueous methanol solution 1 N in KOH to which 20 λ of HTO ($6.3 \times 10^4 \mu$ C/ml) had been added. About 2.3 grams of labeled ascorbic acid ($3.9 \times 10^{-2} \mu$ C/millimole) were obtained after three recrystallizations from acetonitrile.

A preparation of ascorbate-4-d1 is finished. Preliminary experiments were done to determine the optimum conditions for this reaction. Various concentrations of ascorbic acid and KOH were heated in sealed tubes at 100° C for 17 hours. The concentrations ranged from 0.6 M ascorbic acid and 1 N KOH to 15.8 M ascorbic acid and 33.5 N KOH. A tube of intermediate concentration was run with the dipotassium ascorbate and no other KOH. Material from each tube was checked with thin layer chromatography. All showed clean spots for both L-xylo and L-araboascorbic acid.

The final conditions used for the preparation of the labeled material were as follows: A total of 20 grams of ascorbic acid as 3 M dipotassium ascorbate in D_2O was heated in sealed tubes for 20 hours at 100° C. The material was taken to dryness, redissolved in D_2O and the heating was repeated. About three grams of L-xyloascorbic acid has been crystallized from the residue with acetonitrile. This material was twice more recrystallized from the residue with acetonitrile and used to run an NMR spectrum. The remaining residue, rich in L-araboascorbic acid, is being saved for a future work-up.

Basic Studies of Carbohydrates (Cont'd.)

A high specific activity (10 μ C/mg) preparation of ascorbic-4-H^3 is under way. The preparation began with 7.16 grams of dipotassium ascorbate i.. 1.9 ml of H_2O (containing at least one gram of 1000 mC/gram HTO) which was heated for 20 hours at 100° in a sealed tube and then worked up.

Biochemical studies

Biochemistry studies on the enzymic level are being done. Developments include:

The in vitro hog adrenal system of Staudinger has been set up to study the oxidation of NADH by an ascorbic acid intermediate. Staudinger's results on ascorbic acid have been confirmed. In the above system, monodehydroascorbate alone shows no effect, but this cannot be considered a proof of non-involvement for its rate of reaction could be too fast to observe. Dehydroascorbate is currently being tested in this system.

Summary and Conclusions:

Studies have continued on the chemistry of ascorbic acid. The NMR and mass spectra of ascorbic acid have been investigated and reported. Monodehydroascorbic acid has been prepared and studied. An enzyme system has been developed for use in studying the biological role of vitamin C. Investigations on the metabolism of 6-deoxyhexoses and the enzymatic biosynthesis of cellulose are planned for the next year.

List of Publications:

Levandoski, N. G., E. M. Baker and J. E. Canham. A monodehydro form of ascorbic acid in the autooxidation of ascorbic acid to dehydroascorbic acid. Biochem. 3: 1465, 1964.

RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6316	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT	6. SECURITY WORK	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U	U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61145011 3A014501B71P 01 058

10b. PRIOR NUMBER/CODE: 61145011 3A014501B71P 03 04

11. TITLE: (U) Molecular Biochemistry: Proteins, Amino Acids, Nucleotides and Related Cmpds (06)

12. SCIENTIFIC OR TECH. AREA: 002300 Biochem, 003500 Clin. Med., 016800 Toxicology

13. START DATE: 02 64

14. CRIT. COMPL. DATE: NA

15. FUNDING AGENCY: OTHER | DA

16. PROCURE. METHOD: C. In-House

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

18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	2	14
CURRENT FY 66	2	15

19. GOV'T LAB/INSTALLATION/ACTIVITY
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INVESTIGATORS PRINCIPAL: Ziporin, Z. Z.
ASSOCIATE: Morse, Lt. Col., W. C.
TEL: 303 5311 X 24214 TYPE: DA

21. TECHNOLOGY UTILIZATION: Study liver disease, metabolism of nitrogenous compounds

22. COORDINATION: None

23. KEYWORDS: Determination of ammonia; ammonia, blood ammonia; blood chemical analysis; blood; toxicology.

24. (U) Tech Objective: To develop a method for the determination of blood ammonia. This would provide a reliable analytical tool for assessing liver function. Such a method could also be used to measure the formation and metabolism of ammonia. The results of this investigation can be of use in biochemistry and clinical medicine.

25. (U) Approach: Develop a sensitive, reliable procedure for the estimation of ammonia in blood. The procedure should attempt to distinguish between free ammonia in the blood and the ammonia which forms after the blood is drawn. Known constituents of the blood are treated as if they were in the blood, and the amount of ammonia formed is measured. This should reveal some possible sources of ammonia in blood as it forms on standing.

26. (U) Progress: (Oct 64-Jun 65)

A sensitive, reliable procedure for measuring ammonia has been developed. Because the blood ammonia levels measured by this procedure differ significantly from those published, detailed care is being exercised to test each aspect of the assay. Of the known constituents of blood, two-thirds have been tested for ammonia-generating potential. The list of known sources of ammonia from blood should be revised in the light of these data.

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) CPY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) OVER

ABSTRACT

NUTRITIONAL BIOCHEMISTRY OF CHEMOTHERAPEUTICS (FY 1965)
MOLECULAR BIOCHEMISTRY: PROTEINS, AMINO ACIDS, NUCLEOTIDES AND RELATED COMPOUNDS (FY 1966)

An investigation of the relationship between INH and blood ammonia has been suspended until a reliable blood ammonia method could be developed. A quantitative procedure for measuring ammonia has been achieved. The application of this procedure to blood ammonia measurements reveals a significant difference in normal levels when values obtained by the new method are compared with those reported in the literature.

BODY OF REPORT

NUTRITIONAL BIOCHEMISTRY OF CHEMOTHERAPEUTICS (FY 1965)
MOLECULAR BIOCHEMISTRY: PROTEINS, AMINO ACIDS, NUCLEOTIDES AND RELATED COMPOUNDS (FY 1966)

Description:

A chemical procedure for measuring blood ammonia levels has been devised. This procedure eliminates many sources of error in the methods previously used. Venous blood is lysed and precipitated to give a protein-free filtrate. An aliquot is assayed colorimetrically and read in a spectrophotometer. The method is sensitive, reproducible and specific for ammonia, as far as it has been possible to determine.

Progress:

Because of the known tendency for ammonia concentrations to increase in shed blood with increasing time, the problem of measuring ammonia in blood is complex. Furthermore, the customary conditions for measuring ammonia call for the diffusion of the ammonia from a mixture (usually blood) in the presence of an alkali which volatilizes the ammonia. It can be shown that alkali itself may generate ammonia from proteins and thus mask the true value of ammonia in the blood.

Finally, the measurement of the diffused ammonia is usually accomplished by titration with an acid to a visual end-point. When dealing with micro-amounts of ammonia and the subject judgment of the technician as to the end-point, there is an opportunity for a significant error in the estimation of the ammonia.

Our present studies have attempted to prevent the phenomenon of increasing ammonia in blood after being shed by making a protein-free filtrate as soon after drawing the blood as possible. This filtrate is analyzed colorimetrically and read in a spectrophotometer, thus eliminating the titration step and technician's error in estimating the end-point.

Future work will test blood ammonia concentrations using this procedure and comparing it with a chromatographic procedure which has been reported. The blood ammonia values for normals is considerably higher with the new method than those reported by other procedures. The basis for this difference will be investigated.

Summary and Conclusions:

A method has been developed for the quantiative measurement of ammonia in blood. The basis for different values obtained in normal subjects by this method as compared with other methods remains to be investigated.

List of Publications: None.

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RESEARCH AND TECHNOLOGY RESUME

3. GOVT ACCESSION	4. AGENCY ACCESSION	REPORT CONTROL SYMBOL
	DA OA 6317	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U U (RPT) (SUM)	NA	NL	A. WORK UNIT

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

10b. PRIOR NUMBER/CODE: 61145011 3A014501B71P 09 06

11. TITLE: (U) Functional Aspects of Body Composition (06)

12. SCIENTIFIC OR TECH. AREA: 002600 Biology; 012900 Physiology; 003500 Clinical Medicine

13. START DATE: 07 56

14. CNTR. COMPL. DATE: NA

15. FUNDING AGENCY: OTHER | DA

16. PROCURE. METHOD: C. In-House

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

18. RESOURCES EST.	a. PROFESSIONAL MAN/YEARS	b. FUNDS (In thousands)
PRIOR FY 65	1	20
CURRENT FY 66	1	22

19. GOVT LAB/INSTALLATION/ACTIVITY
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21. TECHNOLOGY UTILIZATION: Civilian Medicine, Actuarial Studies, Animal Husbandry

22. COORDINATION: None

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

24. (U) Tech Objective: A simple method to accurately and reliably define the major components of the human body is needed. The few cadavers analyzed, plus animals studied provide only partial and not always applicable information on water, protein, fat and mineral content. No single technique defines all compositional aspects. Techniques, when developed, will permit correlation of body composition to work performance, nutritional status, age and physical conditioning.

25. (U) Approach: Densitometric estimation of body fat by water displacement, further developed at this laboratory, is simple and as accurate as cumbersome underwater weighing techniques commonly used. Potassium-40 whole body counting is presently being compared with volumeter studies wherein total body water and select anthropometric measures were made. Rats are being studied using classical dissection and chemical techniques. The aforementioned body components are compared with creatinine excretion, oxygen consumption and potassium content in an effort to further define the active metabolizing tissue mass of the body.

26. (U) Progress: (Oct 64-Jun 65) Body volumeter studies of two male populations showed increased body fat with age, however, percent body fat was lower in all age groups when residual lung volumes were measured, not estimated. In rats, potassium and creatinine correlated well with water, mineral and the two protein components of the fat free body mass. Reports: Krzywicki, H.J. Densitometric estimation of human body fat by water displacement. Fed. Proc. 24:315, 1965. Chinn, K.S.K. and Plough, I.C. Estimation of fat free mass and its four components from creatinine excretion. Fed. Proc. 24:315, 1965. Krzywicki, H. J. Anthropometric estimation of human body composition. Proc. Colo-Wyoming Acad. Science (in press).

27. COMMUNICATIONS SECURITY	28.	29. OSD CODE	30. BUDGET CODE
[ ] a. COMSEC RELATED [X] b. NOT COMSEC 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 1498 (Items 1 to 24 identical to NASA Form 1122) OVER

ABSTRACT

FUNCTIONAL ASPECTS OF BODY COMPOSITION

The assessment of that most widely variable aspect of body composition, i. e. body fat, still proves most 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, providing permission for waste disposal is granted by the AEC. Two populations were studied extensively in the past year to determine the fewest, most discriminating measurements usable in the estimation of body composition. In the Fort Bragg, North Carolina ration study, D_2O was used to measure total body water and water flux.

Animal studies have shown urinary creatinine excretion correlates very highly with muscle protein and body water in the fat free mass, but the creatine distribution in muscle usually accepted as 98% was determined to be only 78. 81%.

BODY OF REPORT

FUNCTIONAL ASPECTS OF BODY COMPOSITION

Description

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. Animal body composition from direct chemical analysis is not always applicable to humans. No single technique of accurately estimating human body compartments exists, 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. The 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.

Progress

Physiology, Bioenergetics Divisions: A new gas chromatograph for analysis of deuterium containing urine samples for total body water estimates was received and put into operation in April 1965. Despite some instrumental failure and difficulty with the gas generating and delivery systems, preliminary analyses indicate detectable and reproducible deuterium values representing reasonable total body water estimates. Urine samples from the Fort Bragg, North Carolina ration study are being analyzed for D_2O.

Fort Carson survey anthropometric data awaiting computer programming has been held in abeyance pending completion of a report on the human body volumeter. The accuracy and reproducibility of the volumeter was demonstrated to be equal to that of underwater weighing techniques from observations on 14 subjects at 7 intervals during a 24 hour period. Body density varied a maximum of ±0.004 density units with a ±0.62 kg change in body weight, while the minimal variation was ±0.002 density units with ±0.02 kg weight change. An analysis of variance showed the standard deviation of a single observation to vary ±0.002 density units

for the entire group. Siris' critique of densitometry cites ±0.005 density units as the requisite reproducibility in view of the inherent errors of such techniques and the assumptions involved in attempting to quantify per cent body fat.

Unreported early body volume data was analyzed and compared with recent Fort Carson survey data. Computed density was shown to decrease progressively in 5 year age increments from 1.060 ± 0.016 in nine 17 - 19 year olds to 1.017 ± 0.001 in two 65 - 69 year olds. Body fat increased from 19.6 ± 7.0% to 38.7 ± 0.6% in this group of 173 adult males from 17 - 69 years of age. The 105 Fort Carson soldiers showed a similar trend, but were somewhat leaner throughout all age groups and only two soldiers who were beyond age 50 were measured. Residual lung volumes were estimated in the group of 173 males and were measured in the 105 soldiers. Mean body fat of 17 - 25 year olds as well as 20 - 40 year olds agreed with desitometrically observed values reported in the literature. Irrespective of the precision of actual body fat estimates, the fact that body density was shown to decline with 5 year increments in age, is indicative of the simplicity and utility of the volumeter for survey type situations. Per cent body fat of 87 Fort Carson soldiers estimated from body volumes by water displacement also correlated (r = 0.815) with fat estimated from whole body potassium^{40} counting (using Forbes value of 68.1 mEq K/kg lean body mass).

The animal studies have shown some distinct and interesting correlation as follows: The dry protein mass of the rat carcass was dissected into muscle and remaining protein and it was found that muscle protein constituted 11.11 ± 0.42%, and the remaining protein was 12.02 ± 1.55% of the fat free carcass mass. Twenty-four hour urinary creatinine excretion correlated (r = 0.967) with the fat free mass, (r = 0.958) with body water, (r = 0.994) with muscle protein, (r = 0.834) with the remaining protein, and (r = 0.895) with body mineral. Total body potassium correlated at the 0.9 level with the fat free mass and its components, but is less desirable as a reference standard when compared with the aforementioned. The concentration and distribution of potassium and creatine was also measured in muscle and in the remaining body tissues. The data showed potassium distribution to be in close agreement with reported literature values, while only 78.81% of the creatine was found in muscle as opposed to the 98.0% usually reported in the literature. Drafts of 4 papers discussing the above are now in final form and ready for submission to journals.

FUNCTIONAL ASPECTS OF BODY COMPOSITION

Summary and Conclusions

The human body volumeter is simple and as effective as underwater weighing techniques for estimating body density, its precision is lowered when residual lung volume is estimated for purposes of estimating body fat (from 0.50 to 1.62 kg). Per cent body fat from body volume by direct water displacement correlates (r = 0.815) with that obtained from potassium^{40} counting. Body fat increased with age and was not related to body weight in two populations wherein direct body volume measurements were taken. Animal studies have shown 24 hour creatinine excretions to correlate highly with the fat free mass, body water and muscle protein. Body potassium correlated less highly with these components. In all rats 78.81% of the total body creatine was found in muscle.

List of Publications:

1. Krzywicki, H. J.: Densitometric estimation of human body fat by water displacement. Fed. Proc. 24: 315, 1965.

2. Chinn, K. S. K. and Plough, I. C.: Estimation of fat free mass and its four components from creatinine excretion. Fed. Proc. 24: 315, 1965.

3. Krzywicki, H. J.: Anthropometric estimation of human body composition. Proc. Colorado-Wyoming Association Science, 1 May 1965, Denver, Colorado.

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RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA .6325	CSCRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY RPT / SUM	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U / U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 01 040

10b. PRIOR NUMBER/CODE:

11. TITLE: (U) Tissue Ultrastructure in Nutritional Pathology (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002600 Biology	10 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 65	0	25
		CURRENT FY 66	1	28

19. GOV'T LAB/INSTALLATION/ACTIVITY
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TEL: 303 366-5311 X 23230 TYPE: DA

21. TECHNOLOGY UTILIZATION
Research in liver pathophysiology

22. COORDINATION
None

23. KEYWORDS: Choline dificiency; intravenous fat emulsions; electron microscopy; lithocholic acid; perfused liver intravenous fat pigment.

24. (U) Tech Objective. To study morphologic changes in tissue ultrastructure associated with nutritional disorders using electron microscopy.
(U) Approach. The technical problems inherent in the preparation of specimens are many, which begin with the fixation of tissue, and continue through the final phases of photographing the tissue in the electron microscope. Technical competence of the technician and the electron microscopist is being improved by a program of continuing education and on-the-job training which should increase the amount of work that can be done. Electron microscopic examination is being performed on liver whichhas been subjected to: intravenous fat emulsions in conjunction with other drugs; the addition of lithocholic acid in the diet; and to isolation and perfusion with whole blood.
(U) Progress: (Oct 64-July 65) The effect of choline on ultrastructural changes associated with intravenous fat administration has been published in Am. J. Clin. Nutrition, Jan 1965. The results indicated that choline did not offer a protective effect to changes produced by intravenously administered fat emulsions. Livers from chicks fed lithocholic acid are being examined now and a manuscript is being prepared. Ultrastructural changes were notdiscernible after 4-1/2 hours of perfusion of the isolated perfused rat liver. Future plans include: Continued studies of hepatic ultrastructural changes caused by the administration of intravenous fat emulsion, and the possible role of vitamin E, selenium, and emulsifier systems in the production of the hepatic changes; new studies of the etiology of experimentally produced acute pulmonary edema of high altitude in the rabbit.
Reports generated from Oct 64 - July 65:
The Effects of Choline on Hepatic Ultrastructural Changes Associated with the Intravenous Administration of Fat. N. W. King, Capt, VC, Am. J. Clin. Nutr. 16:88, Jan 1965.

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) OVER

ABSTRACT

TISSUE ULTRASTRUCTURE IN NUTRITIONAL PATHOLOGY

An RCA EMU-3G electron microscope is in operation and trained personnel are capable of operating the instrument and auxillary equipment. The continuing study at this laboratory concerns the effect of intravenous fat emulsions on the ultrastructure of the liver. Another study under examination concerns hepatic ultrastructural changes associated with dietary lithocholic acid intake in chickens.

BODY OF REPORT

TISSUE ULTRASTRUCTURE IN NUTRITIONAL PATHOLOGY

Description:

Morphologic studies at the macromolecular level are made possible by electron microscopy. This permits a better understanding of cellular function and response under normal and abnormal circumstances. Beginning with the gross lesion, this Division now has ability to conduct coordinated studies down to the associated sub-organelle changes.

Progress:

The RCA EMU-3G electron microscope utilization is becoming greater as trained personnel become more proficient in the preparation of specimens and operation of the instrument. Three officers (V.C.) use the microscope now about 50% of the machine time, and two technicians use the scope for preliminary examination of specimens about 25% of the machine time. Programmed training schedules will permit greater usage of the scope as other operators are trained.

The major field of interest is in the effect of intravenous fat emulsions on hepatic ultrastructure. One specific area concerns the possible protective effect of dietary choline on ultrastructural changes following intravenous fat administration. This choline study has been completed and results were published in the American Journal of Clinical Nutrition in January 1965.

Research is continuing concerning the hepatic effects of dietary lithocholic acid fed to chicks. Other results are presented under USAMRNL - Chemistry Division, Sub-task 01, Study CH-1. In the present study one group of chicks received lithocholic acid in their diet for three weeks initially and three weeks without, a second group received lithocholic acid for six weeks, and a third group received a standard laboratory diet. Chicks were sacrificed on a sequential schedule and necropsied over a period of six weeks. Light microscopic studies have been completed, and will be reported in a joint Chemistry-Pathology Division publication. Electron microscopic studies are being performed and evaluated.

As new intravenous fat emulsions are tested by investigators of The Surgeon General's Intravenous Nutriment Program electron microscopic studies will be performed on selected tissues. At this time selected tissues from rats which received Intralipid® and various combinations of selenium and Vitamin E are being examined for ultrastructural changes.

Summary and Conclusions:

Different studies of hepatic ultrastructure have been or are being made in relation to hepatic ultrastructure and its response to various nutritional regimens.

The administration of intravenous fat emulsions to rats produces

dilatation of the hepatic rough endoplasmic reticulum similar to that which occurs in choline deficiency. Dilatation of the rough endoplasmic reticulum associated with the intravenous administration of Intralipid was not prevented by dietary supplementation with choline. This indicates that the mechanism by which Intralipid produces dilatation of the hepatic rough endoplasmic reticulum is more complex than simply the production of a relative choline deficiency.

Further electron microscopic studies are being accomplished on the hepatic effect of dietary lithocholic acid in chickens, and the effect of intravenous fat emulsions on the liver.

Publications:

King, N. W., Sasaki, H., Jones, L. D. and Schaffner, F. The effect of choline on ultrastructural liver changes associated with intravenous fat. Am. J. of Clin. Nutrition, 16:88, 1965.

* Vitrum; Stockholm, Sweden.

RESEARCH AND TECHNOLOGY RESUME			3. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
				DA OA .6326	CSCRD 103
4. DATE OF RESUME: 01 07 65	5. KIND OF RESUME: A. NEW	6. SECURITY: U RPT U WRK	7. REGRADING: NA	8. RELEASE LIMITATION: NL	9. LEVEL OF RESUME: A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 01 041

10b. PRIOR NUMBER/CODE: 61130011 3A013001A91C 01 59

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

12. SCIENTIFIC OR TECH. AREA: 002300 (Biochemistry); 002600 (Biology); 010100 (Microbiology)

13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
01 64	NA	OTHER | DA

16. PROCURE. METHOD: C. IN-HOUSE

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

18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	0	25
CURRENT FY 66	1	29

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21. TECHNOLOGY UTILIZATION: Medicine (Infections and malabsorption); nutrition

22. COORDINATION: None

23. KEYWORDS: Symbiosis; intestinal micro-organisms; intestinal diseases; germfree life; laboratory animals.

24. (U) Tech 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: (Oct 64-Jun 65)

Presently, facilities are being developed in terms of both equipment and physical space. Consultations have been held with investigators at Walter Reed Army Institute of Research and at the National Institutes of Health. Standard procedures are being developed for the handling and maintenance of germfree animals and the microbiological examinations essential to the establishment of germfree conditions. Preliminary investigations with the use of germfree animals should be initiated in the near future.

27. COMMUNICATIONS SECURITY: ☐ a. CLASSIFIED ☐ b. UNCLASSIFIED	28.	29. OSD CODE: BR	30. BUDGET CODE: 1
31. MISSION OBJECTIVE: NA		32. PARTICIPATION: NA	
33. REQUESTING AGENCY	34. SPECIAL EQUIPMENT		
35. EST. FUNDS (In thousands) COST:	36.		

DD FORM 1498 (Items 1 to 36 identical to NASA Form 1121) OVER

ABSTRACT

SYMBIOSIS AND INTESTINAL FLORA IN NUTRITION

Personnel associated with the use of germfree animal facilities and studies are undergoing training. Assistance has been provided by members of the staff at Walter Reed Army Institute for Research and the National Institutes of Health. Recommendations by these Institutes has resulted in modifications in germfree isolator equipment and other items. A manual for use of the germfree facilities at USAMRNL is under development. Protocols for several nutrition studies employing germfree rats are in preparation.

BODY OF REPORT

SYMBIOSIS AND INTESTINAL FLORA IN NUTRITION

Description:

Numerous problems in the field of nutrition, malabsorption and infection require the use of germfree animals in order to properly conduct the necessary investigations. Development of the necessary facilities and of the proper techniques to conduct these specialized studies represented the initial objective of this project.

Progress:

Modest physical space has been developed for germfree animal investigations, except for required humidity and temperature control equipment. Eight isolators have been obtained from a commercial source. However, certain features on the isolators are inadequate and the company is in the process of making the necessary modifications.

Personnel to be associated with the germ-free facilities and experiments have received training and guidance from members of the staff at Walter Reed Army Institute for Research and at the National Institutes of Health. Advice provided has led to the modification of our existing sterilizing facilities for more effective use and in the local fabrication of a more satisfactory animal cage. The development of a sterile diet suitable for the type of nutritional studies contemplated at USAMRNL is still in progress. Diets presently employed elsewhere for germfree studies are not entirely satisfactory. Special techniques still need to be developed for some of the balance studies, isotopic experiments, deficiency investigations, etc. that are contemplated. Delays in the installation of adjacent laboratory facilities for the microbiological and biochemical support have delayed finalization of operating procedures for the germfree animal facilities. It is hoped that with conducting simple short-term germfree studies, a manual may be developed for use in conjunction with the facilities at USAMRNL. As experience is gained and techniques projected, studies of a longer-term duration may be undertaken.

Summary:

Facilities, techniques and operating procedures are under development for conducting germfree animal experiments on problems in nutrition and malabsorption.

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RESEARCH AND TECHNOLOGY RESUME

3. GOVT ACCESSION	4. AGENCY ACCESSION	REPORT CONTROL SYMBOL
	DA OA 6327	CSGRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	(U) U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 01 042

10b. PRIOR NUMBER/CODE: 61130011 3A013001A91C 01 60

11. TITLE: (U) Studies in Protein Chemistry (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
014000 Radio and Radiation Chem.;002300 Biochem.; 014100 Radiobiology	09 64	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 65	1	32
		CURRENT FY 66	1	35

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ASSOCIATE: Sauberlich, H.E.
TEL: 303 366 5311 X24214 TYPE: DA

21. TECHNOLOGY UTILIZATION: Devising new or improving existing methods of radiation therapy

22. COORDINATION: None

23. KEYWORDS: Proteins; enzymes;enzymic inactivation; lysozyme; peptide chain; fragmentation; cross-linking; radiation mechanism; radiation protection.

24. (U) Tech Objective.

To determine chemical and physical changes occurring in irradiated enzymes that result in loss of activity.

25. (U) Approach.

Direct effects of gamma rays will be studied in solid, very pure, preparations of egg-white lysozyme. Radiation products from this enzyme will be separated, purified and assayed for enzymic activity. If activity has been lost, detailed study will be made of chemical and physical changes that have occurred. Where possible, existing techniques in peptide chemistry will be adapted to chemical structure studies. New techniques may have to be developed for reducing the larger peptide radiation product molecules to fragments that can be characterized in detail.

26. (U) Progress: (Sept 64-Jun 65)

Molecular weight determinations on several purified radiation products from lysozyme indicate that aggregation above the trimer level results in inactivation. A chromatographic system has been devised for separating 45 of the 47-48 peptides produced from native lysozyme. About 20 of these peptides can be assigned to their origin in the peptide chain of lysozyme. After completing peptide assignments in native lysozyme, the methods evolved will be used to establish the structure of radiation products.

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.
CPYH	

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) OVER

ABSTRACT

STUDIES IN PROTEIN CHEMISTRY

a. Chromatographically homogeneous egg-white lysozyme has been irradiated in a Cesium-137 source with γ-rays. The resulting samples were subjected to amino acid analyses, deuterium exchange measurements and chromatographic examination. After a 26.6 Mrad dose, no significant loss in any amino acid residue is observed even though 40% of the enzymic activity is lost. "Hard-to-exchange-amide" (peptide) hydrogens are lost at least twice as rapidly as enzymic activity as the radiation dose is increased. Chromatographic examination of the phosphate-soluble material reveals 7 active and 1 inactive components. These results indicate that only half the native conformation of lysozyme is essential to enzymic activity.

b. Radiation inactivated lysozyme molecules are generally not soluble in phosphate buffers at neutrality. This material has been separated into 4 fractions. Formation of aggregates accompanies but cannot be solely responsible for inactivation since several active radiation products are also aggregates.

c. Methods are being developed for peptide characterization in partial enzymic hydrolysates of active and inactive lysozyme radiation products. Automatic instrumentation is being employed to measure peptide size and content of several specific amino acids in the 45 peptic peptides which can now be separated chromatographically from native lysozyme.

BODY OF REPORT

STUDIES IN PROTEIN CHEMISTRY

Description:

Many chemical and physical changes have been observed upon irradiating enzymes. However, the relationship of such changes to loss of enzymic activity is not clear at all. This is probably due to the fact that such changes have been measured in the whole irradiated sampled consisting of a mixture of active and inactive radiation products. Therefore, studies have been initiated with these aims:

1. To separate and purify both active and inactive radiation products from enzymes.

2. To characterize physically and chemically these radiation products.

3. To relate loss of enzymic activity to radiation-induced changes in physical and chemical properties.

4. To ascertain radiation changes in physical and chemical properties which permit retention of enzymic activity.

5. To attempt reversal of radiation changes that are deleterious to enzymic activity.

Initial studies have been directed towards elucidating direct effects of gamma irradiation in very pure solid preparations of egg-white lysozyme. At this stage, much work is required on procedural development, particularly in regard to analytical methods and techniques for characterization of large molecular weight radiation products.

Progress:

During the course of earlier work, it was observed that no significant destruction of any amino acid residues occurred in solid chromatographically homogeneous lysozyme receiving a γ-ray dose of 26.6 Mrads. At this radiation level, 40% of the enzymic activity was lost. At higher radiation levels causing almost complete destruction of enzymic activity, it still was not possible to observe significant destruction of amino acid residues. However, physical parameters of the molecule did change at these dose levels. Deuterium exchange measurements showed that hard-to-exchange amide (peptide) hydrogens were lost at least twice as rapidly as enzymic activity up to doses of 30 Mrads. These results indicated that not all of the ordered structure of lysozyme was essential to enzymic activity and, further, that isolation of altered still active enzyme molecules would be possible.

Studies in Protein Chemistry (Cont'd.)

Separation of most of the active lysozyme radiation products has been achieved by precipitating the inactive ones with phosphate at neutral pH. Eight components have been demonstrated in the active portion by chromatography on cation exchange resins and cross-linked dextran columns. Five of these are active and have been purified to a homogeneous state. Two other componenents have not been purified, but do possess activity. Three of the 7 active components are aggregates having molecular weights ranging from 2 to 3 times that of the original unirradiated lysozyme. Exact molecular weights and information on the types of bonds involved in aggregation are being obtained from osmotic pressure measurements in a variety of solvent systems. One of the active radiation-produced aggregates must still be purified further prior to osmotic pressure measurements.

The phosphate insoluble inactive radiation products can be partially solubilized by treatment with concentrated urea solutions. There are 3 components in this soluble material that can be separated on cross-linked dextran columns. One of these components is inactive and about 4 times larger in molecular weight than unirradiated lysozyme. The other two components possess about 10% of the enzymic activity of native lysozyme, but have not been obtained in large enough amounts to allow further purification and characterization.

Thus, molecular weight estimations on the various radiation products have shown that dimer and trimer formation can occur with retention of activity, but tetramer formation results in complete loss of activity. The difference between the trimer and tetramer may be final and complete masking of the active catalytic region of the enzyme towards hydrolysis of its substrate which is a large and bulky bacterial cell wall in our experiments.

Intensive efforts are now directed towards characterization of active and inactive aggregates. Molecular weight and enzymic activity measurements before and after treatment with various reagents should give information on the bond types involved in aggregation and possible ways of reversing enzymic inactivation. Also, in these aggregates, there should be deviations from the characteristic peptide chromatographic patterns obtained by partial peptic or typtic hydrolysis of lysozyme or reduced carboxymethylated lysozyme. Peptic hydrolysis of the latter material should yield 47 characteristic peptides, 45 of which we have been able to separate chromatographically.

From size measurements and partial qualitative amino acid analyses, we have been able to assign about half of the peptides to their original position in the structure of lysozyme. Current efforts are being made to perfect automatic quantitative amino acid determinations on the peptides directly as they are being eluted from the chromatographic column. Knowing the content of 3 amino acids in each peptide and its size should allow rapid assignment of the remainder of the peptides to their positions in

lysozyme's structure. Then, this procedure can be easily applied to radiation products to ascertain where and how radiation induced changes have occurred in the molecule. Though some supplemental manual measurements may be required on certain peptide eluate fractions from the column, most of the necessary information for peptide assignment on a given radiation product will be obtained automatically and rapidly with a minimum of human effort and error involved.

Summary and Conclusions:

Twelve components have been demonstrated in lysozyme after γ-irradiation in the pure solid state. Eight of these components have been extensively purified and subjected to molecular weight and enzymic activity studies. Formation of aggregates occurs, but does not result in complete inactivation until reaching a size 4 times that of unirradiated lysozyme. Apparently, complete masking of the enzyme's active region occurs at this aggregation level.

The bond types involved in aggregation are being studied by molecular weight measurements after treatment with various reagents. Partial peptic hydrolysates of the aggregates and other radiation products are being characterized by an automatic chromatographic method that has been devised and permits separation of and size estimation on 45 of the 47 characteristic peptic peptides obtained from reduced carboxymethylated unirradiated lysozyme. Automatic quantitative methods are being developed for sensitive direct determination of certain amino acid residues in these peptides as they are being eluted from chromatographic columns. Most attention is being given to methods for arginine, carboxymethylcysteine, tyrosine, tryptophane, histidine, glutamine and asparagine.

List of Publications:

1. Tolbert, B. M., M. H. Krinks and C. O. Stevens. Radiation chemistry of amino acids and proteins. Review article in Proc. of the Conference on Methods of Preparing and Storing Marked Molecules, pp. 671-698, published 1964.

2. Stevens, C. O., L. E. Henderson and B. M. Tolbert. Radiation chemistry of proteins. II. Enzymic activity and molecular integrity of lysozyme and α-chymotrypsin. Arch. Biochem. Biophys. 107: 367, 1964.

3. Stevens, C. O. and B. M. Tolbert. Radiation effects in lysozyme. A paper presented before the Colorado-Wyoming Acad. Sci. 36th Annual Meeting, 30 April - 1 May 1965, Denver, Colorado.

Studies in Protein Chemistry (Cont'd.)

4. Stevens, C. O. and B. M. Tolbert. Gaseous effects on radiation products from egg-white lysozyme. A paper in preparation for submission to Radiation Research.

5. Stevens, C. O. and G. R. Bergstrom. Radiation produced aggregates from egg-white lysozyme. A paper in preparation for submission to Arch. Biochem. Biophys.

RESEARCH AND TECHNOLOGY RESUME				DA OA .6328	CSCRD 103
4. DATE OF RESUME: 01 07 65	5. KIND OF RESUME: A. NEW	6. SECURITY: U U	7. REGRADING: NA	8. RELEASE LIMITATION: NL	9. LEVEL OF RES. EF: A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 01 043

10b. PRIOR NUMBER/CODE: 61130011 3A013001A91C 01 66

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

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. ORIG. COMPL. DATE	15. FUNDING AGENCY
010100 Microbiology	10 64	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 65	0	1
	c. TYPE:	d. AMOUNT:	CURRENT FY 66	1	3

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INVESTIGATORS PRINCIPAL: Morse, W.C. Lt.Col
ASSOCIATE: Blair, E.B. Lt Col, Weiser, O.L.
TEL: 303 366 5311 X 25223 TYPE: DA

21. TECHNOLOGY UTILIZATION: All medical laboratories

22. COORDINATION: None

23. KEYWORDS: Mycoplasma; metabolic; staphylococcal culture medium; oxygen tension; pH; anaerobiosis; temperature.

24. (U) Tech Objective.

To define culture media which facilitate the rapid isolation and identification of Mycoplasma and Staphylococcus aureus found in clinical specimens; to determine the nutritional requirements of those organisms in order to develop better culture media.

25. (U) Approach.

Material for study is being obtained through surveys, utilizing throat washings and swabs, nasal swabs and monthly blood specimens. Testing of the efficacy of a new selective medium for staphylococci is in progress. Studies on the effect of variation of oxygen tension, pH and temperature on efficacy of recovery of Mycoplasma pneumoniae from survey specimens is in progress.

26. (U) Progress: (Oct 64-Jun 65)

Results to date are preliminary and do not permit conclusions.

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) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 26 identical to NASA Form 1122) OVER

ABSTRACT

STUDIES IN MICROBIAL METABOLISM

Investigations have been initiated to determine the specific etiologic agent associated with primary atypical pneumonia, and to define the growth requirements of Mycoplasma species.

A new medium, salt mannitol plasma agar (SMPA), for isolation of Staphylococcus aureus from the nose, throat and skin is described. Differentiation of coagulase-positive staphylococci was superior on this medium as compared with mannitol salt agar.

The great variation of grossly observable reactions on SMPA and modifications thereof by staphylococci isolated from carriers or pathogenic processes may provide a means of associating metabolic response with strain differentiation and pathogenesis. Correlation between coagulase and deoxyribonuclease production by members of the Family Micrococcaceae was poor; however, the latter test is most useful for screening out coagulase-negative colonies.

BODY OF REPORT

STUDIES IN MICROBIAL METABOLISM

Description: Mycoplasma in Atypical Pneumonias

a. To determine the specific etiologic agent associated with primary atypical pneumonias and to establish procedures for the rapid isolation and identification of these agents.

Progress:

a. Samples, including blood and throat washings were obtained from volunteers of the 249th General Hospital, from the Medical Technicians' School, selected hospitalized patients, and newborn infants. These samples are being studied by appropriate techniques and results will be evaluated upon completion.

Summary and Conclusions:

a. and b. Work is of a preliminary nature and any conclusions at this time would not be valid or warranted.

Description: Metabolism of Mycoplasma Species

b. Metabolic studies to define the growth requirements of Mycoplasma species.

Progress:

b. Metabolic studies to define or redefine some of the basic growth requirements of the Mycoplasma are underway. An evaluation of optimum pH requirements, atmosphere and temperatures are in progress.

Summary and Conclusions:

See "a. and b.", above.

Description: Staphylococcal Culture Medium

c. A new culture medium for the isolation of Staphylococcus aureus and preliminary observations of reactions on this medium by various strains of Staphylococcus aureus are described.

Progress:

c. Evaluation of two agar media, mannitol salt agar (MSA) and salt milk agar (SMA) for recovery of staphylococci from nasal carriers revealed the MSA medium to be superior. The need for a better medium than MSA became evident because of the difficulty experienced in differentiating Staphylococcus aureus colonies, which are a light yellow color, from white

colonies of coagulase-negative micrococci or staphylococci. Acid production by staphylococci from mannitol in a heavily inoculated medium containing a phenol red indicator often turns the entire medium yellow, thus rendering early colony differentiation almost impossible.

A new medium, salt mannitol plasma agar (SMPA), containing 7.5% NaCl, 1% mannitol, 1% proteose peptone No. 3 (Difco), 0.1% beef extract (Difco), 0.002% brom cresol purple, 10% human plasma and 1.5% agar was compared with MSA for efficacy in recovering Staphylococcus aureus from several hundred nose and throat swabs. With one exception, there was no difference between the two media in demonstrating staphylococci. In this instance the coagulase-positive staphylococcus was non-pigmented and did not produce acid from mannitol, colonies being picked from SMPA on the basis of a color change imparted to the medium. Otherwise, the appearance of staphylococci on one medium to the exclusion of the other could be attributed solely to sampling differences, as there were never more than eight colonies present on the positive plate in these instances.

The SMPA medium was vastly superior in respect to ease of colony differentiation, as well as having the added advantage of forming halos of precipitate around Staphylococcus aureus colonies. The majority of strains tested on SMPA also produced a color change in the medium surrounding colonies of coagulase-positive staphylococci within 24 hours. The latter characteristic enabled us to recover from a nasal culture inoculated to SMPA a coagulase-positive staphylococcus which failed to produce pigment or acid from mannitol, and would probably have been discarded as a micrococcus or Staphylococcus epidermidis on other media.

Summary and Conclusions:

c. A new medium, salt mannitol plasma agar (SMPA), was superior to MSA in recovering staphylococci from nasal and throat swabs. SMPA enabled easier early differentiation of coagulase-positive staphylococci from micrococci and coagulase-negative staphylococci. Halos of precipitate and color changes in the medium around coagulase-positive staphylococci afforded additional selective characteristics not found in other media.

Description: Metabolic Reactions of Staphylococci on a New Culture Medium

d. Studies are in progress using modifications of salt mannitol plasma agar (SMPA) and staphylococci from carriers and disease sources to include: acid production from glucose and mannitol in the presence of NaCl, deoxyribonuclease and coagulase production, and the production of grossly observable reaction on human plasma by extracellular proteins or enzymes.

Progress:

d. Response by various strains of staphylococci obtained from carriers or disease sources to modifications of the SMPA medium have revealed considerable differences in their production of extracellular protein. Variations

in precipitate formation and lysis, pigmentation, color changes in the medium, and acid production in the modified media indicate an acute sensitivity of this genus of bacteria to metabolite alterations. The preliminary nature of these data do not permit conclusions at this time. Studies designed to elucidate the cause and nature of these reactions are in progress.

Correlation of deoxyribonuclease (DNase) and coagulase production was poor. Inasmuch as all coagulase-positive staphylococci were also DNase-positive, the latter reaction was valuable for screening out many pigmented and/or mannitol-positive micrococci. However, many DNase-positive isolates did not produce coagulase.

Summary and Conclusions:

d. Modification of the basic SMPA medium elicited a diversity of metabolic response among staphylococci isolated from carriers as well as from lesions. Studies are in progress in attempts to find unique metabolic activity possibly related to or contributing to pathogenesis of members of this genus.

List of Publications:

None.

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RESEARCH AND TECHNOLOGY RESUME

1.	2. GOVT ACCESSION	3. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6329	CSGRD 103

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	RPT U DOC U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 01 044

10b. PRIOR NUMBER/CODE: 61130011 3A013001A91C 01 67

11. TITLE: (U) Auto-Immunological Aspects of Tissue Transplantation (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
002600 Biology 012900 Physiology	01 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. DATE: b. NUMBER: NA c. TYPE: d. AMOUNT:	PRIOR FY 65	0	1
		CURRENT FY 66	1	3

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INVESTIGATORS PRINCIPAL: Ackerman, L.J. Lt.
ASSOCIATE:
TEL: 303 366 5311 X 23230 TYPE: DA

21. TECHNOLOGY UTILIZATION: Research in organ transplantation

22. COORDINATION: None

23. KEYWORDS: Antigen-antibody reactions; Hypersensitive; Immunosuppression; Gamma globulin; Skin transplantation; Adjuvant.

24. (U) Tech Objective. This is an exploration of the possibility of stimulating host production of immune antibodies which will inactivate or destroy any antibodies which the same host will produce against a subsequently transplanted tissue. This type of auto-immunization will allow a recipient to accept a foreign tissue without the loss of the recipient's protective reticuloendothelial system.

25. (U) Approach. Antisera to the tissues of a donor will be produced by the intraperitoneal injection of lymphoid cells from the donor into the recipient. Since it is possible to produce antibodies against some forms of gamma globulins, the antisera will be injected intradermally into another recipient who will theoretically develop circulating antibodies against the components of the antisera. Lymphoid cells from the first recipient will be injected alone and in combination with the antisera into the ultimate recipient in order to produce a similar auto-immunization. Skin grafts will be used to determine the degree of immunization achieved, since skin grafts are easily observed for determination of the viability of grafted tissue. The major technical problem will be the production of antibodies against the components of the antisera, and it may be necessary to employ the use of haptenes or adjuvants. The production of the antisera by intraperitoneal injection of lymphoid material and the production of antibodies against some forms of gamma globulins have been reported by other investigators. The determination of the viability of grafted skin has also been reported, but the combination of these principles has not been tried.

26. (U) Progress (Jan 65 - Jun 65). The work has only been in progress for 4 months and the first phase has not yet been completed. A group of mice, initial recipients of lymphoid tissue, have been exsanguinated and the globulin faction extracted by dialysis from the serum. The spleens from these mice were also saved at the time of exsanguination. The globulin/lymphoid tissue are being injected intradermally into another group of recipients which will receive skin grafts after completion of the injections.

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) OVER

ABSTRACT

AUTOIMMUNOLOGICAL ASPECTS OF TISSUE TRANSPLANTATION

This study is an exploration of the possibility of stimulating host production of immune antibodies which will inactivate or destroy any antibodies which the same host will produce against a subsequently transplanted tissue.

By intraperitoneal injection of lymphoid cells from the donor into an intermediate recipient, it was attempted to produce antisera to the donor's tissue. The globulin fraction of the intermediate recipient's serum was injected alone and in combination with the intermediate recipient's lymphoid tissue into an ultimate recipient. The ultimate recipient's immunity was then challenged by the use of skin grafts from the donors.

The procedure to date does not appear to be significantly productive, since there was not a substantial difference in the rejection rates between the immunized and the control mice.

BODY OF REPORT

AUTOIMMUNOLOGICAL ASPECTS OF TISSUE TRANSPLANTATION

Description:

This study is to determine if it is possible to stimulate a host to produce immune antibodies which will inactivate or destroy any antibodies which the same host would produce against a subsequently transplanted tissue. The production of antisera to the tissue of the donor was attempted by intraperitoneal injection of lymphoid cells from the donor into the receipient. This antisera was in turn injected intradermally into another receipient who would theoretically develop circulating antibodies against the components of the antisera. Lymphoid cells from the first recipient were injected alone and in combination with the antisera into an ultimate recipient in order to produce an autoimmunization. Skin grafts from the donor were then transplanted to determine the degree of immunization achieved.

Progress:

Sixty Strong-A mice were splenectomized and the splenic material macerated and suspended in physiological saline. The crude splenic suspension was injected into thirty recipient Cb strain mice in a series of three intraperitoneal injections given at seven-day intervals. Seven days after the third injection "pinch" type skin grafts were transferred from the previously splenctomized Strong-A mice to those receipients. Thirty control Cb strain mice also received skin grafts. Following rejection of these grafts, the intermediate recipients were exsanguinated and splenectomized. The globulin fraction of the blood was extracted by dialysis and the splenic tissue was macerated and suspended in normal physiological saline.

A group of fifteen Cb strain mice were then given intradermal injections of the globulin fraction in a series of three injections at seven-day intervals. A second group of fifteen Cb mice were given similar injections in combination with intraperitoneal injections of the splenic suspension. A third group of mice received only the suspension of splenic material. Seven days after the last injection skin grafts were transferred from the original splenectomized mice to all three groups of mice plus an additional fifteen control mice. The mice were then examined daily to determine the rate of rejection of the transplanted tissue.

Summary and Conclusions:

The results are presently being statistically analyzed in order to determine if there is any significant difference between the rate of rejection of the immunized mice as opposed to that of the control group. This approach to the problem of tissue transplantation does not appear to be significantly productive and the approach will have to be altered before future work can be continued. The problem is to determine, first, if the antisera of the intermediate recipient contains antibody against the donor tissue, second, if there is antibody present, it must be determined if the ultimate recipient can produce

antibodies against the components of the antisera.

List of Publications: (None)

RESEARCH AND TECHNOLOGY RESUME			3. GOVT ACCESSION	4. AGENCY ACCESSION	REPORT CONTROL SYMBOL
				DA OA.6330	CSCRD 103
4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	a. CLASS U b. CAT	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE	10b. PRIOR NUMBER/CODE
61130011 3A013001A91C 01 045	61130011 3A013001A91C 01 68

11. TITLE:
(U) Regulation of Thyroid Function (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
012900 Physiology	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 65	0	3
		CURRENT FY 66	0	5

19. GOV'T LAB/INSTALLATION/ACTIVITY	20. PERFORMING ORGANIZATION
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21. TECHNOLOGY UTILIZATION	22. COORDINATION
Protein Purification and Thyroid Function Tests	None

23. KEYWORDS: Thyroid gland; Thyrotropin; Thyroid hormones

24. (U) Objective.

The objective of this study is to develop an experimental model to test the hypothesis that the serum free thyroxin concentration regulates pituitary elaboration of thyrotropin.

25(U) Approach.

Human thyroxine-binding prealbumin will be isolated from serum or plasma and injected into rats. Free thyroxin concentrate and PBI will be measured first. After a fall in free thyroxin concentration is achieved, radioiodine uptake, formation of thyroxin and its precursors, secretion of thyroxin, and thyroid cell height will be measured. These measurements are all influenced by thyrotropin.

26(u) Progress.

Only preliminary studies in this isolation of thyroxin-binding prealbumin have been performed thus far.

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 35 identical to NASA Form 1122) OVER

ABSTRACT

REGULATION OF THYROID FUNCTION

A basic problem in thyroid physiology relates to the nature of the regulation of the thyroid gland by the hypothalamus and anterior hypophysis and the possibility of a negative feed back system between the blood thyroxine concentration and the pituitary similar to that operating for the adrenal gland. Alterations in the total plasma thyroxine concentration, however, do not necessarily induce the changes in thyroid function which are anticipated on the basis of the negative feedback mechanism. It has recently been suggested that the free (unbound) thyroxine rather than the total thyroxine may be of importance in this control system. It is the purpose of this study to design an experimental model in which free thyroxine can be acutely lowered without causing the animal any undue stress. To achieve this aim, human thyroxine-binding prealbumin (TBPA) will be isolated from blood bank plasma by means of DEAE-cellulose chromatography and cellulose column electrophoresis. This protein will then be injected into rats to lower their free thyroxine concentration after which various aspects of thyroid function which are thyrotropin dependent will be measured. At this time, only initial studies in the isolation of TBPA have been completed.

BODY OF REPORT

REGULATION OF THYROID FUNCTION

Description:

It is the purpose of this sub-task to develop an experimental model in which serum free thyroxine can be acutely lowered in the rat without trauma to the animal. In the first stages of this work, human thyroxine-binding realbumin (TBPA) will be isolated from pooled blood bank plasma by means of DEAE-cellulose column chromatography and cellulose column electrophoresis. TBPA will then be added to rat sera in vitro with appropriate measurements of its effect on free thyroxine concentration and on thyroxine binding capacity. When these studies are completed, TBPA will be injected into intact and hypophysectomized rats with suitable controls. Measurements of the thyroid; serum iodide concentration ratio, release of labeled protein-bound iodine and other thyrotropin sensitive functions of the thyroid will be made. It is hoped that these studies will shed light on the role of serum free thyroxine in the regulation of the hypothalamic-anterior hypophyseal-thyroid axis.

Progress:

This is a newly initiated sub-task. At this time, crude TBPA preparations have been prepared by DEAE-cellulose column chromatography of pooled human plasma. Further purification has been hampered by difficulties in the modification of existing equipment to current needs. These problems should be overcome shortly, at which time work on this sub-task will proceed.

Summary and Conclusions:

None

Publications:

None

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RESEARCH AND TECHNOLOGY RESUME

2.	3. GOVT ACCESSION	4. 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 65	A. NEW	a. U b. U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 01 046

10b. PRIOR NUMBER/CODE: 61130011 3A013001A91C 01 69

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	18. RESOURCES EST.	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
C. IN-HOUSE	a. NUMBER: NA c. TYPE: d. AMOUNT:	PRIOR FY 65	0	4
		CURRENT FY 66	1	10

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21. TECHNOLOGY UTILIZATION
Antifatigue Drug Screening

22. COORDINATION
None

23. KEYWORDS
Rats; Fatigue; Drug effects; Endurance; Work Decrement; Work

24.
(U) Tech 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 animal works 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.

From November 1964 to May 1965, the equipment is being built to provide a running wheel with a "titrating" shock source.

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)

OVER

ABSTRACT

DEVELOPMENT OF A MEANS FOR MEASUREMENT OF WORK DECREMENT IN THE RAT

The objective of this sub-task 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 curve may be studied; and only a few animals will be required to obtain reliable results.

BODY OF REPORT

DEVELOPMENT OF A MEANS FOR MEASUREMENT OF WORK DECREMENT IN THE RAT

Description:

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 receives 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.

Progress:

At present the equipment to perform the study is being constructed. The first animals should be run within six months.

Summary and Conclusions:

None

Publications:

None

Previous page was blank, therefore not filmed.

RESEARCH AND TECHNOLOGY RESUME

2.	3. GOVT ACCESSION	4. AGENCY ACCESSION	REPORT CONTROL SYMBOL
		DA OA 6332	CSCRD 193

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	a. U b. U	NA	NL	A. WORK UNIT

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 01 047

10b. PRIOR NUMBER/CODE: 61130011 3A013001A91C 01 70

11. TITLE:
(U) Cardiovascular research (06)

12. SCIENTIFIC OR TECH. AREA	13. START DATE	14. CRIT. COMPL. DATE	15. FUNDING AGENCY
012900 Physiology	12 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 65	0	1
		CURRENT FY 66	1	4

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21. TECHNOLOGY UTILIZATION
Artificial Cardiac Pacing

22. COORDINATION
None

23. KEYWORDS
Heart Block; Heart Catheterization; Heart Conduction System; Artificial Pacemaker.

24. (U) Tech Objective.

25. To establish the importance and/or relative influence of the following factors regarding transvenous unipolar intracardiac electrical pacing of the heart: a) the necessity of endocardial contact of the electrode for reliable and consistent pacings; b) the voltage and amperage requirements necessary for this; c) the importance of "positioning" of the electrode in the right ventricle,and d) the importance of the type and positioning on indifferent electrode for satisfactory pacing.

26. (U) Approach: Complete heart block will be produced in dogs by placing a suture ligature around the bundle of His (according to the method of Starzyl and Gaertner). A unipolar catheter tip will be sheathed in a nonconductive hood of chemically inert material, with holes allowing free flow of blood about the tip but not exposing the tip. The catheter will be placed in the right heart of dogs with an indifferent electrode initially beneath the skin of the chest. Stimulating impulses will be supplied by an external pulse generator. Voltage and amperage requirements will be obtained, and positioning of the catheter tip at various sites in the right heart will be done to determine the ideal placement. Lastly, the most satisfactory location of the indifferent electrode will be determined for consistent and dependable pacing.

(U) Progress. (Dec 64 - Jun 65)

To date three dogs have been operated on and electrically paced. Endocardial contact of the electrode was not necessary in two of the animals. The third case required contact. Voltage requirements, indifferent electrode placement, and intracardial electrode placement data are not available 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) CFY+1	36.

DD FORM 1 AUG 64 1498 (Items 1 to 36 identical to NASA Form 1322)

ABSTRACT

CARDIOVASCULAR RESEARCH

Investigations are being conducted to determine the ideal placement, voltage requirements and reliability of electrical cardiac pacing using unipolar right heart catheters, introduced through a peripheral vein. Surgical complete heart block is produced in dogs by placing a suture around the bundle of His. The unipolar catheter tip is sheathed in a non-conductive hood of chemically inert material with holes allowing free flow about the tip but not exposing the tip. The catheter tip is placed at various positions in the right heart with an indifferent electrode beneath the skin of the chest. Stimulating impulses are supplied by an external pulse generator. Voltage requirements will be obtained, and positioning of the catheter tip at various sites in the right heart will be done to determine ideal placement. Lastly, the most satisfactory location of the indifferent electrode will be determined for consistent and dependable pacing. To date three dogs have been studied revealing that endocardial contact of the intracardiac electrode is not necessary for pacing at low voltages. Electrode placement information is not yet available.

BODY OF REPORT

CARDIOVASCULAR RESEARCH

Study No. 1: UNIPOLAR CARDIAC CATHETER PACING

Description:

The objective is to establish the reliability and clinical usefulness of unipolar right ventricular electrode catheters for treatment of heart block associated with Adams-Stokes attacks and congestive heart failure. Objectives to be determined are: 1) necessity of endocardial contact; 2) voltage requirements; 3) preferential site of catheter tip for dependable pacing; 4) preferential site of indifferent skin electrode.

Progress:

To date, surgical heart block has been produced in three mongrel dogs and attempted in three others. Pacing has been successful using a Medtronic battery pacemaker without endocardial contact of the electrode tip; however, contact was required in one dog. Three dogs died during, or soon after, the surgical heart block procedure. Ideal endocardial placement and indifferent electrode placement has not as yet been determined. Newer techniques for chronic surgical heart block are presently being attempted.

Summary and Conclusions:

Assessment of reliability and evaluation of the methods of pacing by unipolar right heart catheters is in progress. One problem has been the reversion of heart block to sinus rhythm in several surgically blocked dogs. Preliminary studies indicate that endocardial contact of the unipolar electrode is not necessary for adequate pacing at a low voltage, and electrode placement will be evaluated and new techniques for producing heart block are being attempted.

Publications:

None.

Previous page was blank, therefore not filmed.

RESEARCH AND TECHNOLOGY RESUME

4. DATE OF RESUME	5. KIND OF RESUME	6. SECURITY	7. REGRADING	8. RELEASE LIMITATION	9. LEVEL OF RESUME
01 07 65	A. NEW	U U	NA	NL	A. WORK UNIT

2. GOVT ACCESSION: | 3. AGENCY ACCESSION: DA OA 6333 | REPORT CONTROL SYMBOL: CSCRD 103

10a. CURRENT NUMBER/CODE: 61130011 3A013001A91C 01 048

10b. PRIOR NUMBER/CODE:

11. TITLE: (U) The Physiological Role of Cyclic 3',5'-Adenosine Monophosphate in Humans (06)

12. SCIENTIFIC OR TECH. AREA: 012600 Pharmacol. 012900 Physiol.

13. START DATE: 10 64 | 14. CRIT. COMPL. DATE: NA | 15. FUNDING AGENCY: OTHER: 1 DA

16. PROCURE. METHOD: C. IN-HOUSE

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

18. RESOURCES EST.

	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In thousands)
PRIOR FY 65	1	6
CURRENT FY 66	1	6

19. GOV'T LAB/INSTALLATION/ACTIVITY
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21. TECHNOLOGY UTILIZATION: Cardiovascular pharmacology

22. COORDINATION: None

23. KEYWORDS: Adenine nucleotides, catecholamines, hemodynamics, myocardium, cortisone.

24. (U) Objective.

Cyclic 3',5'-AMP has been thought to control several metabolic processes, including glycogenolysis, steroidogenesis, ketogenesis, lipolysis, and antidiuresis. The study is designed to further evaluate the possible physiological role of 3',5'-AMP in man. Pharmacologic application of 3',5'-AMP to such clinical situations as shock and fatigue were to be explored.

25. (U) Approach.

Prior to the use of 3',5'-AMP in humans, toxicological studies were planned in animals. Cardiovascular studies included heart rate, cardiac output and blood pressure. Metabolic studies included measurement of blood glucose, plasma nonesterified fatty acids, plasma cortisol, plasma osmolarity, and blood lipids. Measurements in dogs and humans were to be obtained in the unanesthetized state. A major technical problem was the feasibility of cardiac catheterization in the dog and human.

26. (U) Progress. These investigations were carried out between October, 1964 and June 1965. Toxicological studies failed to reveal any adverse clinical reactions to 3',5'-AMP in rats, guinea pigs, rabbits and dogs. Cardioacceleration and increased cardiac output were demonstrated in dogs and man following administration of 3',5'-AMP. The cardiac actions occurred prior to 3',5'-AMP-induced hyperglycemia and steroidogenesis. These findings support in vitro data that 3',5'-AMP may represent the biochemical basis for the myocardial response to catecholamines. The prompt cardiac actions of 3',5'-AMP appeared to be independent from the observed delayed hyperglycemia.

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ABSTRACT

THE PHYSIOLOGICAL ROLE OF CYCLIC 3',5'-ADENOSINE MONOPHOSPHATE IN HUMANS

These studies have demonstrated in unanesthetized dogs and in human volunteer subjects that 3',5'-AMP induces cardio-acceleration, increased cardiac output, and systolic hypertension. These findings support *in vitro* data that 3',5'-AMP may represent the biochemical basis for the myocardial response to catecholamines. The cardiovascular actions occurred prior to 3',5'-AMP-induced hyperglycemia and steroidogenesis. The increase in plasma cortisol was consistent after constant infusion of 3',5'-AMP and mimicked the effects of an ACTH infusion test. The plasma nonesterified fatty acid response was biphasic, with an initital fall and subsequent rise in concentration. Preliminary studies indicated antidiuresis and an increase in urine osmolarity following acute sustained water loading tests. It is apparent from these studies, that 3',5'-AMP penetrates cells in sufficient concentration to regulate intracellular metabolic events, as reflected by glycogenolysis, antidiuresis, and steroidogenesis. It does not appear that the alterations in heart rate noted were the results of changing levels of circulating catecholamines. The rapidity of the changes which occurred in heart rate would argue against a humoral mechanism.

BODY OF REPORT

THE PHYSIOLOGICAL ROLE OF CYCLIC 3',5'-ADENOSINE MONOPHOSPHATE IN HUMANS (FY 1965)

Description:

It is now established that 3',5'-AMP promotes the accumulation of active phosphorylase in a variety of tissues and as a result glycogenolysis is increased. The response of tissues to increase glycogenolysis is variable, the liver releasing glucose and the adrenal releasing steroids. Studies have been designed to determine the metabolic and cardiovascular effects of cyclic 3',5'-adenosine monophosphate (3',5'-AMP) in human volunteer subjects and unanesthetized dogs.

Progress:

3',5'-AMP was studied in fed and fasted, unanesthetized dogs prepared with catheters chronically implanted in the right atrium and aortic arch. Determination of heart rate, cardiac output, mean blood pressure, total peripheral resistance, stroke volume, pulse pressure, blood glucose, and plasma free fatty acids were recorded. The actions of 3',5'-AMP mimicked those of catecholamines, as reflected by an increase in heart rate, cardiac output and blood glucose. Following single intracardiac doses of 3',5'-AMP (4-8 mg/kg), heart rate increased within seconds. Although there was a slower, transiently significant decrease in mean blood pressure, heart rate remained significantly elevated for 30 minutes. Blood glucose increased to a maximum at 7 minutes and then decreased thereafter, while plasma free fatty acids progressively decreased to a maximum at 15 minutes. From the time sequences of the maximal cardiac and metabolic responses to 3',5'-AMP, there appeared to be no relationship between these actions. No significant cardiac or metabolic changes followed administration of 2',3'-AMP, 5'-AMP, ATP, or saline. Prior treatment with dichloroisoproterenol, in doses which produced selective adrenergic beta receptor blockade with intrinsic stimulation of the heart, however, blocked the cardiovascular but not the metabolic effects of 3',5'-AMP. It was concluded that the chronotropic response of dichloroisoproterenol inhibited a further increase with 3',5'-AMP. Pre-treatment with the beta adrenergic blocking agent propranolol in the dose of 0.1 to 0.5 mg intravenously, failed to block either the cardiovascular or metabolic effects of 3',5'-AMP. It is concluded from these studies that 3',5'-AMP acts on a site distal to that of the beta adrenergic receptor. Pre-treatment with theophylline or imidazole did not consistently alter the cardiac or metabolic responses to 3',5'-AMP.

In vitro experiments and the current experiments in unanesthetized dogs suggest that the glycogenolytic and myocardial responses to catecholamines are mediated by 3',5'-AMP. Single intravenous or intracardiac doses of 3',5'- AMP (8-12 mg/kg) administered to 15 human subjects, including 8 during cardiac catheterization, were attended within seconds by an increase in heart rate. The maximal increase in heart rate averaged 40% above control levels ($P<.005$), with a range of 20 to 150 % of the initial rate,and was accompanied by a mean

increase in cardiac output of 44% ($P<.025$) five minutes after 3',5'-AMP injection. Cardio-acceleration persisted for 15 minutes. There were no significant changes in blood pressure. Mean stroke volume increased from 63 to 69 ml but this was not statistically significant. Five other normal subjects received a constant 3',5'-AMP infusion at the rate of 0.5 mg/kg per minute from 1 to 2 hours which produced persistent tachycardia and an average blood pressure elevation of 22% during the infusion period. Plasma cortisol, measured on the day preceding and the day of infusion, rose from an initial mean value of 11.7 ± 3.5 µg per 100 ml, to 24.6 ± 9.0 µg per 100 ml, ($P<.025$) at the end of the infusion period. In the acute injection experiments, cortisol did not significantly increase in the group and rose in only 6 of 15 subjects. Although hyperglycemia was observed in all 20 subjects, the plasma free fatty acid response was variable. There was an initial 17% fall within the first 5 minutes, followed by a subsequent progressive increase of 35% above control levels at 15 minutes. There were no significant changes in plasma osmolarity, but preliminary data in normal human volunteer subjects indicate that there was antidiuresis and an increase in urine osmolarity following injection of 3',5'-AMP during acute sustained water load tests.

The effects of beta adrenergic blockade were tested by pre-treatment with propranolol (.06 to .1 mg per kg intravenously). Pre-treatment with propranolol caused a mean 12% decrease in heart rate, characteristic of this agent, but no alteration of blood pressure, blood glucose or plasma free fatty acids. Subsequent administration of isoproterenol 30 minutes after propranolol failed to induce a chronotropic response, while it previously increased the heart rate 50 to 100 % above control levels prior to propranolol administration. This finding indicated functional blockade of the cardiac beta adrenergic receptor site. 3',5'-AMP was then infused and, in all instances, induced tachycardia, systolic hypertension, steroidogenesis, hyperglycemia, and a variable plasma free fatty acid response, similar in these 3 subjects tested, to the original action of the cyclic nucleotide alone.

Studies in a patient with anterior pituitary insufficiency secondary to post-partum hemorrhage revealed typical chronotropic and biochemical responses and a plasma cortisol increase following constant infusion with 3',5'-AMP. These studies indicated that 3',5'-AMP could act in the absence of a functioning anterior pituitary gland.

Summary and Conclusions:

These studies have demonstrated in unanesthetized dogs and in human volunteer subjects that 3',5'-AMP induces cardio-acceleration, increased cardiac output, and systolic hypertension. These findings support <u>in vitro</u> data that 3',5'-AMP may represent the biochemical basis for the myocardial response to catecholamines. The cardiovascular actions occurred prior to 3',5'-AMP-induced hyperglycemia and steroidogenesis. The increase in plasma

cortisol was consistent after constant infusion of 3',5'-AMP and mimicked the effects of an ACTH infusion test. The plasma nonesterified fatty acid response was biphasic, with an initial fall and subsequent rise in concentration. Preliminary studies indicated antidiuresis and an increase in urine osmolarity following acute sustained water loading tests. It is apparent from these studies, that 3',5'-AMP penetrates cells in sufficient concentration to regulate intracellular metabolic events, as reflected by glycogenolysis, antidiuresis, and steroidogenesis. It does not appear that the alterations in heart rate noted were the results of changing levels of circulating catecholamines. The rapidity of the changes which occurred in heart rate would argue against a humoral mechanism.

Publications:

Levine, R.A., and Vogel, J.A. Cardiovascular and metabolic effects of adenosine 3',5'-monophosphate in unanesthetized dogs. Fed. Proc. 24: 612, 1965 (abstract).

Levine, R.A. Cardiovascular and metabolic effects of adenosine 3',5'-monophosphate in man. J. Clin. Invest., 1965 (abstract).

Levine, R.A., Dixon, L.M., and Franklin, R.H. The physiological role of adenosine 3',5'-monophosphate in man. I. Regulation of cardiovascular, metabolic and antidiuretic function. Submitted for publication.

Levine, R.A., and Vogel, J.A. Cardiovascular and metabolic effects of adenosine 3',5'-monophosphate in unanesthetized dogs. Submitted for publication.

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13. ABSTRACT

Military Internal Medicine: Microbiological Research in Tuberculosis, Experimental Surgery, Computer Classification, Histopathology of Laboratory Animals, Intravenous Fat Emulsions, Studies of Nutritional Status of Both Civilian and Military Populations, as well as studies of Radiated Foods, together with Amino Acids and Proteins, Carbohydrate studies and Fatigue and Exercise Physiology studies during the FY year.

Military Environmental Medicine: Studies of the interrelationships of hypoxia, diet and temperature on work performance, cardiopulmonary physiology, nutritional status and organ and body metabolism. These studies utilize humans, dogs and small laboratory mammals with various techniques applied.

Basic Research in Support of Military Medicine; Studies of Lipids and Related Compounds, Mineral Metabolism, Carbohydrates, Nutritional Biochemistry of Chemotherapeutics and the Functional Aspects of Body Composition have been intensely studied during the period and the findings in each field have been reported.

In-House Laboratory Independent Research: Total of nine sub-tasks, seven initiated during FY 1965, will be incorporated into the mission program in FY 66 or 67. Several papers which report the findings of investigators working on In-House projects have been published in scientific journals.

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