Form AEC-313 (5-58)	ATOMIC ENERGY COMMISSION **APPLICATION FOR BYPRODUCT MATERIAL LICENSE**	Form approved Budget Bureau No. 38-R027.4

INSTRUCTIONS.--Complete Items 1 through 16 if this is an initial application. If application is for renewal of a license, complete only Items 1 through 7 and indicate new information or changes in the program as requested in Items 8 through 15. Use supplemental sheets where necessary. Item 16 must be completed on all applications. Mail three copies to: U. S. Atomic Energy Commission, Washington 25, D. C. Attention: Isotopes Branch, Division of Licensing and Regulation. Upon approval of this application, the applicant will receive an AEC Byproduct Material License. An AEC Byproduct Material License is issued in accordance with the general requirements contained in Title 10, Code of Federal Regulations, Part 30 and the Licensee is subject to Title 10, Code of Federal Regulations, Part 20.

1. (a) NAME AND STREET ADDRESS OF APPLICANT. *(Institution, firm, hospital, person, etc.)*

Department of the Army
Fitzsimons General Hospital and
U. S. Army Medical Research and
Nutrition Laboratory
Denver, Colorado 80240

(b) STREET ADDRESS(ES) AT WHICH BYPRODUCT MATERIAL WILL BE USED. *(If different from 1 (a).)*

Same as Item 1 (a) and
Summit of Pikes Peak, Colorado
(Condition 11)

2. DEPARTMENT TO USE BYPRODUCT MATERIAL

Radiology Service and USAMRNL

3. PREVIOUS LICENSE NUMBER(S). *(If this is an application for renewal of a license, please indicate and give number.)*

05-00046-13 Renewal

4. INDIVIDUAL USER(S). *(Name and title of individual(s) who will use or directly supervise use of byproduct material. Give training and experience in Items 8 and 9.)*

Users approved by the Radioisotope Committee (See Appendix I for Radioisotope Committee members and Appendix II for individuals approved for human use)

5. RADIATION PROTECTION OFFICER *(Name of person designated as radiation protection officer if other than individual user. Attach resume of his training and experience as in Items 8 and 9.)*

As designated by Radioisotope Committee (WO 2 Charles Spinks, See Appendix I for training and experience)

6. (a) BYPRODUCT MATERIAL *(Elements and mass number of each.)*

		(b) CHEMICAL AND/OR PHYSICAL FORM AND MAXIMUM NUMBER OF MILLICURIES OF EACH CHEMICAL AND/OR PHYSICAL FORM THAT YOU WILL POSSESS AT ANY ONE TIME. *(If sealed source(s), also state name of manufacturer, model number, number of sources and maximum activity per source.)*	
A.	Iodine 131	A. Iodide	A. 250 millicuries
B.	Iodine 131	B. Iodinated Human Serum Albumin	B. 5 millicuries
C.	Iodine 131	C. Labeled Renal Function Compounds	C. 6 millicuries
D.	Iodine 131	D. Rose Bengal	D. 2 millicuries
E.	Iodine 131	E. Labeled Fats and Fatty Acids	E. 2 millicuries
F.	Iodine 131	F. Cholografin	F. 2 millicuries
G.	Iodine 131	G. Thyroxine	G. 2 millicuries
H.	Iodine 125	H. Iodide	H. 1 millicurie
I.	Iodine 125	I. Iodinated Human Serum Albumin	I. 1 millicurie
J.	Iodine 125	J. Labeled Renal Function Compounds	J. 1 millicurie
K.	Iodine 125	K. Rose Bengal	K. 1 millicurie
L.	Iodine 125	L. Labeled Fats and Fatty Acids	L. 1 millicurie
See Appendix III		See Appendix III	

7. DESCRIBE PURPOSE FOR WHICH BYPRODUCT MATERIAL WILL BE USED. *(If byproduct material is for "human use," supplement A (Form AEC-313a) must be completed in lieu of this item. If byproduct material is in the form of a sealed source, include the make and model number of the storage container and/or device in which the source will be stored and/or used.)*

A. Diagnosis of thyroid function and thyroid scanning. Treatment of hyperthyroidism, cardiac conditions and thyroid carcinoma.
B. Determination of plasma volumes and cardiac output. Cardiac scanning. Localization of brain tumors. Placenta localization.
C. Determination of renal function.
D. Determination of liver function. Liver scanning.
E. Determination of fat absorption.
F. Determination of liver and gallbladder function.

See Appendix IV

(Continued on reverse side)

TRAINING AND EXPERIENCE OF EACH INDIVIDUAL NAMED IN ITEM 4 *(Use supplemental sheets if necessary)*

8. TYPE OF TRAINING	WHERE TRAINED	DURATION OF TRAINING	ON THE JOB (Circle answer)	FORMAL COURSE (Circle answer)
a. Principles and practices of radiation protection	Individuals will have appropriate training and experience prior to approval by Radioisotope Committee. (See Appendix I & II for training and experience of Radioisotope Committee members and persons authorized for human use).		Yes No	Yes No
b. Radioactivity measurement standardization and monitoring techniques and instruments			Yes No	Yes No
c. Mathematics and calculations basic to the use and measurement of radioactivity			Yes No	Yes No
d. Biological effects of radiation			Yes No	Yes No

9. EXPERIENCE WITH RADIATION. *(Actual use of radioisotopes or equivalent experience.)*

ISOTOPE	MAXIMUM AMOUNT	WHERE EXPERIENCE WAS GAINED	DURATION OF EXPERIENCE	TYPE OF USE
Same	as Item 8			

10. RADIATION DETECTION INSTRUMENTS. *(Use supplemental sheets if necessary.)*

TYPE OF INSTRUMENTS (Include make and model number of each)	NUMBER AVAILABLE	RADIATION DETECTED	SENSITIVITY RANGE (mr/hr)	WINDOW THICKNESS (mg/cm²)	USE (Monitoring, surveying, measuring)
See Appendix V for Radiation Detection instruments					

11. METHOD, FREQUENCY, AND STANDARDS USED IN CALIBRATING INSTRUMENTS LISTED ABOVE.

See Appendix V

12. FILM BADGES, DOSIMETERS, AND BIO-ASSAY PROCEDURES USED. *(For film badges, specify method of calibrating and processing, or name of supplier.)*

All personnel wear film badges. Badges are processed at 4 week intervals by Lexington Blue Grass Army Depot. Nursing personnel use pocket dosimeters when working near therapy patients. Pocket dosimeters are calibrated at 6 month intervals by Pueblo Army Depot mobile calibration team.

INFORMATION TO BE SUBMITTED ON ADDITIONAL SHEETS

13. FACILITIES AND EQUIPMENT. Describe laboratory facilities and remote handling equipment, storage containers, shielding, fume hoods, etc. Explanatory sketch of facility is attached. (Circle answer) (Yes) No See Appendix VI

14. RADIATION PROTECTION PROGRAM. Describe the radiation protection program including control measures. If application covers sealed sources, submit leak testing procedures where applicable, name, training, and experience of person to perform leak tests, and arrangements for performing initial radiation survey, servicing, maintenance and repair of the source. See Appendix VII

15. WASTE DISPOSAL. If a commercial waste disposal service is employed, specify name of company. Otherwise, submit detailed description of methods which will be used for disposing of radioactive wastes and estimate of the type and amount of activity involved. See Appendix VI

CERTIFICATE (This item must be completed by applicant)

16. THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATE ON BEHALF OF THE APPLICANT NAMED IN ITEM 1, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PART 30, AND THAT ALL INFORMATION CONTAINED HEREIN, INCLUDING ANY SUPPLEMENTS ATTACHED HERETO, IS TRUE AND CORRECT TO THE BEST OF OUR KNOWLEDGE AND BELIEF.

Department of the Army
FGH & USAMRNL, Denver, Colo. 80240
Applicant named in item 1

Date 25 June 1968

By: Edwin L. Overholt
EDWIN L. OVERHOLT
Chief, Radioisotope Committee
Title of certifying official Fitzsimons General Hosp. & US Army Med.Rsch & Nutr Lab.

WARNING.—18 U. S. C., Section 1001; Act of June 25, 1948, 62 Stat. 749; makes it a criminal offense to make a willfully false statement or representation to any department or agency of the United States as to any matter within its jurisdiction.

* U.S. GOVERNMENT PRINTING OFFICE 1961 O—615953

APPENDIX I

Radioisotope Committee

A description of the Radioisotope Committee is attached as Part I of this appendix, and the hospital regulation appointing the Radioisotope Committee is attached as Part II.

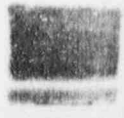
The present membership of the committee is as follows:

Colonel Edwin A. Overholt, MC, Chief, Department of Medicine
* Chief, Department of Surgery
Colonel John E. Canham, MC, Commanding Officer, USAMRNL
Lt. Colonel Paul E. Siebert, MC, Chief, Radiology Service
Major John T. Decker, MC, Chief, Pathology Service
Major Charles G. Liddle, VC, Chief, Radioisotope Section, Physiology Division, USAMRNL
Captain Norman Helman, MC, Chief, Radioisotope Section, Radiology Service
Warrant Officer W-2 Charles L. Spinks, Post Engineers

Curriculum Vitae of the members of the Radioisotope Committee is attached along with the training and experience of those who have previous radioisotope experience.

* Colonel John W. White, MC, Chief, Department of Surgery departed Fitzsimons last week. His replacement has not been assigned as yet. The Curriculum Vitae for his replacement will be included with the next Radioisotope Committee minutes after his arrival.

Appendix I



FITZSIMONS GENERAL HOSPITAL
Denver, Colorado 80240

*CHANGE 1 TO
HOSPITAL REGULATION
NUMBER 15-1

23 April 1968

BOARDS, COMMISSIONS AND COMMITTEES

PROFESSIONAL BOARDS AND COMMITTEES

*Hospital Regulation 15-1, 14 March 1968, is changed as follows:

* * * *

2. Composition.

* * * *

1. Medical Records Committee.

* * * *

(Added) Chief, Department of Dentistry

* * * *

m. Hospital Utilization Board.

* * * *

(Added) Chief, Department of Dentistry

* * * *

MEDEO-XO

FOR THE COMMANDER:

C. E. BRADFORD
Major, MSC
Adjutant

DISTRIBUTION:
"B" and "C"
Plus 10 ea - B8
5 ea - B11, B28, B43, B48

FITZSIMONS GENERAL HOSPITAL
Denver, Colorado 80240

*HOSPITAL REGULATION
NUMBER 15-1

14 March 1968

BOARDS, COMMISSIONS AND COMMITTEES

PROFESSIONAL BOARDS AND COMMITTEES

1. Purpose. To establish the professional boards and committees necessary for the efficient operation of this installation.

2. Composition. (The Senior Officer is Chairman unless otherwise specified.)

a. Profile Classification Board.

Assistant Chief, Department of Medicine
Assistant Chief, Department of Surgery
Chief, Department of Hospital Clinics
Chief, Personnel Mgt Section, Military Personnel Branch
Unit Commanding Officer of individual concerned

b. Rabies Control Board.

Chief, Department of Hospital Clinics (Preventive Medicine Officer) - Chairman
Chief, Department of Surgery
Chief, General Medicine Service
Chief, Pediatric Service
Post Veterinarian (Assistant Preventive Medicine Officer)
Chief, Pathology Division, USAMRNL

c. Sterilization and Therapeutic Termination Board.

Three staff physicians, one of whom will be the Chief or Assistant Chief, OB-GYN Service, or the Chief or Assistant Chief, Urology Service. The second and third members of the board will be designated in each individual case by the Commanding General (in his absence the Executive Officer) with due consideration to the patient's related physical and mental condition. The senior member shall serve as Chairman.

d. Therapeutic Agents Board.

Chief, Professional Services - Chairman
Chief, Department of Surgery
Chief, Department of Medicine
Chief, Department of Psychiatry
Chief, Department of Hospital Clinics
Chief, Pharmacy Service - Recorder & Coordinator
Chief, Pediatric Service

*This Hospital Regulation supersedes HR 15-1, 7 December 1966, with C1, 20 December 1966, C2, 21 February 1967, and C3, 17 March 1967.

e. Tumor Board.

Chief, Department of Surgery - Chairman
Chief, Department of Medicine
Chief, Radiology Service
Chief, Pathology Service
Chief, Department of Dentistry (Dental cases only)
Attending physician concerned

f. Clinical Research Committee.

Chief, Professional Services
Chief, Department of Surgery
Chief, Department of Medicine
Chief, Department of Dentistry
Chief, Department of Hospital Clinics
Chief, Department of Psychiatry
Chief, Administrative Services
Chief, Pathology Service
Chief, Radiology Service
Commanding Officer, USAMRNL
Director, Clinical Research Service

g. Dental Education Committee.

Chief, Department of Dentistry
Chief, Oral Surgery Service
Chief, Periodontia Service
Chief, Operative Service

h. Hospital Education Committee.

Commanding General
Director of Medical Education
Chief, Department of Surgery
Chief, Department of Medicine
Chief, Department of Psychiatry
Chief, Department of Hospital Clinics
Chief, Pathology Service
Chief, Radiology Service
Chief, Cardiology Service
Chief, Dermatology Service
Chief, Pharmacy Service
Chief, Pediatric Service
Chief, General Medicine Service
Chief, Pulmonary Disease Service
Chief, Otolaryngology Service
Commanding Officer, USAMRNL
Chief, Clinical Research Service
Chief, Gastroenterology Service
Asst Chief, Department of Medicine
Chief, Plastic Surgery Service
Chief, Administrative Services/Coordinator, Adm Residents Program
Chief, Anesthesia & Operative Service
Chief, Ophthalmology Service
Chief, General Surgery Service
Chief, OB-GYN Service
Chief, Orthopedic Service
Chief, Neurosurgery Service
Chief, Thoracic Surgery Service
Chief, Urology Service
Chief, Department of Dentistry
Chief, Psychiatry Service
Chief, Neurology Service
Chief, Physical Medicine Service
Chief, Nursing Service
Civilian Education Consultant (on call)
Director, Dietetic Internship
Intern Counselor
Educational Coordinator, Nursing Service

i. Hugh Mahon Lectureship Award Committee.

Chief, Department of Medicine
Chief, Department of Surgery
Chief, Pathology Service
Civilian Education Consultant
Secretary to Chief, Professional Services - Coordinator

j. Infections Committee.

Chief, Department of Surgery - Chairman
Chief, Department of Hospital Clinics (Preventive Medicine Officer)
Chief, Department of Medicine
Chief, Pathology Service
Chief, Microbiology Sub-Section, Pathology Service
Chief, Anesthesia & Operative Service
Chief, OB-GYN Service
Chief, Pediatric Service
Chief, Nursing Service
Assistant Preventive Medicine Officer - Recorder

k. Medical Library Committee.

Chief, Professional Services - Chairman
Chief, Department of Surgery
Chief, Department of Medicine
Chief, Department of Dentistry
Chief, Pathology Service
Chief, Radiology Service
Chief, Nursing Service
Chief, Department of Psychiatry
Adjutant (member w/o vote)
Medical Librarian - Secretary & Coordinator

l. Medical Records Committee.

Chief, Professional Services - Chairman
*Chief, Department of Surgery
*Chief, Department of Medicine
Chief, Department of Psychiatry
*Chief, Pathology Service
Chief, Department of Hospital Clinics
Chief, Nursing Service
Chief, Registrar Division - Recorder
Medical Records Librarian

*These members will also comprise a Tissue and Blood Transfusion Practice Committee. The Chairman will be the Chief, Pathology Service. This committee will present its report during the regularly scheduled meetings of the Medical Records Committee.

m. Hospital Utilization Board.

Chief, Professional Services	- Chairman
Chief, Department of Surgery	
Chief, Department of Medicine	
Chief, Department of Psychiatry	
Chief, Pathology Service	
Chief, Department of Hospital Clinics	
Chief, Nursing Service	
Chief, Registrar Division	- Recorder
Medical Records Librarian	

n. Radioisotope Committee.

*Chief, Department of Medicine	-*Chairman
Chief, Department of Surgery	(Whoever is senior)
*Chief, Radiology Service	
Chief, Pathology Service	
Chief, Radioisotope Section	
Radiation Safety Officer	
Commanding Officer, USAMRNL	
Chief, Radioisotope Sec, Physiology Div, USAMRNL	
Chief, Purchasing & Contracting Br, Supply & Service Div	- Non-voting member

o. Perinatal Mortality Committee.

Chief, Obstetrics & Gynecology Service	- Chairman
Chief, Pediatric Service	
Assistant Chief, Pathology Service	

p. Inhalation Therapy Committee.

Chief, Anesthesiology & Operative Service	- Chairman
Chief, Department of Medicine	
Chief, Department of Surgery	

q. Cardio-Pulmonary Resuscitation Committee.

Chief, Department of Surgery - Chairman	Chief, Nursing Service
Chief, Administrative Service	Chief, Pulmonary Disease Service
Chief, Anesthesia & Operative Service	Chief, Thoracic Surgery Service
Chief, Cardiology Service	

MEDEO-XO

FOR THE COMMANDER:

C. E. BRADFORD
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DISTRIBUTION:
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Plus 10 ea - B8
5 ea - B11,B28,B43,B48

APPENDIX I

Part I

RADIOISOTOPE COMMITTEE

The U. S. Army Medical Research and Nutrition Laboratory operates jointly with Fitzsimons General Hospital under the same General Atomic Energy Commission License. Use of radioisotopes, within the limitations of the AEC License, is controlled by a Radioisotope Committee consisting of Fitzsimons General Hospital personnel as well as U. S. Army Medical Research and Nutrition Laboratory personnel. The persons making up the Radioisotope Committee and the functions of the committee are outlined in AR 40-37. The committee will be responsible for proper handling, storage and disposal of radioactive materials. In addition, the committee will:

1. Recommend changes to the SOP concerning periodic monitoring and enforcement of safety measures in the handling of radioactive material.

2. Review and grant permission for, or disapproval of, the use of radioactive material.

3. Certify individual users for each type of procedure with each individual radioisotope and insure that a copy of such certification is placed in the appropriate users' 201 file. Current records of these approved users, documenting the qualifications and limitations of each, will be maintained.

4. Prescribe special conditions which may be necessary to include and give advice concerning proposed studies where it is needed.

5. Review records and receive reports from the Radiological Protection Officer and recommend corrective action when indicated.

6. Make recommendations for improvement of present laboratory facilities and for expansion of the laboratories in accordance with needs.

7. Hold meetings at the call of the Chairman and report in writing to the Commanding Officer, the results of its deliberation.

CURRICULUM VITAE

Name and Position: Edwin L. Overholt, Colonel, MC
Chief, Department of Medicine
Fitzsimons General Hospital
Denver, Colorado 80240

Education: Franklin-Marshall, B.S. Degree, 1945
Lancaster, Pennsylvania

University of Iowa, M.D. Degree, 1948

Internship: Fitzsimons General Hospital, Denver, Colorado, 1949

Residency: Internal Medicine and Cardiology — Fitzsimons General Hospital, Denver, Colorado, 1949-1952

Military Career:

1950 — Battalion and Regimental Surgeon
21st Infantry Regiment, 24th Division
Korea — Awarded Silver Star

1953-1956: Assistant Chief of Medicine and
Chief of Cardiology
2nd General Hospital
Landstuhl, Germany

1956-1959: Chief, Medical Branch, US Army Medical Unit
Fort Detrick, Frederick, Maryland

1959-1964: Assistant Chief of Medicine and
Chief, General Medicine Service
Walter Reed General Hospital
Washington, D. C.

1964-Present: Chief, Department of Medicine
Fitzsimons General Hospital
Denver, Colorado

Professional Memberships: Fellow, American College of Physicians
American Medical Association

Publications:

1. Roentgenographic Manifestations of Pulmonary Tularemia, Radiology, 74:758, May 1960.

2. Laboratory-Acquired Pneumonic Plague: Report of a Case and Review of Previous Cases, Ann. Int. Med., 56:789, May 1962.

CURRICULUM VITAE
Edwin L. Overholt, Colonel, MC

Publications continued:

3. Pneumonia Caused by Hemolytic Streptococcus, Arch. Int. Med., 111:367, March 1963.

4. Periodic Paralysis Associated with Hyperthyroidism, Arch. Int. Med., 100:132, July 1957.

5. Chronic Effusive Pericarditis: Report of Two Cases, Medical Annals of the District of Columbia, 33:219, May 1964.

6. An Analysis of Forty-Two Cases of Laboratory-Acquired Tularemia: Treatment with Broad Spectrum Antibiotics, Amer. J. Med., 30:785, May 1961.

7. Variants of Kartagener's Syndrome in the Same Family, Ann. Int. Med., 48:574, March 1958.

8. Hereditary Hemorrhagic Telangiectasia in Three Families, Arch. Int. Med., 99:301, February 1957.

9. Antibiotic Management of Overwhelming Infection: Basic Principles of Therapy and Specific Guides to Initial Treatment, Military Medicine, November 1962.

10. Lower Nephron Nephrosis Following Exposure to Carbon Tetrachloride Inhalation, Medical Bulletin of the U.S. Army, Europe.

11. Cholangiolitic Hepatitis: Clinical-Pathologic Studies and Response to Steroid Therapy in Four Cases, Arch, Int. Med., 103:859, June 1959.

12. Acute Pulmonary Renal Syndromes, Diseases of the Chest, 48:68, July 1965.

13. Primary Cutaneous Coccidioidomycosis, Arch. Int. Med., 114:149, July 1964.

14. The Treatment of Tuberculous Pericarditis, Circulation, 9:17, January 1954.

15. Acute Benign Idiopathic Pericarditis, Arch. Int. Med., 99:708, May 1957.

16. Pneumatosis Cystoides Intestinalis: Report of a Case, Ann. Int. Med., 40:618, March 1954.

17. Erythema Nodosum with Bilateral Hilar Adenopathy, Medical Annals of the District of Columbia, 33:217, May 1964.

18. Gram-Negative Septicemia, Military Medicine, 129:1181, December 1964.

19. Xanthoma Diabeticorum, Medical Bulletin of the U.S. Army, Europe, 13:260, November 1956.

CURRICULUM VITAE
Edwin L. Overholt, Colonel, MC

Publications continued:

20. Chronic Constrictive Pericarditis, Medical Bulletin of the U.S. Army, Europe, 12:268, November 1955.

21. The Problem of Chest Pain: Report of Five Cases, Medical Bulletin of the U.S. Army, Europe, 11:90, April 1954.

22. Primary Hypercholesteremic Xanthomatosis, Medical Bulletin of the U.S. Army, Europe, 13:60, March 1956.

23. Immunogenicity of Live Tularemia Vaccine for the Monkey.

24. Aerogenic Immunization of the Monkey and Guinea Pig with Live Tularemia Vaccine, Proc. Soc. Exper. Biol. & Med., 108:732, 1961.

25. Embolization and Nonbacterial Thrombotic Endocarditis, Postgraduate Medicine, 40:571, November 1966.

26. Mucoid Impaction of the Bronchi Associated with Aspergillus, Diseases of the Chest, 52:92, July 1967.

27. Chronic Budd-Chiari Syndrome as a Result of Visceral Thrombophlebitis Migrans Associated with Factor VII Deficiency (publication pending).

28. Stevens-Johnson Syndrome Associated with Mycoplasma pneumoniae Infection, JAMA, 200:79, April 1967.

29. Sarcoidosis and Pleural Effusion, Military Medicine (pending).

30. Phosgene Poisoning, JAMA (pending).

31. Water Intoxication, Its Diagnosis and Management, Military Medicine (pending).

32. Cavernous Sinus Thrombosis, Arch. Otolaryng., 82:303, September 1965.

33. Idiopathic Central Hypoventilation, Diseases of the Chest, 52:553, October 1967.

34. Gram-Negative Septicemia in Urology, Transactions of American Association of Genito-Urinary Surgeons, 56:131, 1964.

35. Relapsing Polychondritis - Report of a Case with Vertebral Column Involvement, JAMA (pending).

CURRICULUM VITAE

6 September 1967

[redacted], John E. Colonel, MC

Position: Commanding Officer
U. S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital
Denver, Colorado 80240

Born: [redacted]

Married:

Military Service:

May 1943 - Mar 1946 Enlisted Service
Jun 1949 - Jun 1950 Intern, Letterman General Hospital San Francisco, California
Jul 1950 - Aug 1950 Medical Resident, Letterman General Hospital, San Francisco, California
Aug 1950 - Feb 1951 TDY to FECOM assigned to 8th Station Hospital, Kobe, Japan. Duty involved care and treatment of patients with surgical and orthopedic conditions and the running of the X-ray department.
Feb 1951 - Dec 1953 Letterman General Hospital, Resident on the Medical Service
Jan 1954 - Jun 1956 Ward Officer, General Medical Service, USAH, Ft. Belvoir, Va.
Jul 1956 - Dec 1956 Student, AMSS, BAMC, Ft. Sam Houston, Texas (Advanced course)
Jan 1957 - Jul 1960 10th Field Hospital (USAH, Wurzburg, Germany)
Duty: Jan 1957 - May 1957 - Asst. Ch., Medical Service
May 1957 - Jul 1960, Chief of Medical Service
Additional Duties:
May 1957 - Jul 1960, Deputy Hospital Commander
Oct 1957 - Jul 1960, Chief of Outpatient and Health Services, Wurzburg Medical Service Area
Oct 1957 - Jul 1960, Chief of Preventive Medicine, Wurzburg Medical Service Area
Sep 1960 - Jun 1961 Biochemistry Dept., Medical School of Vanderbilt University, Nashville, Tenn. for a course entitled, "Nutrition and Metabolism".

Jul 1961 - 31 Oct 1964	Chief of Metabolic Div., USAMRNL, FGH, Denver (Included in this period, was on loan to ICNND to participate in a nutrition survey in Uruguay, S.A. Feb 1962 - May 1962.
Dec 1962 - Jun 1963	Monitor, Utilization Group, Surgeon General's Intravenous Nutriment Groupt.
Jun 1963 - Present	Monitor, Surgeon General's Intravenous Nutriment Program.
31 Oct 1964 - Aug 1966	Commanding Officer, USAMRNL, FGH, Denver, Colorado
6 Jan 1966 - 13 May 66	TDY, Associate Course, Command & Gen. Staff College, Ft. Leavenworth, Kan.
Aug 1966 - Aug 1967	Commanding Officer 121 Evac Hosp, Korea
Aug 1967 - present	Commanding Officer, USAMRNL, FGH, Denver, Colo.

Education:

	Elementary and High School, Barker Central School, Barker, New York
1943 - 1944	The Military School of South Carolina (The Citadel) Charleston, So. Carolina Two semesters, ASTP
1944	The Johns Hopkins University, Baltimore, Maryland, ASTP, 3 Semesters
Oct 1945 - Jun 1949	Columbia University's College of Physicians and Surgeons, New York, N.Y.
Sep 1960 - Jun 1961	Vanderbilt University's School of Medicine, Nashville, Tenn. (Dept of Biochem., - Nutrition and Metabolism)
Jun 66 - May 66	Assoc Course Comd & Gen. Staff College

Special Activities:

1. Associate Guest Editor, Symposium on I.V. Fats-- Am. J. of Clin. Nutrition - Jan 1965.
2. The Surgeon General's liaison representative to:
   a. Nutrition Study Section, NIH (Sep 64 - present)
   b. Food & Nutrition Board, NRC-NAS (Oct 64 to present)
   c. Member, Sub-Committee on Vitamin B of the Committee on Dietary Allowances of the Food and Nutrition Board, NAS-NRC (1965 to present)
3. Member, C.S.U. Radiation Institute, Human Uses Radioisotope Committee, Colo. State University, Fort Collins, Colorado (1964 to present)

Academic Appointments: 1 Jul 1964 — Affiliate Professor of Chemistry, Colorado State University, Fort Collins, Colorado

Memberships in Societies: Diplomate of the American Board of Medical Examiners; American Association for the Advancement of Science; American Medical Association; Am. Institute of Nutr, Am. Soc. for Clin. Nutrition

Special Interests: Nutrition and Nutritional Diseases
Intravenous Fat Emulsions
Liver Disease.

1. Consolazio, C. F., Matoush, L. O., Nelson, R. A., Harding, R. S., and Canham, J. E.: The Dermal Excretion of Minerals and Its Possible Relation to Mineral Balance and Requirements. U. S. Army Medical Research and Nutrition Laboratory Report No. 271, Oct 1962.

2. Consolazio, C. F., Nelson, R. A., Matoush, L. O., Harding, R. S. and Canham, J. E.: The Sweat Excretion of Nitrogen in Relation to Balance, Environment and Physical Activity. U. S. Army Medical Research and Nutrition Laboratory Report No. 270, Oct 1962.

3. Consolazio, C. F., Nelson, R. A., Matoush, L. O., Harding, R. S. and Canham, J. E. Nitrogen Excretion in Sweat and Its Relation to Nitrogen Balance Requirements. J. of Nutr., 79: 399, 1963.

4. Consolazio, C. F., Matoush, L. O., Nelson, R. A., Harding, R. S. and Canham, J. E.: Excretion of Sodium, Potassium, Magnesium and Iron in Human Sweat and the Relation of Each to Balance and Requirements. J. of Nutr., 79: 407, 1963.

5. Nunes, W. T. and Canham, J. E.: The Effect of Varied Periodicity of Eating on Serum Lipid and Carbohydrate Tolerance in Man. Am. J. Clin. Nutr., 12: 334, 1963 (Abstract)

6. Baker, E. M., Sauberlich, H. E. and Canham, J. E.: Vitamin B_6 Requirement of the Human, Fed. Proc., 22: 322, 1963 (Abstract)

7. Canham, J. E., Nunes, W. T. and Eberlin, E. W.: Central Nervous System Manifestations of B_6 Deficiency in Normal Human Adults, Fed. Proc., 22: 322, 1963 (Abstract)

8. Consolazio, C. F., Matoush, L. O., Nelson, R. A., Harding, R. S., and Canham, J. E.: The Excretion of Nitrogen and Minerals in Sweat and Their Relationship to Balance and Requirements. Fed. Proc., 22: 550, 1963 (Abstract)

9. Nunes, W. T. and Canham, J. E.: The Effect of Varied Periodicity of Eating ("Nibbling" vs "Gorging") on Glucose Tolerance in Man. (This manuscript has been prepared for publication in a scientific journal).

10. Nunes, W. T., Canham, J. E., Consolazio, C. F. and Nelson, R. A.: The Effect of Varied Periodicity of Eating on Respiratory Quotient and Oxygen Consumption in Man. (This manuscript has been prepared for publication in a scientific journal).

11. A report by the Interdepartmental Committee on Nutrition for National Defense: Nutrition Survey of the Republic of Uruguay, 1962. United States Government Printing Office, Washington, D. C.

12. Canham, J. E. and Sauberlich, H. E.: Chapter 15 entitled, "Vitamin B_6" for "Handbook of Nutrition" compiled by the Council on Foods and Nutrition of the AMA for publication in 1966.

13. Canham, J. E., Nunes, W. T. and Eberlin, E. W.: Electroencephalographic and Central Nervous System Manifestations of B_6 Deficiency and Induced B_6 Dependency in Normal Human Adults. Proceedings of the 6th International Congress of Nutrition, C. F. Mills & R. Passmore, Eds., Page 587 (Abstract), published by Messrs. E. & S. Livingstone, Ltd., Edinburgh, Scotland, 1964.

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15. Levandoski, N. G., Baker, E. M. and Canham, J. E.: Studies on the Auto-Oxidation of L-Ascorbic Acid. (Abstract). Sixth International Congress of Biochemistry, New York City, N. Y., July 1964., Pub I.U.B., Vol. 32, Sect. V, G 176, p492, 1964.

16. Levandoski, N. G., Baker, E. M. and Canham, J. E.: A monodehydro Form of Ascorbic Acid in the Auto-Oxidation of Ascorbic Acid to Dehydroascorbic Acid. Biochemistry, 3: 1485-1489, Oct, 1964.

17. Baker, E. M., Canham, J. E., Nunes, W. T., Sauberlich, H. E. and McDowell, H. E.: Vitamin B_6 Requirement for Adult Men, Am. J. Clin. Nutr., 16: 59-66, 1964.

18. Jones, L. D., Castleberry, M. W., Canham, J. E., King, N. W.: Toxicity Testing of Fat Emulsions for Intravenous Administration Am. J. Clin. Nutrition, Jan, 1965, Vol 16: 68-74.

19. Levine, R. A., King, N. W. and Canham, J. E.: Hemodynamic Alterations Produced by Artificial Fat Emulsion Perfused Through the Isolated Rat Liver. Vol II, Fette in der Medizin, pp27-31, H. Henning, Ed., Pub. Pallas Verlag, Munich, 1965.

20. Canham, J. E., Jones, L. D., King, N. W. and Levine, R. A.: Metabolic and Toxicity Studies of Intravenously Administered Fat Emulsions, World Fat Congress, Hamburg, Germany, Oct 1964, Abstracts of Papers, pp 258-259, Published by Aschendorffsche, Buchdruckerei, Munster, Westf., Germany, 1964.

21. Baker, E. M., Canham, J. E. and Sauberlich, H. E. : Further Studies on the Vitamin B_6 Requirement for the Young Adult Male. (Abstract) Proc. 38th Ann. Meeting, Colorado-Wyoming Acad. Sci., 1964

22. Harding, R. S., Canham, J. E. and Sauberlich, H. E.: The Free Amino Acids in the Plasma and Urine of Human Subjects on a Vitamin B_6 Deficient Diet. (Ibid.)

23. Guest Editor "Symposium on Intravenous Fat Emulsions": Am. J. Clin. Nutrition, 13: 1-224, 1963.

24. Mueller, J. F. and Canham, J. E.: Editorial - Symposium on Intravenous Fat Emulsions. Am. J. Clin. Nutrition, 15: 1-3, 1964.

25. Canham, J. E., Harding, R. S., Consolazio, C. F. and Witt, M. F.: Gastrointestinal Degradations of Cellulose in the Human. Fed. Proc., 24: 314, 1965.

26. Harding, R. S., Canham, J.E. and Sauberlich, H.E.: The Free Amino Acids in the Plasma and Urine of Human Subjects on a Vitamin B_6 Deficient Diet. In "Automations in Analytical Chemistry", Ed. - Leonard T. Skeggs, Jr., Mediad, Inc. Publishers, 1966.

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28. Matoush, L. O., Nelson, R. A., Consolazio, C. F. and Canham, J. E.: Urine Losses in Relation to Trace Mineral Balances. Fed. Proc., 25: 312, 1966 (Abstract).

29. Baker, E. M. and Canham, J. E.: Xanthurenic Acid Excretions after Loading with Various Forms of Tryptophane in the Evaluation of Vitamin B_6 Status. Fed. Proc., 24: 624, 1965 (Abstract).

30. Canham, J. E. and Consolazio, C. F.: Nutrition and Stress. Accepted for publication in JAMA.

31. Dukes, M. D. and Canham, J. E.: Albumin Metabolism in Man at High Altitudes. Fed. Proc. 25: 399, 1966 (Abstract)

32. Consolazio, C. F., Matoush, L. O., Nelson, R. A., Harding, R. S. and Canham, J. E.: Nutrition Survey - Ranger Department, Fort Benning, Georgia, USAMRNL Report No. 291, January 1966, Denver, Colorado.

33. Canham, J. E., Baker, E. M. and Raica, N., Jr.: Vitamin B_6 Requirement of Adult Men. Proceedings of VII International Congress of Nutrition, Hamburg, Germany, August 1966. (Abstract).

34. Consolazio, C. F., Matoush, L. O., Nelson, R. A., Issac, G. J. and Canham, J. E.: Comparison of Nitrogen, Calcium, and Iodine Excretions in Arm and Total Body Sweat. Am. J. of Clin. Nutrition 16: 443-448, 1965.

Areas of Research: Areas of research acitivty has been in the following fields:

1. The effects of periodicity of eating upon normal intermediate metabolism in humans.

2. The degradation and possible utilization of cellulose in humans.

3. Have supervised the conduct and collaborated with other investigators in the performance of six studies on Vitamin B_6 metabolism in the normal adult male human including deficiency studies. These studies resulted in the original observation that vitamin B_6 deficiency in the adult can produce electroencephalographic abnormalities and convulsive seizures plus the original observation that excessive intake of vitamin B_6 can produce electroencephalographic abnormalities in the adult.

4. Have also participated in studies to determine the usability utilization and toxicity of various intravenous fat preparations.

5. Additional activities include participation with C. F. Consolazio in studies aimed at defining the extent of nutrient loss in perspiration of healthy adult males living in various environmental temperatures.

6. Have been responsible for the planning, coordination and supervision of many nutrition surveys which involved various divisions of the Laboratory.

7. Additional areas of research activity have included the auto-oxidation of ascorbic acid in aqueous solutions and the relationship of these products to the normal metabolic function of ascorbic acid.

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(12-61)
Page 3

UNITED STATES ATOMIC ENERGY COMMISSION

APPLICATION FOR BYPRODUCT MATERIAL LICENSE

SUPPLEMENT A—HUMAN USE

Form approved
Budget Bureau No. 38-R360

This page may be completed by the physician's preceptor (if any) in the medical use of radioisotopes. When the information is not furnished by the preceptor, the name and present address of the preceptor (if any) should be shown in item 12 below.

9. (a) USING PHYSICIAN'S NAME

John D. Conley, M.D.

(b) NAME AND ADDRESS OF APPLICANT (If different from 9(a))

Chief, Metabolic Division
U.S. Army Medical Research and Nutrition Laboratory
Fitzsimons General Hospital, Denver, Colorado 80240

10. CLINICAL TRAINING AND EXPERIENCE OF PHYSICIAN WHO WILL USE BYPRODUCT MATERIAL

(A) ISOTOPE	(B) CONDITION(S) DIAGNOSED OR TREATED	(C) NUMBER OF CASES	(D) TYPE OF PARTICIPATION FOR ALL CASES IN COLUMN B (circle applicable numbers of items in accordance with key set forth below)
I-131	Diagnosis of thyroid function	20-25	(1) 2 3 4
	Treatment of hyperthyroidism		1 2 3 4
	Treatment of thyroid cancer		1 2 3 4
	Treatment of cardiac conditions		1 2 3 4
	Brain tumor localization		1 2 3 4
	Blood determinations		1 2 3 4
	Kidney function		1 2 3 4
	Others:		1 2 3 4
P-32 Soluble	Treatment of polycythemia and leukemia	2	(1) 2 3 4
	Brain tumor localization		1 2 3 4
	Treatment of bone metastases		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
P-32 $CrPO_4$	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
Au-198 Colloid	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
Cr-51	Blood determinations		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
Other Isotopes			1 2 3 4
	Co^{60} and Vitamin B^{12}	24	(1) (2) 3 (4)
			1 2 3 4

Key to above numbers (column D)

Active Participation and Discussion in the:

1. Examination of patients to determine suitability for radioisotope diagnosis and/or treatment and recommendations on dosage to be prescribed.
2. Collaboration in calibration and administration of dosages including related measurements and plotting of data.
3. Active period of training and experience of sufficient duration to permit followup of patients through treatment and posttreatment period including reevaluation as to effectiveness and complications.
4. Study and discussion of case histories to establish most efficacious diagnostic and/or therapeutic techniques for this radioisotope use.

11. TOTAL NUMBER OF HOURS OF PARTICIPATION IN CLINICAL TRAINING _____ hours

THE TRAINING AND EXPERIENCE INDICATED ABOVE WAS OBTAINED UNDER THE SUPERVISION OR GUIDANCE OF

Lt. Col. John Conley M.D. Vanderbilt University
Lt. Col. Richard Donlan School of Medicine
Dr. [illegible] (Preceptor) U.S.A. Med. Res. & Nut. Lab.

(Signature)

Form AEC-313 (5-56)

Page Two

TRAINING AND EXPERIENCE OF EACH INDIVIDUAL NAMED IN 4 (Use supplemental sheets if necessary)

6. TYPE OF TRAINING	WHERE TRAINED	DURATION OF TRAINING	ON THE JOB (Circle answer)	FORMAL COURSE (Circle answer)
a. Principles and practices of radiation protection	U.S. Army Hosp. Marburg & Ft. San Vanderbilt Univ. School of Med.	2½ yrs 13½ mo	yes no	Yes No
b. Radioactivity measurement standardization and monitoring techniques and instruments	Vanderbilt University School of Medicine	4½ mo formal 9 mo on job	Yes No	Yes No
c. Mathematics and calculations basic to the use and measurement of radioactivity	Same as b. above		Yes No	Yes No
d. Biological effects of radiation	Same as b. above		Yes No	Yes No

9. EXPERIENCE WITH RADIATION. (Actual use of radioisotopes or equivalent experience.)

ISOTOPE	MAXIMUM AMOUNT	WHERE EXPERIENCE WAS GAINED	DURATION OF EXPERIENCE	TYPE OF USE
Co^{60}	0.5uc/patient or subject	Nutrition Clinic, Vanderbilt University.	9 months	Studies on Vitamin B12 requirements& half life in humans.
Co^{60}		Dept. Biochemistry Vanderbilt University.	4 months	Relationship B12 to Calcium in B12 absorp.
S-35, P-32, I-131			4 months	Laboratory experience

10. RADIATION DETECTION INSTRUMENTS. (Use supplemental sheets if necessary.)

TYPE OF INSTRUMENTS (Include make and model number of each)	NUMBER AVAILABLE	RADIATION DETECTED	SENSITIVITY RANGE (mr/hr)	WINDOW THICKNESS (mg./cm²)	USE (Monitoring, surveying, measuring)

11. METHOD, FREQUENCY, AND STANDARDS USED IN CALIBRATING INSTRUMENTS LISTED ABOVE

12. FILM BADGES, DOSIMETERS, AND BIO-ASSAY PROCEDURES USED. (For film badges, specify method of calibrating and processing, or name of supplier.)

INFORMATION TO BE SUBMITTED ON ADDITIONAL SHEETS

13. FACILITIES AND EQUIPMENT. Describe laboratory facilities and remote handling equipment, storage containers, shielding, fume hoods, etc. Explanatory sketch of facility is attached. (Circle answer) Yes No

14. RADIATION PROTECTION PROGRAM. Describe the radiation protection program including control measures. If application covers sealed sources, submit leak testing procedures where applicable, name, training, and experience of person to perform leak tests, and arrangements for performing initial radiation survey, servicing, maintenance and repair of the source.

15. WASTE DISPOSAL. If a commercial waste disposal service is employed, specify name of company. Otherwise, submit detailed description of methods which will be used for disposing of radioactive wastes and estimates of the type and amount of activity involved.

CERTIFICATE (This item must be completed by applicant)

16. THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATE ON BEHALF OF THE APPLICANT NAMED IN ITEM 1, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PART 30, AND THAT ALL INFORMATION CONTAINED HEREIN, INCLUDING ANY SUPPLEMENTS ATTACHED HERETO, IS TRUE AND CORRECT TO THE BEST OF OUR KNOWLEDGE AND BELIEF.

Applicant named in item 1

Date

By:

Title of certifying official

WARNING.—18 U. S. C., Section 1001, Act of June 25, 1948, 62 Stat. 749, makes it a criminal offense to make a willfully false statement or representation to any department or agency of the United States as to any matter within its jurisdiction.

U.S. GOVERNMENT PRINTING OFFICE: 1961 O—619965

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UNITED STATES ATOMIC ENERGY COMMISSION

APPLICATION FOR BYPRODUCT MATERIAL LICENSE

SUPPLEMENT A — HUMAN USE

Form approved. Budget Bureau No. 38-R080.1

This page may be completed by the physician's preceptor (if any) in the medical use of radioisotopes. When the information is not furnished by the preceptor, the name and present address of the preceptor (if any) should be shown in item 12 below.

9. (a) USING PHYSICIAN'S NAME	(b) NAME AND ADDRESS OF APPLICANT (If different from 9(a))
PAUL E. SIEBERT, Major MC Fitzsimons General Hospital	Denver 40, Colorado

10. CLINICAL TRAINING AND EXPERIENCE OF PHYSICIAN WHO WILL USE BYPRODUCT MATERIAL

(A) ISOTOPE	(B) CONDITION(S) DIAGNOSED OR TREATED	(C) NUMBER OF CASES	(D) TYPE OF PARTICIPATION FOR ALL CASES IN COLUMN D (circle applicable numbers of items in accordance with key set forth below)
I-131	Diagnosis of thyroid function	161	(1) (2) (3) (4)
	Treatment of hyperthyroidism	9	(1) (2) (3) (4)
	Treatment of thyroid cancer	9	(1) (2) (3) (4)
	Treatment of cardiac conditions	1	(1) (2) (3) (4)
	Brain tumor localization		1 2 3 4
	Blood determinations	22	(1) (2) (3) (4)
	Others:		1 2 3 4
			1 2 3 4
P-32 Soluble	Treatment of polycythemia and leukemia	9	(1) (2) (3) (4)
	Brain tumor localization		1 2 3 4
	Treatment of bone metastases		1 2 3 4
	Others: Eye Tumor Localization	2	(1) (2) (3) (4)
			1 2 3 4
P-32 $CrPO_4$	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
Au-198 Colloid	Treatment of prostatic cancer	3	(1) (2) (3) (4)
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
Cr-51	Blood determinations	7	(1) (2) (3) (4)
	Others:		1 2 3 4
			1 2 3 4
Other Isotopes	Strontium 90	100	(1) (2) (3) (4)
	Cobalt 60 Schilling Test	23	(1) (2) (3) (4)
			1 2 3 4

Key to above numbers (column D) Active Participation and Discussion

1. Examination of patients to determine suitability for radioisotope diagnosis and/or treatment and recommendations on dosage to be prescribed.
2. Collaboration in calibration and administration of dosages including related measurements and plotting of data.
3. Active period of training and experience of sufficient duration to permit followup of patients through treatment and posttreatment period including reevaluation as to effectiveness and complications.
4. Study and discussion of case histories to establish most efficacious diagnostic and/or therapeutic techniques for this radioisotope use.

11. TOTAL NUMBER OF HOURS OF PARTICIPATION IN CLINICAL TRAINING 480 hours

12. THE TRAINING AND EXPERIENCE INDICATED ABOVE WAS OBTAINED UNDER THE SUPERVISION OR GUIDANCE OF

H. F. Hurd, M. C.
Col. John A. Isherwood, M.C., AT Brooke General Hospital, Fort Sam Houston, Texas

(Name of physician (preceptor)) (Institution) (Signature)

UNITED STATES ATOMIC ENERGY COMMISSION

APPLICATION FOR BYPRODUCT MATERIAL LICENSE

SUPPLEMENT A—HUMAN USE

Form approved.
Budget Bureau No. 38-R080.1

This page may be used for providing additional information.

Major Paul E. Siebert's training and experience has been reviewed by the Radioisotope Committee and he has been approved as a user of radioisotopes.

S/S

PHILIP A. BERGMAN
Colonel MC
Chairman, Radioisotope Committee

U.S. GOVERNMENT PRINTING OFFICE: 1960 O - 568572

CURRICULUM VITAE

Name and Position: Paul E. Siebert, Major MC
Chief, Radiology Service
Fitzsimons General Hospital
Denver 40, Colorado

Education: Washington University, St. Louis Mo. AB 1948
MD 1952

Internship: Valley Forge Army Hospital, Phoenixville Pa., 1952-1953.

Residency: Radiology, Brooke Army Hospital, Ft. Sam Houston, Texas, June 1954- 30 June 1957.

Experience: Basic Officers Course 801, MFSS, Ft. Sam Houston, Texas, 1953-1954.

USCOSC Ass. O. Course, Ft. Leavenworth, Kansas, January - May 1961.

Assistant Chief, Radiology, Tripler Army Hospital, APO 438, San Francisco, August 1957 - January 1961

Chief, Radiology Service, Martin Army Hospital, Fort Benning, Georgia, June 1961 - August 1962.

Assistant Chief, Radiology Service, Fitzsimons General Hospital, Denver, Colorado, August 1962 to present.

Professional Memberships: Diplomate American Board of Radiology, May 1958
Member Radiological Society of North America, Inc.
Treasurer, San Antonio Military Radiological Society, 1956-1957
Vice President Radiological Society of Hawaii, 1960
Member Society of Nuclear Medicine, Honolulu, Hawaii, 1960

Radioisotope Experience: Strontium^{90} Applications (1956-1960) 100.

MEDEO-X
Recorder Radioisotope
Committee

Certification
Chief, Radiology Service

31 Mar 1964

PES/bjs
26218

Request I be certified for the following procedures and that a copy of the certification be placed in my 201 file.

I-131	Diagnosis of thyroid function Rx of hyperthyroidism Rx of thyroid Ca Rx of cardiac conditions Blood determinations
P 32 (Soluble)	Rx of polycythemia and leukemia Eye tumor localization
Au 198 (Colloid)	Rx of prostatic Ca
Cr 51	Blood determination
Sr 90	Rx benign skin and eye conditions
Co 60	Schilling test

Enclosed is a copy of my experience.

1 Incl
Form AEC-313a

PAUL E. SIEBERT
Major, MC
Chief, Radiology Service

CURRICULUM VITAE

JOHN T. DECKER
MAJOR MC
CHIEF, PATHOLOGY SERVICE
FITZSIMONS GENERAL HOSPITAL
DENVER, COLORADO

UNDERGRADUATE EDUCATION: Eastern New Mexico University, Portales, New Mexico. B.S. in Biology, 1954

MEDICAL EDUCATION: Stanford University School of Medicine, Palo Alto and San Francisco, California. M.D., 1958.

INTERNSHIP: William Beaumont General Hospital, El Paso Texas. Rotating Internship, July 1958 - June 1959.

RESIDENCY: William Beaumont General Hospital, El Paso Texas. Pathologic Anatomy and Clinical Pathology. September 1959 - August 1963.

BOARD CERTIFICATION: American Board of Pathology, Clinical Pathology and Pathologic Anatomy, October 1964.

POSITIONS: Assistant to Chief, Anatomic Pathology, Walter Reed General Hospital, Washington, D.C. September 1963 - March, 1966

Chief, Anatomic Pathology, Walter Reed General Hospital, Washington, D.C. March, 1966 to July, 1966.

Chief, Pathology Service, Fitzsimons General Hospital, Denver, Colorado. 1 September, 1966 to date.

MEMBERSHIP IN SCIENTIFIC ORGANIZATIONS:

American Medical Association
American Society of Clinical Pathologists
College of American Pathologists
Colorado Society of Clinical Pathologists

TEACHING APPOINTMENTS: Clinical Instructor in Pathology, University of Colorado Medical School, September 1966.

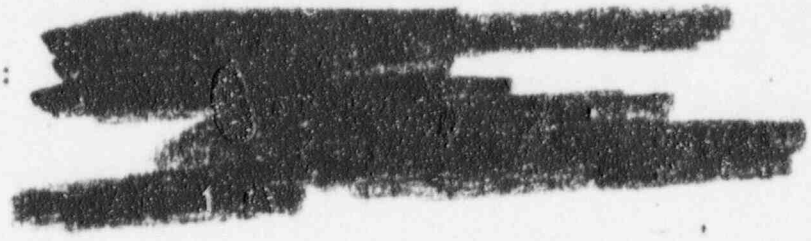
PUBLICATIONS: Reigh, Ernest E. and Decker, John T. "Meningeal sarcoma in a two-week old infant simulating hydrocephalus." Journal of Neurosurgery, 19:427-30 (1962).

CURRICULUM VITAE

Charles G. Liddle June 1968

Personal

Born:
Height and Weight:
Marital Status:



Religion:

Educational Background

Michigan State University, September 1954 - June 1960, BS - 1958, DVM - 1960
University of Rochester, June 1962 - June 1963, MS - Radiation Biology

Professional Positions

1. Veterinarian, Small Animal Practice: Detroit, Michigan, July 1960 - December 1960.

Was one of three veterinarians in a small animal hospital in Detroit, Michigan. The hospital was owned by Dr. W. Clay Young.

2. Chief, Radioisotope Division, Fourth U. S. Army Medical Laboratory, Fort Sam Houston, Texas, January 1961 - May 1962.

After two months of officer basic training at Ft. Sam Houston, I attended a three month "Veterinary Laboratory Procedures" course at the Veterinary Division, Walter Reed Army Institute of Research, Washington, D.C.

Upon returning to Fort Sam Houston, I was assigned as Chief of the Radioisotope Division of the laboratory. A program for environmental monitoring for radioactive fallout in air, food and water was reinstituted during this time. Initiated the radioactive tri-iodothyronine in vitro test for thyroid function to serve smaller army installations with no radioisotope capabilities.

3. Acting Chief, Department of Food Radionuclides, Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D.C. July 1963 - January 1964.

After completing the year of study at the University of Rochester, I was assigned to Walter Reed Army Institute of Research. Was acting chief while the department chief was absent for six months TDY. During this period, was assistant course director for a course entitled "Veterinary Laboratory Procedures". The course

is designed to give training in laboratory administration, food chemistry and radioisotope techniques to veterinary officers who have just completed their basic training and are on their way to army area laboratories. As assistant course director to Lt Col Thomas Murnane, arranged schedules, invited and introduced guest lecturers. gave lectures in nuclear physics and conducted radioisotope laboratory classes.

4. Chief, Small Animal Testing Section, Department of Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, D.C. February - July 1964.

Was assigned to the Department of Medicinal Chemistry under Dr. David Jacobus on a temporary basis to replace Lt Col Edward Henderson until his permanent replacement completed his year of education at the University of Tochester. Was in charge of the small animal testing section which was involved in evaluating radiation protection drugs. Also coordinated with a private research laboratory under government contract engaged in rodent testing and bacteriological screening of potential radiation protection drugs. During this period, also worked on developing an information system for calculating and storing results from both bacteriological and small animal tests on computer punch cards utilizing the IBM 1401 computer at WRAIR.

5. Assistant Chief, Department of Food Radionuclides, Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington, D.C. August 1964 - July 1965.

Returned to the Veterinary Division to work under Capt Stanley Wampler. Again gave the nuclear physics lectures and supervised the radioisotope laboratory experiments for the Veterinary Laboratory Procedures course from September through December. Then began studies to evaluate the degree of induced gamma radioactivity produced from neutron activation of ration items to attempt to determine the possible hazard to foods which might be exposed to neutron radiation from a nuclear device.

6. Chief, Radioisotope Section, Physiology Division, U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado, August 1965 to present.

The Radioisotope Section of the laboratory provides complete radioisotope service to the entire laboratory. This encludes purchase, storage and disposal of all radioisotopes, routine and emergency monitoring for radioactivity, all licensing procedures, and provides a central facility for counting radioactivity. The central counting facility consists of six liquid scintillation counters, two automatic gamma counters, one low background anti-coincidence beta counter, one low background gamma counter and various chromatographic strip and plate scanners, and assorted beta planchet counters. The section also has a 23,000 Curie Cobalt-60 source. The laboratory has an RCA - 301 computer, but a shortage of programmers, so I have studied computer programming (both machine language and Fortran II)

and have written several programs to calculate the results from some of the liquid scintillation counters and the automatic gamma counters and am in the process of writing one general program to provide a complete information system for the results from all of the liquid scintillation counters. This involves efficiency and statistical calculations for single and dual label studies using any of the three methosa for determining liquid scintillation counting efficiencies (internal standard, external standard or channels ratio).

Professional and Scientific Societies

American Veterinary Medical Association

Health Physics Society

Additional Training

"Radionuclides in Foods" Taft Sanitary Engineering Center, Cincinnati, Ohio, July 1961 (two weeks)

"Epidemiology for Veterinarians" CDC, Atlanta, Georgia, February 1965 (one week)

"Radionuclide Analysis by Gamma Spectroscopy" Taft Sanitary Engineering Center, Cincinnate, Ohio, June 1965 (two weeks)

"Veterinary Statistical Procedures" Army Veterinary School, Chicago, Illinois, December 1966 (three weeks)

"Nuclear Hazards Training Course" Sandia Base, New Mexico, August 1967 (one week)

Hobbies

1. Antique Automobiles, Presently Membership Director, Rocky Mountain Packards, a chapter of Packard Automobile Classics.

2. Bowling, Tear captain - Research and Nutrition Lab team, Officers league, Fitzsimons.

3. Camping.

Training and Experience: Charles G. Liddle, Maj VC

Form AEC-313

Item 8 Type of Training:	Where Trained	Duration of Training	
a. Principles and practices of radiation protection	Walter Reed Army Institute of Research	2 weeks	Formal
	Taft Sanitary Engineering Center	4 weeks	Formal
	University of Rochester	1 year	Formal
b. Radioactivity measurement standardization and monitoring techniques and instruments	"	"	"
c. Mathematics and calculations basic to the use and measurement of radioactivity	"	"	"
d. Biological effects of radiation	"	"	"

Item 9 Experience with radiation

Isotope	Maximum amount	Where experience gained	Duration	Type of Use
H^3	1 Mc	Fourth U S Army Med Lab	1 yr	research
		Walter Reed Army Institute of Research	2 yrs	research
		USAMRNL	3 yrs	research
C^{14}	"	"	"	"
P^{32}	"	"	"	"
S^{35}	"	"	"	"
Ca^{45}	"	"	"	"
Cr^{51}	"	"	"	"
Fe^{59}	"	"	"	"
Co^{60}	"	"	"	"
Zn^{65}	"	"	"	"
Sr^{85}	"	"	"	"
Sr^{90}	"	"	"	"
I^{125}	"	"	"	"
I^{131}	"	"	"	"
Cs^{137}	"	"	"	"
$Ba\text{-}La^{140}$	"	"	"	"
Hg^{197}	"	"	"	"
Hg^{203}	"	"	"	"

BIOGRAPHY

July 1, 1967

NORMAN HELMAN, Captain, MC

1. Birthdate: [redacted]

2. Birthplace: [redacted]

3. Education:

Wayne State University - B.S., 1956
University of Michigan College of Medicine - 1955
Wayne University, College of Medicine - M.D., 1958
Internship - Sinai Hospital, Detroit, 1958-1959
Residency Internal Medicine - Sinai Hospital, Detroit, 1959-1962
Residency Hematology - Sinai Hospital, Detroit, 1962-1963

4. Hospital Appointments:

Wayne University, Dept. of Medicine - Research Associate in Hematology.
St. Joseph Mercy, Attending Staff - Chief, Department of Medicine.
Brent, Active Staff - Hematology Consultant.
Crittenton, Courtesy Staff.
North Detroit, Attending Staff.
St. Francis, Associate Staff.
Sinai, Assistant Attending, Section of Hematology.
Detroit Memorial Hospital - Consultant in Hematology.
Saratoga - Consultant in Hematology.
Boulevard General - Consultant in Hematology.
Park Community Hospital - Consultant in Hematology.
Alex. Blain Hospital - Consultant in Hematology.
Cottage Hospital - Consultant in Hematology.
Holy Cross Hospital - Consultant in Hematology.
Visiting Lecture in Nuclear Medicine - Colo. Univ. College of Medicine.

5. Profession Societies:

American Medical Association
Wayne County Medical Society
Michigan State Medical Society
Phi Delta Epsilon
Alpha Omega Alpha
Maimonides Medical Society - President 1966-67.

6. Certified American Board of Internal Medicine, March 1965

7. Publications:

HELMAN, N., BERK, J.E., and GAGLIARDI, R.A. 1959. "Fluid and Electrolyte Changes Following Preparation for and Performance of Barium Enema." Clinical Research. 7:391.

Appendix I

NORMAN HELMAN, Captain,MC

HELMAN, N., 1959,- "Fluid and Electrolyte Changes Following Barium Enema." Bulletin - Sinai Hospital, Detroit. 7:61.

HELMAN, N., 1962,- "Hemophilia and Circulating Anticoagulants." Bulletin - Sinai Hospital, Detroit. 10:58.

HELMAN, N., 1963,- "Understanding Coagulation." Bulletin - Sinai Hospital, Detroit. 11:

WEBER, R.E. and HELMAN, N. 1963,- "The Local Inflammatory Response In Patients with Blood Dyscrasias as Tested by the Skin Window Technic." Bulletin - Sinai Hospital, Detroit. 11:95.

HELMAN, N., 1963, - "The Sideroblastic Anemias." Bulletin - Sinai Hospital, Detroit. 11:67.

HELMAN, N., 1963, - "Platelet Adhesion and Aggregation Studies in Normal Controls." Bulletin Sinai Hospital, Detroit. 11:107.

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(10-61)
Page 3

UNITED STATES ATOMIC ENERGY COMMISSION

APPLICATION FOR BYPRODUCT MATERIAL LICENSE

SUPPLEMENT A—HUMAN USE

Form approved.
Budget Bureau No. 38-R080.1

This page may be completed by the physician's preceptor (if any) in the medical use of radioisotopes. When the information is not furnished by the preceptor, the name and present address of the preceptor (if any) should be shown in item 12 below.

9. (a) USING PHYSICIAN'S NAME	(b) NAME AND ADDRESS OF APPLICANT (If different from 9(a))
NORMAN HELLMAN	FITZSIMONS GENERAL HOSPITAL DENVER, COLORADO 80240

10. CLINICAL TRAINING AND EXPERIENCE OF PHYSICIAN WHO WILL USE BYPRODUCT MATERIAL

(A) ISOTOPE	(B) CONDITION(S) DIAGNOSED OR TREATED	(C) NUMBER OF CASES	(D) TYPE OF PARTICIPATION FOR ALL CASES IN COLUMN B (circle applicable numbers of items in accordance with key set forth below)
I-131	Diagnosis of thyroid function	248	(1) (2) (3) (4)
	Treatment of hyperthyroidism	6	(1) (2) (3) (4)
	Treatment of thyroid cancer		1 2 3 4
	Treatment of cardiac conditions		1 2 3 4
	Brain tumor localization		1 2 3 4
	Blood determinations		1 2 3 4
	Kidney functions Heart Scans	1	(1) (2) (3) (4)
	Others: Lung Scans	5	(1) (2) (3) (4)
P-32 Soluble	Treatment of polycythemia and leukemia		1 2 3 4
	Brain tumor localization		1 2 3 4
	Treatment of bone metastases		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
P-32 $CrPO_4$	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others: INTRACAVITARY		1 2 3 4
	SYNOVIORTHESIS		1 2 3 4
Au-198 Colloid	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others: Liver Scans	18	(1) (2) (3) (4)
			1 2 3 4
Cr-51	Blood determinations		1 2 3 4
	Others: Spleen Scans	6	(1) (2) (3) (4)
			1 2 3 4
Other Isotopes	Hg-197 Kidney Scans	22	(1) (2) (3) (4)
	Tc-99m Brain Scans	38	(1) (2) (3) (4)
	Sr-85 Bone Scans	8	(1) (2) (3) (4)

Key to above numbers (column D)
Active Participation and Discussion in the:

1. Examination of patients to determine suitability for radioisotope diagnosis and/or treatment and recommendations on dosage to be prescribed.
2. Calibration and administration of dosages including related measurements and plotting of data.
3. Active period of training and experience of sufficient duration to permit followup of patients through treatment and posttreatment period including reevaluation as to effectiveness and complications.
4. Study and discussion of case histories to establish most efficacious diagnostic and/or therapeutic techniques for this radioisotope use.

11. TOTAL NUMBER OF HOURS OF PARTICIPATION IN CLINICAL TRAINING 248 hours

12. THE TRAINING AND EXPERIENCE INDICATED ABOVE WAS OBTAINED UNDER THE SUPERVISION OR GUIDANCE OF

DAVID P. PRESTON, MAJOR, MC FITZSIMONS GENERAL HOSPITAL
DENVER, COLORADO 80240

(Name) (Signature)

UNITED STATES ATOMIC ENERGY COMMISSION
APPLICATION FOR BYPRODUCT MATERIAL LICENSE
SUPPLEMENT A—HUMAN USE

Form approved.
Budget Bureau No. 38-R0203

This page may be used for providing additional information.

10(a), I-131, Continued ...

I-131 Renograms	22	(1)(2)(3)(4)
Rose Bengal Liver Scans	2	(1) (2)(3)(4)
Placentograms	7	(1)(2)(3)(4)

10(a), Other Isotopes, Continued ...

CO-57 Schillings	3	(1)(2)(3)(4)

U.S. GOVERNMENT PRINTING OFFICE : 1961 O—615963

The Radiological Training, Experience, and Background of WO-1 Charles L. Spinks is as follows:

Successful completion of the Nuclear Power Plant Operator's Course (52 weeks), conducted by the U.S.A.E.R.D. at Fort Belvoir, Virginia, October 1960 - October 1961, followed by 6½ years' utilization in the Nuclear Power Program. This school contained 32 hours of Nuclear Physics, 64 hours of Nuclear Engineering, and 46 hours of Health Physics.

Successful completion of the Radiation Safety Course conducted at the U. S. Army Chemical School, Fort McClellan, Alabama (3½ hours).

APPENDIX II

Attached is the training and experience of the individuals approved for human use of radioisotopes with the exception of Col. Canham and Capt. Helman. Their training and experience is included in Appendix I.

Appendix II

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UNITED STATES ATOMIC ENERGY COMMISSION

APPLICATION FOR BYPRODUCT MATERIAL LICENSE

SUPPLEMENT A—HUMAN USE

Form approved. Budget Bureau No. 33-R060

This page may be completed by the physician's preceptor (if any) in the medical use of radioisotopes. When the information is not furnished by the preceptor, the name and present address of the preceptor (if any) should be shown in item 12 below.

9. (a) USING PHYSICIAN'S NAME	(b) NAME AND ADDRESS OF APPLICANT (if different from 9(a))
Ralph Lazzara	

10. CLINICAL TRAINING AND EXPERIENCE OF PHYSICIAN WHO WILL USE BYPRODUCT MATERIAL

(A) ISOTOPE	(B) CONDITION(S) DIAGNOSED OR TREATED	(C) NUMBER OF CASES	(D) TYPE OF PARTICIPATION FOR ALL CASES IN COLUMN B (circle applicable numbers of items in accordance with key set forth below)
I-131	Diagnosis of thyroid function		(1) 2 (3) (4)
	Treatment of hyperthyroidism		(1) 2 (3) (4)
	Treatment of thyroid cancer		1 2 3 4
	Treatment of cardiac conditions		1 2 3 4
	Brain tumor localization		(1) 2 (3) (4)
	Blood determinations		1 2 3 4
	Kidney function		1 2 3 4
	Others:		1 2 3 4
P-32 Soluble	Treatment of polycythemia and leukemia		(1) 2 (3) (4)
	Brain tumor localization		1 2 3 4
	Treatment of bone malignancy		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
P-32 $CrPO_4$	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
Au-198 Colloid	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others: Liver Scan		(1) 2 (3) (4)
			1 2 3 4
Cr-51	Blood determinations		(1) 2 (3) (4)
	Others:		1 2 3 4
			1 2 3 4
Other Isotopes	Xe-133 H^3OH Na-22 Mg-28		(1) (2) (3) (4)
	Technetium Brain Scane		(1) 2 (3) (4)
			1 2 3 4

Key to above numbers (column D)

Active Participation and Discussion in the:

1. Examination of patients to determine suitability for radioisotope diagnosis and/or treatment and recommendations on dosage to be prescribed.
2. Calibration and administration of dosages including related measurements and plotting of data.
3. Active period of training and experience of sufficient duration to permit followup of patients through treatment and posttreatment period including reevaluation as to effectiveness and complications.
4. Study and discussion of case histories to establish most efficacious diagnostic and/or therapeutic techniques for this radioisotope use.

11. TOTAL NUMBER OF HOURS OF PARTICIPATION IN CLINICAL TRAINING 200 + hours

12. THE TRAINING AND EXPERIENCE INDICATED ABOVE WAS OBTAINED UNDER THE SUPERVISION OR GUIDANCE OF

G.E. Burch at Tulane Medical School

(Name of physician (preceptor)) (Institution) (Signature)

TRAINING AND EXPERIENCE OF EACH INDIVIDUAL NAMED IN ITEM 4 (Use supplemental sheets if necessary)

8. TYPE OF TRAINING	WHERE TRAINED	DURATION OF TRAINING	ON THE JOB (Circle answer)	FORMAL COURSE (Circle answer)
a. Principles and practices of radiation protection	Tulane Medical School	1960-64	(Yes) No	(Yes) No
b. Radioactivity measurement standardization and monitoring techniques and instruments	Tulane Medical School	1960-64	(Yes) No	(Yes) No
c. Mathematics and calculations basic to the use and measurement of radioactivity	Tulane Medical School	1960-64	(Yes) No	(Yes) No
d. Biological effects of radiation	Tulane Medical School	1960-64	(Yes) No	(Yes) No

9. EXPERIENCE WITH RADIATION. (Actual use of radioisotopes or equivalent experience.)

ISOTOPE	MAXIMUM AMOUNT	WHERE EXPERIENCE WAS GAINED	DURATION OF EXPERIENCE	TYPE OF USE
Mg 28	500μc	Tulane Medical School	4 years	Experimental
H3OH	10μc	Tulane Medical School	4 years	Experimental
Na22	500μc	Tulane Medical School	4 years	Experimental
Xe133	5μc	Ochsner Clinic, New Orleans	6months	Experimental

10. RADIATION DETECTION INSTRUMENTS. (Use supplemental sheets if necessary.)

TYPE OF INSTRUMENTS (Include make and model number of each)	NUMBER AVAILABLE	RADIATION DETECTED	SENSITIVITY RANGE (mr/hr)	WINDOW THICKNESS (mg/cm²)	USE (Monitoring, surveying, measuring)

11. CALIBRATION FREQUENCY, AND STANDARDS USED IN CALIBRATING INSTRUMENTS LISTED ABOVE.

12. FILM BADGES, DOSIMETERS, AND BIO-ASSAY PROCEDURES USED. (For film badges, specify method of calibrating and processing, or name of supplier.)

INFORMATION TO BE SUBMITTED ON ADDITIONAL SHEETS

13. FACILITIES AND EQUIPMENT. Describe laboratory facilities and remote handling equipment, storage containers, shielding, fume hoods, etc. Explanatory sketch of facility is attached. (Circle answer) Yes No

14. RADIATION PROTECTION PROGRAM. Describe the radiation protection program including control measures. If application covers sealed sources, submit leak testing procedures where applicable, name, training, and experience of person to perform leak tests, and arrangements for performing initial radiation survey, servicing, maintenance and repair of the source.

15. WASTE DISPOSAL. If a commercial waste disposal service is employed, specify name of company. Otherwise, submit detailed description of methods which will be used for disposing of radioactive wastes and estimates of the type and amount of activity involved.

CERTIFICATE (This item must be completed by applicant)

16. THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATE ON BEHALF OF THE APPLICANT NAMED IN ITEM 1, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PART 30, AND THAT ALL INFORMATION CONTAINED HEREIN, INCLUDING ANY SUPPLEMENTS ATTACHED HERETO, IS TRUE AND CORRECT TO THE BEST OF OUR KNOWLEDGE AND BELIEF.

Ralph Lazzara

Applicant named in item 1

Date _____ By: _____

Title of certifying official

WARNING—18 U. S. C., Section 1001, Act of June 25, 1948, 62 Stat. 749, makes it a criminal offense to make a willfully false statement or representation to any department or agency of the United States as to any matter within its jurisdiction.

* U.S. GOVERNMENT PRINTING OFFICE : 1961 O—616963

Form AEC-313 (16-61) Page 3

UNITED STATES ATOMIC ENERGY COMMISSION

APPLICATION FOR BYPRODUCT MATERIAL LICENSE

SUPPLEMENT A—HUMAN USE

Form approved. Budget Bureau No. 38-R080.1

This page may be completed by the physician's preceptor (if any) in the medical use of radioisotopes. When the information is not furnished by the preceptor, the name and present address of the preceptor (if any) should be shown in item 12 below.

9. (a) USING PHYSICIAN'S NAME: Richard P. Carson, Capt MC USAMRNL Denver, Colo.

(b) NAME AND ADDRESS OF APPLICANT (If different from 9(a))

10. CLINICAL TRAINING AND EXPERIENCE OF PHYSICIAN WHO WILL USE BYPRODUCT MATERIAL

(A) ISOTOPE	(B) CONDITION(S) DIAGNOSED OR TREATED	(C) NUMBER OF CASES	(D) TYPE OF PARTICIPATION FOR ALL CASES IN COLUMN B (circle applicable numbers of items in accordance with key set forth below)
I-131	Diagnosis of thyroid function	24	(1) (2) 3 (4)
	Treatment of hyperthyroidism	6	(1) (2) (3) (4)
	Treatment of thyroid cancer	3	(1) (2) (3) (4)
	Treatment of cardiac conditions	1	1 2 3 (4)
	Brain tumor localization		1 2 3 4
	Blood determinations	3	(1) (2) 3 (4)
	Kidney function		1 2 3 4
	Others:		1 2 3 4
P-32 Colloid	Treatment of polycythemia rubra vera and leukemia	3	(1) (2) (3) (4)
	Brain tumor localization		1 2 3 4
	Treatment of bone metastases		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
P-32 $CrPO_4$	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
Au-198 Colloid	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
Cr-51	Blood determinations		1 2 3 4
	Others:	3	(1) (2) 3 (4)
			1 2 3 4
Other Isotopes	Hg^{197} Brain and Renal scanning	15	(1) (2) 3 (4)
	Na^{24} Dilution study	1	(1) (2) 3 (4)
			1 2 3 4

Key to above numbers (column D)

Active Participation and Discussion in the:

1. Examination of patients to determine suitability for radioisotope diagnosis and/or treatment and recommendations on dosage to be prescribed.
2. Calibration and administration of dosages including related measurements and plotting of data.
3. Active period of training and experience of sufficient duration to permit followup of patients through treatment and posttreatment period including reevaluation as to effectiveness and complications.
4. Study and discussion of case histories to establish most efficacious diagnostic and/or therapeutic techniques for this radioisotope use.

11. TOTAL NUMBER OF HOURS OF PARTICIPATION IN CLINICAL TRAINING 160 hours

12. THE TRAINING AND EXPERIENCE INDICATED ABOVE WAS OBTAINED UNDER THE SUPERVISION OR GUIDANCE OF

Wm. H. Beierwaltes MD - Univ. of Mich. Med Center

(Name of Physician (print or type)) (Address)

TRAINING AND EXPERIENCE OF EACH INDIVIDUAL NAMED IN ITEM 4 *(Use supplemental sheets if necessary)*

8. TYPE OF TRAINING	WHERE TRAINED	DURATION OF TRAINING	ON THE JOB (Circle answer)	FORMAL COURSE (Circle answer)
a. Principles and practices of radiation protection	Nuclear Medicine Dept Univ. Hospital, Ann Arbor, Mich.	1 mo.	(Yes) No	(Yes) No
b. Radioactivity measurement standardization and monitoring techniques and instruments	"	1 mo.	(Yes) No	(Yes) No
c. Mathematics and calculations basic to the use and measurement of radioactivity	"	1 mo.	(Yes) No	(Yes) No
d. Biological effects of radiation	"	1 mo.	(Yes) No	(Yes) No

9. EXPERIENCE WITH RADIATION. *(Actual use of radioisotopes or equivalent experience.)*

ISOTOPE	MAXIMUM AMOUNT	WHERE EXPERIENCE WAS GAINED	DURATION OF EXPERIENCE	TYPE OF USE
I^{131}	10 Mc	Univ. of Mich. Med. Center	1 Month	Diagnostic and Therapeutic
Xe^{133}	1 Curie	Univ. of Mich. Med. Center	1 Year	Research

10. RADIATION DETECTION INSTRUMENTS *(Use supplemental sheets if necessary.)*

TYPE OF INSTRUMENTS (Include make and model number of each)	NUMBER AVAILABLE	RADIATION DETECTED	SENSITIVITY RANGE (mr/hr)	WINDOW THICKNESS (mg/cm^2)	USE (Monitoring, surveying, measuring)

11. METHOD, FREQUENCY, AND STANDARDS USED IN CALIBRATING INSTRUMENTS LISTED ABOVE.

12. FILM BADGES, DOSIMETERS, AND BIO-ASSAY PROCEDURES USED. *(For film badges, specify method of calibrating and processing, or name of supplier.)*

INFORMATION TO BE SUBMITTED ON ADDITIONAL SHEETS IN DUPLICATE

13. FACILITIES AND EQUIPMENT. Describe laboratory facilities and remote handling equipment, storage containers, shielding, fume hoods, etc. Explanatory sketch of facility is attached. *(Circle answer)* Yes No

14. RADIATION PROTECTION PROGRAM. Describe the radiation protection program including control measures. If application covers sealed sources, submit leak testing procedures where applicable, name, training, and experience of person to perform leak tests, and arrangements for performing initial radiation survey, servicing, maintenance and repair of the source.

15. WASTE DISPOSAL. If a commercial waste disposal service is employed, specify name of company. Otherwise, submit detailed description of methods which will be used for disposing of radioactive wastes and estimates of the type and amount of activity involved.

CERTIFICATE (This item must be completed by applicant)

16. THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATE ON BEHALF OF THE APPLICANT NAMED IN ITEM 1, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PART 30, AND THAT ALL INFORMATION CONTAINED HEREIN, INCLUDING ANY SUPPLEMENTS ATTACHED HERETO, IS TRUE AND CORRECT TO THE BEST OF OUR KNOWLEDGE AND BELIEF.

Richard P. Carson, Capt., M.C.
Applicant named in item 1

Date _____ By: _____

Title of certifying official

WARNING.—18 U. S. C., Section 1001, Act of June 25, 1948, 62 Stat. 749, makes it a criminal offense to make a willfully false statement or representation to any department or agency of the United States as to any matter within its jurisdiction.

Form AEC-313 a (10-61) Page 3

UNITED STATES ATOMIC ENERGY COMMISSION

APPLICATION FOR BYPRODUCT MATERIAL LICENSE

SUPPLEMENT A—HUMAN USE

Form approved. Budget Bureau No. 38-R080.1

This page may be completed by the physician's preceptor (if any) in the medical use of radioisotopes. When the information is not furnished by the preceptor, the name and present address of the preceptor (if any) should be shown in item 12 below.

9. (a) USING PHYSICIAN'S NAME

Alfonso H. Janoski Capt MC
USAFRNL
Denver, Colo. 80240

(b) NAME AND ADDRESS OF APPLICANT (If different from 9(a))

10. GIVE TRAINING AND EXPERIENCE OF PHYSICIAN WHO WILL USE BYPRODUCT MATERIAL

(A) ISOTOPE	(B) CONDITION(S) DIAGNOSED OR TREATED	(C) NUMBER OF CASES	(D) TYPE OF PARTICIPATION FOR ALL CASES IN COLUMN B (circle applicable numbers of items in accordance with key set forth below)
I-131	Diagnosis of thyroid function	50	(1) 2 (3) (4)
	Treatment of hyperthyroidism	15	(1) 2 (3) (4)
	Treatment of thyroid cancer	4	(1) 2 (3) (4)
	Treatment of cardiac conditions		1 2 3 4
	Brain tumor localization	5	1 2 3 (4)
	Blood determinations		1 2 3 4
	Kidney function		1 2 3 4
	Others:		1 2 3 4
P-32 Colloid	Treatment of polycythemia vera and leukemia	10	(1) 2 3 (4)
	Brain tumor localization		1 2 3 4
	Treatment of bone metastases	2	1 2 3 (4)
	Others:		1 2 3 4
			1 2 3 4
P-32 $CrPO_4$	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites		1 2 3 4
	Others:		1 2 3 4
			1 2 3 4
Au-198 Colloid	Treatment of prostatic cancer		1 2 3 4
	Treatment of cervical cancer		1 2 3 4
	Treatment of pleural effusions and/or ascites	2	1 2 3 (4)
	Others:		1 2 3 4
			1 2 3 4
Cr-51	Blood determinations	25	(1) 2 (3) (4)
	Others:		1 2 3 4
			1 2 3 4
Other Isotopes	Tritium labelled & Carbon-14 labelled	6	(1) (2) 3 (4)
	Steroids - Research		1 2 3 4
			1 2 3 4

Key to above numbers (column D)

Active Participation and Discussion in the:

1. Examination of patients to determine suitability for radioisotope diagnosis and/or treatment and recommendations on dosage to be prescribed.
2. Collaboration in calibration and administration of dosages including related measurements and plotting of data.
3. Active period of training and experience of sufficient duration to permit followup of patients through treatment and posttreatment period including reevaluation as to effectiveness and complications.
4. Study and discussion of case histories to establish most efficacious diagnostic and/or therapeutic techniques for this radioisotope use.

11. TOTAL NUMBER OF HOURS OF PARTICIPATION IN CLINICAL TRAINING 240 hours

12. THE TRAINING AND EXPERIENCE INDICATED ABOVE WAS OBTAINED UNDER THE SUPERVISION OR GUIDANCE OF

Dr Sergei Feitelberg - Mt Sinai Hosp New York; Dr Sidney Werner - Columbia-Presbyterian Med Center N.Y.; Dr Nicholas P. Christy - Barnes Hospital, St. Louis, Mo.

(Physician (preceptor)) (Institution) (Signature)

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Page Two

7. TRAINING AND EXPERIENCE OF EACH INDIVIDUAL NAMED IN ITEM 4 (Use supplemental sheets if necessary)

8. TYPE OF TRAINING	WHERE TRAINED	DURATION OF TRAINING	ON THE JOB (Circle answer)	FORMAL COURSE (Circle answer)
a. Principles and practices of radiation protection	Columbia Univ College of Physicians & Surgeons	2 yrs	(Yes) No	(Yes) No
b. Radioactivity measurement standardization and monitoring techniques and instruments	Mt Sinai Hosp (Radiophysics Dept)	1 yr	(Yes) No	(Yes) No
c. Mathematics and calculations basic to the use and measurement of radioactivity	"		(Yes) No	(Yes) No
d. Biological effects of radiation	"		Yes (No)	(Yes) No

9. EXPERIENCE WITH RADIATION. (Actual use of radioisotopes or equivalent experience.)

ISOTOPE	MAXIMUM AMOUNT	WHERE EXPERIENCE WAS GAINED	DURATION OF EXPERIENCE	TYPE OF USE
H^3	5 Mc	Columbia Univ College of Physicians & Surgeons	2 yrs	Purification, Human metabolism, Isolation & Synthesis
C^{14}	10 Mc	"	"	"

10. RADIATION DETECTION INSTRUMENTS. (Use supplemental sheets if necessary.)

TYPE OF INSTRUMENTS (Include make and model number of each)	NUMBER AVAILABLE	RADIATION DETECTED	SENSITIVITY RANGE (mr/hr)	WINDOW THICKNESS (mg/cm^2)	USE (Monitoring, surveying, measuring)

... METHOD, FREQUENCY, AND STANDARDS USED IN CALIBRATING INSTRUMENTS LISTED ABOVE.

... BADGES, DOSIMETERS, AND BIO-ASSAY PROCEDURES USED. (For film badges, specify method of calibrating and processing, or name of supplier.)

INFORMATION TO BE SUBMITTED ON ADDITIONAL SHEETS

12. FACILITIES AND EQUIPMENT. Describe laboratory facilities and remote handling equipment, storage containers, shielding, fume hoods, etc. Explanatory sketch of facility is attached. (Circle answer) Yes No

13. RADIATION PROTECTION PROGRAM. Describe the radiation protection program including control measures. If application covers sealed sources, submit leak testing procedures where applicable, name, training, and experience of person to perform leak tests, and arrangements for performing initial radiation survey, servicing, maintenance and repair of the source.

14. WASTE DISPOSAL. If a commercial waste disposal service is employed, specify name of company. Otherwise, submit detailed description of methods which will be used for disposing of radioactive wastes and estimates of the type and amount of activity involved.

CERTIFICATE (This item must be completed by applicant)

16. THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATE ON BEHALF OF THE APPLICANT NAMED IN ITEM 1, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PART 30, AND THAT ALL INFORMATION CONTAINED HEREIN, INCLUDING ANY SUPPLEMENTS ATTACHED HERETO, IS TRUE AND CORRECT TO THE BEST OF OUR KNOWLEDGE AND BELIEF.

Alfonso H. Janoski Capt MC

Applicant named in item 1

Date _____ By: _____

Title of certifying official

WARNING.—18 U. S. C., Section 1001, Act of June 25, 1948; 62 Stat. 749; makes it a criminal offense to make a willfully false statement or representation to any department or agency of the United States as to any matter within its jurisdiction.

U.S. GOVERNMENT PRINTING OFFICE : 1961 O-615985

Form AEC-313 a (10-61) Page 3	UNITED STATES ATOMIC ENERGY COMMISSION APPLICATION FOR BYPRODUCT MATERIAL LICENSE SUPPLEMENT A—HUMAN USE	Form approved. Budget Bureau No. 38-R060.1

This page may be completed by the physician's preceptor (if any) in the medical use of radioisotopes. When the information is not furnished by the preceptor, the name and present address of the preceptor (if any) should be shown in item 12 below.

9. (a) NAME OF PHYSICIAN
Robert E. [illegible]
Major, MC
[illegible], [illegible], Denver, Colo.

(b) NAME AND ADDRESS OF APPLICANT (if different from 9(a))

10. CLINICAL TRAINING AND EXPERIENCE OF PHYSICIAN WHO WILL USE BYPRODUCT MATERIAL

(A) ISOTOPE	(B) CONDITION(S) DIAGNOSED OR TREATED	(C) NUMBER OF CASES	(D) TYPE OF PARTICIPATION FOR ALL CASES IN COLUMN B (circle applicable numbers of items in accordance with key set forth below)
I-131	Diagnosis of thyroid function	Approx 50	(1) 2 (3)(4)
	Treatment of hyperthyroidism	Approx 50	(1) 2 ( )(4)
	Treatment of thyroid cancer		1 2 3 4
	[illegible]		1 2 3 4
	[illegible]		1 2 3 4
	[illegible]		1 2 3 4
	[illegible]		1 2 3 4
	[illegible]		1 2 3 4
[illegible]	[illegible]	Approx 4[illegible]	(1) 2( )(4)
	[illegible]		1 2 3 4
	[illegible]		1 2 3 4
	[illegible]	Approx 1	(1) 2 (3)(4)
			1 2 3 4
[illegible]	[illegible]		1 2 3 4
	[illegible]		1 2 3 4
	[illegible]		1 2 3 4
	Other		1 2 3 4
			1 2 3 4
[illegible]	[illegible]		1 2 3 4
	[illegible]		1 2 3 4
	[illegible]	Approx 1	(1)(2)( )(4)
	Other		1 2 3 4
			1 2 3 4
Cr-51	[illegible]	Approx 20	(1)(2)( )( )
	Other		1 2 3 4
			1 2 3 4
Other Isotopes			1 2 3 4
			1 2 3 4
			1 2 3 4

Key to column numbers (column D)
[illegible]
1. [illegible] of patients to determine suitability for radioisotope diagnosis and/or treatment and recommendations [illegible] to be prescribed.
2. [illegible] in calibration and administration of dosages [illegible] measurements and plotting of data.
3. [illegible] of sufficient duration to permit followup of patients through treatment and post-treatment period [illegible] and complications.
4. Study and discussion of case histories to establish most efficacious diagnostic and/or therapeutic techniques for this [illegible].

11. [illegible] At least 100 hrs over a 3 year [illegible] [illegible] Clin. Invest. in metabolic & endocrine [illegible]

12. [illegible] Appropriate staff

[illegible] Hospital.

[illegible signature]

(Date) (Signature)

8. TYPE OF TRAINING	WHERE TRAINED	DURATION OF TRAINING	ON THE JOB (Circle answer)	FORMAL COURSE (Circle answer)
a. Principles and practices of radiation protection	Walter Reed Army Institute of Research	1 yr	(Yes) No	(Yes) No
b. Radioactivity measurement standardization and monitoring techniques and instruments	Walter Reed Army Institute of Research	1 yr	(Yes) No	(Yes) No
c. Mathematics and calculations basic to the use and measurement of radioactivity	Mass. Instit. of Technology	4 yrs	Yes (No)	(Yes) No
d. Biological effects of radiation	Walter Reed Army Institute of Research & Walter Reed Gen Hosp	(incl. rng in intended)	(Yes) No	(Yes) No

9. EXPERIENCE WITH RADIATION (Actual use of radioisotopes or equivalent experience)

ISOTOPE	MAXIMUM AMOUNT	WHERE EXPERIENCE WAS GAINED	DURATION OF EXPERIENCE	TYPE OF USE
[illegible]		WRAIR	9 mos	Lab. res.
^{59}Fe		WRAIR	1 month	Lab. res.
^{51}Cr		Walter Reed Genl Hosp.	3 years	Blood vol. deter. in patients

TYPE OF INSTRUMENTS	NUMBER AVAILABLE	RADIATION DETECTED	SENSITIVITY RANGE	WINDOW THICKNESS (mg/cm^2)	USE (Monitoring, surveying, measuring)
[illegible]	[illegible]	[illegible]	[illegible]		Measuring
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Measuring
[illegible]	[illegible]	[illegible]	[illegible]		Measuring
[illegible]	[illegible]	[illegible]			Measuring
[illegible]	[illegible]	[illegible]			Measuring

11. METHOD, FREQUENCY, AND STANDARDS USED IN CALIBRATING INSTRUMENTS LISTED ABOVE.

[illegible] U.S. Army Institute of Research and Univ. of Pennsylvania.

12. FILM BADGES, DOSIMETERS, AND BIO-ASSAY PROCEDURES USED. (For film badges, specify method of calibrating and processing, or name of supplier.)

Film badges provided by radiation safety office at WRAIR and the Univ. of Penn.

INFORMATION TO BE SUBMITTED ON ADDITIONAL SHEETS

13. FACILITIES AND EQUIPMENT. Describe laboratory facilities and remote handling equipment, storage containers, shielding, fume hoods, etc. Explanatory sketch [illegible]

14. RADIATION PROTECTION PROGRAM. Describe the radiation protection program including control measures. If application covers sealed sources, submit [illegible] and experience of person to perform leak tests, and arrangements for performing initial radiation survey, [illegible]

15. WASTE DISPOSAL. If a commercial waste disposal service is employed, specify name of company. Otherwise, submit detailed description of methods which will be used for disposing of radioactive waste and estimates of the type and amount of activity involved.

CERTIFICATE (This item must be completed by applicant)

16. THE APPLICANT AND ANY OFFICIAL EXECUTING THIS CERTIFICATE ON BEHALF OF THE APPLICANT NAMED IN ITEM 1, CERTIFY THAT THIS APPLICATION IS PREPARED IN CONFORMITY WITH TITLE 10, CODE OF FEDERAL REGULATIONS, PART 30, AND THAT ALL INFORMATION CONTAINED HEREIN, INCLUDING ANY SUPPLEMENTS ATTACHED HERETO, IS TRUE AND CORRECT TO THE BEST OF OUR KNOWLEDGE AND BELIEF.

Applicant named in Item 1

[illegible] — Date

By: ______________________

Title of certifying official

WARNING.—18 U.S.C., Section 1001; Act of June 25, 1948; 62 Stat. 749; makes it a criminal offense to make a willfully false statement or representation to any department or agency of the United States as to any matter within its jurisdiction.

9. Experience with radiation (Continued)

Isotope	Maximum Amount	Where Experience Gained	Duration	Type of Use
^{14}C-progesterone		WRAIR and Walter Reed General Hospital	1 month	Metabolism of adrenal steroids in patients with metabolic disease
^{14}C-glucose	22,200 dpm/flask	WRAIR USAMRNL	4 yrs 1 yr	Lab research Lab research
^{14}C-Cholesterol	Approx. 10^6 dpm	Univ. of Penn. Dept of Biochemistry	3 yrs	Lab research
$^{14}CO_2$		WRAIR	4 yrs	Lab research
^{14}C-Steroids (Various kinds)	Approx. 10^6 dpm	Univ. of Penn. Dept of Biochemistry	3 yrs	Lab research
^{14}C-Iodobutyric acid	Approx 10^7 dpm	Univ. of Penn. Dept of Biochemistry	3 yrs	Chemical synthesis of ^{14}C-labeled steroids

Continuation of Item 6

Appendix III

M.	Iodine 125	M. Cholografin	M. 1 millicurie
N.	Iodine 125	N. Thyroxine	N. 1 millicurie
O.	Phosphorus 32	O. Soluble Phosphate	O. 25 millicuries
P.	Phosphorus 32	P. Colloidal Chromic Phosphate	P. 25 millicuries
Q.	Gold 198	Q. Colloidal	Q. 250 millicuries
R.	Chromium 51	R. Sodium Chromate and Chromic Chloride	R. 10 millicuries
S.	Cobalt 58 and Cobalt 60	S. Vitamin B 12	S. 10 millicuries
T.	Iron 59	T. Ferric Chloride and Ferrous Citrate	T. 1 millicurie
U.	Mercury 197 and Mercury 203	U. Chlormerodrin	U. 10 millicuries
V.	Hydrogen 3	V. Water	V. 25 millicuries
W.	Sodium 24	W. Sodium Chloride	W. 1 millicurie
X.	Xenon 133	X. Gas	X. 2 curies
Y.	Strontium 85	Y. Nitrate and Chloride	Y. 3 millicuries
Z.	Strontium 90	Z. Tracerlab Model RA-1 Sealed Medical Applicator	Z. 25 millicuries
AA.	Any byproduct material with Atomic Nos. 1-83, inclusive	AA. Any	AA. 500 millicuries of each except Hydrogen 3 5 curies. Total not to exceed 10 curies
BB.	Iodine 131	BB. Macro Aggregated Human Serum Albumin	BB. 20 millicuries
CC.	Strontium 90	CC. U. S. Radium Corporation Model LAB-369-1	CC. 1 source of 13 millicuries
DD.	Molybdenum 99	DD. E. R. Squibb Model 08871 Generator	DD. 600 millicuries
EE.	Technetium 99m	EE. Pertechnetate	EE. 300 millicuries
FF.	Calcium 45	FF. Chloride	FF. 10 millicuries
GG.	Calcium 47	GG. Chloride	GG. 10 millicuries
HH.	Xenon 133	HH. Gas dissolved in saline	HH. 50 millicuries
II.	Not renewed		
JJ.	Not renewed		
KK.	Carbon 14	KK. Vitamins, Carbohydrates, Amino Acids, Lipids, Acetate	KK. 10 millicuries of each
LL.	Hydrogen 3	LL. Vitamins, Water	LL. 50 millicuries of each
MM.	Magnesium 28	MM. Oxide, Chloride, Citrate	MM. 10 millicuries of each
NN.	Chromium 51	NN. Labeled Human Serum Albumin	NN. 4 millicuries
OO.	Cesium 137	OO. Any	OO. 1 millicurie

Appendix III

[redacted]

Continuation of Item 7 Appendix IV

G. Determination of thyroxine turnover.
H. Diagnosis of thyroid function and thyroid scanning.
I. Determination of plasma volumes.
J. Determination of renal function.
K. Determination of liver function.
L. Determination of fat absorption.
M. Determination of liver and gallbladder function.
N. Determination of thyroxine turnover.
O. Treatment of polycythemia vera, leukemia and bone metastases.
P. Intracavitary treatment of malignant effusions.
Q. Intracavity treatment of malignant effusions. Interstitial treatment of prostate carcinoma. Liver scanning.
R. Determination of red cell mass, red cell survival time and gastrointestinal bleeding. Spleen scanning.
S. Diagnosis of pernicious anemia.
T. Determination of iron turnover.
U. Kidney and brain scanning. Brain scanning with Mercury 203. Brain scanning and kidney scanning with Mercury 197.
V. Determination of total body water.
W. Determination of total exchangeable sodium.
X. Determination of pulmonary function.
Y. Bone scans in patients with diagnosed cancer.
Z. Treatment of superficial eye conditions.
AA. Laboratory research in vitro and in lower animals.
BB. Lung scanning for diagnosis of massive pulmonary embolism.
CC. For use in Glowall Corporation Model AD-10 Ionization Detector in a Glowall Corporation gas chromatograph.
DD. Source of Technetium 99m Pertechnetate.
EE. Brain scanning.
FF. Metabolic and physiological tracer studies in volunteers, in accordance with application submitted November 18, 1966.
GG. Metabolic and physiological tracer studies in volunteers, in accordance with application submitted November 18, 1966.
HH. Measurement of myocardial blood flow.
II. Not renewed
JJ. Not renewed
KK. Metabolic and physiological tracer studies in volunteers, in accordance with application submitted November 18, 1966.
LL. Metabolic and physiological tracer studies in volunteers, in accrodance with application submitted November 18, 1966.
MM. Metabolic and physiological tracer studies in volunteers, in accordance with application submitted November 18, 1966.
NN. Determination of gastrointestinal protein loss.
OO. Standard for assay of molybdenum content of eluate of molybdenum generator.

Appendix IV

[redacted]

DIAGNOSTIC PROCEDURES, DOSE SCHEDULE AND THERAPEUTIC DOSAGES

All procedures listed in HR-40-601. Dose schedule and additional procedures listed below:

DIAGNOSTIC PROCEDURE	ISOTOPE USED	DOSAGE RANGE
1) Lung Scan	Macroaggregated Human Serum Albumin I-131	300 uc
2) Brain Scan	Technetium 99m Hg-197	8 MC 700 uc
3) Liver Scan	Colloidal Au-198	150 uc
4) Spleen Scan	Cr-51	200 uc
5) Bone Scan	Sr-85	100 uc
6) Thyroid Scan	I-131	60 - 100 uc
7) Cardiac Scan	Iodinated Human Serum Albumin I-131	300 uc
8) Renal Scan	Hg-197	150 uc
9) Renogram	I-131 and I-125 labeled renal function compounds	5 uc/Kg of body weight
10) Blood Volume	Iodinated Human Serum Albumin I-131 Iodinated Human Serum Albumin I-125 Cr-51	5 uc 5 uc 50 uc
11) Placenta Localization	Iodinated Human Serum Albumin I-131	5 uc
12) Liver Function Study with scan	Rose Bengal I-131	150 uc
13) Red Blood Cell Survival	Cr-51	150 uc
14) Platelet Survival	Cr-51	150 uc
15) Schilling Test	Co-57	0.2 - 0.5 uc
16) Thyroid Uptake	I-131	10 uc
17) PBI Conversion Ratio	I-131	100 uc
18) Thyroid Rate Studies	I-131	100 uc
19) Cardiac Output Determination	Iodinated Human Serum Albumin I-131	5 - 15 uc

DOSAGE SCHEDULE CONTINUED.....

DIAGNOSTIC PROCEDURE	ISOTOPE USED	DOSAGE RANGE
20) Iron Studies	Fe-59	50 uc
21) Fat Absorption Studies	I-131 Labeled Fats and Fatty Acids	25 - 60 uc
22) Determination of Gastro-intestinal Bleeding	Cr-51 Labeled Human Serum Albumin	50 - 75 uc
23) T-3 <u>in-vitro</u> Test	I-125 & I-131	less than 0.1 uc
24) T-4 <u>in-vitro</u> Test	I-125	less than 0.05 uc

25) Childrens doses calculated by formula

$$\frac{(\text{Age of child})\ (\text{Adult Dose})}{\text{Age} + 12} = \text{Child's Dose}$$

THERAPEUTIC USES	ISOTOPE USED	DOSAGE RANGE
1) Hyperthyroid Treatment	I-131	5 - 10 MC
2) Thyroid Carcinoma	I-131	75 - 150 MC
3) Cardiac Diseases	I-131	5 - 30 MC
4) Leukemia	P-32	5 - 10 MC
5) Polycythemia Vera	P-32	5 - 10 MC

APPENDIX V

TYPE OF INSTRUMENTS	NUMBER AVAILABLE	RADIATION DETECTED	SENSITIVITY RANGE(mr/hr)	WINDOW THICKNESS	USE
1. Packard Mod. 314EX Liquid Scintillation Counting System	1	Alpha,Beta Gamma	N/A	N/A	measuring
2. Packard Mod. 3375 Liquid Scintillation Counting System	1	Alpha,Beta Gamma	N/A	N/A	measuring
3. Packard Mod. 3314 Liquid Scintillation Counting System	1	Alpha,Beta Gamma	N/A	N/A	measuring
4. Nuclear-Chicago Mod. 6801 Liquid Scintillation Counting System	1	Alpha,Beta Gamma	N/A	N/A	measuring
5. Nuclear-Chicago Mod.Mark I Liquid Scintillation Counting System	1	Alpha,Beta Gamma	N/A	N/A	measuring
6. Tracerlab Versa matic V Scaler with GM tube or Sodium Iodide Crystal	1	Beta, Gamma	N/A	150 mg/cm^2	measuring
7. Atomic Ass. Chromatograph Plate Scanner	1	Beta	N/A	N/A	measuring
8. Nuclear-Chicago Survey Meter Mod. 2612	1	Beta, Gamma	0-20	1.4 mg/cm^2	surveying
9. IM-154/PDR-54	1	Alpha	N/A	UNK	surveying
10. Nuclear-Chicago Labitron Mod. 1619A	2	Beta, Gamma	N/A	100 mg/cm^2	surveying

Appendix V

APPENDIX V (Continued)

TYPE OF INSTRUMENTS	NUMBER AVAILABLE	RADIATION DETECTED	SENSITIVITY RANGE(mr/hr)	WINDOW THICKNESS	USE
11. Nuclear-Chicago Mod. 4351 Tobar Gamma Counting System	1	Gamma	N/A	N/A	measuring
12. Beckman Low Beta II	1	Alpha, Beta	N/A	80 ug/cm^2	measuring
13. Eberline Mod. PAC 3G with Beta Probe	1	Beta	N/A	0.85 mg/cm^2	surveying

Items 1 through 5 are calibrated as necessary with Nuclear Chicago C-14 and H-3 quench correction standards.

Items 6 through 10 and item 13 are calibrated at 6 month intervals by the Pueblo Army Depot Mobile Calibration Team.

Items 11 and 12 are calibrated with the appropriate standards when needed.

FGH DIAGNOSTIC SECTION

Form 313 #10 Radiation Detection Instruments

TYPE OF INSTRUMENT	NUMBER AVAILABLE	RADIATION DETECTED	SENS. RANGE	USE
1) PHO/GAMMA CAMERA II NUCLEAR CHICAGO model #6401 with strip chart recorder attachment N.C. model #3445	1	Gamma		Measuring
2) Nuclear Chicago Uptake Unit includes:		Gamma		Measuring
Analyzer Input model #8742	1			
Ultrascaler II model #8276	1			
Sample Changer model #813050	1			
Autosubtract model #8721	2			
3) Picker Nuclear Magnascanner II (3 inch crystal)	1	Gamma		Measuring
4) Picker Nuclear Magnascanner V (5 inch crystal)	1	Gamma		Measuring
5) Picker Nuclear Twinscaler II (model #600-125) with floor model well	1	Gamma		Measuring
6) Picker Nuclear Autowell with Twinscaler II model #600-125	1	Gamma		Measuring
7) Picker Nuclear Dual Probe System includes:		Gamma		Measuring
Dual Rate Computer model #60082	1			
Dual Channel Analyzer model #600145	1			
Strip Chart Recorder model #PWD 600-092	1			
Digitape 4 (tape recorder) #626155	1			
Monroe Data/log model #MC10-40	1			
8) Scintillation Detector Nuclear Chicago model #DS5 with probe	1	Gamma		Measuring
9) Ames Atomium Volemetron with memory unit	1	Gamma		Measuring
10) Mediac Nuclear Chicago Dose Calibrator model #6362	1	Gamma	75KEV-3MEV	Measuring & Bio-Assay
11) Nuclear Chicago Labitron model #D[illegible]9	1	Gamma	up to 20,000 cpm	Measuring, Surveying, Monitoring
12) Picker Nuclear Laboratory Monitor model #600081 with GM Tube	1	Gamma	up to 30,000 cpm	Surveying & Monitoring
13) Radiac Survey Meter IM-7A/PDR-27C	1	Beta, Gamma	up to 500 mr/hr	Monitoring& Surveying

FGH DIAGNOSTIC SECTION

Form 313 #11 Method, Frequency, and Standards Used in Calibrating Instruments listed in #10

#9 the Ames Atomium Volemetron is calibrated with a Bal33 reference standard kit before each use of the machine. All other instruments calibrated daily or as needed with Cesium or other sources. All survey and monitoring instruments are calibrated at 6 month intervals by Pueblo Army Depot mobile calibration team.

#12 Film Badges, Dosimeters, and Bio-Assay Procedures Used

Film badges are worm by all personnel using radioisotopes, changed monthly and obtained from U.S. Army Signal Corps. Radiacmeter pocket dosimeters (IM 9E/PD) which read up to 200 mr/hr are available for monitoring. Bio-assay of Technetium eluate of Molybdenum-99 Generator is carried out using a dose calibrator or by the dilution method described in attached E.R. Squibb literature.

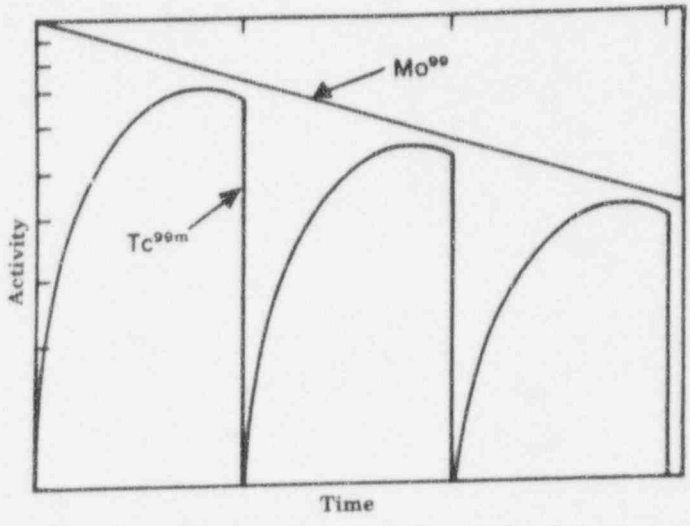
Issued September 1967

CAUTION: NEW DRUG–Limited by Federal law to investigational use

TECHNETOPE®
Squibb Technetium-99m
STERILE GENERATOR

The Technetope (Squibb Technetium-99m) Sterile Generator provides a means of obtaining a sterile, non-pyrogenic supply of Technetium-99m (Tc^{99m}), a versatile scanning agent that can be administered intravenously or orally. Tc^{99m}, the short-lived daughter ($T_{1/2}$ = 6 hours) of Molybdenum-99 (Mo^{99}; $T_{1/2}$ = 67 hours), is obtained from the generator by periodic elution. The amount (in millicuries) of Tc^{99m} obtained in the initial elution will depend on the original potency of the generator, while the activity obtained from subsequent elution[s] will depend on the time interval between elutions (see Graph).

Eluting the generator every 24 hours will provide optimal amounts of Tc^{99m}. Most laboratories therefore will find it convenient to elute the generator each day at a specific time. However, the generator may be eluted whenever sufficient amounts of Tc^{99m} have accumulated within the column.



Mo^{99} decay and Tc^{99m} growth after daily elutions

DESCRIPTION

The Technetope (Squibb Technetium-99m) Sterile Generator has been sterilized by autoclaving. The Molybdenum-99 used in the generator meets or exceeds the purity requirements of the Atomic Energy Commission with respect to allowable levels of Ruthenium-103, Tellurium-132, and Iodine-131 contamination. At the time of initial elution, the alumina concentration is less than 0.5 mg. per 10 millicuries of Tc^{99m} activity. The generator consists of a specially designed lead shield containing an alumina-packed glass column which releases Tc^{99m} upon elution. The lead shield has two access ports to the rubber closures at the top and bottom of the glass column, allowing aseptic elution and storage under conditions of constant shielding. Additional shielding during shipment is provided by a removable lead sleeve which surrounds the entire assembly.

Supplied with the generator are 6 bottles of sterile, non-pyrogenic eluent, and suitable equipment for eluting, collecting, and assaying the Technetium-99m.

WARNING

Maintain proper radiation safety precautions at all times. The glass column containing Mo^{99} need not be removed from the lead shield at any time. The radiation field surrounding an unshielded column is quite high. Solutions of Tc^{99m} withdrawn from the generator should always be adequately shielded. The early elutions from the generator are highly radioactive.

IMPORTANT

Since material obtained from the generator may be intended for intravenous administration, aseptic technique must be strictly observed in all handling. The stoppers of the eluent bottle and the collecting vial, and both rubber closures in the generator column should be swabbed with a suitable germicide before each entry. All entries into the generator column must be made with sterile needles. Either the eluent provided, or Sodium Chloride Injection U.S.P. should be used to elute the generator. Use a fresh disposable syringe, milking tube, breather needle, bottle of eluent, and collecting vial for each elution; sufficient equipment is provided for this purpose. All equipment used to collect or administer the Tc^{99m} must be sterile. Do not administer material eluted from the generator if there is any evidence of foreign matter.

DIRECTIONS FOR ASSAYING Tc^{99m} ACTIVITY
Using the Conometry™ Unit and the Squibb Cobalt-57 Standard

The Conometry Unit consists of a device which standardizes counting geometry when inserted into any standard well counter, and a lead absorber for obtaining a suitable counting rate. The Cobalt-57 Standard provides an appropriate amount of Cobaltous Chloride Co 57 Solution for comparison with an appropriately diluted 0.1 cc. aliquot of Tc^{99m}. Special bottles for assaying the Tc^{99m} are also provided.

1. Place the Conometry Unit in a well-type counter. See photo below.
2. Place the vial of Co^{57} Standard in the cup of the Conometry Unit and determine the activity in the 0.04–0.2 Mev range. Record net counts/minute.
3. Prepare a Tc^{99m} assay sample as follows:
   Put 5.0 cc. of tap water in one of the assay bottles.
   Aseptically withdraw 0.1 cc. from the vial of Tc^{99m} eluate and add this to the water in the assay bottle. (The lead collar may be fitted over the top of the collecting bottle for additional shielding during withdrawal).
   Swirl the assay bottle gently to assure uniform mixing of the Tc^{99m} assay sample.
4. Remove the Co^{57} Standard from the Conometry Unit and replace it with the Tc^{99m} sample. *Maintaining constant geometry* with the previous count, determine the activity in the 0.04–0.2 Mev range. Record net counts/minute.
5. Calculate Tc^{99m} activity using the following formula:

$$Tc^{99m}\ \text{activity (mc/cc.)} = \frac{A \times B \times 10 \times 0.91}{C}$$

Where:

A = net cpm of Tc^{99m} sample
B = activity (in millicuries) of Co^{57} Standard (taken from label and corrected for decay)
C = net cpm of Co^{57} Standard
10 = dilution factor for Tc^{99m}
0.91 = factor for converting Co^{57} activity to equivalent Tc^{99m} activity (corrections for difference in photon yield and attenuation of Co^{57} and Tc^{99m} photons are included)

Conometry Unit in well-type counter

COBALT-57 DECAY FACTORS

To obtain B in the formula at left, multiply the assay value stated on the label of the Cobalt-57 Standard by the appropriate factor in the following table:

Weeks	Factor	Weeks	Factor	Weeks	Factor	Weeks	Factor
1	0.980	14	0.776	27	0.614	40	0.486
2	0.962	15	0.762	28	0.603	41	0.477
3	0.945	16	0.748	29	0.592	42	0.469
4	0.928	17	0.735	30	0.582	43	0.461
5	0.911	18	0.722	31	0.571	44	0.452
6	0.895	19	0.709	32	0.561	45	0.444
7	0.880	20	0.696	33	0.551	46	0.436
8	0.864	21	0.684	34	0.541	47	0.429
9	0.848	22	0.672	35	0.532	48	0.421
10	0.833	23	0.660	36	0.522	49	0.414
11	0.819	24	0.648	37	0.513	50	0.406
12	0.804	25	0.636	38	0.504	51	0.399
13	0.790	26	0.625	39	0.495	52	0.392

DIRECTIONS FOR ASSAYING Mo^{99} ACTIVITY

1. Place the collecting vial containing the total Tc^{99m} eluate in a ¼-inch lead container and set this on the surface of a well-type scintillation detector or scintillation probe. (The lead container is provided on request with the Cesium-137 Standard mentioned below.)
2. Determine the activity in the 0.6–1.0 Mev range. Record net counts/minute.
3. Remove the vial of Tc^{99m} from the lead container and replace it with a Cesium-137 Standard. Determine the activity in the 0.6–1.0 Mev range, maintaining constant geometry with previous count. Record net counts/minute. (The Cesium-137 Standard is provided on request in a Technetope collecting vial with a total volume of 25 cc.)
4. Calculate Mo^{99} activity using the following formula:

$$Mo^{99}\ \text{Activity}\ (\mu c/cc.) = \frac{A \times B \times 4.5}{C}$$

Where:

A = net cpm of Tc^{99m} sample
B = activity (in microcuries per cc.) of Cs^{137} Standard
C = net cpm of Cs^{137} Standard
4.5 = factor for converting Cs^{137} activity to equivalent Mo^{99} activity

NOTE: It is not possible to completely shield out spurious Mo^{99} counts which are due to the presence of large amounts of Tc^{99m}. Hence, Tc^{99m} eluates that are totally free of Mo^{99}, when assayed in this manner, will appear to contain approximately 0.1 microcurie of Mo^{99} per millicurie of Tc^{99m}.

Tc^{99m} DECAY FACTORS

The activity of Tc^{99m} at the time of administration can be determined by multiplying the calculated activity by the appropriate factor in the following table:

Hours	Factor	Hours	Factor	Hours	Factor
½	0.944	4½	0.595	8½	0.375
1	0.891	5	0.561	9	0.354
1½	0.841	5½	0.530	9½	0.334
2	0.794	6	0.500	10	0.315
2½	0.749	6½	0.472	10½	0.297
3	0.707	7	0.445	11	0.281
3½	0.667	7½	0.420	11½	0.265
4	0.630	8	0.397	12	0.250

PHYSICAL PROPERTIES

Technetium-99m has a half-life of 6 hours. The scintillation spectrum of Tc^{99m} shows a photopeak at 0.14 Mev from its gamma emission; it does not emit a beta particle.

Molybdenum-99 has a half-life of 67 hours. Its two major photopeaks are at 0.74 Mev and 0.78 Mev, from two of its numerous gamma emissions; in addition, the isotope emits several beta particles.

The half-life of Cobalt-57 is 270 days. The scintillation spectrum of Co^{57} shows essentially only one photopeak at 0.123 Mev from its principal gamma emission. Co^{57} also emits an X-ray, but has no beta emission.

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INC.

12-422D

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APPENDIX VI

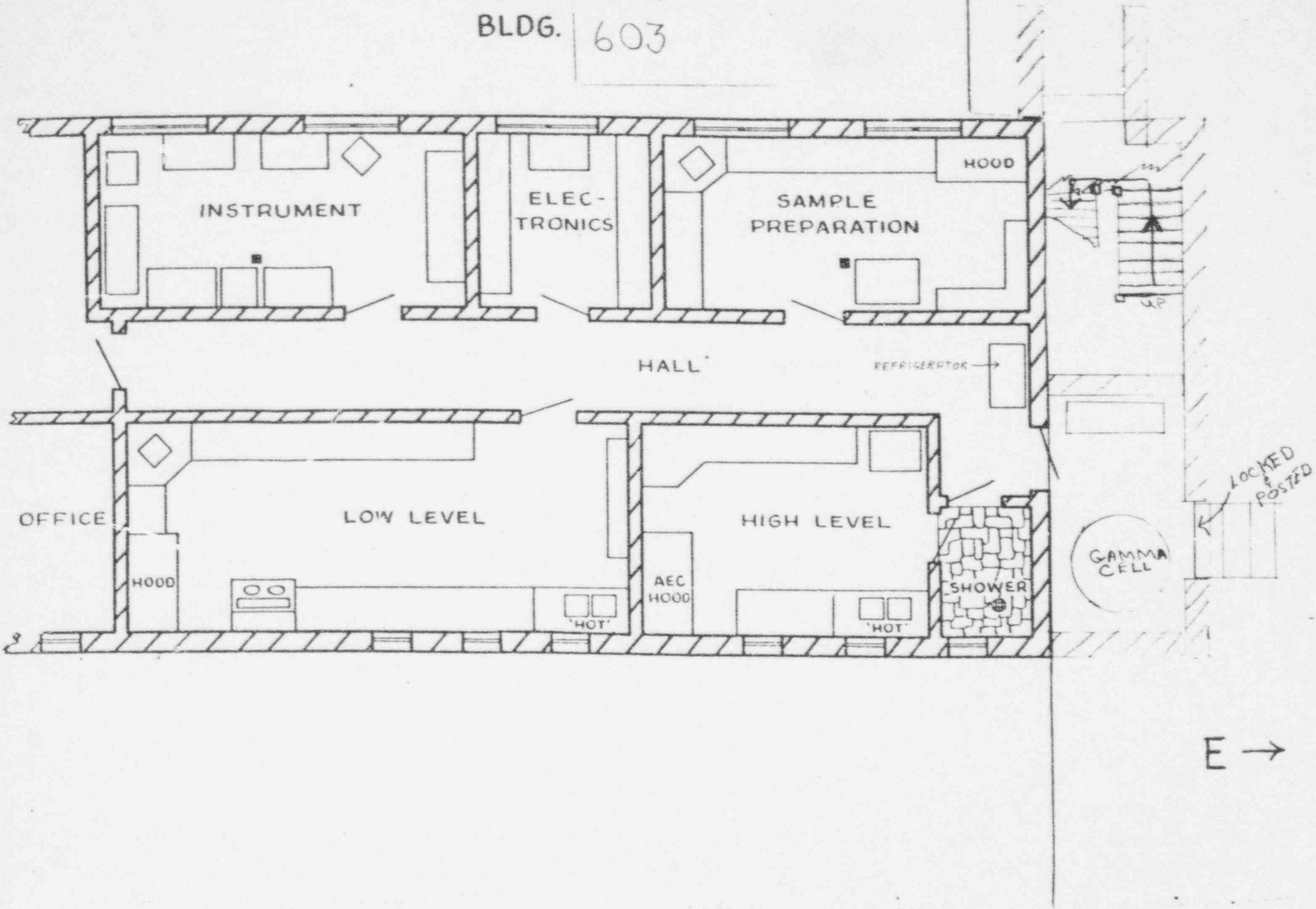
Attached is a map of the Radioisotope Section of the U. S. Army Medical Research and Nutrition Laboratory. The laboratory which represents the east wing of the building is operated as a restricted area with controlled access. Low energy beta emitting radioisotopes are stored in the refrigerator at the east end of the hall. This refrigerator is kept locked to provide additional protection. High energy beta emitters and gamma emitters are stored in the AEC hood in the high level room. This hood is surrounded by one inch of lead shielding and built up at the hood opening with lead bricks.

Liquid waste disposal is carried out in the "hot" sink in the high level room as described in Appendix VII. An outside walk-in freezer is located alongside building S 602 (not shown) and is used to store solid radioactive waste. It is locked and posted.

Also attached is a map of the Radioisotope Clinic and a description of the facilities.

Appendix VI

RADIOISOTOPE LABORATORY
USAMRNL
BLDG. 603



LEGEND FOR FLOOR PLAN OF DIAGNOSTIC RADIOISOTOPE SECTION

Room 1 - Scan room for special studies
A - Profile scanner with tape recorder attachment

Room 2 - Laboratory
B - Hood
C - Hot sink
D - Radioisotope storage (lead brick enclosure with lead sheet bottom)
D_1 - Dose calibrator
E - Cold sink
E_1 - Cold storage radioisotopes. Radioisotopes to be refrigerated are kept in original shipping lead containers.

Room 3 - Laboratory office

Room 4 - Camera room
F - Pho/Gamma Camera

Room 5 - Latrine

Room 6 - Store room

Room 7 - Renogram room
G - Dual probe machine
H - Tape recorder attachment
I - Digital printout

Room 8 - Doctors office

Room 9 - Scan room
J - Magnascanner V

Room 10 - Decay storage
K - Metal container for storage

Room 11 - Well counting
L - Volemetron
M - Autowell
N - Refrigerator
O - Dual channel scaler and floor model well

Room 12 - Doctors office

Room 13 - Scan room
P - Magnascanner II

Room 14 - Uptake room
Q - Scaler
R - Burdick Photomotograph and Electrocardiograph machine for ART

Room 15 - Supervisors office

LEGEND OF DIAGNOSTIC SECTION CONTINUED.....

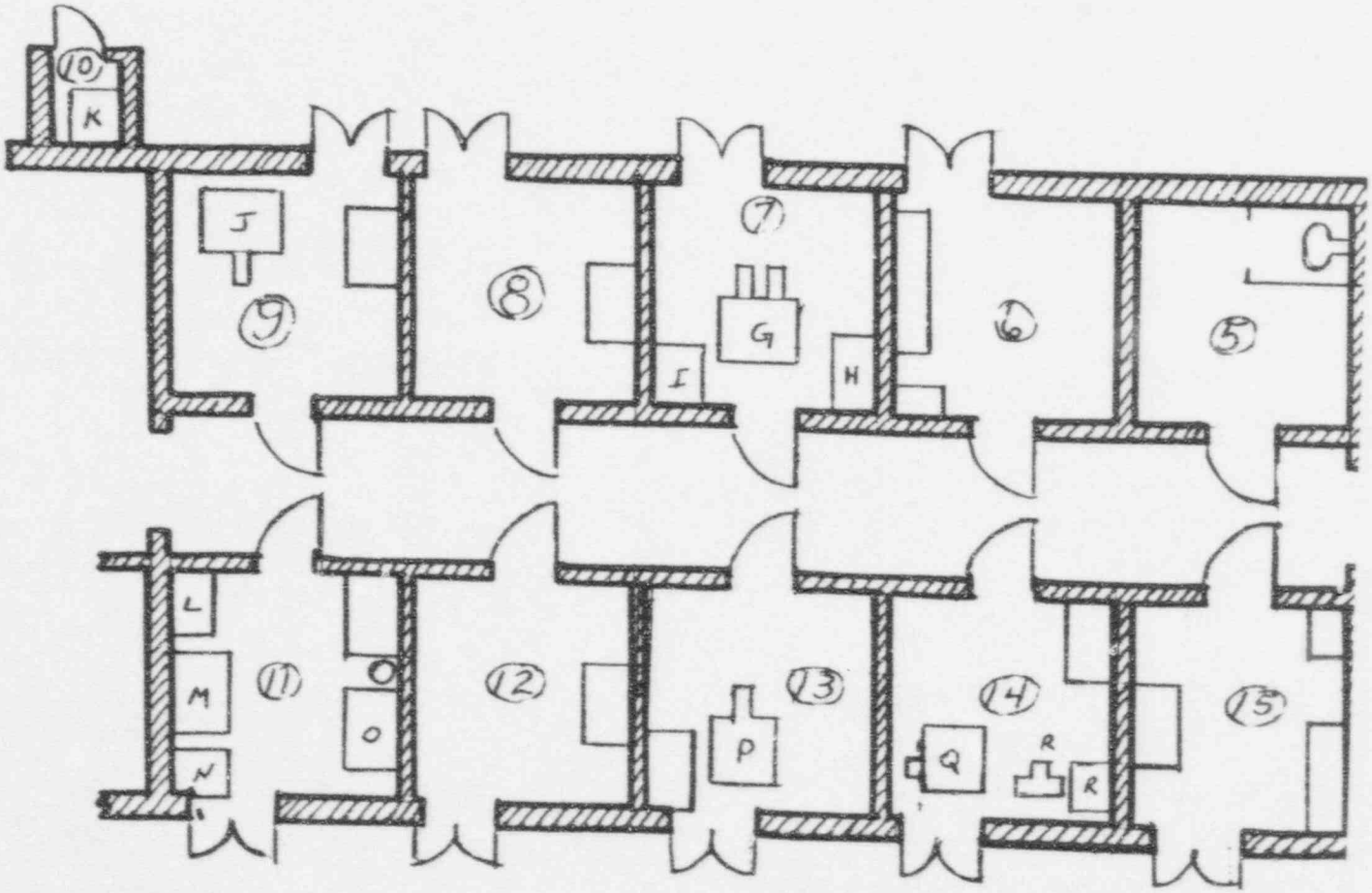
Room 16 - Doctors office

Room 17 - Reception area and secretaries office

The floor plan and accompying legend details space and instrumentation as of 24 June 1968. All radiopharmaceuticals are procured and received accorging to HR 40-602 and stored in designated area in laboratory. All doses are prepared and administered in laboratory area. Books are kept on each radioisotope for procurement, use, and disposal. The decay storage room is kept locked at all times. All other doors are kept locked when no one is in attendance.

RADIOISOTOPE (DIAGNOSTIC) SECTION
FITZSIMONS GENERAL HOSPITAL
24. JUNE 1968

C3583



APPENDIX VII PART I

Surgeon. Sec.

FITZSIMONS GENERAL HOSPITAL
Denver, Colorado 80240

HR 40-602

*HOSPITAL REGULATION
NUMBER 40-601

16 December 1966

MEDICAL SERVICE - RADIOLOGICAL SERVICES AND PROCEDURES

OPERATION OF RADIOLOGY SERVICE

SECTION I - GENERAL

1. Purpose. To furnish information and guidance concerning the operation of the Radiology Service and The Surgeon General's policy for Medical Roentgenography.

SECTION II - RESPONSIBILITIES

2. Responsibility of All Medical Personnel. (Reference paragraph 1d - SGO Policy.) An increasing awareness of the hazards encountered in medical roentgenography requires that all personnel of the Medical Corps participate actively in the reduction of those hazards and in the employment of all means to protect from unnecessary exposure all patients requiring X-ray examination and all personnel working with X-ray equipment. That such hazards exist and the fact that reduction of hazards is imperative shall in no way interfere with good medical practice, but shall only emphasize that the elimination of unnecessary hazards is mandatory. The responsibility for the reduction of hazards and the employment of protective measures does not rest with one individual in a medical treatment facility but with all persons concerned with the care of patients.

3. Responsibility of the Chief, Radiology Service.

a. Precautionary Measures. (Reference paragraph 1d - SGO Policy.) The Chief, Radiology Service, will make periodic inspections of radiographic equipment to determine whether or not unusual hazards exist, and he shall take immediate action to eliminate dangerous conditions. He shall employ all means at his disposal to reduce hazards by:

(1) The minimum addition of 2 mm aluminum filter (to include inherent filtration of the collimator) to all fixed medical radiographic and fluoroscopic X-ray tubes in use at this installation.

(2) The proper use of cones and collimators designed to limit the X-ray beam to the part under investigation.

(3) The proper use of protective materials and apparel by personnel of the X-ray department engaged in making fluoroscopic and radiographic examinations and for the protection of anatomical structures adjacent to or remote from the part under investigation.

*This Hospital Regulation supersedes HR 40-601, 23 February 1965, as amended.

Appendix VII

[redacted]

(4) The employment of techniques designed to reduce exposure to the patient; i.e., immobilization of the part, use of intensifying screens, and the use of optimum processing procedures.

(5) The conduct of periodic inspections of technical procedures and conduct of a continuing program of instruction for X-ray technicians.

(6) Consultation with other physicians or dentists concerning special diagnostic procedures that may be required and to warn of unusual hazards resulting from repeated examinations.

b. Authorizing Use of Equipment. The use of radiographic or fluoroscopic equipment will not be undertaken by any individual, other than members of the Radiology Service, without prior approval of the Chief, Radiology Service. Individuals desiring to use such equipment will submit a Disposition Form (in duplicate) to the Chief, Radiology Service, stating their training and/or experience in the use of such equipment.

c. Monitoring and Recording Radiation Exposure. Personnel monitoring will be performed by the Chief, Radiology Service for all individuals occupationally exposed to ionizing radiation. Periodically, at appropriate times, and at least quarterly the Chief, Radiology Service, will send individual exposure records to the Chief of Personnel Division for entry on individual DD Form 1141 (Record of Occupational Exposure to Ionizing Radiation). The custodian of the medical records shall prepare and maintain DD Form 1141 for each person occupationally exposed to ionizing radiation.

4. Responsibility of the Attending Physician and Dentist. (Reference paragraph 1d - SGO Policy.)

a. Determining X-ray Requirements. Requests for X-ray examination will be made only after the physician or dentist has examined the patient and thereby determined that X-ray examination is necessary for the proper diagnosis and treatment of the individual. Each examination will be made at the specific request of the physician or dentist having knowledge of the responsibility for the welfare of the patient.

(1) Sound medical judgment is necessary to determine whether or not roentgen pelvimetric examination of pregnant women is necessary. Such an examination should not be a routine procedure, but should be made only after the attending physician has determined its necessity. It may be considered advisable to prepare a report of pelvic measurements to be given to the patient for preservation with her personal records. Such a record may obviate the necessity for pelvimetric examinations in future pregnancies.

(2) Films accompanying a patient transferred from one hospital to another will be reviewed by the attending physician and by the radiologist at the receiving hospital in order to determine the diagnostic value of the films and to determine whether re-examination of the patient is necessary.

b. Preparing Examination Requests. Requests properly prepared must correctly identify the patient as to name, rank, serial number (when applicable), age, and status; indicate the part to be examined; show the clinical diagnosis or impression; and bear the personal signature of the physician or dentist requesting the examination.

c. Using X-ray Equipment. Physicians and dentists desiring to use fluoroscopic or radiographic equipment shall receive instruction in the use of equipment and the employment of protective devices by a qualified radiologist before authorization is granted.

SECTION III - GENERAL INFORMATION

5. Hours of Operation.

a. Normal Hours of Operation. Mondays through Fridays from 0745 to 1200 hours, and 1245 to 1630 hours. Only emergency patients will be handled at other times. Except for true emergencies, no film reports or film files will be given out on Saturdays. If films are required for conferences and ward rounds Saturday mornings, they must be requested prior to 1200 hours Friday. Patients should not be sent to the clinic after 1600 hours to allow for completion of work for those already waiting and permit the technicians to clean the machines and prepare for the next day's work.

b. Service After Normal Duty Hours. To obtain X-ray service at other than normal duty hours, a call will be made to Extension 21140 before the patient is sent to the clinic. If there is no answer, the Operator will be called to locate the technician on duty. Female patients and children will not be sent to the X-ray Clinic unless accompanied by a female attendant after normal duty hours or on Sundays or holidays.

6. X-ray Reports and Films.

a. Loan of X-ray Films. X-ray films may be withdrawn from the central files for short periods of time by an medical officer provided a proper receipt is given (FAH Form 222-W, Request for X-ray Film, prepared by or for the requesting officer). The individual requesting the films will be held responsible for the loss or damage of films while outside the department, and will also be responsible for the prompt return of films. Under no circumstances will films be removed from their jacket and retained by individuals or a new jacket prepared while outside the Radiology Service. The TB Service may, if they desire, receipt for films of their patients to be retained on the wards as long as the patient is in the hospital, but all films must be returned to the Radiology Service when new films are requested. Additional examinations will not be done unless borrowed films accompany the request. When a patient is transferred from ward to ward, films must be returned to the central files. Except under very unusual conditions, and then only when cleared by the Chief, Radiology Service, films may not be withdrawn from the central files until

they have been rejected. Films accompanying patients to this hospital are to be sent directly to the Radiology Service except those transferred with a diagnosis of tuberculosis, in which case they are sent to the TB Service. If films are inadvertently sent directly to the ward, the medical officer will review them and send them immediately to the Radiology Service. Films which have been coded for teaching purposes and identified as "Teaching File" on the jacket may be receipted for only by a Medical or Dental Corps Officer and will be inventoried on leaving and return to the central files by number and size of films. Such films will not be loaned for periods in excess of 48 hours.

b. X-ray Films from Other Hospitals. Requests for films from other hospitals will be made to the Chief, Radiology Service (by signing X-ray Film Record Card FAH 1451-X on file in Outf lm Section). When borrowed films have served their purpose, they will b returned promptly to the Radiology Service for return to the loaning hospital.

c. Photographs and Duplication of X-ray Films. All requests for photographs and duplication of X-ray films will be submitted to the Radiology Service. Requests must be approved by the chief of the department or service concerned. Requests for photography will be made on SG Form 416 (in duplicate), one form for each patient, with desired films attached to the request. The Radiology Service will forward the request and films to the Medical Illustration and Audio Visual Activity, from where the requesting official will receive completed reproductions. Requests for duplication (solarization) of films will be made to the Radiology Service, using a Disposition Form (one copy only), signed by the requesting officer. This service will not be performed for individual collections.

SECTION IV - DIAGNOSTIC PROCEDURES

7. General. The interpretation of films is not a detached study of shadows to be made without consideration of the clinical findings, and it is therefore necessary for the clinician to furnish all pertinent information as to diagnosis and, in the case of injuries, to state the specific area involved. This system will result in much more efficient and reliable film diagnosis and react to the benefit of all patients and physicians. The Radiology Service reserves the right to refuse to report on films when such information is not furnished.

8. Examinations.

a. Requests for Examination. Diagnostic procedures will be accomplished on requests prepared and personally signed by medical and dental officers of the Command. Requests will be prepared in duplicate on SF 519A and must be typewritten or clearly written in ink. It is imperative that the duplicate copy be legible. The patient's full name, rank or status, serial number (if any), age, race, ward or department; the part to be X-rayed;

and clinical diagnosis will be entered. The name of the hospital will be shown in the space provided on the form. When possible the examination should be requested by part or area rather than specific views. If non-standard views are desired, the requesting physician should accompany the patient to the Radiology Clinic. S.O.P.'s for X-ray examinations are available for distribution from the Radiology Service. "Routine" or "Observation" will not suffice for clinical diagnosis on requests other than for PA chests. "Possible fracture 5th left rib," "Suspected tuberculosis," "Food Handler," etc., are examples of appropriate entries. Requests for routine examination of inpatients will be delivered to the department, and patients will be called in order to prevent unnecessary waiting if the examination cannot be accomplished immediately. All requests for examinations in connection with the immediate discharge from the Service will bear the notation "For Discharge," "For Discharge and Reenlistment," "For Discharge for Disability," etc. Information similar to that required for discharge will be furnished on all requests for enlistment, appointment in ORC, appointment to OCS, etc. Improperly prepared and unsigned requests will not be honored.

b. Emergency Examinations. Emergency examinations will be made at any hour. In such cases a "Wet Reading" should be requested. This procedure must not be abused, and no examination will be designated as emergency unless the proper treatment of the patient is contingent on immediate films and immediate interpretation. The telephone number to be called will be entered on the request. The handling of pre-operative chest X-rays is covered in paragraph 12. Films obtained at the request of OPD physicians at times when no Radiologist is in the hospital will be sent to the OPD with the patient. These are to be returned to the X-ray Clinic as soon as possible. Emergency readings will be given to Clinic Patients to be hand-carried to the requesting physician. If the films are also desired, it should be indicated on the request. Emergency readings on ward patients will be called to the wards. Patients with requests marked "Ortho Wet" will be retained in the X-ray Clinic until their films have been interpreted and returned with the report and films to the referring physician.

c. Bedside Examination. Requests for bedside examination will be marked "Bedside." If an emergency bedside examination is needed, the request will be marked "Bedside - Emergency." In such cases, the ward will either send the request to the Radiology Service or telephone that office giving the necessary information, so that proper film identification markers can be taken to the ward. Phone number and name of person to be called will be entered on the request form.

d. Special Examinations. Special examinations such as fluoroscopy, cholecystograms, laminograms, gastrointestinal series, barium enemas, angiograms, myelograms, bronchograms, sinus tract injections, encephalograms, chest or heart fluoroscopy, examinations during surgery, etc., will be made by appointment only. If in doubt, the requesting individual should inquire of the Radiology

Service.

e. Urological Examinations. Requests for intravenous pyelograms and other urological procedures will be made directly to the Chief of Gastro-Urinary Service, and not to the Radiology Service. Special intravenous pyelograms with nephrotomography or urea washouts in hypertensive patients will be scheduled with the Radiology Service.

f. Multiple Examinations. Multiple examinations on the same individual require careful planning. Members of the radiologic staff will assist in arranging the sequence of such examinations. If barium or other radiopaque substances are given, it may take several days for them to disappear sufficiently to eliminate confusing shadows in roentgenograms of the abdomen. As a rule, contrast studies should be done last. Cholecystography should ordinarily be done before a gastrointestinal examination except when the small bowel is to be studied. Due to the irritative effect of gallbladder visualization preparations, it is best to do cholecystography last when a small bowel study is contemplated. A gastrointestinal series should be done before a barium enema. When numerous examinations of the same area are likely, total skin dosage must be considered. A member of the radiology staff will assist in determining if a dangerous dosage is probable. All anticipated thyroid studies should be performed before any contrast procedures, other than upper GI or BE, are done.

g. Progress Examinations. Progress films or examinations should be requested only after consideration of likelihood of change since the last examination. Consultation with the radiologist should be obtained to determine the optimal time for follow-up examination.

9. Patient Handling.

a. Patients with Contagious Diseases. All patients with contagious diseases will have this warning properly inscribed in BOLD PRINT on their X-ray blanks. It is the responsibility of the ward officer to see that this procedure is carried out.

b. Nonambulatory Patients. Unless the Radiology Service is warned to the contrary, it will be assumed that every patient brought into the department may be handled as ambulatory. If other than this type of care is expected, the request blank must properly show that the patient is to be moved carefully or is not to be moved at all.

c. Removal of Casts and Braces. If a cast or brace may be removed for the examination, a statement to that effect must be entered on the request.

d. Female Patients. All female patients who are not ambulatory must be dressed in an operating room gown, tied in the back, before leaving the ward.

Under no conditions will female patients or children be sent to the X-ray Clinic unless accompanied by a female attendant after normal duty hours or on Sundays and holidays.

10. Preparation of Patient for Special Examinations.

a. GI Series or Small Bowel Examination. Nothing by mouth after midnight before the examination. This is to include water, gum chewing and medications. No smoking until after the examination.

b. Barium Enema. In the 24 hours before the examination the diet should be entirely non-residue - clear juices, coffee, tea, gelatin, clear soups. Eat and drink nothing on the morning of the examination. Dairy products are high residue foods and must be avoided. Two ounces of castor oil will be taken between 1600 and 1800 hours of the day before the examination. Children between 6 and 14 should receive one ounce of castor oil. Young children should receive no castor oil but are to be given saline enemas until clear within two hours of the examination.

c. Oral Cholecystogram. The patient will report to the X-ray Department one or more days before the examination for a preliminary film. On the evening before the examination a fat-free meal containing lean meat (but no pork products), vegetables, fruit, toast, or bread without butter, coffee or tea may be eaten. Nothing except water should be taken after this meal. At approximately 2100 hours the contrast material will be taken according to instructions given by the Radiology Service. On the following morning omit breakfast until after the examination. If the gallbladder is not visualized after a single dose of the contrast material, a reinforcing dose may be given. If nonvisualization is due to diarrhea, 4-8 cc. of paregoric may be given 30 minutes before a single dose of contrast material.

d. Special Procedures. Specific instructions for other special procedures may be obtained by contacting an officer of the Radiology Service.

11. Pre-operative Chest X-rays.

a. Surgical Emergencies.

(1) This classification shall apply only to those patients who are true surgical emergencies and shall not be used to cover administrative lapses of responsible medical officers.

(2) Pre-operative chest X-rays on surgical emergency patients, if required, may be requested at any time. The responsible medical officer will call the Radiology Service (Ext 21151 during duty hours, Ext 21140 after duty hours) to determine when the patient may be sent to the Clinic.

(3) At the time of this original call, the responsible medical officer will inform the X-ray receptionist, if during duty hours, to what telephone extension the film reading is to be reported.

(4) If the call is being made after duty hours, the responsible medical officer will notify the emergency X-ray technician where he may be reached when the film is ready for viewing.

(5) If, after 2000 hours, there is a question about the film interpretation which cannot be resolved by the senior surgical officer on the case, consultation may be obtained with the radiology officer on call.

(6) X-ray requests on these patients will be given top priority and will have recorded across the top of the form the following legend: "Surgical Emergency, Immediate Reading." All other sections of the X-ray request will also be completed, and the signature of the requesting medical officer will appear in the appropriate space on the form.

(7) Reports will be submitted through routine channels.

b. Routine Pre-operative Chest X-rays.

(1) The medical officer to whom the patient is assigned will be responsible for seeing that pre-operative chest X-rays are ordered when indicated.

(2) All patients requiring pre-operative chest X-rays in elective surgical procedures will be referred to the Radiology Service for these studies no later than 1200 hours on the last duty day preceding the date of the scheduled operation.

(3) Patients will not be referred for routine pre-operative chest X-rays after 1200 hours on Saturday or on Sundays and holidays. Any chest X-ray within 7 days prior to scheduled surgery will be considered adequate for pre-operative clearance unless there is some clinical indication for a more recent film.

(4) Patients to be admitted over the weekend for surgery on a Monday will be referred for their chest X-ray during the week prior to admission.

(5) In the event of an administrative oversight resulting in failure to obtain the pre-operative chest film in the prescribed manner, the responsible medical officer will call the emergency technician to determine if and when such an examination can be made. No priority will be given these cases; and if the X-ray technician is busy with other emergency duties, then it may be necessary to postpone the scheduled surgical procedure.

(6) X-ray requests for pre-operative chest films on elective surgical cases will have recorded in the space provided for pertinent clinical history the following legend: "Routine Pre-operative Chest." In addition, the pertinent clinical information will be recorded, and all sections of the X-ray request will be properly completed, including the signature of

the requesting medical officer.

(7) All routine pre-operative chest film reports will be given by telephone to the requesting ward not later than 1600 hours of the day of the examination. If a ward is not designated on the request, the report will be given to the Office of the Chief of Surgery. It will then become the responsibility of the medical officer concerned to take whatever steps are necessary to either cancel the surgical procedure or to clear the patient with the Anesthesia Service.

(8) Wet-film reports on patients from the Thoracic Surgery Service will note only if there has been a change from the last examination. If a change has occurred, the report will be given to the ward concerned.

(9) Typewritten reports will be submitted through routine channels.

SECTION V - RADIATION THERAPY PROCEDURES

12. Requests for Radiation Therapy. Requests for consideration for radiation therapy will be submitted to the Radiation Therapy Section on SF 513 (Consultation Form), in duplicate. History in some detail must be entered. Treatment of malignant conditions will be undertaken only after consideration of the patient by the Tumor Board.

13. Care of Patients Receiving Roentgen Therapy. The care of patients receiving radiation therapy will be under the direction of the Radiation Therapy Section. Any alterations or interruptions in therapy will be made only after consultation with the Chief, Radiation Therapy Section. No cytotoxic drugs or chemotherapeutic agents will be administered or prescribed for patients undergoing radiation therapy without prior consultation with Chief, Radiation Therapy because of the increased incidence of adverse reactions detrimental to the treatment of patients.

a. A white blood count, differential and hematocrit will be secured at least once each week by the Radiation Therapy Section and subsequently forwarded to the patient's hospital chart.

b. The diet will be supplemented by interval feedings of fruit juices, milk shakes, etc. Moderate doses of sodium bicarbonate immediately after each treatment may be indicated. If the patient is nauseated, "Dramamine" 50-100 milligrams t.i.d., "Bonamine" 50 milligrams t.i.d., "Compazine" 5 milligrams q.i.d., or 10 milligrams "Spansule" b.i.d. may be given.

c. A representative of the Radiology Service will prescribe the care of skin reactions and no other treatment should be given.

14. Care of Patients Receiving Radium Therapy. The routine care of patients receiving radium therapy can be carried out by the ward personnel without hazard of significant radiation exposure if done in an expeditious manner. As a general rule, each individual should limit the time spent with a radium patient to one hour per day while the radium is in the patient.

SECTION VI - RADIOISOTOPE PROCEDURES

15. Requests for Radioisotope Study, Consultation, or Therapy. Requests for radioisotope study, consultation, or therapy should be submitted to the Radioisotope Section (Bldg T-511W) on SF 513 (Consultation Form), in duplicate. This request should indicate the study or therapy to be considered, and the clinical history of the patient, and must be completed by the physician responsible for the patient's care.

16. Routine Procedures.

a. Thyroid Studies (available only through the Thyroid Clinic or by special arrangement.) In vivo uptake and scan, conversion radio, urinary radioactivity, triiodothyronine resin uptake.

b. Hematologic Studies. Total blood volume, red cell volume, plasma volume, red cell survival and sequestration studies, Schilling test, ferrokinetics.

c. Placental Localization.

d. Gastrointestinal Studies. Triolein and oleic acid absorption, albumin kinetics, polyvinylpyrrolidone excretion, rose bengal liver studies.

e. Renal Studies. Renogram, renal uptake of neohydrin, renal scan

f. Scintiscan. Thyroid, renal, liver, spleen, pancreas, brain, precordium, bone.

g. Therapy.

(1) Radioiodine - hyperthyroidism, thyroid carcinoma, advanced cardiac or pulmonary insufficiency.

(2) Radiophosphorus - polycythemia, leukemia, bone metastases.

(3) Colloidal Radigold or Radiophosphorus - malignant pleural effusion, peritoneal effusion, pericardial effusion.

17. Special Procedures. Special procedures may be arranged by consultation with the Radioisotope Section.

18. Loan of Radioisotope Studies. Certain studies may be withdrawn from the Radioisotope Section by any medical officer provided a proper receipt is given (FGH Form 222-W, X-ray Film Request and Receipt). This request should indicate the patient's name and the study desired, and must be prepared and signed by the medical officer requesting the study. The individual requesting the study will be held responsible for loss or damage of the study and will also be responsible for the prompt return of the study. Studies must be returned to the Radioisotope Section within 72 hours of their receipt. Studies may be photographed or reproduced with the prior approval of the Chief, Radioisotope Section. Any medical officer, who fails to comply with these requirements, will not be permitted to withdraw further studies from the Radioisotope Section.

19. Preparation of Patient. The following procedures require preparation by ward personnel:

a. Renogram. Nothing by mouth for 6-8 hours preceding the examination.

b. Schilling Test. Nothing by mouth for 6-8 hours preceding the examination and for 2 hours following the dose of radioactivity. Collect complete 24 hour urine specimen (begin and end at exact time of dose of radioactivity) and send to Radioisotope Section.

c. Ferrokinetics. Nothing by mouth for 6-8 hours preceding the examination.

d. Triolein, Oleic Acid Tests. Nothing by mouth for 6-8 hours preceding the examination and for 2 hours following the dose of radioactivity. Collect complete 72 hour stool specimen and send to Radioisotope Section.

e. Brain Scintiscan. Administer Mercurhydrin 1 cc. intramuscularly 24 hours prior to the examination.

f. Rose Bengal Liver Studies. Nothing by mouth for 6-8 hours preceding the examination. No BSP dye the morning of the examination.

g. Red Cell Survival. Patient must be available for 2-3 weeks.

h. Polyvinylpyrrolidone (PVP) Excretion. Collect complete 4-day stool specimen and send to Radioisotope Section.

i. Pancreas Scintiscan. Special instructions will be provided by radioisotope personnel.

j. Therapy. Patient should have private room, preferably with private bath. Further instructions will be provided by radioisotope personnel. Under certain circumstances, radioiodine, radiophosphorus, and

chromic radiophosphorus may be administered to an outpatient.

20. References.

a. TB MED 62.

b. AR 40-14.

c. The Surgeon General's Policy.

MEDEO-X

FOR THE COMMANDER:

THOMAS M. DOYLE
LTC, MSC
Adjutant

DISTRIBUTION:
"D"
Plus 5 ea - D43

APPENDIX VII PART II

FITZSIMONS GENERAL HOSPITAL
Denver, Colorado 80240

* HOSPITAL REGULATION
NUMBER 40-602 14 December 1966

MEDICAL SERVICE - RADIOLOGY SERVICE AND PROCEDURES

PROCUREMENT, RECEIPT AND ISSUE OF RADIOACTIVE ISOTOPES

1. Purpose. To establish policies, procedures and responsibilities for the procurement, receipt and issue of radioactive isotopes.

2. Procurement. All purchase requests will be reviewed and countersigned by the Radiological Safety Officer prior to purchase. An exception to this procedure is procurement of specific isotopes against previously established Blanket Purchase Requests submitted by the Fitzsimons General Hospital Radioisotope Section or the US Army Medical Research and Nutrition Laboratory. When the requirement exists, the chiefs of the respective isotope sections will notify the Radiological Safety Officer by telephonic communication.

3. Duty Hour Responsibilities.

a. Purchasing and Contracting Branch. Will insure that purchase order indicates whether consignee is USAMRNL or the Radioisotope Section, Radiology Service, Fitzsimons General Hospital, in order to expedite accurate and efficient delivery.

b. Transportation Branch. Upon notification, personnel of the Transportation Branch will pick up radioactive isotopes at the airport and deliver the unopened shipping container to the Storage Section, Supply Control Branch, for identification purposes. If the shipping container shows any evidence of damage, Transportation Service personnel will notify the Radiological Safety Officer, Extension 22133, immediately.

c. Storage Section, Supply Control Branch. Will determine whether the material is for USAMRNL or Radioisotope Section, Radiology Service. Upon determination, they will immediately deliver to Radioisotope Section or notify USAMRNL for immediate pickup. If USAMRNL cannot pick up material immediately, the Storage Section will deliver the items. The individual in USAMRNL or the Radioisotope Section who receives the radioactive isotopes will open the package and sign one copy of the billing document, returning the document to Storage Section personnel

*This Hospital Regulation supersedes HR 40-602, 2 October 1963.

making the delivery. UNDER NO CIRCUMSTANCES WILL THE RADIOACTIVE ISOTOPE SHIPPING CONTAINER BE OPENED IN THE RECEIVING SECTION BY SUPPLY PERSONNEL.

d. Radioisotope Section and USAMRNL. Will be responsible for safe storage of radioactive isotopes. Personnel will maintain DA Form 8-235 (Pharmacy-Drug and Narcotic Stock Record), in accordance with paragraph 5, AR 40-61.

4. After Duty Hour Responsibilities.

a. The Administrative Officer of the Day, upon receiving a call from the airport, will arrange for pickup of radioactive isotopes and take the unopened shipping container to the Radioisotope Clinic, Building 511. The container should be placed in the center of the counter immediately inside the front door where Radioisotope Clinic personnel will check it in the following duty day. The Radioisotope Clinic front door should be locked after delivery. The AOD will notify Storage personnel, Supply Control Branch, Extension 22210, at 0745 hours the following morning that radioactive material has been received.

b. All radioisotopes must be placed in safe storage (Radioisotope Clinic Counter, Building 511) without delay, and under no circumstances will they be permitted to remain in any other area after arrival from the airport.

c. In the event of damage to the shipping container, action will be taken as required in paragraph 3b, above.

5. References.

a. HR 40-604.
b. TB MED 249.
c. AR 40-61.
d. AR 711-16.
e. AR 725-50.

MEDEO-X

FOR THE COMMANDER:

Thomas M. Doyle
THOMAS M. DOYLE
LTC, MSC
Adjutant

DISTRIBUTION:
"B"

APPENDIX XII PART III

FITZSIMONS GENERAL HOSPITAL

NURSING CARE OF A PATIENT RECEIVING A RADIOISOTOPE

SECTION I

DEFINITION OF TERMS

1. Contamination Precautions: Using whatever precautions may be necessary to avoid direct or indirect contamination with a radioisotope. This may be effected by use of gloves, by handling contaminated materials with tongs, etc.

2. Radiation Exposure Precautions: Precautions involving time of exposure, distance from the radio-active material and use of shielding.

3. Radioisotope: An unstable element used in medicine as a source of internal and/or external radiation due to its emission of energy, principally in the form of alpha, beta and gamma radiations.

   a. Alpha radiation: Highly ionizing but non-penetrating.

   b. Beta radiation: Less ionizing than alpha radiation, but slightly more penetrating.

   c. Gamma radiation: Less ionizing than beta radiation but highly penetrating. (Similar to x-ray).

4. Radio-active Half-life: Length of time required for a radioactive substance to lose fifty per cent of its activity.

5. Sealed Source: A radioactive source that is firmly bonded within a material or sealed in a cover of sufficient strength to exclude the possibility of direct contact with that source.

6. Internal Radiation: Radiation received by the body from a radio-active source within it.

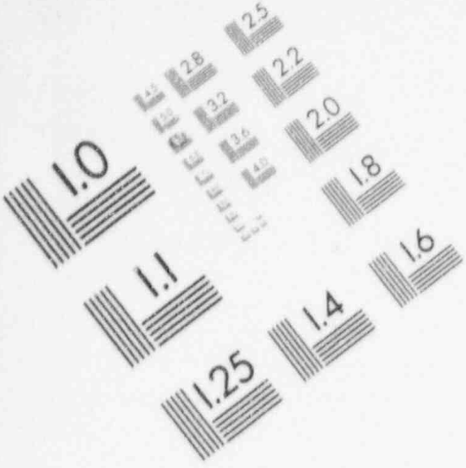
7. External Radiation: Radiation received by the body from a radio-active source or sources which are external to it.

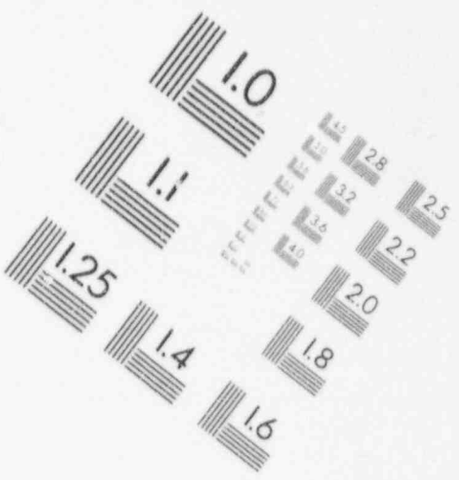
8. Ionizing Radiation: Radiation which causes changes in the molecular structure of the atom resulting in alteration or destruction of the cell in living tissue.

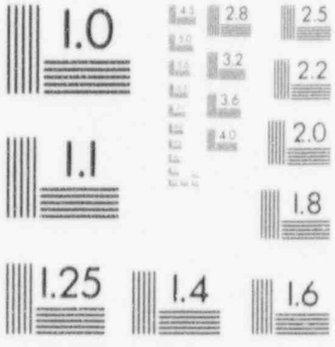
9. Radioactive Contamination: The undesired presence of a radioactive substance in or on any material.

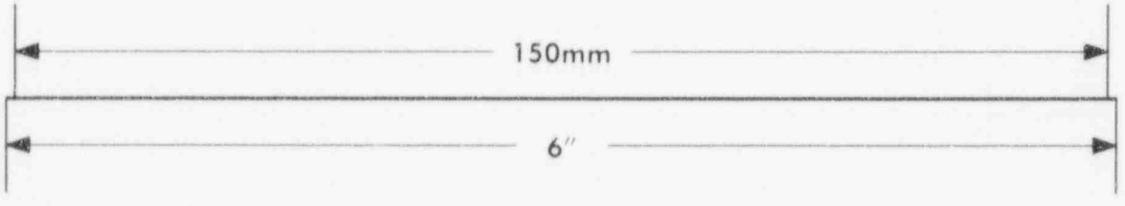
2

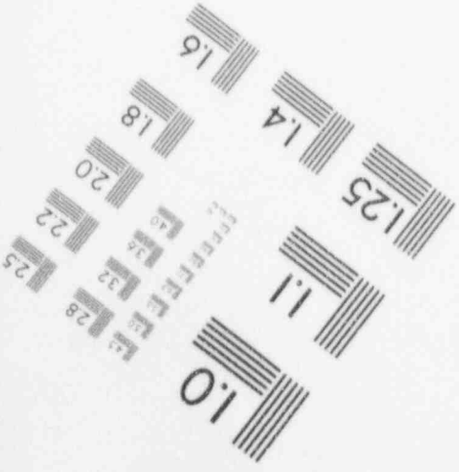
IMAGE EVALUATION TEST TARGET (MT-3)

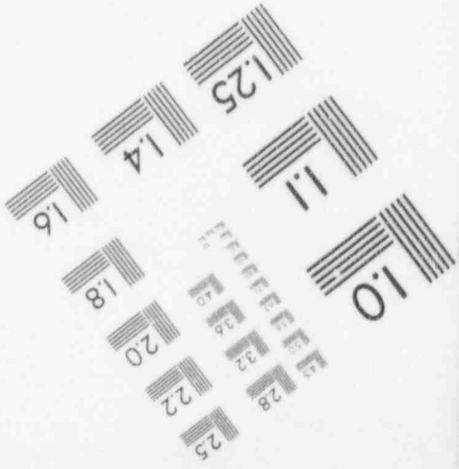












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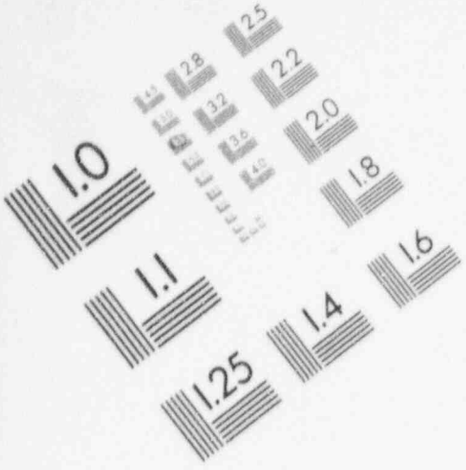
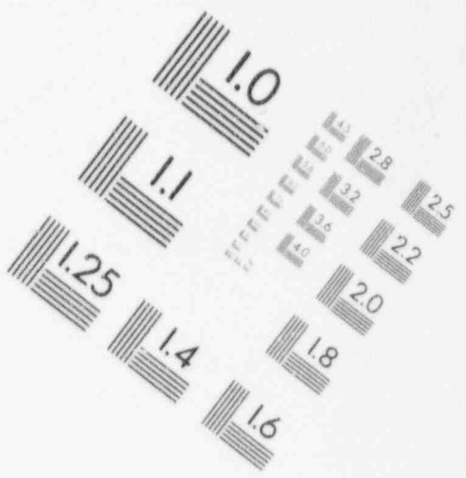
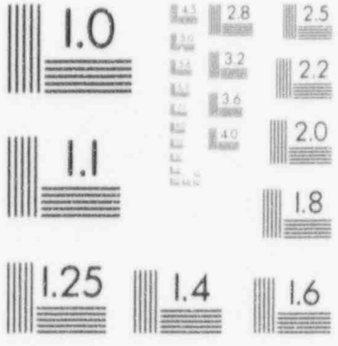
WEBSTER, NEW YORK 14580
(716) 265-1600

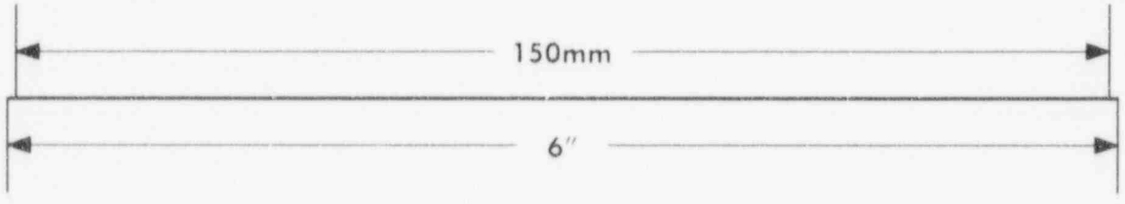
2

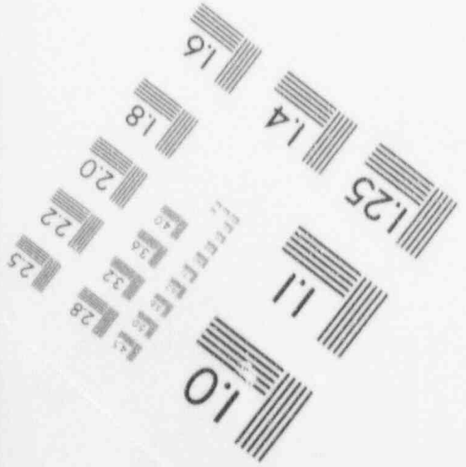
IMAGE EVALUATION TEST TARGET (MT-3)

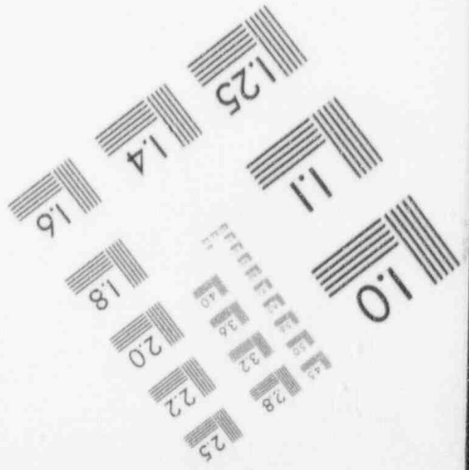












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10. Adequate Protection: Protection from internal or external radioactive sources such that the amount of radiation received by any person does not exceed the maximum permissible levels of exposure recommended by the Atomic Energy Committee on Radiation Protection.

11. Radiological Isolation: Isolation when radiation exposure precautio and/or contamination precautions are essential in the nursing care o the patient.

NURSING CARE OF A PATIENT RECEIVING A RADIOISOTOPE

SECTION II

CLASSIFICATION OF PATIENTS

CLASS I: No precautions.

1. All patients who receive a radioisotope for diagnostic purpose will be placed in this category.

CLASS II: Contamination Precautions only.

1. Intracavitary injection of Chromium Phosphate (P32).

CLASS III: Major Radiation Exposure Precautions only.

1. Intracavitary insertion of radium needles or capsules.
2. Interstitial implant of cobalt needles or capsules.
3. Interstitial implant of iridium seeds.
4. Interstitial implant of radium needles.
5. Interstitial implant of radon seeds.
6. Surface application of radium needles.

CLASS IV: Contamination and Moderate Radiation Exposure Precautions.

1. Hyperthyroidism treated with radioactive iodine.
2. Treatment of cardiac patients with radioactive iodine.

CLASS V: Contamination and major Radiation Exposure Precautions.

1. Thyroid carcinoma treated with radioactive iodine.
2. Intracavitary injection of Radioactive Gold (Au 198).

NURSING CARE OF A PATIENT RECEIVING A RADIOISOTOPE

SECTION III

NURSING CARE OF CATEGORIES I THRU V

CLASS I: No Precautions.

1. No change in nursing care or nursing procedures are necessary.

CLASS II: Contamination Precautions Only.

1. Radioactive phosphorus (P32) presents no external radiation hazard to ward personnel. Therefore, there need be no restriction as to the patient's placement on the ward nor to the time spent in caring for the patient.

2. If there is leakage from the injection site the material presents a major radiation hazard.

   a. Do not attempt to clean up leakage or change linens.
   b. Notify radioisotope clinic immediately.

3. If the patient should expire do not remove any indwelling tubes, do not prepare for removal from ward. Notify Radioisotope Clinic. A clearance must be obtained from the Radioisotope physician before any steps may be taken to do anything other than close door of patients room.

CLASS III: Major Radiation Exposure Precautions only.

1. Contact Radiology Service for specific instructions on Nursing care of these patients.

CLASS IV: Contamination and Moderate Radiation Exposure Precautions.

1. The radioactive iodine with which the patient is treated is administered orally. The amounts given will not present a radiation hazard unless the patient vomits within the first 24-hours after treatment. If this should happen:

   a. Notify isotope clinic immediately.
   b. Do not attempt to clean up vomitus, do not handle any material which has vomitus on it.
   c. If patient vomits on personnel they should:

(1) Remove contaminated clothing and place in plastic bag for further handling by radioisotope personnel.
(2) Wash contaminated exposed skin areas with mild soap and water. DO NOT SCRUB WITH BRUSH.
DO NOT IRRITATE SKIN.

2. There will be no restriction as to placement of patient on ward.

3. The urine will be considered as a moderate radiation hazard. If any urine is spilled do not attempt to clean it up - notify the isotope clinic. If the patient is confined to bed a bedpan for the use of that patient only should be kept in the room and handled with gloves. Discarded gloves should be kept in a bag in the patients area to be disposed of by the isotope personnel.

4. Should the patient who received treatment expire the patient is not to be prepared for removal from the Ward without clearance from the Radioisotope physician.

CLASS V: Contamination and Major Radiation Exposure Precautions.

1. Thyroid Carcinoma Treated with Radioactive Iodine.

a. The patient should be in a private room with a private toilet.

b. The pillow and mattress should be protected by plastic coverings.

c. Gloves and gowns worn by personnel and the linens used by the patient should be placed in a linen hamper, kept in the patient's room, for survey by radioisotope personnel.

d. No blood or urine specimens are to be sent to the laboratory without clearance from Radioisotope physician.

e. Nursing care will be under supervision of radioisotope clinic.

f. If patient vomits within first twenty-four hours after administration of radioactive iodine:

(1) Notify isotope clinic immediately.
(2) Do not attempt to clean up vomitus, do not handle any material which has vomitus on it.

(3) If patient vomits on personnel they should:
First: Remove contaminated clothing and place it in a plastic bag for further handling by radioisotope personnel.
Second: Wash contaminated exposed skin areas with mild soap and water. DO NOT SCRUB WITH BRUSH
DO NOT IRRITATE SKIN

g. Pocket dosimeters will be issued by the radioisotope clinic to personnel who will be caring for this class of patient. Dosimeters will be marked with the individuals name and will be worn during his or her entire duty tour. It will be left in the medicine cabinet when that person is off duty. It will not be worn by anyone except the person to whom it is issued. Radiation exposure record will be placed in 201 file of personnel when applicable.

h. Pregnant personnel will not be assigned to care for Class V patients.

i. No visitors will be allowed in the patients room until clearance is given by the radioisotope clinic.

j. Dishes are not considered contaminated unless patient vomits on them.

k. If patient is incontinent special instructions will be issued as to nursing care by the radioisotope physician.

l. Thermometer and such other equipment used by the patient should be used only by that patient and when not in use should be kept in the Radiological isolation area.

m. Urine will be considered a major radiation hazard. Gloves will be worn when handling the bedpan. In the event that urine is spilled notify the isotope clinic immediately. Do not attempt to clean up the spill.

n. Should the patient expire notify the Isotope Clinic immediately. No preparation of patient for removal from ward may be made without clearance from the radioisotope physician.

o. The patient will receive radiation clearance from the Radiation Safety Officer of the hospital when the danger no longer exists. Until Radiation Safety Clearance is given, the Isotope Clinic personnel will be available at all times to be of assistance.

(1) During duty hours call: 25259
22133

(2) During off duty hours the Information Desk at the Hospital can give you the home telephone number of the person who is "on call" for the radioisotope clinic.

2. Intracavitary Injection of Radioactive Gold:

a. No contamination precautions are necessary unless there is leakage from the injection site. In this event

(1) Notify radioisotope clinic immediately.
(2) Do not attempt to clean up leakage or change linens.

b. Patient should be in a private room but does not need a private toilet.

c. Mattress and pillow should be protected with a plastic cover.

d. Urine and blood specimens may be sent to the laboratory as ordered.

e. Pocket dosimeters will be issued by radioisotope clinic to personnel who will be caring for this class of patient. Dosimeters will be marked with individual's name and will be worn during his or her entire duty tour. It will be left in the medicine cabinet when that person is off duty. It will not be worn by anyone except the person to whom it is issued. Radiation exposure record will be placed in 201 file of personnel when applicable.

f. Pregnant personnel will not be assigned to care for a Class VI, patient.

g. No visitors will be permitted in the patient's room until clearance is given by the Isotope Clinic.

h. Body wastes will not be considered as radioactively contaminated.

i. Thermometer and bedpan isolation is not necessary.

j. Patient will be monitored daily by Radioisotope Clinic personnel.

k. Should a Class VI patient expire notify the Radioisotope Clinic immediately. Do not prepare patient for removal from ward without clearance from the Radioisotope physician. Do not remove any indwelling tubes or catheters.

NURSING CARE OF A PATIENT RECEIVING A RADIOISOTOPE, Cont'd . . .

l. Nursing care of a Class VI patient will be under the supervision of the Radioisotope Clinic.

m. The patient will receive radiation clearance from the Radiation Safety Officer of the hospital when the danger no longer exists. Until Radiation Safety Clearance is given the Isotope Clinic personnel will be available at all times to be of assistance.

(1) During duty hours call: 25259
22133

(2) During off duty hours the Information Desk at the Hospital can give you the home telephone number of the person who is "on call" for the radioisotope clinic.

DAVID P. PRESTON
Major, MC
Chief, Radioisotope Section

APPENDIX VII PART IV

U. S. ARMY MEDICAL RESEARCH AND NUTRITION LABORATORY
FITZSIMONS GENERAL HOSPITAL
DENVER, COLORADO

PROCEDURES FOR USE OF RADIOACTIVE MATERIAL

Radioactive Material SOP, USAMRNL

References:

Title 10 C. F. R., Part 20
AR 40-14, 40-37, 40-440, 55-55, 70-25, 385-30, 711-16, 755-15, 700-52, 700-63, 700-64.

National Bureau of Standards Handbooks

No. 48 Control and Removal of Radioactive Contamination in Laboratories
No. 49 Recommendations for Waste Disposal of Phosphorus-32 and Iodine-131 for Medical Users
No. 51 Radiological Monitoring Methods and Instruments
No. 53 Recommendations for the Disposal of Carbon-14 Wastes
No. 59 Permissible Dose from External Sources of Radiation
No. 65 Safe Handling of Bodies Containing Radioactive Isotopes
No. 69 Maximum Permissible Body Burdens and Maximum Permissible Concentrations of Radionuclides in Air and in Water for Occupational Exposure
No. 78 Report of the International Commission Radiological Units and Measurements, 1959.
No. 80 A Manual of Radioactive Procedures

SECTION I

GENERAL

1. The purpose of this memorandum is to insure the safe handling of all radioactive materials within the United States Army Medical Research and Nutrition Laboratory.

2. The Radiation Safety Officer of Fitzsimons General Hospital and U. S. Army Medical Research and Nutrition Laboratory (hereafter designated only "Radiation Safety Officer") shall have the responsibility for the enforcement of all phases of radiation safety within USAMRNL.

3. These procedures are published as a guide and must not be construed to be an amendment or change to any existing federal regulation, Army regulation, or local regulation governing the use of radioactive material.

SECTION II

RADIOISOTOPE COMMITTEE

4. The U.S. Army Medical Research and Nutrition Laboratory operates jointly with Fitzsimons General Hospital under the same General Atomic Energy Commission License. Use of radioisotopes, within the limitations of the AEC License, is controlled by a Radioisotope Committee consisting of Fitzsimons General Hospital personnel as well as U.S. Army Medical Research and Nutrition Laboratory personnel. The persons making up the Radioisotope Committee and the functions of the committee are outlined in AR 40-37. The committee will be responsible for proper handling, storage and disposal of radioactive materials. In addition, the committee will:

a. Recommend changes to the SOP concerning periodic monitoring and enforcement of safety measures in the handling of radioactive material.

b. Review and grant permission for, or disapproval of, the use of radioactive material.

c. Certify individual users for each type of procedure with each individual radioisotope and insure that a copy of such certification is placed in the appropriate users' 201 file. Current records of these approved users, documenting the qualifications and limitations of each, will be maintained.

d. Prescribe special conditions which may be necessary to include and give advice concerning proposed studies where it is needed.

e. Review records and receive reports from the Radiological Protection Officer and recommend corrective action when indicated.

f. Make recommendations for improvement of present laboratory facilities and for expansion of the laboratories in accordance with needs.

g. Hold meetings at the call of the Chairman and report in writing to the Commanding Officer, the results of its deliberation.

SECTION III

HAZARD CONTROL

5. The Chief of the Radioisotope Branch, USAMRNL, shall instruct, direct, and supervise all individuals at USAMRNL working with or near radioactive materials in the observance of radiological safety.

6. Each individual working within or near radioactive material (or any employee who will possibly be exposed to ionizing radiation) will be issued a film badge. Before a film badge will be issued, each individual must read both CFR, Title 10, Part 20, and the laboratory SOP, and certify in writing that he has read and understands both.

7. Permission to handle, administer, or assist in the administration of radioactive materials under AEC General License in USAMRNL may be granted only by the Radioisotope Committee. This permission may be denied or withdrawn from any person who, in the opinion of the Radioisotope Committee or on the advice of the Radiation Safety Officer, is inadequately trained in the handling and use of radioactive materials, or is guilty of any breach of discipline as concerns the handling and use of radioactive materials so as to incur real or possible hazard to himself or others.

8. The safety rules listed hereinafter are to be observed. However, it is emphasized that mere following of the rules will not eliminate all possible hazards associated with the handling of radioactive materials.

9. The protection rules are based upon assumed long-term whole-body exposure to ionizing radiation by personnel whose duties involve regular handling of radioactive material or regular use of x-ray equipment. These rules apply to all persons occupationally employed using any source of ionizing radiation in a controlled area or those incidentally exposed as a result of such use, under any condition. A controlled area is one in which the occupational exposure of personnel to radiation or to radioactive material is under the supervision of a Radiation Protection Officer. (This implies that a controlled area is one that requires control of access, occupancy and working condition for radiation protection purposes.)

SECTION IV

PROCUREMENT, STORAGE AND ADMINISTRATION

10. All radioactive materials for use in USAMRNL will be procured through the Radioisotope Branch by the Supply Officer, USAMRNL.

11. The Supply Officer, USAMRNL, will be responsible for the storage and handling of the contents of each shipment of radioactive material until such time as the shipment is delivered to the Chief, Radioisotope Branch, who will be responsible for the maintenance of records pertaining thereto.

12. The Radiation Safety Officer will direct the storage and handling of the contents of each shipment of radioactive material after it has been delivered to him or his designated representative in the Radioisotope Laboratory, and will be responsible for the maintenance of the records pertaining thereto.

13. The storage area will be neat and segregated by type emission. Gamma emitting isotopes will be stored so that the radiation level at the edge of the storage area does not exceed 2 mr/hr.

14. The Radiation Safety Officer will be responsible for the handling and disposal of radioisotope-contaminated liquid and solid wastes of the Radioisotope Laboratory in accordance with the recommended procedures found in Part 20, Title 10 and Army regulations concerning such matters.

SECTION V

SAFETY RULES

15. In order to avoid undue exposure to ionizing radiation, unauthorized personnel will not enter the Laboratory of the Radioisotope Branch except when accompanied by an authorized person.

16. Only persons specifically authorized to do so by the Radioisotope Committee will handle any shipment of radioactive material or any part thereof after it has been delivered to the Radioisotope Branch.

17. Only persons specifically authorized to do so by the Radioisotope Committee and/or under the supervision of the Radiation Safety Officer will prepare or administer a dose of any radioactive material after it has been delivered to the Radioisotope Laboratory.

18. In all rooms where radioactive materials are being used, the following regulations shall be in effect:

a. There will be no eating or drinking, and no application of cosmetics.

b. Smoking is not permitted while active material is being handled.

c. There will be absolutely no mouth pipetting of radioactive material in the laboratory under any circumstances.

d. Under no circumstances will radioactive waste be handled or disposed of by the janitorial staff.

e. Rubber gloves will be worn at all times when radioactive material is being handled. (Except sealed, or capped containers of radioactive materials.)

f. All gloves, protective clothing, instruments, and glassware will be checked for radioactive-contamination with a laboratory monitor after using, and, if contaminated, will be placed in the appropriate receptacle to await decontamination.

g. All contaminated glassware, instruments, pipettes, and waste incurred in any radioisotope experiment or study will be policed and placed in an appropriate receptacle or sink by the persons performing the experiment or study.

h. At the end of each work period the hands shall be carefully washed and tested for contamination with an instrument of suitable sensitivity.

i. Before placing radioactive material in any container, the container will be clearly labeled with radioactive caution tape of yellow and magenta to show the particular radioactive material, the concentration in microcuries or millicuries per unit volume weight as of some particular date, and the identifying initials of the person preparing the material.

j. Work surfaces will be covered with absorbent paper. The work in hoods will be similarly performed with absorbent paper. The work bench will be equipped with wiping papers for the prompt removal of spills.

k. When using radioactive material, special equipment suitable for the type and level of activity being used will be used for each type of operation. This will include handling tools such as tongs, forceps, trays, and mechanical holders. When the isotopes concerned are primarily beta emitters, efficient use can be made of transparent plastic shields. Containers for liquid samples will be reinforced by an outer unbreakable container.

l. No individual shall knowingly expose himself, or cause others to be exposed, to more than 0.02 rem in any working day.

m. All laboratory operations with more than low level activity will be conducted in hoods.

19. The sinks in the laboratory portion of the Radioisotope Laboratory will not be used for purposes of performing personal toilets, except that the non-contaminated sinks may be used for the purpose of hand washing after the removal of rubber gloves.

20. No water for drinking purposes will be obtained from the laboratory portion of the Radioisotope Branch.

SECTION VI

RADIOACTIVE WASTE

21. The Radiation Safety Officer is responsible for the disposal of all radioactive waste within USAMRNL. Such disposal shall be accomplished under all existing regulations listed in Part 20, Title 10, NBS Handbooks, and Army Regulations.

22. For persons other than Radioisotope personnel:

a. Solid radioactive waste shall be placed in waterproof disposable containers and deposited in the container marked with a Radiation Caution symbol and wording "Danger Radioactive Material." The radiation level outside the receptacle should not exceed 1.0 milliroentgens per hour. When full, the bag will be labeled as to content, isotope present, approximate amount of microcuries (or millicuries) and the date. These waste bags will then be collected by personnel of the Radioisotope Branch.

b. All liquid wastes shall be placed in appropriate containers and marked with radioactive caution tape as to isotope content, approximate amounts (in microcuries or millicuries), and the date of collection. This contaminated liquid waste will then be delivered to the Radioisotope Laboratory for disposition.

c. Carcasses of animals containing radioactive material will be marked with radioactive caution tape and delivered to the Radioisotope Laboratory in a container properly marked as to date, isotope content, and approximate amounts in microcuries or millicuries.

d. Fecal material containing radioactive material similarly will be marked with radioactive caution tape, marked as to date, isotopic content, and approximate activity and delivered to the Radioisotope Laboratory.

23. Radioisotope Personnel:

RADIOACTIVE WASTE WILL BE DISPOSED OF ONLY BY PERSONNEL OF THE RADIOISOTOPE LABORATORY

Liquid:

a. Liquids containing short lived radioisotopes will be held in storage until the activity is essentially background. (The material will be stored in such a way that the radiation level outside the storage area will not exceed 1.0 milliroentgen per hour.)

b. All contaminated liquid waste may be disposed of in the "hot" sink provided the quantity which, if diluted by the average daily quantity of sewage (sanitary sewage flow per 24 hours is 525,000 gallons) released into the sewer by the licensee, will not result in an average concentration in excess of values specified in Appendix B, Table I, Column 2 of CFR, Title 10, Part 20; or

c. Ten times the quantity of such material specified in Appendix C of same; and

d. The quantity of any licensed or other radioactive material released in any one month, if diluted by the average monthly quantity of water released by the licensee, will not result in an average concentration exceeding the limits specified in Appendix B, Table I, Column 2 of same; and

a. The gross quantity of licensed and other radioactive material released into the sewage system by the licensee does not exceed one curie per year.

b. All liquid wastes which are held for decay must be placed in appropriate containers and marked as to isotope content, approximate amounts, and the date of collection. The radiation level outside the storage area will not exceed 1.0 milliroentgens per hour.

c. Solid radioactive waste shall be stored in such a way that the radiation level outside the storage area will not exceed 1.0 milliroentgens per hour.

d. All clothing that is known or suspected of being contaminated with a short half-life radioactive isotope or long half-life isotope will be placed in suitable containers and later destroyed or decontaminated as determined by the Radiation Safety Officer.

e. Disposal of solid radioactive waste will be carried out under the direction of:

Radioactive Material Disposal Facility
U.S. Army Edgewood Arsenal
Edgewood Arsenal, Maryland 21010

Under no circumstances will waste be incinerated.

SECTION VII

DECONTAMINATION OF GLASSWARE

32. All glassware which is utilized directly with radioactive material shall be deemed "contaminated." The decontamination of such glassware is important not only in the interests of radiation safety but also in the minimization of additional experimental data.

33. Contaminated glassware will be placed in a "hot" sink where it will be immediately rinsed and then undergo continuous washing. As required, the glassware will be washed in detergent and rinsed in hot water. The wash and rinse cycle will be repeated until three washings have been completed. Washed glassware may be oven or air dried.

26. Pipettes will be rinsed immediately after use and placed in a pipette soaker containing detergent. Washing shall be done by continuous washing in a pipette washer for a minimum of two hours.

27. Syringes shall be disassembled when placed in the sink. As required, syringes will be washed, dried, and monitored before return to central material or put into reuse. When possible the use of disposable syringes and needles is suggested.

28. All glassware which has been decontaminated from gamma radiation will be monitored by an appropriate detector of suitable sensitivity to prevent recontamination during another course of study or experiment. Always monitor after drying, never wet. Decontaminated glassware for low energy beta radiation will be periodically spot checked by the Radioisotope Branch.

29. All glassware which upon monitoring proves to be still contaminated will immediately be placed back into the appropriate washing cycle. If the monitoring again indicates any level of radioactivity, the glassware shall be delivered to the Radioisotope Laboratory for further decontamination.

SECTION VIII

RADIOACTIVE SPILL

30. All radioactive material, when spilled, constitutes a hazard, either to personnel or to equipment. If a spill of radioactive material occurs in Group I (Appendix No. 1) turn off all fans in the immediate area and notify all other personnel in the controlled area. If the spill is liquid, drop absorbent paper on the spill and mark off the area with chalk or cord. If the spill is dry, proceed in the same manner, but convert the dry spill to liquid spill by applying wet absorbent paper over the area.

31. If a spill of radioactive material occurs in Group II (Appendix No. 1), hazard control is of first importance. In order to accomplish this, the person responsible for the spill will:

a. Notify the Radiation Safety Officer or his designated representative.

b. Be prepared to evaluate the hazard by knowing at all times which radioisotope is being handled, its chemical form, and the approximate amount being used (in millicuries or microcuries).

c. See that all personnel in the area are notified and that they leave the immediate area of the spill without delay.

32. In the event of a spill of radioactive material in Group III (Appendix No. 1), the procedure listed above in "a", "b", and "c" should be carried out, plus the following:

a. Determine the extent of personal contamination by inspection and monitoring of the involved personnel.

b. Remove contaminated clothing.

c. Rinse the contaminated body parts with water, if applicable (making use of the sinks located in the area or the emergency shower if the spill took place in the high level room of the Radioisotope Laboratory), and then wash with soap and water, monitoring the contaminated body part after each washing.

33. Decontamination of the area of the spill will be carried out under the supervision of the Radiation Safety Officer, but only after the personnel contamination problem has been disposed of. As a general rule, the work associated with the decontamination is performed by the person responsible for the spill.

34. If ingestion or inhalation is suspected from a spill of radioactive material, AR 40-582 will be complied with, and the following will be accomplished by the Radiation Safety Officer:

a. Evacuate the area of the original contamination.

b. Personal decontamination will be carried out by washing external parts to prevent additional exposure or ingestion.

c. Decontaminate the film badge (when necessary) and forward it by Air Mail Special Delivery to the Lexington Signal Depot; Lexington, Kentucky, with all data concerning the incident (i. e., isotope and its chemical form, amount ingested, date, names, etc.).

d. Carry out all routine decontamination of clothing, work spaces, etc., which were involved.

e. Notify the Surgeon General, Department of the Army, Washington D. C., ATTN: MEDCE-OH, by telegram, of possible internal exposure. Complete DA Form 285 (Accident Report).

f. In the event a very dangerous radioisotope is involved such as H^3, Ca^{45}, Fe^{55}, Sr^{90}, Y^{91}, Zr^{95}, Ce^{144}, Pm^{147}, or Bi^{210} (refer to

AR 40-582), immediately notify the Surgeon General, Department of the Army, Preventive Medicine Division by telephone of:

(1) Time and date of incident
(2) Millicurie strength of isotope and its chemical form.
(3) Name of individual and treatment already undertaken. Include a statement indicating the treatment rendered (or that no treatment has been rendered).
(4) Extent of individual contamination as determined by immediate monitoring.

Telephone notifications will be confirmed by telegraphic notifications.

g. A 24-hour urine sample will be collected under the direction of the Radiation Safety Officer from the person concerned. The collection shall be in a polyethylene liter bottle which will have a card attached containing the following data:

From:

(1) Name, grade and serial number
(2) Date of incident
(3) Inclusive dates of collection
(4) Isotope and chemical form

Reverse:

A 24-hour urine sample will be collected as follows:

(1) Wash hands before collecting a portion of sample.
(2) Void urine at 0800 hrs (or any other convenient time) and discard it. Do not collect it in the bottle.
(3) Collect all urine from that time up to and including the corresponding hour the following day. ALL URINE MUST BE COLLECTED. LOSS OF A SIGNIFICANT AMOUNT MAY RENDER THE SAMPLE USELESS.

h. Samples will be held until further instructions are received from the Surgeon General. If so directed, forward:

THRU: Commanding General
Walter Reed Army Medical Center
Washington, D. C.

TO: Director
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
Washington, D. C.

ATTN: Department of Biophysics

i. If an overexposure to ionizing radiation occurs, DD Form 1141 (Report of Exposure to Ionizing Radiation) must be completed in accordance with AR 40-431. A brief description of the condition or act which resulted in the overexposure will be attached to the DD Form 1141.

SECTION IX

RADIATION SAFETY MONITORING

35. Area Monitoring:

a. Routine monitoring will be accomplished according to the following time schedule:

Weekly

(1) Radioisotope Laboratory (according to diagram in Appendix 2).

Monthly

(1) USAMRNL (According to diagrams in Appendix 2).

b. Other areas will be monitored when deemed necessary by the Radiation Safety Officer.

c. Readings obtained during the surveys will be recorded and retained as a permanent record.

d. Routine monitoring in USAMRNL (including blowers on roof of Radioisotope Laboratory) will be done, using a portable PAC3G gas proportional counter with a beta detection probe and a GM counter. If contamination is detected, the area will be immediately decontaminated. If any gamma reading, with the GM counter, exceeds a value of 2.0 milliroentgens per hour, the Radiation Safety Officer will be notified. The area will be marked as to reading in milliroentgens/hour and the working time limit.

e. Swipe tests will be routinely conducted and when contamination is suspected. The swipes will be counted in the liquid scintillation counter. Any activity above background will be considered a contaminated area. Readings obtained will be recorded and retained as a permanent record.

f. Any areas of previously undetected contamination will be promptly removed by those persons responsible for the contamination, under the supervision of the Radiation Safety Officer.

36. Personnel Monitoring:

a. Film badges are provided for persons working with radioactive material in USAMRNL. These film badges will be worn during normal working hours and are not to be removed from USAMRNL. Care of the film badge will be the responsibility of the individual user.

b. Badges will be collected monthly by the Radioisotope Laboratory personnel. The collected badges will be sent to Lexington Signal Depot, Lexington, Kentucky for processing and reading. The returned values will be permanently recorded in Radioisotope Branch files on AEC Form Nos. 4 and 5 and DD Form 1141.

c. A thorough medical examination should be made of each individual potentially exposed to significant amounts of radiation before employment and annually ther after.

d. Those persons working with millicurie amounts of Tritium shall have periodic urine checks for radioactivity.

SECTION X

LOGS AND RECORDS

37. AEC Form 3 (Notice to Employees - Standards for Protection

Against Radiation) will be posted in a conspicuous location.

38. AEC Forms 4 and 5 (History of Exposure and Record of Exposure to Ionizing Radiation) will be kept. This record will also be entered on DD Form 1141 in accordance with AR 40-431.

39. The USAMRNL SOP will be posted and the AEC licenses will be readily available.

40. Radioisotope inventory balance will be determined monthly. (Radioisotope inventory records are kept on Forms DA 8-235 and DA 8-212).

41. Instrument logs will be maintained indicating calibration and maintenance of the portable survey instruments.

42. Records of surveys (including wipe tests) will be kept.

43. Caution signs, labels, and signals will be utilized according to CFR, Title 10, Part 20, para. 20.203.

44. A report covering the period of each calender quarter will be prepared by the Commander of Fitzsimons General Hospital in accordance with AR 40-37. This report will be dispatched to the Surgeon General, ATTN: MEDPS-PO, by the fifteenth working day following the close of the report period and will contain the following information as a minimum:

a. Copy of minutes of each Radioisotope Committee meeting, including a record of all actions taken by the Committee.

b. Copy of the training and experience of each newly approved user of radioisotopes or any change in qualifications or certifications of previously approved user (for human use, AEC Form 313a, page 3).

c. Radioisotope inventory, including data on quantities of radioisotopes procured, used, or disposed of, or currently in storage.

d. List of procedures with dosage for each radioisotope used in humans during the reporting period.

e. Information on unsolved problems, new or improved developments, or other comments of interest to, or having a bearing on, support rendered by the Surgeon General.

f. Notification of all changes in membership of Radioisotope Committee.

SECTION XI

OTHER ROUTINE LABORATORY PROCEDURES

45. Neatness in the laboratory is a prime requisite for elimination of the spread of contamination. The work area should be free of equipment and materials not required for the experiment at hand, and equipment used will be decontaminated and stored in a controlled location after use.

46. Floors in the Radioisotope Laboratory should be cleaned frequently by wet mopping. Brooms and mops will not be transferred to other area unless they are free from radioactive contamination.

47. Table tops, equipment, or any surface within the Radioisotope Laboratory will be kept clean. Under no circumstances will there be an accumulation of dust and/or possible contamination.

48. Floors will be waxed and buffed on a monthly basis.

49. Air condi' ner filters, glove box filters, and hood filters will be checked quarterly and properly cleaned or replaced when necessary.

50. Desiccant in the liquid scintillation counter will be checked weekly and changed when necessary.

51. The emergency shower will be checked weekly.

52. The survey meters will be calibrated at least every six months and after every maintenance procedure or battery change.

53. Batteries in the survey meters will be checked monthly, and (anged when necessary.

Safe Handling Level For Some Representative Radioisotopes
Authorized For Use In USAMRNL

GROUP I **No special handling required in normal laboratory procedures		GROUP II **Not dangerous, but unnecessary exposure is to be avoided		GROUP III **Dangerous, should be handled with utmost caution		
Isotope	Maximum Amount	Isotope	Maximum Amount	Isotope		Amount
Au^{198}	0.025mc	Au^{198}	1.000mc	Au^{198}	over	1.000mc
Br^{82}	0.300mc	Br^{82}	5.000mc	Br^{82}	"	5.000mc
Be^{7}	0.005mc	Be^{7}	0.100mc	Be^{7}	"	0.100mc
*C^{14} Urea	0.050mc	C^{14} Urea	1.000mc	C^{14} Urea	"	1.000mc
*C^{14} all other	0.025mc	C^{14} all other	1.000mc	C^{14} other	"	1.000mc
Ca^{45}	0.005mc	Ca^{45}	0.100mc	Ca^{45}	"	0.100mc
Co^{60}	0.025mc	Co^{60}	1.000mc	Co^{60}	"	1.000mc
Cr^{51}	0.025mc	Cr^{51}	1.000mc	Cr^{51}	"	1.000mc
Fe^{55}	0.005mc	Fe^{55}	0.100mc	Fe^{55}	"	0.100mc
Fe^{59}	0.025mc	Fe^{59}	1.000mc	Fe^{59}	"	1.000mc
*H^{3} Water	0.025mc	H^{3} Water	10.000mc	H^{3} Water	"	10.000mc
*H^{3} Thymidine	0.001mc	H^{3} Thymidine	0.050mc	H^{3} Thymidine	"	0.050mc
*H^{3} all other	0.005mc	H^{3} all other	0.100mc	H^{3} other	"	0.100mc
I^{131}	0.025mc	I^{131}	1.000mc	I^{131}	"	1.000mc
Na^{22}	0.025mc	Na^{22}	1.000mc	Na^{22}	"	1.000mc
P^{32}	0.025mc	P^{32}	1.000mc	P^{32}	"	1.000mc
S^{35}	0.025mc	S^{35}	1.000mc	S^{35}	"	1.000mc
Se^{75}	0.025mc	Se^{75}	1.000mc	Se^{75}	"	1.000mc
Sr^{85}	0.025mc	Sr^{85}	1.000mc	Sr^{85}	"	1.000mc
Sr^{89}	0.025mc	Sr^{89}	1.000mc	Sr^{89}	"	1.000mc
Sr^{90}	0.005mc	Sr^{90}	0.100mc	Sr^{90}	"	0.100mc
Zn^{65}	0.005mc	Zn^{65}	0.100mc	Zn^{65}	"	0.100mc

* Group classification dependent upon chemical form.

**It must be remembered that these limits are by no means fixed and that any undue exposure is undesirable. Therefore, when working with the above radioisotopes, the physical characteristics, half-life, the internal and external hazard, and the radiative properties of the radioactive material must be considered. If in doubt, always consult the Chief, Radioisotope Branch.

APPENDIX VII PART V

RADIOACTIVE WASTE TREATMENT AT THE U.S. ARMY MEDICAL RESEARCH AND NUTRITION LABORATORY

I. SOURCES: Biological and metabolic experiments using primarily animals and occasionally humans.

II. COLLECTION:

A. Responsibility for collecting waste, rests with each investigator.

B. Methods for the collection of radioactive waste are the following:

1. Each sector dealing with radioactive material has radioactive waste receptacles with waxed bag inserts for collection of waste material. These are collected periodically and placed in polyethylene bags.

2. Solids are placed in polyethylene bags.

3. Liquids are sealed in glass bottles.

4. Animal carcasses and tissues are put in polyethylene bags and frozen immediately.

5. All radioactive waste is brought to the Radioisotope Section for disposal. Each package is labeled as follows:

a. Isotope

b. Activity in microcuries

c. Material, e.g. glass, paper

d. Date

e. Investigator's name

6. Also, each package has affixed to it, radioactive tape with the wa..ing "Caution Radioactive Material."

C. Local handling is carried out in the following manner:

1. Non-combustible and combustible waste is segregated into fifty-five gallon drums and sealed. These are stored in the outside freezer located north of Building 602, West Wing.

2. Animal carcasses and tissues are sealed in fifty-five gallon drums and stored in the outside freezer.

3. Animal carcasses from studies conducted in the Small Animal Room may be stored in polyethylene bags in the Small Animal Room freezer. When gamma-emitters are used, the freezer is monitored once a week; otherwise, monitoring is performed once a month.

4. Edgewood Arsenal is notified that there is waste to be disposed of with a listing of the approximate amount of activity and the type of waste material that has accumulated.

5. A disposition date is obtained from Edgewood Arsenal.

6. If an escort from Edgewood Arsenal is not available to pick up the waste then the Radioisotope Section arranges with the Transportation Office to provide local carriers to ship the waste.

III. PACKAGING

A. Wastes are sorted on the basis of:

1. Combustibility vs. non-combustibility of the material involved.

2. The type of isotope and the activity contained within the package.

B. Packaging practices followed are:

1. Solids are placed in two polyethylene bags, one enclosed in the other.

2. Liquids are stored in large glass bottles until disposition. After sewage disposal glass containers are treated as non-combustible radioactive waste.

C. Shielding is carried out as follows:

1. No special shielding for beta-emitters up to the millicurie range is required.

2. Storing gamma-emitters in the outside freezer provides shielding and distance for laboratory personnel.

3. The high-level non-perishables are stored behind lead bricks in a room that is separated from the main working area.

D. The only local waste treatment performed is the disposal of liquid waste into the sanitary sewage system.

1. Where the amounts of the isotope to be disposed are less than the amount allowed in paragraph 20.303 and Appendix C of Title 10 of AEC Regulations, then the radioactive waste may be flushed into the sewage system with copious amounts of water.

2. The disposal is scheduled so that the sewage from USAMRNL, as well as that of the Radioisotope Clinic, FGH, will not exceed the total amount allowed. This is accomplished by scheduling specific days for each laboratory to dispose of its waste.

3. All liquid disposal at USAMRNL is accomplished in the sink located in the High Level Laboratory.

E. Solid wastes are disposed of by packaging in fifty-five gallon drums sealed air tight, and stored in the outside freezer until enough waste is collected to justify a shipment.

F. The procedures of the U.S. Army Nuclear Defense Laboratories are followed to facilitate interstate transportation by a local carrier to Edgewood Arsenal.

IV. STORAGE

A. Storage is provided by an outside freezer maintained at temperatures below 0° F and by a lead-lined hood in the High Level Laboratory. Both places are adequately sealed and are designated as "Radiation Areas."

B. The combustible and non-combustible beta-emitters and low activity gamma-emitters are stored in the outside freezer.

C. In the event that high activity wastes should occur, then the perishables, e.g. animal carcasses, are stored in the outside freezer, while the non-perishables are stored in the High Level Laboratory.

D. Local storage space is sufficient to accommodate approximately

fifteen fifty-five gallon drums. This amount may be collected during a three-month period prior to shipment.

V. SHIPPING

A. Ten to fifteen fifty-five gallon drums are shipped approximately every three months.

B. The waste is shipped via refrigerated trucks obtained from Edgewood Arsenal or by local carrier.

C. Release through local environment is accomplished only by discharging liquid waste as described under "Packaging." There is no incineration or burial of waste materials.

VI. MONITORING

A. Drums are monitored to provide readings at the surface of the drums.

B. Swipe tests are performed on the surface of the drums to ensure that no incidental contamination has occurred.

C. The following safeguards are taken:

1. Animals stored in the freezer in the Small Animal Room are monitored weekly if gamma-emitters are present; monthly, if only beta-emitters are present.

2. The outside freezer is monitored weekly.

3. High level material stored in the High Level Laboratory is monitored weekly.

VII. AUTHORITY

A. U.S. Atomic Energy Commission Byproduct Material License No. 5-46-13.

B. Atomic Energy Commission Rules and Regulations, Title 10, Part 20.

C. Army Regulation No. 55-55.

D. Title 49, Parts 71-78, Code of Federal Regulations, Transportation

E. Army Regulation No. 755-15.

F. Procedures for Use of Radioactive Material, USAMRNL, Fitzsimons General Hospital.

APPENDIX VIII

This appendix includes copies of the protocols for the studies utilizing radioisotopes in human volunteers. Part A represents the revised protocol which was submitted in November 1966, entitled "Request for Approval for Human Use of Radioisotopes in Tracer Amounts in Volunteer Experimental Research Subjects." Part B includes excerpts from the annual progress reports for fiscal year 1967 and 1968, and other reports which represent the work completed to date.

Section C is the protocol submitted in July of 1967, entitled "Endocrine Effects fo Altitude." The study is a long way from being completed, but the portion of the study involving administration of radioisotopes to human volunteers has been completed. For this reason, renewal of this portion of the license has not been requested. A summary of the progress to date is included as Section D.

Section E is the protocol submitted in April of 1967 entitled "Effects of Selective Coronary Arteriography on Myocardial Blood Flow in Man." A report of the progress to date is attached as Section F.

APPENDIX VIII PART A

REQUEST FOR APPROVAL FOR HUMAN USE OF RADIOISOTOPES IN TRACER AMOUNTS IN VOLUNTEER EXPERIMENTAL RESEARCH SUBJECTS

Submitted by:

U. S. Army Medical Research and Nutrition Laboratory
Denver, Colorado

J. C. Syner, M.D., Col., MC; Commanding Officer and Director
R. H. Herman, M.D., Ph.D., Lt. Col., MC; Chief, Metabolic Division
H. E. Sauberlich, Ph.D.; Chief, Chemistry Division
C. G. Liddle, D.V.M., Capt., VC; Chief, Radioisotope Branch Physiology Division
E. M. Baker, Ph.D., Lt. Col., MSC; Assistant Chief, Chemistry Division
Z. Z. Ziporin, Ph.D.; Assistant Chief, Chemistry Division
D. Zakim, M.D., Capt., MC; Metabolic Division
J. W. Anderson, M.D., Capt., MC; Metabolic Division
R. P. Carson, M.D., Capt., MC; Physiology Division
A. H. Janoski, M.D., Capt., MC; Physiology Division
B. M. Tolbert, Ph.D., Professor of Chemistry, University of Colorado and Consultant to USAMRNL
J. E. Canham, M.D., Lt. Col., MC; Consultant and former Commanding Officer and Director of USAMRNL

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Section I. General Introduction

1. Purpose of request

a. Par. 3b(3), AR 40-37, 'Radioisotope License Program (Human Use),' dated 12 August 1963, requires that written approval be obtained from the Secretary of the Army prior to the submission of license application (through channels to AEC) for human use (of radioisotopes), when volunteers are to be used as experimental research subjects. This paragraph (Par. 3b(3), AR 40-37) cites AR 70-25, 'Research and Development: Use of Volunteers as Subjects of Research,' dated 26 March 1962 as the basis for the requirement.

b. AR 70-25 prescribes policies and procedures governing the use of volunteers as subjects, including research in nuclear, biological and chemical warfare, wherein human beings are deliberately exposed to unusual or potentially hazardous conditions. Par. 6 of this AR requires approval of the Chief of Research and Development prior to the research and, in the case of nuclear, biological or chemical agents, approval of the Secretary of the Army is required.

c. The Atomic Energy Commission will license the use of tracer amounts of radioisotopes in physiological studies in normal human beings done by competent medical research scientists. Such licenses have been granted to members of this organization in the past.

d. To comply with the requirements of Par. 3b(3), AR 40-37 and Par. 6, AR 70-25, this request is submitted for approval for human use of stated radioisotopes in tracer amounts in volunteer experimental research subjects at U. S. Army Medical Research and Nutrition Laboratory and Fitzsimons General Hospital and in field studies conducted by USAMRNL.

2. Scope of request

a. Experiments included in this request are not, in and of themselves, unusual or potentially hazardous under the definitions of AR 70-25. They would be considered potentially hazardous (and minimally so) only to the extent that radioactive isotopes in tracer quantiti are used.

b. Therefore, this request seeks approval only for use of the specified radioisotop , the experiments otherwise not requiring individual approval under AR 70-25. However, sufficient description is furnished to indicate importance of the studies in warranting use of radioisotopes.

c. For any studies later contemplated under the general description given in this request which would, in and of themselves (apart from the use of radioisotope tracers), constitute unusual or hazardous experiments, specific approval (directed to the non-isotope aspects) would then be requested per AR 70-25.

d. This request gives consideration to the health physics aspects of the radioisotope tracers required (Section II). Additional information as to health physics aspects was presented in the previous license amendment, including details as to research methods and approaches. Since no request has been made for the use of additional radioisotopes, these details will not be repeated.

3. General guidelines for requested studies

a. The administered radioactive material would, in no case, exceed a radiation dose high enough to approach the permissible dose indicated in CFR Title 10, Part 20, RC-12, "The Medical Use of Radioisotopes--Recommendations and Requirements by the Atomic Energy Commission." In fact, in no case will the dose exceed one-half that

of the permissible dose, and every attempt will be made to use even lesser amounts of isotope when compatible with obtaining reliable data. Acquisition of the most recently developed counting equipment enables minimal use of the isotopes.

b. All policies, procedures and regulations prescribed in AR 70-25 and AR 40-37 will be rigidly adhered to in all investigations.

c. The person in charge of each specific phase of the studies proposed herein will be formally designated prior to the research by the Commanding Officer, USAMRNL, from the Government scientists listed in this application, and the attending physician will similarly be designated from the Medical Officers among them. Medical officers are assigned a minimum of three years to USAMRNL. The staff of the Laboratory is approximately equally divided between military and civilian personnel.

4. History of USAMRNL isotope usage

a. This Laboratory has employed radioactive labeled compounds in studies with human subjects under AEC License Number 5-46-5 since 17 December 1957. Authorization was given initially to use ^{131}iodine-labeled human serum albumin to measure the turnover rate of albumin of ten normal young men in various nutritional states.

b. USAMRNL staff members have had experience in use of various radioisotopes in a number of chemical forms in collaborative clinical investigations with Fitzsimons General Hospital, involving the basic disease process or new treatment procedures. Such work has been carried out under the Fitzsimons General Hospital's AEC License 5-46-9 and has included the use of ^{131}iodine, ^{32}phosphorus, ^{51}chromium, ^{60}cobalt and ^{59}iron.

c. On 11 December 1959, authorization was granted in License No. 5-46-12(L 61) for the use of carbon-14 labeled glucose, glucuronic acid, glucuronolactone and ascorbic acid to measure the pool size and turnover rate of body ascorbic acid in normal human subjects and for investigation of the possibility that humans may be able to synthesize small amounts of ascorbic acid.

d. License No. 5-46-12(L 61) was renewed on 24 October 1961 and expanded to include carbon-14 labeled glycine, cholesterol, mevalonic acid, acetate and carbon monoxide, in addition to the compounds previously authorized, for use in metabolism and physiological tracer studies in humans.

e. License No. 5-46-12 (including prior approval by the Secretary of the Army) was amended to permit the use of tritiated water for the determination of total body water in 112 human volunteers at Ft. Carson, Colorado.

f. Until recently, nutrition and metabolism tracer studies in human volunteers were conducted under AEC Radioisotope License No. 5-46-13(A 66), Amendment No. 1. This amendment was in effect for a period of eighteen months (31 July 1964 through 31 January 1966). Studies conducted during this limited period are indicated in Section III. Such studies have been discontinued until authorization has been received.

5. Specific radioisotopes to be used

a. Use of the following radioisotopes in volunteer human research in tracer dosages is requested:

By-product Material	Chemical and/or Physical Form
Carbon-14	Vitamins
	Carbohydrates
	Amino acids
	Lipids
	Acetate
Hydrogen-3	Vitamins (vitamin C, folacin, vitamin B_6)
	Water
Magnesium-28	MgO, $MgCl_2$, Mg citrate
Calcium-47	$CaCl_2$
Calcium-45	$CaCl_2$

b. All the labeled compounds to be employed are naturally occurring nutrients or metabolites for the human.

c. Except for hydrogen-3, the maximum amount of radioactivity which the licensee requests permission to possess at any one time is 10 millicuries of the individual chemical forms indicated. For hydrogen-3, 50 millicuries are requested.

d. Authorization is requested to use tracer quantities of ^{14}C-labeled ascorbic acid and ^{3}H-labeled ascorbic acid in human volunteers at the University of Iowa Medical School, Iowa City, Iowa, in the study outlined in this document.

e. According to current plans, personnel of USAMRNL will be associated with studies in Iran and Thailand involving the use of the above indicated radioisotopes in human volunteer tracer investigations on nutrition problems.

Section II. General Health Physics for Requested Isotopes

6. Carbon-14

Carbon-14 has a soft beta emission that lends itself to tracer studies. Fat in the body is usually considered the critical organ. The biological half-life for carbon-14 in fat is given as 35 days. The National Bureau of Standards Handbook No. 69 lists the maximum permissible burden in fat as 300 mc. Constants for calculating maximum permissible internal concentration of radioisotopes assume that 50% of the carbon-14 that is present in the blood is transferred to the critical organ, fat. However, based on animals, it can also be assumed that few of the carbon-14 labeled compounds proposed to be used would approach this retention in the critical organ. The majority of the compounds proposed are readily metabolized and removed from the body as expired CO_2 or metabolites in the urine, and would reduce even further the body burden of irradiation. Flushing procedures could also be employed in the case of the labeled vitamins to hasten their removal from the body upon completion of the studies. In all investigations, balances will be performed that will permit careful knowledge of the extent of retention and turnover of the labeled compound administered.

7. Hydrogen-3

Hydrogen-3 emits only a very soft beta particle, but with present counting instruments is a very useful isotope for tracer studies. The entire body is generally considered the critical organ, and the isotope has a biological half-life of approximately 12 days. The maximum permissible body burden is 1-2 mc. This approximate amount has been used

routinely in numerous laboratories for the determination of total body water in the human. Previously, permission has been granted this Laboratory to use this technique utilizing one millicurie of tritiated water on volunteers at Ft. Carson.

The use of tritiated vitamins is proposed since several vitamins are available only as the tritiated compounds. Because of the considerably smaller pool size, the dosage of tritium employed as a vitamin will be much less than that employed in the measurement of total body water. Amounts less than 0.1 mc are anticipated. Tritiated folic acid and pyridoxine are presently employed at a number of laboratories for studying malabsorption syndromes in humans such as may be encountered in tropical sprue.

8. Magnesium-28

This isotope is available as a cyclotron produced element. It has a very short half-life of only 21 hours. Magnesium-28 has been used in a number of laboratories with humans. Dr. J. K. Aikawa, Department of Medicine, University of Colorado School of Medicine, Denver, has administered 90 μc of Mg-28 to normal subjects and patients and found essentially no activity in the urine or plasma after 40 hours. By this time, approximately 90% of the Mg-28 was accounted for in the feces and urine. (Peaceful Use of Atomic Energy, Vol. 24, p. 148, 1958; The Role of Magnesium in Biological Process. J. K. Aikawa, 1963, C. C. Thomas, Publisher, Springfield, Ill.)

9. Calcium-47

This isotope with a half-life of only 4.9 days has seen use in a number of studies with human subjects. The maximum permissible burden when the total body is considered the critical organ is approximately 10 μc; with bone the critical organ, a permissible burden of 5 μc is allowed. For the proposed studies, a dose not to exceed 5 μc would be used, with an anticipation that a dose of only 2 μc may be sufficient.

10. Calcium-45

If the use of calcium-47 should prove not feasible because of the short half-life and transportation or delivery difficulties, calcium-45 would be employed instead. Calcium-45, with a soft beta emission and a half-life of 163 days, has a maximum permissible burden in bone of 30 μc or 200 μc for the total body. The dosage proposed for the anticipated studies would not exceed 15 μc.

All use of radioisotopes in humans would be in accordance with the following:

a. Use will be confined to metabolic and physiological tracer studies.

b. The licensee shall comply with the provisions of Title 10, Part 20, Code of Federal Regulations, Chapter 1, "Standards for Protection Against Radiation," and RC-12, "The Medical Use of Radioisotopes--Recommendations and Requirements by the Atomic Energy Commission."

c. Radioisotopes for use in humans shall be acquired from a supplier other than an Atomic Energy Commission facility, who certifies the pharmaceutical quality and assay of such material.

d. The licensee, except as otherwise specifically provided for in the license, shall possess and use the material as described in this license in accordance with statements, representations and procedures contained in supplementary sheets attached to the application.

e. All rules, regulations and limitations set forth by Army, AEC and local authorities (including those set forth in AR 70-25, AR 40-37 and Handbook 69 of the National Bureau of Standards) will be complied with.

Section III. Proposed Research: Nutrition and Metabolism Tracer Studies

One of the missions of the U. S. Army Medical Research and Nutrition Laboratory (USAMRNL) has been to investigate the nutritional requirements of the human, both micro- and macronutrients, and to study factors that may influence these requirements. Factors under study include dietary imbalances; interactions of vitamins, minerals and macronutrients; malabsorption; disease; adaptation and stress, including altitude, cold and heat. The studies have included the development of techniques for the evaluation of nutritional status and requirements. The initial phases of the studies have employed laboratory animals and non-radioisotopic human volunteer studies. The studies have been conducted at the Denver Laboratory, as well as at several continental U.S.A. locations and several foreign countries.

Following these initial phases, the use of tracer studies was essential in order to obtain additional desired information. During the past recent years, a limited number of such label experiments have been successfully conducted. In view of the expanding nutrition research program at USAMRNL, both domestic and international, it is essential that limited tracer studies be made available as a technique to aid the program in obtaining required answers to problems of both military and civilian importance. In addition to the studies in progress at the Denver Laboratory, personnel of USAMRNL are currently conducting nutrition research at other locations within the U.S.A., as well as in several other countries, with plans to conduct studies in several additional countries in the near future.

Indicated briefly below are current interests and recent activities in human nutrition and metabolism research at USAMRNL that have involved tracer studies.

11. Vitamins: Investigations on the vitamin requirements of the human and factors that influence the requirement with the use of carbon-14 or hydrogen-3 labeled vitamins or related compounds.

a. All studies on vitamins, as well as for the other indicated compounds, would be conducted in terms of dosage and administration of the labeled material in accordance with the procedures outlined in the document submitted for the previous license application. Every effort will be made to use the minimum dosage necessary to obtain valid and significant data, with oral administration whenever suitable for the study. Similarly, subjects, medical supervision, sample handling and other techniques as previously indicated would be adhered to in proposed studies. No changes or modifications appear to be necessary. An exception would be the request outlined below for the use of ^{14}C-labeled ascorbic acid and ^{3}H-labeled ascorbic acid in the study at the University of Iowa. As indicated earlier, personnel of USAMRNL would be associated overseas in studies involving the use of tracers in human volunteers.

b. The preliminary use of ^{14}C-labeled thiamine has already provided information as to the pool size, turnover rate and urinary metabolites of this vitamin. Thus, orally ingested 2-^{14}C-thiazole labeled thiamine has demonstrated, for the normal young adult human, the following: (a) that the half-excretion time of the thiazole-labeled thiamine was shown to be 18 days, as compared to 9 days in the rat; (b) that no decarboxylation of the thiazole-labeled thiamine was seen; (c) that thiazole-labeled thiamine is efficiently absorbed; (d) that free thiamine was the only metabolite that was positively identified; and (e) that the total number of metabolites, as determined by column and paper chromatography, were 27 in number, of which 8 appear to be major metabolites. Current work is now in progress in an attempt to

identify the 27 labeled metabolites found in the urine. Future studies must include at least one more human study using the pyrimidine-^{14}C labeled thiamine to determine if the metabolic pattern is similar to that seen in the thiazole-labeled thiamine studies in man. This Laboratory has recently spent $6,000 for custom-synthesized pyrimidine-^{14}C labeled thiamine for use in future human studies. These studies will be required to provide information on the metabolism of thiamine and an estimation of the pool size and turnover rate of the vitamin. Such information is needed to evaluate the human requirement for vitamin B_1 (thiamine). The nature of the metabolites, their function and biosynthetic sources remain under study. The relationship of the metabolites to thiamine requirements and to adaptations is also under investigation and requires the use of ^{14}C-labeled thiamine.

c. The human requirement for riboflavin (vitamin B_2) has received increasing attention at USAMRNL because of the lack of satisfactory information pertaining to needs and the worldwide occurrence of deficiencies of this vitamin. Studies thus far have been restricted to animal studies with the use of riboflavin-2-^{14}C or to non-label human studies. Investigations in man employing ^{14}C-labeled riboflavin are planned for the immediate future. The objectives of the studies would be similar to those associated with the ^{14}C-thiamine experiments. The animal studies have been completed and techniques for the isolation and characterization of the urinary metabolites have been fully worked out. From the preliminary data obtained on the rat, it would appear that the metabolite pattern of riboflavin will not be as complex as that seen in the ^{14}C-labeled thiamine and pyridoxine studies. In the projected foreign studies, ^{14}C-labeled riboflavin is anticipated to be employed in Iran and

Thailand. Such studies will be conducted in cooperation with the governments of the respective countries. The investigations will involve administering small amounts of the ^{14}C-labeled riboflavin (20 to 50 uc) to individual volunteers resident in these countries with known intakes of the vitamin. The studies will be designed to evaluate the influence of deficiency, adaptation, stress, climate and other factors peculiar to the region on riboflavin requirements. Currently, uniformly labeled ^{14}C-riboflavin is in the process of being produced by fermentation. This has involved obtaining permission from both the U. S. Department of Agriculture and the State of Colorado to import a culture of Ashbya gossypii, the organism used in the fermentation. Approximately $10,000 of radioactive precursors for the culture medium have been purchased. Low level fermentations have been conducted and the isolation techniques developed. The high specific activity riboflavin to be produced is intended for human nutrition investigations.

d. Of the B-complex vitamins under study at USAMRNL, vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine) has received the greatest attention in the past several years. These studies have received international attention since they have established, within reasonable limits, the young adult human requirement for vitamin B_6. Current studies are aimed at investig ting nutritional interrelationships and other factors that may influence the requirement. The use of ^{14}C-labeled pyridoxine has been of assistance in this respect. Orally ingested ^{14}C-pyridoxine has demonstrated for the normal young adult human the following: (a) pyridoxine is efficiently absorbed; (b) the vitamin is not metabolized to

CO_2; (c) the half-time excretion of pyridoxine is approximately 15 to 20 days, subject to the level of intake of vitamin B_6; (d) the body pool of vitamin B_6 is approximately 22 to 27 mg, with normal intakes of the vitamin; and (e) the urinary metabolites of vitamin B_6 are numerous, with 4-pyridoxic acid representing approximately one-third of the vitamin B_6 ingested.

To date, only two human subjects have been studied; in both cases, the subjects received an oral dose of 30 μc of the ^{14}C-labeled pyridoxine. Further studies will be required to provide information on the metabolism of pyridoxine and an estimation of the pool size and turnover rate of the vitamin. Such information would be valuable in evaluating the human requirement for vitamin B_6 and in studying nutritional and metabolic interactions that may influence the requirement. Studies on vitamin B_6 requirement are projected for both Iran and Thailand.

e. Vitamin C has received at USAMRNL the most intensive study of all the vitamins currently under investigation. Various facets are under study, including functions, metabolism, requirements, interactions, methodology and adaptation. Numerous reports have appeared as a result of the investigations and are indicated in the appendixes. Using the techniques of investigation as set forth in our previous human isotope license application, the following summation of the results obtained from isotope studies on vitamin C metabolism in the human is presented:

(1) Studies of body composition and the use of ^{14}C isotopes have resulted in a method for stating the actual utilization of ascorbic acid by healthy men. In studies of 29 men of diverse body weight and

degree of fatness, it was found that ascorbate utilization, as expressed in terms of ^{14}C-oxalate excretion, occurred at a rate of 11 to 37 mg/day, with a mean of 21.5 mg and a standard deviation of 8.1 mg. If expressed on a fat-free, lean body basis, the rate would be 0.4 mg/day/kilogram of fat-free body weight. Rarely, if ever, do adult males exceed 90 kilograms in lean body mass. Therefore, 40 mg/day intake would exceed the greatest quantity of ascorbate metabolized by the largest healthy man.

(2) It has been shown that dehydroascorbic acid (dAs) is partially reduced to L-ascorbic acid in both animals and man. It has been further shown that only pure reduced L-ascorbic acid (AsH_2), and not any of its oxidized forms, is incorporated into the body ascorbate pool.

(3) It has been shown that the only known metabolic products of vitamin C labeled with ^{14}carbon in the one position in man are oxalate, AsH_2 and dAs. In young, healthy male volunteers studied, the AsH_2 pool size is 2 to 3 grams, and the turnover half-time is about 20 days on AsH_2 intakes of about 100 mg/day.

(4) The kinetic and metabolic fate of ascorbic-4-^{3}H acid has been studied in a human subject. The radioactive label does not enter the body water pool, but instead is excreted as organic-bound tritium. The excretion products were found to be ascorbic acid and its immediate oxidation products, and an unknown organic compound(s). Kinetic analysis of the data shows half-times of 2 days and 46 days for turnover of the labeled ascorbic acid and the unknown compound, respectively. These results, combined with previous ascorbate-1-^{14}C studies, indicate that the

unknown metabolite(s) is probably a derivative(s) of L-threose or L-threonic acid.

(5) Recent experiments using L-ascorbic-4-^{3}H acid fed to a human volunteer subject have indicated that the tritium-labeled ascorbate may serve as a better method of evaluating the actual vitamin C metabolism and requirement in the human subjected to any stress situation.

(6) Currently, L-ascorbic-6-^{14}C acid is being synthesized at USAMRNL. From the results of studies conducted, it was essential that this material be prepared in order to further the investigations on the fate, function and requirement of vitamin C. Considerable time, effort and expense have gone into developing synthetic procedures to produce L-ascorbic-6-^{14}C acid. The synthesis of the compound is almost complete and, upon purification and characterization, the material will be available for human studies.

Immediate and future proposed studies employing the use of carbon-14 or hydrogen-3 labeled ascorbic acid (vitamin C):

Upon the availability of the ascorbic-6-^{14}C acid, studies would be conducted with this material in the same manner as have been previously reported and as outlined in the previous license application. These studies are part of a series that represent an attempt not only to prescribe requirements, but to elucidate the metabolism and function of this unique vitamin.

All studies on the vitamin C requirement of humans, to date, have been conducted on normal young adult males. There have been no studies

performed on individuals who are existing on sub-optimal or deficient intakes of vitamin C. From the data obtained by the Nutrition Section, Office of International Research, National Institutes of Health during the nutrition surveys of Turkey and Iran, it would appear as though the human could adapt to very low intakes of vitamin C without developing scurvy. The question as to whether humans, adapted to low dietary intakes of vitamin C, would in a stressful situation develop frank scurvy, or other metabolic abnormalities, is academic because no work has been performed in this area in an attempt to define vitamin C requirement or metabolism. Therefore, the following studies are proposed.

(1) A joint study between Robert E. Hodges, M.D., of the University Hospitals, University of Iowa, Iowa City, and the Chemistry Division of USAMRNL, Denver, Colorado, is proposed to begin in November 1966. The objectives of this study are: To induce scurvy in six prison volunteers in order to permit a study of the clinical characteristics and accurate measurements of the pool size and utilization of vitamin C by means of isotope. The recovery phase of the study will be designed to provide evidence of the true requirements for vitamin C. Initially, the six subjects will receive orally 50 µc of L-ascorbic-1-^{14}C acid to determine their pool sizes and rate of utilization of vitamin C. They will then be placed on a vitamin C-deficient diet and studied by measuring the rate of decrease of their pool sizes until such time as a decrease of 50% of their initial pool size is noted; at this time, they will be resupplemented with vitamin C. The subjects will then be relabeled with 50 µc of ascorbic-4-^{3}H acid at the time they are resupplemented with vitamin C. The subjects will be given different controlled

intakes of ascorbic acid, varying from 2 to 70 mg per day, in order to determine the influence of these varying levels of vitamin C intake on the rate of saturation of the individual ascorbate pool size.

It is anticipated that the depletion phase of this study will take between 3 to 4 months, since the average individual has an ascorbate pool size of 2 to 3 grams and a daily utilization of 10 to 20 mg. The repletion phase will probably require a period of 2 months on the varied levels of ascorbate intake to obtain saturation of the ascorbate pool to its initial control level. The total amount of isotope to be received by each subject will not exceed 50 μc of ascorbic-1-^{14}C acid and 50 μc of ascorbic-4-^{3}H acid.

(2) Foreign studies: Studies similar to the above Iowa City study are proposed for the country of Iran. In areas of Iran that are found to have a populace with a deficient or low intake of vitamin C, it is proposed to label a small number of these people with 50 μc of ascorbic-1-^{14}C or ascorbic-4-^{3}H acid to determine their ascorbate pool size and actual rate of utilization. Such studies will be conducted in cooperation with authorities of the foreign government involved. research project in Iran will represent a program of a minimum of three years' duration.

Health physics were not discussed here since they were thoroughly discussed and considered in our original human isotope request. There have been no changes made in the methods and techniques as set forth in the original protocol in terms of isotope dosage and disposition.

12. Carbohydrates

As was indicated in the previous license application, this Laboratory has been investigating for some time the digestibility of cellulose and other complex carbohydrates. The earlier studies were concerned with the digestibility of ^{14}C-labeled cellulose, hemicellulose and various uncommon sugars by laboratory animals such as the rat, hamster and guinea pig. These studies were extended to include germ-free rats. Following completion of the animal studies, attention was directed to the digestibility of these complex carbohydrates by the human. Time has permitted the completion of only a single human experiment employing ^{14}C-labeled cotton cellulose. The labeled cotton was produced at USAMRIL by growing cotton plants in a controlled environment. The harvested mature cotton bolls were processed and the resulting pure alpha-cellulose was prepared for feeding studies.

In related studies, modified cellulose (Avicel-R), prepared by the American Viscose Company, has been investigated for nutritional properties in human volunteers. A portion of the ^{14}C-labeled cotton cellulose has been processed for us by American Viscose Company. In order to complete these studies on cellulose and related carbohydrates, additional tracer experiments need to be conducted.

In addition to the above studies, other human studies are being conducted on malabsorption and atherosclerosis which involve the use of carbohydrates. Studies on the interrelationship of various types of carbohydrates and other dietary components on serum triglycerides and cholesterol in the [illegible] have advanced to the state where tracer levels of common sugars are necessary to provide the desired information. Details as to procedures employed in the use of ^{14}C-labeled carbohydrates were included in the previous license application.

13. Minerals: Studies on mineral metabolism, interactions and requirements in the human with the use of radioisotopes.

The research program at USAMRNL includes investigations on the metabolism, interactions and requirements for the majority of both the macro- and micro-mineral nutrients. These investigations have employed the use of both laboratory animals and human volunteer subjects. Current major emphasis in the area of minerals, involving human subjects, has been related to magnesium, calcium, phosphorus, sodium and potassium balance studies and the sweat losses of all minerals. Special attention has been given magnesium because of the interest in determining the human requirement for this mineral and its relationship, along with calcium and phosphorus, to renal calculi formation. The previous license application outlined the background information on the renal calculi problem and the nature of the investigations being conducted at USAMRNL, requiring the need for the use of magnesium-28, calcium-45 and calcium-47. Although permission to use these isotopes was granted, they were not employed during the past eighteen months for several reasons. The availability of magnesium-28 is limited and its use must be timed exceedingly close with the experimental situation. Unfortunately, suitable patients with renal calculi problems did not become readily available for study locally during this period. With the present modifications in the Metabolic Ward of the Metabolic Division at USAMRNL, it is now anticipated that patients can be maintained readily locally for investigation. The procedures previously outlined would be followed. For each case study, considerable preliminary clinical and biochemical evaluations must be performed in order to ensure that the use of the radioisotopes of magnesium or calcium will provide the desired data.

Plans are also projected to study further calcium and magnesium metabolism and requirements in Thailand and possibly Iran. The studies in Thailand would be in support of studies (a) on the possible role of minerals in the etiology of bladder stones and (b) the influence of adaptation on calcium requirements. These studies would be conducted in cooperation with Thai investigators in collaboration with the SEATO Clinical Research Center, Bangkok, Thailand.

14. Other

Depending upon the rate of progress of the above indicated studies and the development of other current investigations, the use of additional carbon-14 or hydrogen-3 labeled biological compounds is desired. These compounds include additional vitamins, lipids and related compounds such as acetate, and amino acids. The use of tritiated water is desired for body water measurements, since it is more convenient and satisfactory to use than water labeled with deuterium. The malabsorption studies would be assisted if routinely employed test labeled compounds, including tritiated folacin and pyridoxine, could be permitted. As mentioned above, studies are being conducted on atherosclerosis which could be enhanced by the availability of ^{14}C-labeled lipids or ^{14}C-labeled acetate. The previous license application outlined several experiments that would have utilized carbon-14 labeled amino acids. These experiments were not conducted because a sufficient number of suitable volunteer subjects was not available at appropriate times during the period while licensed. The need to conduct the indicated studies remains.

Appendix I. References on General Health Physics

1. Recommendations of the International Commission on Radiological Protection, ICRP Publication 2, Report of Committee II on Permissible Dose for Internal Radiation, Pergamon Press, 1959.

2. Radiological Health Handbook, U. S. Department of Health, Education and Welfare, Sept. 1960.

3. Radioactive Isotopes in Medicine and Biology: Medicine, S. Silver; Lea and Febiger, Publishers, 1962.

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5. Use of Radioisotopes in Animal Biology and the Medical Sciences, Vol. 1 and 2, Academic Press, 1962.

6. Maximum Permissible Amounts of Radioisotopes in the Human Body and Maximum Permissible Concentrations in Air and Water. Handbook 52, U. S. Dept. of Commerce.

7. Progress in Nuclear Energy: Series VI, Biological Sciences, J. G. Bugher, J. Coursaget and J. F. Loutit, Editors, Pergamon Press, 1959.

8. Maximum Permissible Body Burdens and Maximum Permissible Concentrations of Radionuclides in Air and in Water for Occupational Exposure. Handbook 69, U. S. Dept. of Commerce.

9. Progress in Nuclear Energy: Series VII, Medical Sciences, J. G. Bugher, J. Coursaget and J. F. Loutit, Editors, Pergamon Press, 1959.

10. Peaceful Uses of Atomic Energy: Vol. 22, "Biological Effects of Radiation," 1958; United Nations Publication.

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12. Radioisotope Studies of Fatty Acid Metabolism, J. F. Mead and D. R. Howton, Pergamon Press, 1960.

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14. Clinical Use of Radioisotopes. W. H. Beierwaltes, P. C. Johnson and A. J. Solari. W. B. Saunders Co., Publishers, 1957.

15. The Use of Isotopes in Nutrition Research with Special Reference to Tritium. J. Dono and P. R. Payne. World Review of Nutrition and Dietetics: Vol. 1, p. 207, 1959, Hafner Publishing Co.

V O L U N T A R Y C O N S E N T S T A T E M E N T

Military_____ Military Patient_____ Civilian_____ Civilian Patient_____

I, _____, having the capacity to consent, voluntarily and without force or duress consent to participate in research involving the use of tracer amounts of radioisotopes. I have been informed of, and understand, the nature, duration, and purpose of the experiment, the method and means by which it is to be conducted, the inconveniences and hazards to be expected, and the effects upon my health and person which may possibly come from participation in the experiment.

Specifically, I agree to receive (intravenously) (orally) a small quantity of _____ _____ containing _____ microcuries of _____. I also agree to furnish urine and stool samples for the period following until no detectable radioactivity is present. I submit to measurements of expired gases if Carbon-14 has been received.

I understand that I may at any time during the course of the experiment revoke my consent and withdraw from the experiment without prejudice.

I do not at this time have any physical diseases, except for the following _____, or mental disease, to the best of my knowledge.

_____ DATE

_____ SIGNATURE

_____ SIGNATURE OF WITNESS

APPROVAL

I have personally ascertained that the quality of the foregoing consent is sufficient to permit the volunteer to participate in the experiment.

_____ ATTENDING PHYSICIAN

_____ PROJECT LEADER

Appendix III. Publications by Personnel of USAMRNL Since 1962 Involving the Use of Tracer Studies in Human Volunteers

1. Baker, E. M., H. E. Sauberlich, S. J. Wolfskill, W. T. Wallace and E. E. Dean. Tracer studies of vitamin C utilization in man; metabolism of D-glucuronolactone-(-^{14}C, D-glucuronic-6-^{14}C acid and L-ascorbic-1-^{14}C acid. Proc. Soc. Exp. Biol. Med., 109: 737, 1962.

2. Baker, E. M., N. G. Levandoski and H. E. Sauberlich. Respiratory catabolism in man of the degradative intermediates of L-ascorbic-1-^{14}C acid. Proc. Soc. Exp. Biol. Med., 113: 379, 1963.

3. Respiratory catabolism of L-ascorbic acid. Reviewed in Nutrition Rev., 22: 7, 1964.

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5. Tolbert, B. M., E. M. Baker and J. C. Saari. Ascorbic acid metabolism in man. Fed. Proc., 25: 218, 1966 (Abstract).

6. Baker, E. M., H. E. Sauberlich, W. H. Amos and J. A. Tillotson. Use of carbon-14 labeled vitamins in human nutrition studies: pyridoxine and L-ascorbic acid. Am. J. Clin. Nutr., 18: 302, 1966 (Abstract).

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9. Amos, W. H., Jr., M. Balaghi, O. Ramirez and H. E. Sauberlich. Metabolism of ^{14}C-riboflavin in the rat. Fed. Proc., 25(2): 245, 1966 (Abstract).

10. Reica, N., Jr., Y. F. Herman, W. H. Amos, Jr. and H. E. Sauberlich. Riboflavin nutrition in the germ-free and pathogen-free rat. Fed. Proc., 25(2): 245, 1966 (Abstract).

11. Tillotson, J. A., H. E. Sauberlich, E. M. Baker and J. E. Canham. Use of carbon-14 labeled vitamins in human nutrition studies: pyridoxine. Proc. VIIth Inter. Congress of Nutrition, Hamburg, Germany (In press).

12. Sauberlich, H. E. Biochemical alterations in thiamine deficiency--their interpretation (In press).

13. Baker, E. M., J. C. Saari and B. M. Tolbert. Ascorbic acid metabolism in man. Am. J. Clin. Nutrition (In press).

14. Tolbert, B. M., A. W. Chen, E. M. Bell and E. M. Baker. Metabolism of L-ascorbic-4-^{3}H acid in man. Am. J. Clin. Nutrition (In press).

15. Saari, J. C., E. M. Baker and H. E. Sauberlich. Thin-layer chromatographic separation of the oxidative degradation products of ascorbic acid. Anal. Biochem. (In press).

ABSTRACT

PROJECT NO. 3A025601A822 Military Internal Medicine

TASK NO. 01

WORK UNIT NO. 072 Studies in Human Nutrition

The following investigations have been conducted under this work unit:

STUDY NO. 1 Ascorbic acid metabolism and requirement in the human male

STUDY NO. 2 Ascorbic-4-^{3}H acid metabolism in man

STUDY NO. 3 Further studies on the vitamin B_6 requirements of young adult male humans

STUDY NO. 4 Thiamine metabolism and requirements

STUDY NO. 5 Metabolism of ^{14}C-labeled cotton cellulose by man

STUDY NO. 6 Other nutrition activities, national and international

1. The preliminary phase of a study on the production of experimental human vitamin C deficiency is completed. The objective of the study was to induce scurvy in healthy men under controlled conditions in an attempt to evaluate the human requirement for this vitamin and to study its metabolism and dietary interrelationships. Numerous observations have been reported as a result of this initial study.

2. Vitamin C metabolism in man was also studied with the use of L-ascorbic-4-^{3}H acid. It was observed that the radioactive label does not enter the body water pool but, instead, is excreted as organic-bound tritium. The results of these studies indicate the presence of more than one kinetically distinguishable pool in the metabolism of ascorbic acid. The unknown metabolites observed are possibly derivatives of L-threose or L-threonic acid.

3. The results of a recent experiment in a series of studies on the human requirement for vitamin B_6 demonstrated that there is no apparent effect of increased caloric utilization upon vitamin B_6 requirement. Under the conditions of this experiment, the minimum daily requirement of the young adult male human for vitamin B_6 is 1.25 milligrams.

4. Additional studies were conducted on thiamine metabolism and requirements. The yeast method for measuring some of the metabolites of thiamine in urine appears to offer a specific assay for thiamine nutriture in the human. The effect of caloric intake and exercise on the thiamine requirement of the human

has been investigated in a study employing adult males. Preliminary evaluation of the data indicates that increased caloric expenditure, employing carbohydrate as the source of calories, increases the human requirement for thiamine.

5. ^{14}C-labeled cotton cellulose was modified to form microcrystalline ^{14}C-cellulose (Avicel-R). This material has been fed to a volunteer human subject in an attempt to determine whether or not the human is capable of utilizing cellulose. Although the study is not entirely completed, evidence would indicate that the human cannot utilize cellulose to any significant degree.

6. Continuing support was provided national and international nutrition agencies and their basic or applied nutrition programs and training activities.

BODY OF REPORT

WORK UNIT NO. 072 — Studies in Human Nutrition

STUDY NO. 1 — Ascorbic acid metabolism and requirement in the human male

PROBLEM:

The preliminary phase of a study on human scurvy was completed on 31 May 1967. This study, a joint project between the USAMRNL Chemistry Division and the Metabolic Ward at the University Hospitals, University of Iowa, has led to exciting new concepts of vitamin C requirements and metabolism in man. Dr. R. E. Hodges of the Department of Internal Medicine, University Hospitals, is the chief medical investigator for this study. The objectives of these studies are as follows:

1. To induce in healthy men (prison volunteers) a deficiency of ascorbic acid.

2. To label their ascorbic acid pools with L-ascorbic-1-^{14}C acid in order to study (a) total body pool size; (b) rate of depletion; (c) minimal requirements for vitamin C; (d) the relationship between symptoms and signs of scurvy and levels of the vitamin body pool; (e) the amount of ascorbic acid necessary to replete the body pool and to alleviate signs and symptoms of deficiency; and (f) theories concerning the physiologic functions of ascorbic acid based on metabolites in blood and urine.

RESULTS AND DISCUSSION OF THE RESULTS:

The pilot study began with six healthy men (prison volunteers from Iowa State Penitentiary) who were housed on the Metabolic Ward of University Hospitals and were fed a diet totally deficient in vitamin C, but adequate in all essential nutrients and containing sufficient calories to maintain body weight. This diet, a formula composed of vitamin-free casein, purified carbohydrates and fats, was fed in three equal portions daily through a naso-gastric tube. The formula was continued throughout depletion and for the first 15 days of the repletion period. On the 99th day, repletion with vitamin C was commenced, and on the 115th day the diet was changed to solid foods. This diet, composed of a soybean food, casein products, fats and carbohydrates, was found by thin-layer chromatographic assay to furnish only 2.5 mg of ascorbic acid daily.

WORK UNIT NO. 072 - BODY OF REPORT

Throughout the study, detailed clinical, biochemical and radioisotopic studies were performed. Photographs were taken of the eyes, mucous membranes and skin at regular intervals.

To date, only a small portion of these results is available for interpretation, but those which are available are shown below:

1. Depletion Period. Following the administration of 1.3 mg of L-ascorbic-1-^{14}C acid containing 39.77 μc to each subject, the ^{14}C-ascorbate metabolites excreted were followed daily. From the total cumulative plot of the daily radiocarbon excretion, it appeared that more than 97% of the ^{14}C-labeled ascorbate was absorbed and retained in a deep "physiologic pool" by all subjects. Once the incorporation of the ^{14}C-labeled ascorbate had taken place, the rate of urinary ^{14}C-labeled organic metabolites of ascorbate occurred as a logarithmic function at a rate of one-half of one per cent of the total pool of ascorbic acid per day. This implies that the catabolism of ascorbic acid from the "physiologic pool" is a first order exponential process; therefore, the summation of all metabolic products derived from this pool must also be a first order process.

Estimates were made of the initial pool size and the rate of utilization of vitamin C in each man. By the 99th day of depletion, the average man had reduced his pool size to approximately 170 mg, with a utilization rate of 2.5 mg daily. Had the project been continued for an additional 32 days, the vitamin C pool size would have been depleted to less than 100 mg total, with a daily utilization of 1 mg; at that point, frank and possibly dangerous scurvy might have ensued.

2. "Physiologic Ascorbate Pool." The exact chemical nature of this pool is as yet unknown. We do know that the organic ^{14}C-labeled material is not L-ascorbic acid or any of its commonly recognized derivatives. The urinary metabolites of this physiologic ascorbate pool are water soluble and chemically stable. We have been able to isolate, on chromatographic columns, some of these materials. Thus far, it appears there are two major metabolites and three minor ones. Work is in progress to isolate and identify the chemical nature of these compounds. The two major metabolites have been found to be reproducibly homogeneous in three different solvent systems, thus indicating that they constitute the major metabolites of this pool. Even though chemical identification of these compounds in the urine has not yet been accomplished, we believe that characterization of them will not explain the physiologic functions of vitamin C. Identification of these compounds will, however, direct attention to other systems which must be studied in order to understand the physiological functions of vitamin C.

3. Repletion Period. After a depletion period of 99 days, during which the diet contained no ascorbate, each of the men was repleted with labeled ascorbic acid in amounts of 4, 8, 16, or 32 mg daily. The administered ascorbic acid had a specific activity of 0.05 μc/mg, and permitted tabulation of a cumulative material balance for each subject throughout the repletion period. The subject who received the 8 mg daily supplement did so for a period of 57 days, and then was placed on an intake of 64 mg daily for a period of 20 days. The rate of repletion in all subjects was a zero order linear function of the intake of ascorbate. The data indicated that the rate of incorporation into the "deep pool" occurred at the rate of 80-90% of the administered dose within the range of 4-64 mg intake daily. Repletion continues until the "deep pool" becomes fully saturated. During the repletion phase, there was no free ascorbic acid excreted in the urine until the "deep pool" had been repleted fully. Once this occurred, urinary spilling of ascorbic acid did take place and the rate of incorporation of ascorbic acid into the deep pool fell accordingly. Once urinary spillage of ascorbic acid occurred in any of the subjects, labeled ascorbate supplementation was discontinued and the subject then received the same level of ascorbate supplementation as "cold" ascorbate.

4. Minimal and Optimal Ascorbic Acid Requirements. Preliminary data indicate, as of the 97th day of repletion, that the minimal daily requirement of ascorbic acid necessary to prevent scurvy is approximately 3.8 mg (as compared with the British estimate of 3 to 4 mg daily). The data further imply that the optimal daily intake would be 10 mg if we assume that the physiologic pool is already saturated. This is in accord with the observation that the rate of depletion is an exponential process occurring at the rate of 0.5% daily and that the average subject has a saturated pool size of 2,000 mg. Thus, the normal rate of catabolism would be 10 mg daily.

It was of interest to note that simple counting of radiocarbon-labeled products in the urine each day provides the best indication of a physiologic, metabolic, or emotional stress as these influence vitamin C catabolism. As an example, one of the subjects underwent a 21-day period of severe emotional stress. His emotional stress was clearly reflected in an increased rate of excretion of ascorbate metabolites, an observation that strongly suggested an increased requirement for ascorbate under these conditions.

5. Other Vitamin Interrelationships. Additional interesting observations have been noted that relate to other essential nutrients. It was observed that the level of vitamin A in serum of these men (who were receiving supplements of 5,000 IU of vitamin A daily) fell approximately 20 to 30% during the deficiency period and began to rise again during repletion with vitamin C. In

addition, there were changes in the nonpolar lipids of the erythrocytes which suggest the possibility of an effect on vitamin E metabolism resulting from an ascorbic acid deficiency (despite an intake of 80 mg of alpha-tocopherol daily). Also, there may be a relationship of vitamin C to pyridoxine metabolism, since the subjects were found to have a three-fold increase in the rate of urinary excretion of "free" pyridoxine during an ascorbic acid deficiency. These subjects were on a constant daily intake of 1.7 mg of pyridoxine during the entire experiment.

6. Clinical Observations. During the deficiency period, three of the four men complained of fatigue and mild malaise. All four developed some degree of follicular hyperkeratosis. Hemorrhagic manifestations included the appearance of a positive Rumple-Leeds test (petechiae of the skin following venous occlusion) in three men and bleeding of the gums and minor bruising tendencies in all four. A puzzling and possibly new observation was the appearance of tiny triangular hemorrhages in the bulbar conjunctiva of the eye. These appeared near the end of the deficiency period or early in the repletion period, and the severity of their occurence was inversely proportionate to the dose of vitamin C given. Thus, they were most severe in the subject given only 4 mg of ascorbic acid; less severe in the man receiving 8 mg; mild in the subject who received 16 mg; and absent in the person who received 32 mg of ascorbic acid daily. Despite these evidences of hemorrhagic tendencies, blood studies performed failed to show any evidence of impairment of any of the blood clotting mechanisms, which are measurable by modern techniques.

Physiologic tests, including basal metabolic rates and electroencephalograms, failed to demonstrate any significant departure from normal. Electrocardiograms showed minor changes, which are yet to be interpreted.

Throughout the study, each of the men was required to walk for an estimated 10 miles daily. Three miles of this was provided by an escorted walk. The men wore pedometers and were instructed to walk an additional 7 miles, for a total of 10 miles. Although this was an imprecise measure of exercise, they did maintain a rather high degree of energy expenditure, as evidenced by the fact that their weight remained constant on a caloric intake designed to meet this level of energy expenditure.

7. Infections. One man had three episodes of infection of the external ear canals with accompanying fever and regional lymphadenopathy, yet there was no apparent change in his rate of utilization of vitamin C.

8. Wound Healing. At the beginning of the repletion period, each of the men was subjected to a surgical laceration on the lateral aspect of the thigh, extending through the dermis and the fascia. One week later, a punch biopsy was taken from the healing wound margin and processed for electron microscopic examination, standard hematoxylin and eosin histologic examination and ^{14}Carbon vitamin C content. A second punch biopsy was similarly processed at the end of 2 weeks. The rate of healing of these wounds appeared to be directly proportional to the dose of vitamin C administered, yet all of the wounds eventually healed satisfactorily.

CONCLUSIONS:

1. It would appear that the physiologically functional pool of ascorbic acid is not vitamin C as we chemically identify it, but rather a stable derivative of L-ascorbic acid.

2. When subjects are placed on a zero vitamin C intake, the extent ascorbate pool is catabolized exponentially at a very low rate (27 day half-time), and this rate of catabolism is a function of the pool size.

3. The rate of repletion of ascorbic acid in deficient subjects is a zero order linear function of the intake of ascorbate. The rate of incorporation into the physiologic pool occurs at the rate of 80-90% of the administered dose within the range of 4-64 mg intake daily. Repletion continues until the ascorbate pool becomes fully saturated. During the repletion phase, there is no free ascorbic acid excreted in the urine. Once the pool has been fully repleted, urinary spilling does occur, and the rate of incorporation of ascorbate into the physiologic pool falls accordingly.

4. The clinical responses pertaining to hemorrhagic manifestations, physiologic tests, stress, infections and wound healing have been discussed.

PUBLICATIONS:

1. Baker, E. M., J. C. Saari and B. M. Tolbert. Ascorbic acid metabolism in man. Am. J. Clin. Nutr., 19: 371-378, 1966.

2. Saari, J. C., E. M. Baker and H. E. Sauberlich. Thin-layer chromatographic separation of the oxidative degradation products of ascorbic acid. Anal. Biochem., 18: 173, 1967.

3. Baker, E. M. Vitamin C metabolism in stress. Am. J. Clin. Nutr. (in press).

WORK UNIT NO. 072 - BODY OF REPORT

STUDY NO. 2 Ascorbic-4-^{3}H acid metabolism in man

PROBLEM:

Recently, 4-^{3}H-labeled ascorbic acid has been prepared, having a specific activity of 9.5 μc/mg. This material was prepared for metabolic studies in both animal and man.

RESULTS AND DISCUSSION OF THE RESULTS:

The kinetic and metabolic fate of ascorbic-4-^{3}H acid have been studied in a human subject. The radioactive label does not enter the body water pool but, instead, is excreted as organic bound tritium. The excretion products were found to be ascorbic acid and immediate oxidation products, and unknown organic compound(s). Kinetic analysis of the data shows half-times of 2 days and 46 days for turnover of the labeled ascorbic acid and the unknown compound, respectively. These results, combined with previous ascorbate-1-^{14}C studies, indicate that the unknown metabolite(s) are probably derivative(s) of L-threose or L-threonic acid.

CONCLUSIONS:

The metabolism of L-ascorbic-4-^{3}H acid has been studied in man, with the following results:

1. The radioactive label does not enter the body water pool but, instead, is excreted as organic bound tritium.

2. These data indicate the presence of more than one kinetically distinguishable pool in the metabolism of ascorbic acid.

3. The unknown metabolite(s) are probably derivative(s) of L-threose or L-threonic acid.

PUBLICATIONS:

1. Tolbert, B. M., A. W. Chen, E. M. Bell and E. M. Baker. Metabolism of L-ascorbic-4-^{3}H acid in man. Am. J. Clin. Nutr., 20: 250, 1967.

2. Tolbert, B. M., S. C. March, D. B. Karr, W. Scharf and E. M. Baker. Ascorbate function: donor of a four carbon moiety? Fed. Proc., 26: 85[illegible] 1967 (abstract).

WORK UNIT NO. 072 - BODY OF REPORT

STUDY NO. 3 — Further studies on the vitamin B_6 requirements of young adult male humans

PROBLEM:

In the course of a previous study, the question was raised as to whether or not the level of exercise affected the human requirement for vitamin B_6. Further, at recent NAS-NRC Food and Dietary Allowances Committee meetings, the question was raised as to whether there is a caloric effect upon the vitamin B_6 requirement of the human. In order to answer these questions, the following study was proposed in an effort to determine if there is any relationship between vitamin B_6 requirement and caloric utilization.

RESULTS AND DISCUSSION OF THE RESULTS:

A study was performed to determine if there was any relationship between vitamin B_6 requirement and caloric utilization. Eight subjects were placed on a vitamin B_6-free formula diet providing daily 100 g protein and 2800 calories for a period of 2 weeks. That a vitamin B_6 deficiency was produced was demonstrated by increased urinary xanthurenic acid excretion following a 5 g L-tryptophan load and by reduced urinary excretion of vitamin B_6.

Following depletion, the subjects were divided into two groups. Group A was given a processed natural diet that provided 100 g protein, 0.90 mg vitamin B_6 and 2800 calories per day. Group B received the same basic diet, but at the level of 3600 calories per day. All subjects received a pyridoxine supplement to provide a total controlled daily intake of 1.25 mg of vitamin B_6. All subjects were exercised to constant weight. The subjects were maintained on the respective diets for 6 weeks. During this period, urinary excretion of vitamin B_6 and of xanthurenic acid following L-tryptophan loads was studied. The results demonstrated that (a) there is no apparent effect of increased caloric utilization upon vitamin B_6 requirement; and (b) the daily minimum requirement of vitamin B_6 is 1.25 mg under the conditions employed.

CONCLUSIONS:

The results of a recent experiment in a series of human vitamin B_6 requirement studies demonstrated that (a) there is no apparent effect of increased caloric utilization upon vitamin B_6 requirement; and (b) the daily minimum requirement, in young adult male humans, of vitamin B_6 is 1.25 mg under the conditions employed in the experiment.

WORK UNIT NO. 072 - BODY OF REPORT

PUBLICATIONS:

1. Baker, E. M., Y. F. Herman and H. E. Sauberlich. Vitamin B_6 requirement of young adult male humans. Fed. Proc., 26: 413, 1967 (abstract and presentation).

2. Canham, J. E., E. M. Baker, N. Raica and H. E. Sauberlich. Vitamin B_6 requirement of adult men. Proc. VIIth Int. Cong. Nutr., Hamburg, Germany (in press).

3. Tillotson, J. A., H. E. Sauberlich, E. M. Baker and J. E. Canham. Use of carbon-14 vitamins in human nutrition studies: Pyridoxine. Proc. VIIth Int. Cong. Nutr., Hamburg, Germany (in press).

STUDY NO. 4 Thiamine metabolism and requirements

PROBLEM:

Various techniques are under study to investigate the human metabolism and requirement for thiamine. Urinary metabolites and ^{14}C-labeled thiamine are employed in addition to human volunteer studies. For example, a method has been published describing a procedure whereby metabolites of thiamine may be measured in human urine. The procedure depends upon the ability of yeast to synthesize thiamine from thiamine-related compounds. Following the synthesis, the urine is analyzed for thiamine by the conventional thiochrome procedure. The increase in thiamine following incubation with yeast is taken as the amount of thiamine metabolites in the urine.

In a recent study with human subjects on a restricted intake of thiamine, it was shown that the excretion of thiamine reached such low levels in 12 days as to be undetectable by the thiochrome assay. Yet, at the same time, the metabolite excretion increased to a constant level during the deficiency. This increase has led to speculation that the yeast was synthesizing substances which acted like thiamine in the yeast assay but were not directly related to thiamine. Thus, the validity of the method was in doubt.

In order to identify the thiamine-related compounds obtained from the incubation of urine with yeast, the following standards were synthesized: pyrimidine sulfonic acid, pyrimidine carboxylic acid, thiochrome, N-methylnicotinamide, thiazole and hydroxyethylthiamine. With the development of a thin-layer chromatographic procedure which could separate the

above compounds from each other and from thiamine, it is now possible to identify the compounds being measured as fluorescent substances in the thiochrome assay following the incubation of human urine with yeast.

RESULTS AND DISCUSSION OF THE RESULTS:

Preliminary results indicate that the fluorescent substances found in the thiochrome assay are in fact, thiochrome. If future work should confirm this finding, it would establish the specificity of the yeast method in measuring thiamine derivatives in the urine. This would assure those interested in assessing thiamine nutriture in various populations that the use of this method is valid for measuring thiamine metabolites in the urine.

No additional metabolic studies have been performed during the past year using ^{14}C-labeled thiamine. However, ^{14}C-pyrimidine labeled thiamine has been obtained, and it is projected that a human study utilizing this material will take place this fall.

The effect of caloric intake and exercise on thiamine requirement has just been completed in a study employing young adult healthy male human subjects. The data are being processed. Preliminary evaluation of the findings indicates that increased caloric expenditure, employing carbohydrate as the source of calories, increases the human requirement for thiamine.

CONCLUSIONS:

1. The yeast method for measuring some of the metabolites of thiamine in urine appears to offer a specific assay for thiamine nutriture in humans.

2. Results indicate that increased caloric expenditure, employing carbohydrate as the source of calories, increases the human requirement for thiamine.

PUBLICATIONS:

1. Sauberlich, H. E. Biochemical alterations in thiamine deficiency -- their interpretation. Am. J. Clin. Nutr. (in press).

2. Waring, P. P., W. C. Goad and Z. Z. Ziporin. The use of thin-layer chromatography to separate thiamine and related compounds as well as N-methylnicotinamide and related compounds (manuscript in preparation).

WORK UNIT NO. 072 - BODY OF REPORT

STUDY NO. 5 Metabolism of ^{14}C-labeled cotton cellulose by man

PROBLEM:

The objective of these studies was to determine whether or not the human subject is capable of digestion of cellulose and, if so, to what extent and what factors may influence such an ability.

RESULTS AND DISCUSSION OF THE RESULTS:

Cotton labeled with carbon-14, grown at USAMRNL, and nonlabeled cotton were converted into microcrystalline cellulose, "Avicel-R," by the American Viscose Corporation. Avicel-R is similar to the Avicel preparation which was previously used at USAMRNL, with the exception that it is of a smaller and more uniform particle size. The emulsification and other properties of Avicel-R gels are also more superior to the earlier product.

A human volunteer subject was placed on a 15-day preconditioning control period during which time he was fed a total of 150 g (dry weight) of nonlabeled microcrystalline cellulose per day in two equal portions. The cellulose was consumed in the form of milk shakes or sherbets. After the control period, he was then fed the ^{14}C-labeled cellulose (approximately 30 μc) without any nonlabeled cellulose as carrier added. Following the ingestion of the ^{14}C-labeled cellulose, 24-hour fecal and urine collections were obtained until such time as a complete ^{14}C-material balance was obtained. During the same experimental period, expired $^{14}CO_2$ was monitored daily.

The subject received the ^{14}C-labeled cellulose on 19 May 1967. Thus far, there has been no detectable $^{14}CO_2$ expired via the lung. Further, there has been no measurable ^{14}C activity in the urine over the past 11 days. At this time, the amount of ^{14}C activity excreted in the feces has not been ascertained. This information will be forthcoming. There is no reason not to believe that in excess of 98% of the ^{14}C-labeled cellulose will be excreted via the feces, as was the case in the animal studies.

CONCLUSIONS:

Although the study is not entirely completed, available evidence indicates that the human cannot utilize cellulose to any significant degree.

WORK UNIT NO. 072 - BODY OF REPORT

PUBLICATIONS:

None

STUDY NO. 6 — Other nutrition activities, national and international

PROBLEM:

Assistance and cooperation are provided in support of the mission of USAMRNL to extend nutritional and medical research, recommendations and training to U.S. military and civilian groups and to civil and military populations of other countries as judged important and appropriate.

RESULTS AND DISCUSSION OF THE RESULTS:

Members of the Chemistry Division have assisted in the training program of the Reserve Officers' groups and of individual foreign officers assigned to USAMRNL for special programs or training. Similarly, assistance has been provided in research, training and consulting to regional, national and international educational or government institutes, including FAO of the United Nations. Locally, cooperative support has been provided the University of Colorado, University of Colorado Medical School and Colorado State University. Assistance has been provided the Office of International Research, Nutrition Section, of the National Institutes of Health, as requested, in support of nutrition programs in Central America and South East Asia. Recently, a biochemist from USAMRNL spent 3 months at the Institute of Nutrition of Central America and Panama (INCAP), located in Guatemala, in support of the nutrition survey of Honduras. Blood samples from the recent nutrition survey of Panama were sent to USAMRNL for transketolase assay to assist in thiamine nutriture evaluation. Consulting and analytical services have been provided nutrition programs in Thailand, Malaysia and Indonesia. Currently, the Advanced Research Program Agency (ARPA) is being assisted in an evaluation of Thai military rations. Assistance was provided in conducting the nutrition survey of Ft. Campbell, Kentucky. Professional assistance has been provided by staff members by serving as members of editorial boards of nutrition journals and as members of various scientific committees, including NAS-NRC Committee on Dietary Allowances. Currently, additional studies have been proposed for Iran, Panama and Thailand and are pending approval.

WORK UNIT NO. 072 - BODY OF REPORT

CONCLUSIONS:

Continuing support was provided national and international nutrition agencies and their basic or applied nutrition programs and training activities.

PUBLICATIONS:

1. Sauberlich, H. E. World nutrition problems. Proc. Western Section of the Am. Soc. of Animal Sci., 1966.

2. "Requirements of Vitamin A, Thiamine, Riboflavine and Niacin," Report of a Joint FAO/WHO Expert Group. Rome, Italy, 1967.

ABSTRACT

PROJECT NO. 3A025601A822 Military Internal Medicine

~~TASK NO.~~ ~~01~~

WORK UNIT NO. 072 Studies in Human Nutrition

The following investigations have been conducted under this work unit:

STUDY NO. 1 Thiamine metabolism in man.

STUDY NO. 2 Riboflavin metabolism and requirements.

STUDY NO. 3 Vitamin C metabolism and requirement in man.

STUDY NO. 4 Measurement of carbon-14 in fecal samples.

STUDY NO. 5 The effects of short-term ingestion of contraceptive steroids on tryptophan metabolism in female subjects.

STUDY NO. 6 Development of a rapid, thin-layer, chromatographic procedure for measurement of various urinary metabolites of tryptophan.

1. Thiamine metabolism was investigated in human volunteer adult male subjects using 2-^{14}C-thiazole labeled thiamine and 2-^{14}C-pyrimidine labeled thiamine. The orally administered labeled thiamine was almost completely absorbed with no measurable amount of $^{14}CO_2$ detected in the respiratory air. The half excretion time of the ingested label occurred within 9 1/2 to 18 1/2 days and was related to the weight of the subject and the daily dietary intake of thiamine. The urinary metabolites of thiamine were found to be numerous. Several of the metabolites were isolated and identified. Additional studies were conducted on the metabolites employing a yeast assay method.

2. Uniformly labeled ^{14}C-riboflavin was synthesized by the organism, Ashbya gossypii, which was grown in a defined medium composed of labeled compounds that were incorporated into both the isoalloxazine ring and ribitol side chain of riboflavin. Riboflavin with a specific activity of 21 μc/mg. was extracted and purified. The uniformly labeled ^{14}C-riboflavin will be utilized in animal and human metabolic studies.

3. Studies were continued on evaluating the human requirement for ascorbic acid (vitamin C). Five human volunteer subjects were given a diet sufficient in

all nutrients except ascorbic acid, which was absent. The subjects remained on this diet until obvious signs of clinical scurvy appeared. At this time cscorbic acid was added to their diet in varying amounts and the effects of the graded levels on various clinical and biochemical parameters were studied. Labeling doses of radio-ascorbic acid were given during both the deficiency phase and during the ascorbic acid repletion phase. Since this study is still in progress, no conclusions can be presented at this time.

4. A simple, rapid method was developed for the measurement of ^{14}C-radio-activity in fecal samples. Dried aliquots of the fecal material is pressed into a disk and placed in a planchet and counted in a low-background, thin-window, gas-flow proportional counter.

5. Recent work has suggested that continual ingestion of contraceptive steroids leads to an increase in urinary tryptophan metabolites. This study, conducted jointly with the OB-GYN Clinic of Fitzsimons General Hospital, is an attempt to study the effects of contraceptive steroids on urinary levels of Xanthurenic Acid, kynurenic acid, 5-OH-tryptophan and 5-OH-Indole-3-Acetic Acid. The effects of oral ingestion of graded levels of pyridoxine on the urinary excretion of these metabolites is also under investigation. In limited studies, with female subjects, it has been observed that the use of contraceptive steroids increased the urinary excretion of several tryptophan metabolites. Oral ingestion of 10 mg. of pyridoxine per day prevented these increases.

6. A rapid method has been developed for semi-quantitation of the urinary metabolites of tryptophan. The metabolites measured include xanthurenic acid, kynurenic acid, 5-OH-tryptophan and 5-OH-indole-3-acetic acid. The method is based upon the use of thin layer chromatography separation of the urinary metabolites, followed by automatic scanning of the plates and recording of results with a microfluorometer. Further improvement in the system is anticipated to yield a rapid, reliable quantitative procedure.

BODY OF REPORT

WORK UNIT NO. 072 Studies in Human Nutrition

STUDY NO. 1 Thiamine metabolism in man

(a) Identification of thiamine and thiamine-like compounds extracted from yeast following incubation with urine.

PROBLEM:

In 1962 a publication from this Laboratory described a method for determining the metabolites of thiamine in urine. It was established that baker's yeast could quantitatively convert the pyrimidine and thiazole moieties of thiamine to the intact vitamin B_1. This method was applied to the urines of subjects placed on a diet with a restricted intake of the vitamin at levels below those deemed sufficient. After 18 days on the diet the level of thiamine excreted in the urine dropped to below limits detectable by thiochrome assay. At the same time the metabolite excretion increased from 588 µg/day to 913 µg/day.

Because of the rise in thiamine metabolites at a time when the subject was deficient or nearly deficient in thiamine, the question of specificity of the yeast resynthesis method and therefore its validity was raised. It is apparent that the usefulness of the yeast resynthesis method for studying thiamine metabolism via urinary metabolite excretion patterns would be seriously impaired if the products being measured bore little or no relation to thiamine ingested or metabolites excreted. On the other hand, establishing the validity of the yeast resynthesis method would assure investigators that the use of this method would provide a quantitative and specific tool for studying the metabolism of thiamine in the human.

RESULTS AND DISCUSSION OF THE RESULTS:

Our studies thus far have shown that there is no measurable quantity of pyrimidine and thiazole synthesized by baker's yeast from amino acids during the incubation period of the yeast assay.

In order to isolate and identify the thiamine products formed by the yeast during incubation, it has been necessary to develop a method whereby this isolation is accomplished. Toward this end we have devised a thin layer chromatographic procedure which is rapid and provides adequate resolution of products which may be found in yeast and are related to thiamine or interfere with the thiochrome assay of thiamine. We have been able to separate thiamine, α-hydroxyethylthiamine, pyrimidine carboxylic acid, pyrimidine sulfonic acid, the pyrimidine moiety of thiamine, the thiazole moiety of thiamine, thiochrome, N'-methylnicotinamide and the alkaline ferricyanide fluorescent product of N'-methylnicotinamide oxidation. Attempts to achieve chemical isolation of the products extracted from baker's yeast following incubation with urine have not yet been successful because these compounds are at the microgram level in a very concentrated salt solution. Efforts are still continuing. Attempts will be made to desalt by Sephadex columns although the molecular weight differences between thiamine and the salt are not very large.

CONCLUSIONS:

None at present.

Thiamine metabolites present in urine are under study using a yeast assay procedure. Conclusion cannot be made from the results thus far obtained.

PUBLICATIONS:

Waring, P.P., W.C. Goad and Z.Z. Ziporin. The use of thin-layer chromatography to separate thiamine and related compounds as well as N-methylnicotinamide and related compounds. To be published in Analytical Biochemistry (accepted for publication).

(b) The metabolism of ^{14}C-labeled thiamine in man.

PROBLEM:

Although considerable efforts have been directed toward the elucidation of thiamine metabolism in the rat, relatively little is known of its catabolic fate in

man. Investigations were designed to study the nature of the urinary of thiamine catabolism in man using two types of radioactive thiamine. Information obtained from such studies would assist in evaluating human requirements for thiamine.

RESULTS AND DISCUSSION OF THE RESULTS:

Absorption, Excretion and Oxidation of the Labeled Thiamine in Man. Four healthy young male volunteers were placed on a cyclic diet which provided a constant thiamine intake. After the consumption of this diet for three days, the labeled thiamine was ingested in a fasting state. Three of the subjects received a single dose of 50 mCi 2-^{14}C-thiazole labeled thiamine (0.67 mg). The fourth subject received 2-^{14}C-pyrimidine labeled thiamine. Because of the lower specific activity of the pyrimidine labeled thiamine, administration of this material was modified in order to adequately label the body thiamine pool and thereby provide sufficient radioactivity in the urine. This subject, therefore, was given each morning in a fasting state for five consecutive days 5.6 mCi of 2-^{14}C-thiazole labeled thiamine (1.04 mg) for a total of 28 mCi. In this manner, the total daily thiamine intake for each subject was maintained within normal dietary and physiological levels.

The measurement of radioactivity present in the fecal samples obtained from two subjects which received 2-^{14}C-thiazole labeled thiamine, demonstrated the presence of less than 1% of the administered radioactive dose in the first and second day fecal samples indicating almost complete absorption. On the third day, however, a significant increa was observed in fecal radioactivity, which may be due to re-excretion of thiamine or its metabolites into the intestinal lumen. Less than 5% of the administered dose of radioactivity was accounted for in the 5-day fecal collection.

The expired $^{14}CO_2$ was followed on a Vibrating Reed Respiration Pattern Analyzer, three times a day, one hour each time, for five days, after the ingestion of labeled thiamine in each of the subjects. No measurable amount of $^{14}CO_2$

could be detected in the respiratory air of any of the subjects who had received either 2-^{14}C-thiazole labeled or ^{14}C-pyrimidine labeled thiamine, indicating less than 1% conversion of the administered dose to $^{14}CO_2$.

Half Excretion Time of 2-^{14}C-Thiazole Labeled Thiamine. A 0.5 ml aliquot of the daily urine was counted in a liquid scintillation spectrometer for the measurement of the total radioactivity present in 24 hour urine. Subject A had a half excretion time of 18 1/2 days. He weighed 90 kg and was consuming daily 1.35 mg thiamine in his diet. Subject B weighed 95 kg and had a constant daily dietary intake of 1.8 mg of thiamine. The half excretion time of the ingested label for this subject was 13 days. The excretion curve of Subject C, however, indicated a half excretion time of 9 1/2 days. This subject weighed only 69 kg and the amount of unlabeled thiamine in his diet accounted for a daily intake of 2.1 mg. Thus, the higher intake level of thiamine and a smaller body size caused a faster turnover rate in this subject. It is perhaps noteworthy to mention that changes in the slope of the excretion curve appeared to occur on days 1, 2 or 3, and probably 7 which suggests the presence of more than one miscible pool of thiamine in the human.

Studies On the Urinary Metabolites of 2-^{14}C-Thiazole and 2-^{14}C-Pyrimidine Labeled Thiamine in Man. The urinary radioactive metabolites of 2-^{14}C-thiazole labeled thiamine and 2-^{14}C-pyrimidine labeled thiamine were first concentrated and then fractionated by Amberlite CG-50 column chromatography. Two large and two small peaks were obtained from 2-^{14}C-thiazole labeled thiamine and two large and one small peak from 2-^{14}C-pyrimidine labeled thiamine. These peaks accounted for 98-100% of the radioactivity applied to the column. The content of the tubes collected from the columns were combined into the indicated respective peak fractions, mixed, and a small aliquot was taken for liquid scintillation counting. The radioactivity of each peak, as the per cent of the total radioactivity applied to the Amberlite column was then calculated.

More than 90% of the radioactivity in peak IV was accounted for as free thiamine. Peak II was observed to contain more than 10 metabolites of thiamine.

In one subject, approximately 46% of the total radioactivity present in the first 24-hour urine collection when applied on the Amberlite CG-50 column appeared in peak IV, while in a second subject similarly studied, this peak contained 41% of the total radioactivity. The per cents of radioactivity present in peak III for these subjects were 41% and 52%, respectively. In the second 24-hour urine collection, however, the percentage of radioactivity in peak IV, in both subjects, decreased to 26% of the total radioactivity, while the percentage of radioactivity in peak III increased to approximately 61% and this proportion remained constant thereafter. Apparently, after the excess thiamine had been excreted into the urine during the first 24-hour period along with attainment of thiamine equilibrium with the deep pool, each day thereafter, a portion of the radioactive thiamine present in the body was converted into its metabolites and excreted subsequently into the urine. Also, it should be mentioned that the percentage of radioactivity that appeared in peak III in the first 24-hour urine excretion of the heavier subject was more than that noted for the lighter subject (52% in comparison to 41%). This difference may be due to the differences in body sizes and the levels of thiamine intake.

Microbiological Assay of the Radioactive Peaks. The radioactive peaks obtained from Amberlite CG-50 column chromatography were reduced in volume, under vacuum, and an aliquot was taken from each peak for L. viridescens assay. It was mentioned previously that peak IV contains mainly free thiamine. However, the sum of the biological activity for L. viridescens obtained from peaks II and III, separately, was equal to that of peak IV. Thus, when urine is assayed microbiologically for its thiamine content, it should be recognized that only 50% of the activity present may be actually free thiamine. This is, indeed, in agreement with the observations of Balaghi and Pearson who found that some of the urinary thiamine metabolites in rat are not thiochrome positive but have some activity for this organism.

Autoradiography. Similar concentrated radioactive peaks obtained from the subjects who had received either ^{14}C-thiazole or ^{14}C-pyrimidine labeled thiamine were spotted side by side, in a linear fashion, on cellulose thin layer plates for development in a solvent system consisting of n-propanol/acetate buffer/water. The chromatograms were then marked with radioactive ink on the corners and left in contact with x-ray films for preparation of autoradiograms.

When ^{14}C-pyrimidine labeled thiamine was used, peak II contained one radioactive metabolite which has an R_f value identical to that of authentic pyrimidine carboxylic acid when chromatographed in the n-propanol/acetate buffer/water solvent system. This metabolite accounted for 1.64% of the total administered radioactive dose in the first 24 hours' excretion following the ingestion of labeled thiamine.

Isolation and Identification of 4-Methyl-Thiazole-5-Acetic Acid as a Urinary Metabolite of Thiamine in Man. One of the major metabolites in peak II, resulting from the ingestion of 2-^{14}C-thiazole labeled thiamine, accounted for more than 70% of the radioactivity present in this peak. This metabolite appeared near the solvent front when the n-propanol/acetate buffer/water solvent system was used in the thin layer chromatography separation. This compound was isolated in sufficient quantity from the urine by thin layer chromatography and cochromatographed in four different solvent systems with an authentic sample of 4-methyl-thiazole-5-acetic acid. After development, the plates were reviewed under ultraviolet light in order to lactate the authentic sample. The thin layer chromatography plates were then exposed to x-ray film to locate the position of the unknown metabolite. This unknown compound had an R_f value identical to that of the authentic sample in all solvent systems used. On the basis of co-chromatographic and recrystallization evidences, the identity of this metabolite as that of 4-methyl-thiazole-5-acetic acid was proved beyond any reasonable doubt. From a quantitative viewpoint, this compound accounted for, during the first and second day following ingestion of the label, only 0.2% and 0.12%, respectively, of the radioactivity administered.

Studies on the Metabolites Present in Peak III.

(1) 4-Methyl-5-B-Hydroxyethyl-Thiazole: A Urinary Metabolite of Thiamine in Man? As was mentioned previously, a major portion of the ingested radioactivity appears in the urine in the fraction termed "peak III." This peak contained numerous metabolites following the ingestion of either of the forms of labeled thiamine.

One of the metabolites of ^{14}C-thiazole labeled thiamine in peak III migrates with the solvent front in the n-propanol/acetate buffer/water solvent system. This metabolite, which was not present in peak III when ^{14}C-pyrimidine labeled thiamine was administered, was scraped and eluted from the cellulose thin layer chromatography plates and re-chromatographed in four different solvent systems in comparison with an authentic sample of 4-methyl-5-B hydroxyethyl thiazole, i.e., the thiazole moiety of thiamine. The autoradiographs obtained from these co-chromatograms showed identical R_f values for both the unknown metabolite and the authentic sample, which was localized on the plates by its ultraviolet quenching characteristics. Thus, on the basis of co-chromatographic evidence, this metabolite was tentatively identified as the thiazole moiety of thiamine. This metabolite, however, accounted in the first 24-hour urine excretion for only 0.05% of the radioactivity of the ^{14}C-thiazole labeled thiamine ingested. Thus, it would appear that the major portion of the thiazole moiety of thiamine produced by the cleavage of thiamine in the body undergoes oxidation to thiazole acetic acid. A similar finding has been noted for the rat.

(2) Is Thiamine Pyrophosphate a Urinary Metabolite of Thiamine in Man? One of the metabolites in peak IV, which was observed when either ^{14}C-pyrimidine or ^{14}C-thiazole labeled thiamine was ingested, appeared near the origin in thin layer chromatograms developed in the n-propanol/acetate buffer/water solvent system. This metabolite, tentatively named Metabolite III-1, was isolated both

from the urine of a subject labeled with ^{14}C-pyrimidine thiamine and the urine of a subject labeled with ^{14}C-thiazole thiamine. The metabolites isolated from these two sources were co-chromatographed by thin layer chromatography in different solvent systems and showed identical R_f value in all solvent systems used. Thus, it was established that the metabolite contained both moieties of thiamine in the molecule. In addition, L. viridescens assay of this metabolite revealed a biological activity similar to that of free thiamine (on the basis of specific activity). This metabolite was then re-chromatographed by thin layer chromatography in various solvent systems in comparison to that of authentic samples of thiamine monophosphate and thiamine diphosphate. For further confirmation, a mixture of Metabolite III-1 and 10 mg of unlabeled thiamine diphosphate was re-crystallized four times from a solution of acetone and absolute ethanol. Although the specific activities in the last two recrystallizations remained almost constant, a slight gradual decrease was noticed. This slight decrease in specific activity, however, might have been due to a slow decomposition of the labile thiamine diphosphate under the rather rigorous conditions of recrystallization. Thus, Metabolite III-1 has been tentatively designated as a phosphorylated derivative of thiamine.

(3) Studies on Metabolite III-5. As the urinary radioactivity decreased during the days after the ingestion of the labeled thiamine, most of the urinary radioactive metabolites tended to disappear. However, one of the catabolic products of thiamine in peak III, which was tentatively named Metabolite III-5 continued to contain a high level of radioactivity throughout the experiment. The amount of this metabolite in the first 24-hour urine collection represented 2% of the total administered radioactive dose. Thus, efforts were made to identify this particular major metabolite. This metabolite was present in peaks III obtained from urine of subjects administered either form of the labeled thiamine and thus the metabolite apparently contained both moieties of the thiamine molecule. To

further verify the presence of both moieties of the thiamine molecule in the metabolite, the metabolite was re-chromatographed in various solvent systems. In addition, co-chromatography of the metabolite isolated from urines obtained from the two specific types of thiamine labeling were performed. Identical R_f values were obtained with all solvent systems used and, thus, it was concluded that Metabolite III-5 contained both moieties of the thiamine molecule. Metabolite III-5 did not possess a biological activity for L. viridescens. Ultraviolet absorption spectra and infrared spectroscopy studies indicated that Metabolite III-5 had a complex structure. During the course of purification of this metabolite by Sephadex G-10 gel filtration (column 1 x 40 cm), it was found that the elution volume for this metabolite was almost equal to that of the void volume of the column. In other words, the ratio of the elution volume (Ve) to the void volume (Vo) was 1 ($\frac{Ve}{Vo} = 1$). The same ratio for thiamine hydrochloric (mol. wt. = 237.27) was found to be 4.5 ($\frac{Ve}{Vo} = 4.5$). This suggested that Metabolite III-5 should have a molecular size much larger than that of a simple thiamine derivative. The molecular weight of Metabolite III-5 was estimated to be in the vicinity of 25,000. Thus, it appeared that this metabolite was a combination of thiamine (or a derivative of thiamine) and a high molecular weight compound, perhaps a peptide or protein. To investigate this possibility, a new batch of this metabolite was isolated from peak III and purified twice by cellulose thin layer chromatography and four times, by recycling on Sephadex G-10 and G-100 columns. Each time the radioactive peak was collected, its volume reduced under vacuum and reapplied to another column. The final preparation was hydrolyzed in 6 N hydrochloric acid at 6.8 kg pressure and 121° C for 8 hours. The hydrolyzed material gave a positive ninhydrin reaction. A quantity of the hydrolysate was then analyzed for its amino acid constituents. Except for cystine and methionine, all commonly occurring amino acid were present. It was of interest to note that a considerable amount of histidine and lysine were present, providing probably a rather basic compound.

CONCLUSIONS:

The metabolism of 2-^{14}C-thiazole labeled thiamine and 2-^{14}C-pyrimidine labeled thiamine was studied in four healthy young adult men. The orally administered labeled thiamine was almost completely absorbed with no measurable amount of $^{14}CO_2$ detected in the respiratory air. The half excretion time of the ingested label occurred within 9 1/2 days to 18 1/2 days and was related to the weight of the subject and the daily dietary intake of thiamine.

The urinary radioactive metabolites of thiamine were fractionated by Amberlite CG-50 column chromatography. Four radioactive peaks were obtained from the urine of three subjects who had ingested 2-^{14}C-thiazole labeled thiamine and three peaks from the urine of a subject who had received 2-^{14}C-pyrimidine labeled thiamine. Each peak was tested separately for its biological activity for L. viridescens. Approximately 50% of the thiamine activity present in the urine was thus found due to metabolites other than that of free thiamine.

The urinary radioactive metabolites resulting from both types of labeled thiamine administration were further separated by thin layer chromatography and autoradiography of the radioactive peaks obtained from the column chromatography. The use of 2-^{14}C-pyrimidine labeled thiamine resulted in 13 metabolites, of which four were major, while 2-^{14}C-thiazole labeled thiamine produced 31 metabolites, of which six were major. One of the major urinary metabolites of 2-^{14}C-thiazole labeled thiamine was positively identified as 4-methyl-thiazole-5-acetic acid. Also the thiazole moiety of thiamine and thiamine diphosphate were tentatively identified as two minor catabolic products of thiamine. One of the major urinary metabolites of thiamine, which continued both the pyrimidine and the thiazole moieties, appeared to be a conjugation of thiamine or a derivative with a peptide moiety.

Studies in Human Nutrition

PUBLICATIONS:

1. Ariaey-Nejad, M.R., M. Balaghi, E.M. Baker, and H.E. Sauberlich. Studies on thiamine metabolism in man (Submitted OTSG for clearance prior to submission for publication).

2. Canham, J.E., E.M. Baker, N.Raica, Jr., and H.E. Sauberlich. Vitamin B_6 requirement of adult men. Proc. Seventh Int. Cong. Nutr., 5, 558 (1967).

3. Tillotson, J.A., H.E. Sauberlich, E.M. Baker and J.E. Canham. Use of carbon-14 labeled vitamins in human nutrition studies: pyridoxine. Proc. Seventh Int. Cong. Nutr., 5, 554 (1967).

4. Sauberlich, H.E. Biochemical alterations in thiamine deficiency -- their interpretation. Am. J. Clin. Nutr., 20, 528 (1967).

STUDY NO. 2 Riboflavin metabolism and requirements

PROBLEM:

In a previous study (Amos, W.H. Jr., M. Balaghi, O. Ramirez, Jr. and H.E. Sauberlich. Metabolism of ^{14}C-riboflavin in the rat. Fed. Proc 25:245, 1966) riboflavin with the activity on the second carbon (2-^{14}C-riboflavin) of the isoalloxazine ring failed to be metabolized to $^{14}CO_2$ in the rat. Therefore, in order to study the metabolic fate of ^{14}C-riboflavin it was necessary to synthesize uniformly labeled riboflavin. If the uniformly labeled riboflavin provides the same labeled metabolites as the 2-^{14}C-riboflavin, then the less expensive 2-^{14}C-riboflavin can be utilized in future metabolic studies related to this nutrient.

RESULTS AND DISCUSSION OF THE RESULTS:

Ashbya gossypii, ATTC #10895, was cultured and maintained on a medium containing 2.0% glucose, 1.0% peptone, 0.5% yeast extract and 1.8% agar. Slants of the medium were made after autoclaving at 15 lbs pressure for 30 minutes. Cultures were incubated at 28° C, ±1°, for 4 days and then refrigerated.

To prepare seed cultures for use as inocula for the defined liquid medium, highly pigmented areas of the cultures were serially transferred, using a sterile spatula, so that cultures after 24-48 hours incubation at 28° C, ±1°, consistently produced pigmentation. After 4 days growth these cultures were used to inoculate the various liquid media which are listed in tables 1, 2, 3 and 4. Table 1 shows the defined minimal liquid medium, initially used, for growth and flavogenesis of A. gossypii.

TABLE 1

Defined Minimal Liquid Medium for Growth and Flavogenesis of Ashbya gossypii

A.	Basal Salts	
	KH_2PO_4	0.1%
	K_2HPO_4	0.1%
	NaCl	0.05%
	$MgSO_4 \cdot 7 H_2O$	0.05%
B.	Glucose	2.0%
C.	Vitamin Mixture (Add per 100 ml medium)	
	Inositol	3.0 mg
	Thiamine-HCl	0.1 mg
	D-biotin	2.0 µg
D.	Amino Acids (per 100 ml medium)	
	L-Aspartic acid	210 mg
	L-Arginine-HCl	10 mg
	Glycine	30 mg
	L-Histidine	33 mg
	L-Leucine	25 mg

Improvements of this medium are shown in tables 2, 3 and 4 which show results of the effects of amino acid combinations, folic acid, sodium formate, glucose concentration and traces of peptone and yeast extract.

Table 2

Effect of Amino Acid Combinations on Growth and Flavogenesis of Ashbya gossypii

Medium	Growth	Flavogenesis
DMLM- Complete[1]	++ (average)	+ (slight)
" less histidine	++ (average)	+ (slight)
" less leucine, aspartic acid plus 210 mg glutamic acid	+ (minimal)	0 (none)
" less histidine, leucine	+++ (good)	+++ (good)
" less histidine, aspartic acid	++ (average)	+ (slight)
" less histidine, leucine,	++ (average)	++ (average)
Complex[2] (Control)	++++ (very good)	++++ (very good)

1. DMLM is the defined minimal liquid medium of Table 1 composed of A. Basal Salts; B. Glucose; C. Vitamin mixture; D. Amino acids.

2. Complex control medium composed of A. as above; B. as above; C. 0.5% yeast extract; D. 0.5% peptone.

TABLE 3

Effect of Folic Acid and Sodium Formate on Growth and Flavogenesis of Ashbya gossypii

Medium	Growth	Flavogenesis
DMLM-Complete[1] plus folic acid plus sodium formate	+++ (good)	++++ (good)
" less folic acid	+++ (good)	++ (average)
" less folic acid and sodium formate	+++ (good)	++ (average)
Complex[2] (Control)	++++ (very good)	++++ (very good)

1. DMLM is the defined minimal liquid medium of Table 1 with changes in the composition as follows: A. Basal Salts; B. 1.0% glucose; C. Vitamin mixture plus 0.1 mg folic acid; D. Amino Acids, total of 5 changed by deleting histidine and leucine. Aspartic acid decreased to 150 mg; E. 37.8 mg sodium formate/100 ml medium.

2. Complex control medium composed of A. as above; B. as above; C. 0.5% yeast extract; D. 0.5% peptone.

TABLE 4

Effects of Glucose Concentration and Traces of Peptone and Yeast Extract on Growth and Flavogenesis of Ashbya gossypii

Medium	Glucose Conc.	Growth	Flavogenesis
DMLM-Complete[1] plus peptone plus yeast extract	1.0%	+++ (good)	+++ (good)
" less peptone	1.0%	+++ (good)	+++ (good)
" less peptone less yeast extract	1.0%	++ (average)	++ (average)
DMLM-Complete[2] plus peptone plus yeast extract	0.5%	+++ (good)	++ (average)
" less peptone	0.5%	++ (average)	++ (average)

1. DMLM is the defined minimal liquid medium of Table 3. In addition 0.1% peptone; 0.1% yeast extract. 2. DMLM is the defined minimal liquid medium, as above, except glucose concentration is 0.5%.

Table 5 lists the components of the improved liquid medium which was used finally for production of the uniformly labeled ^{14}C-riboflavin.

TABLE 5

Composition of the Defined Liquid Medium Used for the Production of Uniformly Labeled ^{14}C-Riboflavin by Ashbya gossypii

Component	Wt/100 ml Medium	Total ^{14}C (μc)	Specific Activity μc/mg C
D-glucose-U-^{14}C	1000 mg	10,000	25.0
Glycine-1,2-^{14}C	30 mg	240	25.0
L-aspartic acid-U-^{14}C	150 mg	1,350	25.0
L-arginine-U-^{14}C (mono HCl)	11.9 mg	1,000	25.0
Sodium formate-^{14}C	38 mg	168	25.0
Yeast Extract, Difco	100 mg	-	-
Inositol	3 mg	-	-
D-Biotin	0.002 mg	-	-
Thiamine·HCl	0.10 mg	-	-
Folic acid	0.10 mg		
KH_2PO_4	100 mg	-	-
K_2HPO_4	100 mg	-	-
NaCl	50 mg	-	-
$MgSO_4 \cdot 7 H_2O$	50 mg	-	-

Fifty ml of the medium (Table 5) was added into each of two 250 ml Ehrlenmeyer flasks which were set up with a compressed air circulation system containing a 10% NaOH inlet trap to remove CO_2 from the compressed air, sterile cotton air filters, and two 1 liter outlet traps containing methyl cellosolve:monoethanolamine (2:1 v/v) to trap expired $^{14}CO_2$. The inoculum was prepared by aseptically transferring the

pigmented mycelial mat of fresh 4 day old cultures into sterile water and macerating this with a pipette. The slightly turbid, very yellow supernatant was inoculated into the flasks which were then incubated in a 28° C shaking water bath for 7 days in a darkened room with continuous aeration. At the end of the incubation period 12.5 ml of 0.123 M sodium acetate buffer, pH 4.6, was added to each flask, after which the flasks were autoclaved and the cells removed by filtration. The filtrate was concentrated to approximately 15 ml on the flash evaporator under subdued light. All procedures henceforth were carried out in a darkened room.

One hundred fifty ml of boiling acetone was added to the concentrate and proteins were precipitated overnight in the freezer compartment of the refrigerator. The supernatant was concentrated and again treated with acetone for removal of residual protein. The supernatant was then extracted with ethyl ether to remove lipids and the aqueous phase chromatographed on a 2 X 20 cm florosil column, which was eluted with water, 2% acetic acid, additional water and pyridine-water mixtures from 1 to 10% pyridine. Riboflavin was eluted with 5-10% pyridine in water. This eluate was concentrated to about 100 ml. Chloroform was used to remove the pyridine in the eluate, leaving the aqueous phase containing the riboflavin. This aqueous phase was filtered on a moistened filter paper to remove suspended chloroform and again concentrated to approximately 30 ml. The preparation, divided into two 50 ml centrifuge tubes, was placed in the freezing compartment of the refrigerator. A gel-like material separated out and this was centrifuged off.

The clear riboflavin containing supernatant was applied to a 1 mm thick layer of silica gel G and chromatographed in glacial acetic acid:Acetone:methanol:benzene (5:5:20:70 v/v). Riboflavin with a R_f of about 0.3 was scraped off the plate and eluted with water. Two minor bands of a higher R_f were later identified as lumiflavin and lumichrome by paper chromatography in butanol:acetic acid:water (4:1:5 v/v). The eluted riboflavin was concentrated and the $CaSO_4$ binder from the silica gel G

removed by centrifugation. The concentrated riboflavin was chromatographed on Whatman number 3 mm paper in butanol:acetic acid:water (4:1:5) and eluted with water. The purified riboflavin band was concentrated to a small volume and crystallized from water-acetone mixture. The crystals were redissolved in water and concentrated to exactly 100 ml from which a 1 ml aliquot was removed for microbiological assay of riboflavin and for radioactivity determination. The remaining 99 ml riboflavin solution was divided into 5 ampoules, 4 containing 20 ml each, and 1, 19 ml. These ampoules were lyophilized and sealed in vacuo. Lactobacillus casei, ATTC #7469, was employed as the test organism in determining the total yield of 1.46 mg riboflavin. The specific activity of the riboflavin was 21.0 μc/mg.

Examination of the lumiflavin and lumichrome bands, artifacts of the isolation procedure, confirmed that both the ribitol side chain and the isoalloxazine ring contained ^{14}C.

CONCLUSIONS:

Riboflavin ^{14}C, uniformly labeled, with a specific activity of 21 μc/mg was prepared and is available for future use in animal riboflavin nutrition studies.

PUBLICATIONS:

None at present; manuscript in preparation.

STUDY NO. 3 Vitamin C metabolism and requirements in man

PROBLEM:

Studies were designed cooperatively between the Metabolic Ward at the University Hospitals, University of Iowa and the Chemistry Division, USAMRNL, to study vitamin C (ascorbic acid) requirements and metabolism in man.

The objective of these studies are:

a. To induce in healthy men (prison volunteers) a deficiency of ascorbic acid.

b. To label their ascorbic acid pools with L-ascorbic-1-^{14}C acid in order to study (a) total body pool size; (b) rate of depletion; (c) minimal requirements for vitamin C; (d) the relationship between symptoms and signs of scurvy and levels of the vitamin body pool; (e) the amount of ascorbic acid necessary to replete the body pool and to alleviate signs and symptoms of deficiency; and (f) theories concerning the physiologic functions of ascorbic acid based on metabolites in blood and urine.

RESULTS AND DISCUSSION OF THE RESULTS:

The initial vitamin C deficiency study conducted on prison volunteers has been completed and the findings have been submitted for publication (see "Publications"). The results and conclusion of this study were presented in the 1967 Annual Progress Report.

A second and more extensive study on ascorbic acid metabolism and deficiency was initiated this past year. This study began on 17 October 1968 with six human volunteers from the Iowa State Prison. The subjects are housed at the Metabolic Ward, Department of Internal Medicine, University Hospital, University of Iowa, Iowa City, Iowa. The subjects were conditioned on a normal diet for one week. At the end of this period each man received 40.1 μCi of L-Ascorbic Acid-1-^{14}C. Twenty-three days later, at the time when urinary free ascorbic acid fell to low levels, all subjects received 121 μCi of radioascorbic acid. It was hoped that the delay in relabeling would allow a more complete retention of the labeled ascorbic acid. Shortly after the second labeling the subjects went on a 30 day mineral depletion. The effect of this depletion on rate of urinary ^{14}C excretion was studied. After approximately 100 days of deficiency, at the point when marked clinical signs of scurvy appeared, labeled ascorbic acid, 1μc/day, in amounts of 4, 64 and 128 mg per day were added to the ascorbic acid free diet. Two men on 4 mg/day, two on 64 mg/day and one on 128 mg/day.

This graded supplement period will continue to 1 June 1968 at which time very high levels, 250 and 500 mg/day will be fed. In addition to the labeled supplementary ascorbic acid, during the last part of the graded supplement phase, the diet was changed. The new diet provides approximately 2.5 mg/day of ascorbic acid.

Routine clinical studies are being carried out by the metabolic ward staff at the University of Iowa. This laboratory is responsible for radio assay of urine and feces, vitamin levels in urine and blood, proximate analysis of feces and urine creatine, nitrogen and oxalic acid and several other assays. All assays are proceeding at this time.

Only the preliminary results can be given at this time.

a. Urine ^{14}C excreting pattern in this study follows closely that of the previous vitamin C deficiency study.

b. The presence of radioactive material in the feces derived from the ingested labeled ascorbic acid has been confirmed.

c. No marked change in plasma vitamin A levels have been noted during the deficiency or repletion of ascorbic acid.

d. There have been no marked changes in the urinary nitrogen or creatinine excretion during the study.

e. The formula diet used in this study will induce scurvy in 100 to 110 days, quite independent of body size, age, or other physical parameters.

f. Preliminary results indicate that a dietary intake of ascorbic acid of between 4 and 6.4 mg/day will not alleviate all of the serious symptoms of scurvy in 60 days.

CONCLUSIONS:

Since this second vitamin C study has not been completed, final conclusion cannot be presented at this time. However, the initial study conducted on experimental human scurvy resulted in the following conclusions:

a. Labeling the body ascorbic acid pool during the depletion phase resulted in no detectable urinary excretion of ^{14}C-labeled reduced or dehydroascorbic acid.

b. Urinary Excretion of ^{14}C by all subjects occurred as a first order process during the depletion phase. The urinary-^{14}C excretion curves of the four subjects studied did not differ from the average man by more than ±3%, despite marked differences in body weight and age.

c. First symptoms of mild scurvy appeared in the subjects when their body ascorbic acid pool had been reduced to approximately 300 mg.

d. Once the body pool of ascorbic acid was repleted to a level of 1.5 g, urinary loss of reduced ascorbic acid occurred.

e. The rate of repletion of ascorbic acid was found to be a zero order process and proportional to the level of daily ascorbic acid intake.

f. When the subjects were fed a high intake of ascorbic acid, only a limited quantity of the ingested vitamin was equilibrated with the ^{14}C-labeled ascorbate pool.

g. All of the radioactivity excreted during the depletion phase was in the form of stable organic material that did not behave as ascorbic acid. This organic material was separated into four unknown compounds.

h. This study would indicate that a daily intake of less than 6.5 mg per day of ascorbic acid is sufficient to alleviate and prevent scurvy in man.

i. The four subjects who completed the study developed clinical signs compatible with a diagnosis of scurvy: swollen bleeding gums, conjunctival hemorrhages, and follicular hyperkeratosis of the thighs, buttocks, calves and the posterior aspects of the arms.

j. Blood counts, numerous biochemical measurements, the rate of wound healing and physiologic tests including electrocardiograms, electroencephalograms, basal-metabolic rates and blood coagulation studies showed no specific abnormalities as a result of induced scurvy.

k. Ascorbic acid requirement was increased by emotional stress, but was not influenced by other forms of stress investigated.

PUBLICATIONS:

1. Hodges, R.E., M. Baker, J. Hood, H.E. Sauberlich and S.C. March. Experimental scurvy in man. (Submitted for publication).

2. Hodges, R.E., et al. Metabolism of Ascorbic-^{14}C acid in experimental human scurvy (Submitted for publication).

3. Hood, J. and R.E. Hodges. Ocular lesions in scurvy (Submitted for publication).

4. Van Reen, R., N. Raica, Jr. and R.A. Nelson. Chapter 6. "Nutrition Studies" in Studies of the Naval Facilities Engineering Command Protective Shelter. II Summer Trials. NRL Report 6656, March 29, 1968.

STUDY NO. 4 Measurement of carbon-14 in fecal samples

PROBLEM:

The various ^{14}C isotopic labeling studies conducted at this laboratory required the development of a rapid, reliable method to determine the ^{14}C-activity present in fecal samples. The wet combustion method commonly employed was too cumbersome and time-consuming for application to the large number of samples requiring analysis.

RESULTS AND DISCUSSION OF THE RESULTS:

As a part of another study carried out in this laboratory a method of assaying for ^{14}C-labeled compounds in feces was developed. The method is an adaptation of a technique previously employed for the assay of high energy beta emmitters. The technique requires a very dry aliquot of the fecal material to be analyzed. Routinely the fecal material is homogenized and a weighed aliquot is removed and lyophilized to dryness. The dry cake is broken up and pressed into a 1 7/8" diameter disk in a steel die at pressure of 15,000 p.s.i. for one minute. Disks have been made

from weighed aliquots in the range of 0.9 to 2.0 gms. The pressed disk is not too fragile and can be weighed and handled if care is used. The weighed disk is then placed in a planchet and counted in a low-background, thin-window, gas-flow proportional counter. All disks prepared in the range 0.9 - 2.0 gms are more than infinitely thick to beta radiation emitted within the sample. Efficiency of counting the infinitely thick layer varies only slightly with varying weight disks. After counting, the total radioactivity contained in the disks is determined by reference to a standard curve prepared from feces of high, constant radioactivity prepared in the above manner. After radioassay the disk can, of course, be used in other types of fecal assays.

CONCLUSIONS:

The method described here does provide a rapid convenient method for assaying for ^{14}C in fecal material. Because of the large figure of merit (E^2/B) of low-background beta counters and the relatively large amount of sample counted the sensitivity of this method compares very favorably with liquid scintillation counting of combusted samples.

PUBLICATIONS:

None.

APPENIX VIII PP T B-3

STUDIES ON THIAMINE METABOLISM IN MAN[1,8]

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SHORT TITLE: THIAMINE METABOLISM IN MAN

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Although considerable efforts (1-5) have been directed toward the elucidation of thiamine metabolism in the rat, relatively little is known of its catabolic fate in man. Dorsook et al. (6) studied the metabolism of thiamine in man by injecting ^{35}S-labeled thiamine into a young healthy man for 15 days and found that a considerable amount of radioactivity appeared in his urine as inorganic sulfur. Despite extensive efforts, only two metabolites of thiamine have been positively identified in the urine of man and rat (2,3). These compounds are pyrimidine carboxylic acid and free thiamine. Recently, however, 4-methyl thiazole-5-acetic acid has been reported to be a urinary metabolite of thiamine and oxythiamine in the rat (7).

The present investigation was designed to study the nature of the urinary products of thiamine catabolism in man using two types of radioactive thiamine.

MATERIALS AND METHODS

Labeled Thiamine

2-^{14}C-thiazole labeled thiamine, having a specific activity of 25 mCi/mM was obtained from Nuclear Chicago Inc.[5] and 2-^{14}C-pyrimidine labeled thiamine was synthesized by Nuclear Research Chemical Inc.[6], and had a specific activity of 2.06 mCi/mM. The structure of thiamine and position of the labeled carbon atoms are shown in Figure 1.

Measurement of Radioactivity

A Packard Tricarb Liquid Scintillation Spectrometer, Model 3375, was used throughout the experiment. Bray's solution (8) was used as scintillator and the counts were corrected for blank and quenching by external standard techniques. For detection of radioactive peaks eluted from either Amberlite CG-50 or Sephadex columns, a small aliquot was taken from each fraction and counted in a Nuclear Chicago Mark 1 Liquid Scintillation Spectrometer. The

expired $^{14}CO_2$ following ingestion of the labeled thiamine was measured by a Cary 3802 Vibrating Reed Respiration Pattern Analyzer. For the measurement of radioactivity in feces, the 24-hour fecal samples were homogenized in water (10 times of its volume) and a portion was lyophilized overnight. A thin pellet (5.03 cm diameter; approximately 0.7 - 2.0 gm in weight) was subsequently made from the lyophilized material and its radioactivity measured in a Beckman Low Beta-II Thin-Window Gas-Flow Proportional Counter. The total radioactivity was then calculated for the 24-hour sample. Essentially identical results were obtained if the fecal samples were ground, lyophilized and the radioactivity determined by measuring the $^{14}CO_2$ evolved upon combustion.

Yeast and Microbiological Assay

Thiamine activity in urine and in the radioactive fractions obtained from urine by Amberlite CG-50 column chromatography was assayed with the use of Lactobacillus viridescens (ATCC 12706) (9, 10). Saccharomyces cerevisiae (Baker's yeast obtained from Standard Brands) was used in thiamine metabolite recoupling procedure (11).

Collection and Preservation of Urine and Feces

Complete 24-hour urinary collections were made for 18 days following the ingestion of the labeled thiamine. The urine was preserved by the addition of 1 ml of concentrated hydrochloric acid per liter of urine, layering with toluene, freezing, and storing frozen until analyzed. The fecal samples were collected in plastic bags, on the first through fifth days following ingestion of the labeled thiamine. The fecal samples were also kept frozen until analyzed.

Concentration of the Urinary Thiamine Metabolites

The urinary radioactive metabolites of thiamine were concentrated by the charcoal treatment and subsequent elution of the charcoal with a mixture of

pyridine/ethanol/water (2). The eluate was then reduced in volume, under vacuum, in a rotary evaporator to 5 ml and fractionated further by Amberlite CG-50 column chromatography.

Column Chromatography

For fractionation of the urinary radioactive metabolites of thiamine, Amberlite CG-50 ion exchange resin columns (1 x 40 cm; 200-400 mesh, H^+ form) were used. The columns were developed with water (200 ml) followed by a mixture of pyridine, acetic acid and water (7.5:1:91.5, by volume). Purification and estimation of the molecular weight of the thiamine metabolites were performed with the use of Sephadex G10 and G100 columns (1 x 40 cm) (12).

Thin Layer Chromatography

The radioactive peaks obtained from the Amberlite CG-50 columns were reduced in volume, under vacuum, in a rotary evaporator to 1-3 ml and subjected to cellulose thin layer chromatography for further separation of the radioactive metabolites. The following solvent systems were used for the development of cellulose thin layer chromatograms.

(a) n-propanol, 1M acetate buffer pH 5.0, water (7/1/2; V/V)
(b) 2-propanol, 0.2M acetate buffer pH 6.0, water (85/20/26; V/V)
(c) 2-propanol, 0.1 N Hcl (110.20; V/V)
(d) 2-propanol, ammonium hydroxide, water (20/1/2; V/V)
(e) chloroform, methanol, 0.1 N Hcl (41/81/10; V/V)
(f) chloroform, methanol, ammonium hydroxide (43/81/5; V/V)
(g) acetonitrile, water, formic acid (100/25/ up to pH 2.57)
(h) ethanol, ammonium hydroxide, water (20/1/2; V/V)

Ascending thin layer chromatography was carried out from 3-8 hours depending upon the solvent system used. Autoradiograms were made by exposing the chromatograms to Eastman Kodak No-Screen-X-ray films (20 x 25 cm) from

3-15 days. The radioactive bands were marked on the cellulose thin layer plates and were subsequently scraped and collected by a microsuction vacuum device. The radioactive metabolites were then eluted with distilled water from the cellulose with 1 to 2 ml of distilled water and saved for further characterization.

Ultraviolet and Infrared Spectroscopy

The ultraviolet spectra of the metabolites were obtained with the use of either a Beckman DK-2A Spectrophotometer or a Beckman DB attached to a continuous recording device. The infrared spectra were obtained with the use of a Perkin-Elmer 221 Spectrophotometer and the potassium bromide pellets were made in the conventional manner.

Amino Acid Analysis

A Beckman/Spinco amino acid system, modified for accelerated micro analyses, was used for the determination of amino acids in the urinary metabolites. The samples were hydrolyzed in 6 N hydrochloric acid at 6.8 kg pressure and 121° C for 8 hours. The procedure of Stein and Moore (13) was used for amino acid analysis of the samples.

For the purpose of clarity, some additional experimental procedures will be introduced in the following section.

RESULTS AND DISCUSSION

Absorption, Excretion and Oxidation of the Labeled Thiamine in Man

Four healthy young male volunteers were placed on a cyclic diet which provided a constant thiamine intake. After the consumption of this diet for three days, the labeled thiamine was ingested in a fasting state. Three of the subjects received a single dose of 50 mCi 2-^{14}C-thiazole labeled thiamine (0.67 mg). The fourth subject received 2-^{14}C-pyrimidine labeled thiamine. Because of the lower specific activity of the pyrimidine labeled thiamine, administration of this material was modified in order to adequately label the

body thiamine pool and thereby provide sufficient radioactivity in the urine. This subject, therefore, was given each morning in a fasting state for five consecutive days 5.6 mCi of 2-^{14}C-thiazole labeled thiamine (1.04 mg) for a total of 28 mCi. In this manner, the total daily thiamine intake for each subject was maintained within normal dietary and physiological levels.

The measurement of radioactivity present in the fecal samples obtained from two subjects which received 2-^{14}C-thiazole labeled thiamine, demonstrated the presence of less than 1% of the administered radioactive dose in the first and second day fecal samples indicating almost complete absorption. On the third day, however, a significant increase was observed in fecal radioactivity, which may be due to re-excretion of thiamine or its metabolites into the intestinal lumen. Less than 3% of the administered dose of radioactivity was accounted for in the 5-day fecal collection.

The expired $^{14}CO_2$ was followed on a Vibrating Reed Respiration Pattern Analyzer, three times a day, one hour each time, for five days, after the ingestion of labeled thiamine in each of the subjects. No measurable amount of $^{14}CO_2$ could be detected in the respiratory air of any of the subjects who had received either 2-^{14}C-thiazole labeled or ^{14}C-pyrimidine labeled thiamine, indicating less than 1% conversion of the administered dose to $^{14}CO_2$.

Half Excretion Time of 2-^{14}C-Thiazole Labeled Thiamine

A 0.5 ml aliquot of the daily urine was counted in a liquid scintillation spectrometer for the measurement of the total radioactivity present in 24 hour urine. The cumulative excretion curves of 2-^{14}C-thiazole labeled thiamine for the three subjects are shown in Figure 2. Subject A had a half excretion time of 18 1/2 days. He weighed 90 kg and was consuming daily 1.35 mg thiamine in his diet. Subject B weighed 95 kg and had a constant daily dietary intake of 1.8 mg of thiamine. The half excretion time of the ingested label for this subject was 13 days. The excretion curve of Subject C, however, indicated a half excretion time of 9 1/2 days. This subject weighed only 69 kg and the

amount of unlabeled thiamine in his diet accounted for a daily intake of 2.1 mg. Thus, the higher intake level of thiamine and a smaller body size caused a faster turnover rate in this subject. It is perhaps noteworthy to mention that changes in the slope of the excretion curve appeared to occur on days 1, 2 or 3, and probably 7 which suggests the presence of more than one miscible pool of thiamine in the human.

Studies On the Urinary Metabolites of 2-^{14}C-Thiazole and 2-^{14}C-Pyrimidine Labeled Thiamine in Man

The urinary radioactive metabolites of 2-^{14}C-thiazole labeled thiamine and 2-^{14}C-pyrimidine labeled thiamine were first concentrated and then fractionated by Amberlite CG-50 column chromatography as described previously (see Methods). Two large and two small peaks were obtained from 2-^{14}C-thiazole labeled thiamine and two large and one small peak from 2-^{14}C-pyrimidine labeled thiamine (Fig. 3). These peaks accounted for 90-100% of the radioactivity applied to the column. The content of the tubes collected from the columns were combined into the indicated respective peak fractions, mixed, and a small aliquot was taken for liquid scintillation counting. The radioactivity of each peak, as the per cent of the total radioactivity applied to the Amberlite column was then calculated.

Figure 4 is a bargraph showing the proportion of radioactivity in each of these peaks observed in urine obtained from two subjects for four consecutive days following the administration of 2-^{14}C-thiazole labeled thiamine. More than 90% of the radioactivity in peak IV was accounted for as free thiamine. Peak II was observed to contain more than 10 metabolites of thiamine. In one subject, approximately 46% of the total radioactivity present in the first 24-hour urine collection when applied on the Amberlite CG-50 column appeared in peak IV, while in a second subject similarly studied, this peak contained 41% of the total radioactivity. The per cents of radioactivity present in peak III for these subjects were 41% and 52%, respectively. In the second

24-hour urine collection, however, the percentage of radioactivity in peak IV, in both subjects, decreased to 21% of the total radioactivity, while the percentage of radioactivity in peak III increased to approximately 61% and this proportion remained constant thereafter. Apparently, after the excess thiamine had been excreted into the urine during the first 24-hour period along with attainment of thiamine equilibrium with the deep pool, each day thereafter, a portion of the radioactive thiamine present in the body was converted into its metabolites and excreted subsequently into the urine. Also, it should be mentioned that the percentage of radioactivity that appeared in peak III in the first 24-hour urine excretion of the heavier subject was more than that noted for the lighter subject (52% in comparison to 41%). This difference may be due to the differences in body sizes and the levels of thiamine intake.

Microbiological Assay of the Radioactive Peaks

The radioactive peaks obtained from Amberlite CG-50 column chromatography were reduced in volume, under vacuum, and an aliquot was taken from each peak for L. viridescens assay (9, 10). It was mentioned previously that peak IV contains mainly free thiamine. However, the sum of the biological activity for L. viridescens obtained from peaks II and III, separately, was equal to that of peak IV. Thus, when urine is assayed microbiologically for its thiamine content, it should be recognized that only 50% of the activity present may be actually free thiamine. This is, indeed, in agreement with the observations of Balaghi and Pearson (4) who found that some of the urinary thiamine metabolites in rat are not thiochrome positive but have some activity for this organism.

Yeast Resynthesis Assay

An aliquot of the same radioactive peaks which were used for L. viridescens assay were taken for yeast resynthesis assay (11). The results

obtained were in agreement with those found with the use of L. viridescens. For example, L. viridescens assay indicated the presence of 10.8 µg% of thiamine activity per ml of peak III obtained from column chromatography of the urine of the subject who had ingested 2-^{14}C-thiazole labeled thiamine, while the value obtained by the yeast resynthesis method for the same peak was 12 µg per ml. Repeated experiments gave similar results. Since L. viridescens cannot couple thiamine moieties to form thiamine (9, 10), the above data indicate that apparently yeast was not able to recouple the metabolites of thiamine in the individual peaks. The values obtained by Ziporin et al. (14) using the yeast assay might be due to either other compounds present in the urine rather than thiamine metabolites or to catabolic products of thiamine present in the different peaks which when combined and assayed can be coupled by yeast to form thiamine compounds.

Autoradiography

Similar concentrated radioactive peaks obtained from the subjects who had received either ^{14}C-thiazole or ^{14}C-pyrimidine labeled thiamine were spotted side by side, in a linear fashion, on cellulose thin layer plates for development in a solvent system consisting of n-propanol/acetate buffer/water. The chromatograms were then marked with radioactive ink on the corners and left in contact with x-ray films for preparation of autoradiograms.

A schematic representation of autoradiograms obtained from the chromatography of peaks II obtained from the urine of the subjects ingesting either ^{14}C-thiazole or ^{14}C-pyrimidine labeled thiamine is shown in Figure 5. When ^{14}C-pyrimidine labeled thiamine was used, peak II contained one radioactive metabolite which had an R_f value identical to that of authentic pyrimidine carboxylic acid when chromatographed in the n-propanol/acetate buffer/water solvent system. Further work was not attempted on this metabolite since it had been reported by Neal and Pearson (3) to be present in rat and human urine as a catabolic product of thiamine. This metabolite, however,

accounted for 1.64% of the total administered radioactive dose in the first 24 hours' excretion following the ingestion of labeled thiamine.

Isolation and Identification of 4-Methyl-Thiazole-5-Acetic Acid as a Urinary Metabolite of Thiamine in Man

One of the major metabolites in peak II, resulting from the ingestion of 2-^{14}C-thiazole labeled thiamine, accounted for more than 70% of the radioactivity present in this peak. This metabolite appeared near the solvent front when the n-propanol/acetate buffer/water solvent system was used in the thin layer chromatography separation. This compound was isolated in sufficient quantity from the urine by thin layer chromatography and co-chromatographed in four different solvent systems with an authentic sample of 4-methyl-thiazole-5-acetic acid, which had been prepared by the method of Curcedo and Teplin (15). After development, the plates were reviewed under ultraviolet light in order to locate the authentic sample. The thin layer chromatography plates were then exposed to x-ray film to locate the position of the unknown metabolite. This unknown compound had an R_f value identical to that of the authentic sample in all solvent systems used. The results are shown in Table 1.

To provide further evidence as to the identity of this metabolite as that of 4-methyl thiazole-5-acetic acid (TAA), approximately 100 µg of the unknown metabolite (calculated on the basis of radioactivity) were mixed with 10 mg of unlabeled TAA. The mixture was chromatographed on an Amberlite CG-50 column and the radioactive peak, which was eluted with distilled water, collected and its volume reduced, under vacuum, to 2 ml. The solution was left in a refrigerator overnight and the crystals obtained were harvested by centrifugation. A sample of the crystals were dissolved in 1 ml of 0.01 N HCl by warming and a 0.1 ml aliquot of this solution was diluted to 2 ml with another portion of 0.01 N HCl. The concentration of TAA in the final solution was determined spectrophotometrically and 1 ml of

this solution was counted in a liquid scintillation counter for 100 minutes. The counts were corrected for blank and quenching. The remaining crystals were dissolved in 0.3 ml boiling water and recrystallized first at room temperature and finally in a refrigerator. This procedure was repeated five times. The results are shown in Table II. After the first crystallization, the specific activity decreased, but remained constant with subsequent recrystallizations. Thus, on the basis of co-chromatographic and recrystallization evidences, the identity of this metabolite as that of 4-methyl-thiazole-5-acetic acid was proved beyond any reasonable doubt. From a quantitative viewpoint, this compound accounted for, during the first and second day following ingestion of the label, only 0.2% and 0.12%, respectively, of the radioactivity administered.

Studies on the Metabolites Present in Peak III

(1) 4-Methyl-5-β-Hydroxyethyl-Thiazole: A Urinary Metabolite of Thiamine in Man? As was mentioned previously, a major portion of the ingested radioactivity appears in the urine in the fraction termed "peak III." This peak contained numerous metabolites following either of the forms of labeled thiamine. A schematic representation of autoradiographs of the thin layer chromatograph plates of peaks III, that were isolated from urine of subjects administered either ^{14}C-thiazole labeled thiamine or ^{14}C-pyrimidine labeled thiamine, is shown in Figure 6.

One of the metabolites of ^{14}C-thiazole labeled thiamine in peak III migrates with the solvent front in the n-propanol/acetate buffer/water solvent system. This metabolite, which was not present in peak III when ^{14}C-pyrimidine labeled thiamine was administered, was scraped and eluted from the cellulose thin layer chromatography plates and re-chromatographed in four different solvent systems in comparison with an authentic sample of 4-methyl-5-β hydroxyethyl thiazole, i.e., the thiazole moiety of thiamine. The autoradiographs obtained from these co-chromatograms showed identical R_f values for both the unknown metabolite and the authentic sample, which was localized on the plates by its

ultraviolet quenching characteristics. These results are shown in Table III. It should be mentioned that on Amberlite CG-50 column chromatography the thiazole moiety of thiamine was observed to be eluted in peak III. Thus, on the basis of co-chromatographic evidence, this metabolite was tentatively identified as the thiazole moiety of thiamine. This metabolite, however, accounted in the first 24-hour urine excretion for only 0.05% of the radioactivity of the ^{14}C-thiazole labeled thiamine ingested. Thus, it would appear that the major portion of the thiazole moiety of thiamine produced by the cleavage of thiamine in the body undergoes oxidation to thiazole acetic acid. A similar finding has been noted for the rat (7).

(2) Is Thiamine Pyrophosphate a Urinary Metabolite of Thiamine in Man?

One of the metabolites in peak IV, which was observed when either ^{14}C-pyrimidine or ^{14}C-thiazole labeled thiamine was ingested, appeared near the origin in thin layer chromatograms developed in the n-propanol/acetate buffer/water solvent system. This metabolite, tentatively named Metabolite III-1, was isolated both from the urine of a subject labeled with ^{14}C-pyrimidine thiamine and the urine of a subject labeled with ^{14}C-thiazole thiamine. The metabolites isolated from these two sources were co-chromatographed by thin layer chromatography in different solvent systems and showed identical R_f value in all solvent systems used. Thus, it was established that the metabolite contained both moieties of thiamine in the molecule. In addition, L. viridescens assay of this metabolite revealed a biological activity similar to that of free thiamine (on the basis of specific activity). This metabolite was then re-chromatographed by thin layer chromatography in various solvent systems in comparison to that of authentic samples of thiamine monophosphate and thiamine diphosphate. The results of the chromatograms are shown in Table IV. For further confirmation, a mixture of Metabolite III-1 and 10 mg of unlabeled thiamine diphosphate was recrystallized three times from a solution of acetone and absolute ethanol. The specific activities of these recrystallization products, which had been obtained

in a manner described previously for identification of thiazole acetic acid, are shown in Table V. Although the specific activities in the last two recrystallizations remained almost constant, a slight gradual decrease was noticed. This slight decrease in specific activity, however, might have been due to a slow decomposition of the labile thiamine diphosphate under the rather rigorous conditions of recrystallization. Thus, Metabolite III-1 has been tentatively designated as a phosphorylated derivative of thiamine. Although it has been reported (2) that phosphorylated forms of thiamine do not normally occur in urine, the amount of this metabolite in the first 24-hour urinary collection represented only .25% of the administered radioactive dose. It is possible that an overflow of the circulating phosphorylated forms of thiamine might occur in the kidney. This indeed, is in agreement with the recent observation of Rindi et al. (16) who found that thiamine monophosphate is present in the plasma of the normal rat.

Studies on Metabolite III-5. As the urinary radioactivity decreased during the days after the ingestion of the labeled thiamine, most of the urinary radioactive metabolites tended to disappear. However, one of the catabolic products of thiamine in peak III, which was tentatively named Metabolite III-5 (Fig. 6), continued to contain a high level of radioactivity throughout the experiment. The amount of this metabolite in the first 24-hour urine collection represented 2% of the total administered radioactive dose. Thus, efforts were made to identify this particular major metabolite. Figure 6 clearly demonstrates that this metabolite was present in peaks III obtained from urine of subjects administered either form of the labeled thiamine and thus the metabolite apparently contained both moieties of the thiamine molecule. To further verify the presence of both moieties of the thiamine molecule in the metabolite, the metabolite was re-chromatographed in various solvent systems. In addition, co-chromatography of the metabolite isolated from urines obtained from the two specific types of thiamine labeling were performed. Table VI shows the R_f values obtained by co-chromatography of Metabolite III-5 obtained from the two sources. Identical

R_f values were obtained with all solvent systems used and, thus, it was concluded that Metabolite III-5 contained both moieties of the thiamine molecule.

(4) Thiochrome Reactivity and L. Viridescens Assay Activity of Metabolite III-5. An aliquot of this metabolite was spotted on a cellulose thin layer chromatography plate and chromatographed in a n-propanol/acetate buffer/water solvent system. The location of the radioactive spot was then determined by autoradiography and marked. When this metabolite was sprayed with an alkaline solution of potassium ferricyanide, no thiochrome type of compound was formed, indicating either the absence of an intact thiazole ring or a substituted C-2 in the thiazole ring. In addition, a free amino group at the C-4 position in the pyrimidine ring is also required for the formation of thiochrome type compounds. This metabolite did not possess any biological activity for L. viridescens.

(5) Sodium Metabisulfite Cleavage Studies. In order to study the nature of the linkage between the two ring systems present in thiamine and related derivatives, metabolite III-5, derived from both ^{14}C-thiazole and ^{14}C-pyrimidine labeled thiamine, was subjected to the bisulfite cleavage procedure (17). In addition, equal amounts of 2-^{14}C-pyrimidine labeled thiamine and 2-^{14}C-thiazole labeled thiamine (on the basis of radioactivity) isolated from urine, were subjected simultaneously to the same procedure. ^{14}C-pyrimidine and ^{14}C-thiazole labeled thiamine were both cleaved quantitatively, while Metabolite III-5 derived from either form of labeled thiamine remained unchanged; hence, Metabolite III-5 was not susceptible to the nucleophilic attack of sodium metabisulfite. The failure of the metabolite to react may be due to some changes in thiazole moiety of the molecule producing an alteration in the quaternary characteristics of the nitrogen in the thiazole ring. Another possibility is that of a conjugation at either the C-2 position of the thiazole moiety or the amino group at C-4 position of the pyrimidine ring to a bulky compound causing a steric hinderance of the nucleophilic attack of sodium metabisulfite on the methylene bridge of the molecule.

(6) Ultraviolet Absorption Spectra of Metabolite III-5. An ultraviolet spectrum of this metabolite was obtained according to the procedure of Balaghi and Pearson (5). The ultraviolet spectra of this metabolite, in comparison to that of thiamine and thiamine disulfide, is shown in Figures 7 and 8. The absorption peak at approximately 275 - 280 mμ is more pronounced after acidification. This peak was first interpreted as the absorption peak of the pyrimidine moiety of Metabolite III-5. Later studies, however, revealed that this metabolite is apparently conjugated to a protein moiety and, therefore, this peak may be related to the absorption peak of proteins at 280 mμ. In fact, this is very probable since the major portion of the Metabolite III-5 molecule appears to be that of a protein or polypeptide (see below).

(7) Infrared Spectroscopy Studies on Metabolite III-5. Metabolite III-5, providing 500,000 dpm from ^{14}C, was purified by cellulose thin layer chromatography employing a n-propanol/acetate buffer/water solvent system. The final preparation was dissolved in 1 ml of distilled water and purified further with the use of a Sephadex G-10 column. The radioactive peak was collected and its volume reduced, under vacuum, to 2 ml and recycled on a Sephadex G-100 column. The final preparation was reduced in volume to 2 ml and 300 mg of potassium bromide was added and the mixture was lyophilized overnight. A pellet was made from the lyophilized mixture and an infrared spectrum of the unknown metabolite was obtained with the use of a Perkin-Elmer 221 Infrared Spectrophotometer. Repeated procedures gave similar results. The IR spectrum of this metabolite is shown in Figure 9. As might be judged from the nature of the IR spectra, it would appear that the purification procedures yielded a single substance with a complex structure.

(8) Presence of Disulfide Bond(s) in Metabolite III-5. The presence of disulfide bond(s) in the molecule was investigated by the procedure of Karush et al. (10). This method is based on fluorescence quenching characteristics of disulfide bonds when sprayed with an alkaline solution of fluorescene mercuric

acetate (FMA). A mixture of metabolite III-5 derived from both 2-^{14}C-pyrimidine and 2-^{14}C-thiazole labeled thiamine was prepared and spotted on cellulose thin layer chromatography plates along with an authentic sample of thiamine disulfide as a reference. The solvent systems used to develop the chromatograms consisted of (a) 2-propanol/0.1 N HCl, and (b) chloroform/methanol/0.1 N HCl. The location of Metabolite III-5 on the chromatograms was determined by autoradiography and marked, while thiamine disulfide was located by viewing the plates under ultraviolet light. The chromatograms were then sprayed with an alkaline solution of FMA and viewed under ultraviolet light. The location of the quenched areas were identical to that of the radioactive bands and the thiamine disulfide bands. Thus, the disulfide bridge(s) appeared to be present in the molecule. The FMA reagent, however, is not entirely specific for the disulfide bridge and therefore no final conclusion could be drawn. It should be mentioned that thiamine disulfide and metabolite III-5 had different Rf values when co-chromatographed in several solvent systems.

(2) Determination of the Molecular Weight of Metabolite III-5. During the course of purification of this metabolite by Sephadex G-10 gel filtration (column 1 x 40 cm), it was found that the elution volume for this metabolite was almost equal to that of the void volume of the column. In other words, the ratio of the elution volume (Ve) to the void volume (Vo) was 1 ($\frac{Ve}{Vo} = 1$). The same ratio for thiamine hydrochloric (mol. wt. = 237.27) was found to be 4.5 ($\frac{Ve}{Vo} = 4.5$). This suggested that Metabolite III-5 should have a molecular size much larger than that of a simple thiamine derivative. For estimation of the molecular weight of Metabolite III-5, the method of Andrews (12) was used with the following slight modification: The column size was 1 x 40 cm and distilled water was used instead of a buffer. Distilled water was used to avoid the addition of any salts or extraneous material to this metabolie and at the same time prevent the breakdown of this metabolite at an elevated pH.

Bovine serum albumin (mol. wt. = 67,000), ovine serum albumin (mol. wt. = 45,000) and chymotrypsinogen A (mol. wt. = 25,000) were used as markers. Using the above procedure, the molecular weight of Metabolite III-5 was estimated to be in the vicinity of [illegible],000. Thus, it appeared that this metabolite was a combination of thiamine (or a derivative of thiamine) and a high molecular weight compound, perhaps a peptide or protein. To investigate this possibility, a new batch of this metabolite was isolated from peak III and purified twice by cellulose thin layer chromatography and four times, by recycling on Sephadex G-10 and G-100 columns. Each time the radioactive peak was collected, its volume reduced under vacuum and reapplied to another column. The final preparation was hydrolyzed in 6 N hydrochloric acid at 6.8 kg pressure and 161° C for 6 hours. The hydrolyzed material gave a positive ninhydrin reaction. An aliquot of the hydrolysate was then analyzed for its amino acid constituents. The results are presented in Table VII. Neither methionine nor cystine (cystine) was detected with certainty indicating either the absence or presence of little of these amino acids in the metabolite. It was of interest to note the considerable amount of histidine and lysine present, providing probably a rather basic compound. In this respect it should be mentioned that Somogyi (19) isolated an antithiamine factor from carp viscera, which contained a protein and a non-protein moiety. The non-protein moiety was subsequently identified by Kuhelj and Somogyi (20) as hemin or a closely related substance. The antithiamine activity of the non-protein moiety was found to be much greater than that of the protein moiety. These investigators postulated that the carp antithiamine factor conjugates with thiamine rather than cleave thiamine into its respective moieties. However, the detailed mechanism of action of this substance has not been established.

The possible relationship of a similar factor in man, as might be suggested by the properties of Metabolite III-5, requires further investigation. It is of interest to note, however, that when 2-^{14}C-thiazole labeled oxythiamine,

an antimetabolite of thiamine, was administered to rats, Metabolite III-5 was not observed in the urine.[7] This would suggest that for the formation of Metabolite III-5 the presence of the amine group on carbon 4 of the pyrimidine moiety of the thiamine molecule is required.

COMMENTS

The ingestion of ^{14}C-labeled thiamine by normal adult human subjects has established that better than 95% of the thiamine was absorbed. No measurable amount of $^{14}CO_2$ could be detected in the respiratory air indicating with the limits of the techniques employed that less than 1% of the ingested ^{14}C-labeled thiamine was converted into $^{14}CO_2$. This observation was in contrast to the report of Balaghi and Pearson (4) who noted that 7-24% of the injected ^{14}C-thiazole labeled thiamine appeared as $^{14}CO_2$ in the respiratory air of rats.

The half-time excretion of ingested labeled thiamine ranged from 9 1/2 days to 18 1/2 days and, as expected, was related to the level of dietary intake of thiamine. The half-time excretion is consistent with observations that symptoms of a thiamine deficiency can be induced in human subjects within 14 to 21 days when ingesting diets devoid of thiamine (14,21). The half-time of thiamine in rats receiving adequate levels of the vitamin has been reported to be 9 days (4).

It is of interest to note that the pattern of urinary thiamine metabolites for the human resembles that observed for the rat (2,4). The results of studies with human subjects suggest that over 30 urinary thiamine metabolites may exist; six of which appear as major metabolites. Although care was taken to prevent the production of artifacts during the separation of the metabolites, such destruction may have occurred, and if so, the actual number of metabolites could be smaller. Nevertheless, a similar number of thiamine metabolites have been reported for the rat.

The observation that growth of L. viridescens is supported by a number of thiamine metabolites present in human urine other than free thiamine, limits the use of this microbiological assay for assessing urinary thiamine levels as may be applied in nutrition studies and surveys. Guides currently used to interpret urinary thiamine excretion in terms of dietary intakes (22) appear applicable when the thiochrome procedure is employed, but would need to be revised for use with the L. viridescens microbiological assay.

The present studies demonstrate the complexity of the metabolism of thiamine in the human. It would appear from this and other studies performed at this laboratory (14, 21) that a certain amount of thiamine is catabolized by the body at a relatively constant rate regardless of the level of intake of thiamine. This would suggest that this catabolism is not a function of the actual biochemical requirement for thiamine, but rather representative of a separate degradative pathway not related to physiological function. The mechanisms involved in this catabolism, whether enzymic, non-enzymic, or both requires further investigation. Nevertheless, this catabolism represents an obligatory requirement for thiamine that must be provided by dietary means.

SUMMARY

The metabolism of 2-^{14}C-thiazole labeled thiamine and 2-^{14}C-pyrimidine labeled thiamine was studied in four healthy young adult men. The orally administered labeled thiamine was almost completely absorbed with no measurable amount of $^{14}CO_2$ detected in the respiratory air. The half excretion time of the ingested label occurred within 9 1/2 to 10 1/2 days and was related to the weight of the subject and the daily dietary intake of thiamine.

The urinary radioactive metabolites of thiamine were fractionated by Amberlite CG-50 column chromatography. Four radioactive peaks were obtained from the urine of three subjects who had ingested 2-^{14}C-thiazole labeled thiamine and three peaks from the urine of a subject who had received 2-^{14}C-pyrimidine labeled thiamine. Each peak was tested separately for its biological

activity for L. viridescens. Approximately 50% of the thiamine activity present in the urine was thus found due to metabolites other than that of free thiamine.

The urinary radioactive metabolites resulting from both types of labeled thiamine administration were further separated by thin layer chromatography and autoradiography of the radioactive peaks obtained from the column chromatography. The use of 2-^{14}C-pyrimidine labeled thiamine resulted in 13 metabolites, of which four were major, while 2-^{14}C-thiazole labeled thiamine produced 31 metabolites, of which six were major. One of the major urinary metabolites of 2-^{14}C-thiazole labeled thiamine was positively identified as 4-methyl-thiazole-5-acetic acid. Also the thiazole moiety of thiamine and thiamine diphosphate were tentatively identified as the minor catabolic products of thiamine. One of the major urinary metabolites of thiamine, which contained both the pyrimidine and the thiazole moieties, appeared to be a conjugation of thiamine or a derivative with a peptide moiety.

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FOOTNOTES

1. Preliminary reports were given at the Federation meetings in 1966 and 1968.
2. Visiting Research Fellow, Imperial Iranian Army. Present address: Imperial Iranian Army Nutrition Committee, Army Medical Department, Aziz Khan Crossroad, Hafiz Avenue, Tehran, Iran.
3. Assistant Chief, Chemistry Division, U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado 80240.
4. Chief, Chemistry Division, U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado 80240.
5. Nuclear-Chicago Inc., 333 E. Howard Avenue, Des Plain, Illinois 60018.
6. Nuclear Research Chemicals Inc., P.O. Box 6453, Orlando, Florida.
7. Unpublished data: W.W. Ashley-Nejad, Ph.D. Thesis, Vanderbilt University, 1968, entitled, "The Metabolism of Oxythiamine in the Rat."
8. All radioisotope usage was conducted in accordance with the policies and procedures outlined in Army Regulations AR40-37 and AR 70-25.

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LEGENDS

Figure 1: Structural formula of thiamine by hydrochloride indicating the positions of the carbon-14 labeling in the molecule.

Figure 2: Cumulative excretion curve of 2-^{14}C-thiazole labeled thiamine in three adult human males. Subject A weighed 90 kg and consumed a cyclic diet providing 1.35 mg of thiamine per day. Subject B weighed 95 kg and received diets that provided 1.8 mg thiamine per day. Subject C weighed 69 kg and consumed a diet containing 2.1 mg thiamine per 24 hours.

Figure 3: Comparison of the radioactive peaks obtained by column chromatography of urine obtained from subjects who had ingested either 2-^{14}C-thiazole labeled thiamine or 2-^{14}C-pyrimidine labeled thiamine. (Amberlite-CG-50 column, 1 x 40 cm, 200-400 mesh, in the H+ form; flow rate 10 ml/hr; each fraction represents 5 ml of effluent).

Figure 4: Distribution of radioactivity among the peaks obtained by Amberlite CG-50 column chromatography of urine of two subjects collected the four days following the ingestion of 2-^{14}C-thiazole labeled thiamine.

Figure 5: Schematic representation of autoradiograph of thin layer chromatography (TLC) separations of peak II obtained by column chromatography of urine of two subjects who had ingested either 2-^{14}C-pyrimidine or 2-^{14}C-thiazole labeled thiamine. TLC solvent system: n-propanol/1 M acetate buffer (pH 5.0)/water (7-1-2; V/V).

Figure 6: Schematic representation of autoradiograph of thin layer chromatography (TLC) separations of peak III obtained by column chromatography of urine of subjects who had ingested either 2-^{14}C-pyrimidine or 2-^{14}C-thiazole labeled thiamine. TLC solvent systems: n-propanol/1 M acetate buffer (pH 5.0)/water (7-1-2; V/V).

Figure 7: A comparison of the ultraviolet spectra (at pH 6.0) of metabolite III-5, thiamine disulfide and thiamine hydrochloride.

Figure 8: A comparison of the ultraviolet spectra (after acidification) of Metabolite III-5, thiamine disulfide and thiamine hydrochloride.

Figure 9: Infrared spectrum of Metabolite III-5 obtained with the use of a Perkin-Elmer 221 Spectrophotometer. Potassium bromide pellets were made in the conventional manner. The broad band at approximately 3500 cm^{-1} (a) may be attributed to amide group(s), OH binding, and carboxyl groups in the molecule. The band at 1700 cm^{-1} (b) represents more than one carbonyl group. The broad band at approximately 1050 cm^{-1} (c) may be interpreted as the existence of C-O stretch in this metabolite.

TABLE I

Comparison of thin layer chromatography R_f values of authentic 4-methyl-thiazole-5-acetic acid and that of an unknown metabolite obtained from peak II of the urine of subjects who had received 2-^{14}C-thiazole labeled thiamine.

Solvent systems employed	R_f values of authentic TAA[a]	R_f values of the unknown metabolite	R_f values of mixture[b]
Ethanol/NH_4OH/H_2O	0.62	0.62	0.62
Methanol/chloroform/NH_4OH	0.59	0.57	0.57
2-Propanol/NH_4OH/H_2O	0.27	0.27	0.27
n-Propanol/1M acetate buffer, pH 6/H_2O	0.89	0.89	0.89

a. TAA = unlabeled authentic 4-methyl-thiazole-5-acetic acid.

b. Mixture indicates a combination of unknown metabolite and authentic sample.

TABLE II

The specific activities of five recrystallizations of a mixture of authentic 4-methyl-thiazole-5-acetic acid and the unknown radioactive urinary metabolite.

Crystallization No.	^{14}C Activity dpm/ml[a] of collection	Optical density at 256 mμ	TAA in[b] sample (μg)	^{14}C Activity (dpm/μg TAA)	^{14}C Activity (dpm/μm TAA)
1	9[illegible]	0.22	8.5	11.1	1733
2	122	0.06	15.0	8.1	1265
3	139	0.[illegible]	[illegible]	8.7	1352
4	1[illegible]	0.03	16.[illegible]	8.4	1310
5	173	0.[illegible]	21.3	8.3	1293

a. Radioactivity was determined in a Packard Tricarb Scintillation Spectrometer, Model 3375, using Bray's solution as phosphor.

b. Determined spectrophotometrically in a Beckman DB-G Spectrophotometer. TAA = 4-methyl-thiazole-5-acetic acid.

TABLE III

Comparison of the R_f values of 4-methyl-5-β hydroxyethyl-thiazole and the unknown metabolite obtained from peak III of the urine of subjects administered 2-^{14}C-thiazole labeled thiamine.

Solvent systems employed	R_f values of unknown metabolite[a]	R_f values of authentic thiazole[b]	R_f values of Mixture[c]
n-Propanol/acetate buffer/water	0.94	0.94	-
2-Propanol/0.1N HCl	0.91	0.91	-
Chloroform/methanol/0.1N HCl	0.98	0.96	0.97
Acetonitrile/water/formic acid	0.78	0.78	0.78

a. This metabolite was located on the chromatography plates by autoradiography.

b. The authentic sample was located on the chromatography plates by its ultraviolet quenching characteristics.

c. Mixture indicates a combination of unknown metabolite and authentic samples.

TABLE IV

Comparison of the thin layer chromatography R_f values of thiamine mono and diphosphates with that of Metabolite III-1, which was obtained from the column chromatographic peak III of urine of subjects who had received 2-^{14}C-thiazole or 2-^{14}C-pyrimidine labeled thiamine.

Solvent systems employed	R_f values of Metabolite III-1 obtained from administration of ^{14}C-thiazole-thiamine[a]	R_f values of Metabolite III-1 obtained from administration of ^{14}C-pyrimidine-thiamine[a]	R_f values of thiamine diphosphate[b]	R_f values of thiamine monophosphate
Chloroform/methanol/0.1N HCl	0.13	0.13	0.13	0.26
2-Propanol/0.1N HCL	0	0	0	0
n-Propanol/1M acetate buffer (pH 5.0) H_2O	0.10	0.11	0.08	0.15
2-propanol/0.2M acetate buffer (pH 6.0) H_2O	0.08	0.08	0.08	0.14

a. Obtained by autoradiography; ^{14}C-thiazole thiamine = 2-^{14}C-thiazole labeled thiamine; ^{14}C-pyrimidine thiamine = 2-^{14}C-pyrimidine labeled thiamine.

b. Thiamine mono and diphosphates were located on the chromatography plates by their ultraviolet quenching characteristics.

TABLE V

The specific activities of four recrystallizations of a mixture of radioactive Metabolite III-1 and an authentic sample of thiamine diphosphate.[a]

Crystallization Number	dpm/ml of solution[b]	Optical density at 245 mμ	Concentration of TDP in sample (μg)[b, c]	^{14}C activity (dpm/μg of TDP)[b, c]	^{14}C activity (dpm/μM of TDP)[b, c]
1	80	0.36	10.5	7.6	3883
2	143	0.80	24.0	5.6	2681
3	118	0.83	25.0	4.7	2250
4	46	0.34	10.0	4.6	2202

a. A mixture of 10 mg of thiamine diphosphate and "98,000 dpm" of the unknown Metabolite III-1 was recrystallized four times from a solution of acetone and absolute ethanol (V/V) as follows: The crystals were dissolved in 0.1 ml distilled water by slight warming and the solution of acetone and ethanol was added drop by drop until the crystals were formed.

b. Each sample was counted in a Mark I Nuclear Chicago Liquid Scintillation Spectrometer for 100 minutes and corrected for blank and quenching.

c. TDP = thiamine diphosphate (measured spectrophotometrically).

TABLE VI

Comparison of the thin layer chromatography R_f values of Metabolite III-5 which was obtained from the column chromatographic peak III of the urine of subjects who had received 2-^{14}C-thiazole labeled thiamine or 2-^{14}C-pyrimidine labeled thiamine.

Solvent systems employed	R_f values of Metabolite III-5 obtained from administration of ^{14}C-thiazole thiamine[a]	R_f values of Metabolite III-5 obtained from administration of ^{14}C-pyrimidine thiamine[a]	Mixture of both Metabolites
n-Propanol/1M acetate buffer (pH 5.0) H_2O	0.50	0.50	0.50
Chloroform/methanol/0.1N HCl	0.55	0.59	0.59
2-Propanol/0.1 N HCl	0.23	0.25	0.23
Acetonitrile/water/formic acid	0.61	0.61	0.61

a. 2-^{14}C-thiazole labeled thiamine and 2-^{14}C-pyrimidine labeled thiamine. The metabolites were located on the chromatography plates by autoradiography.

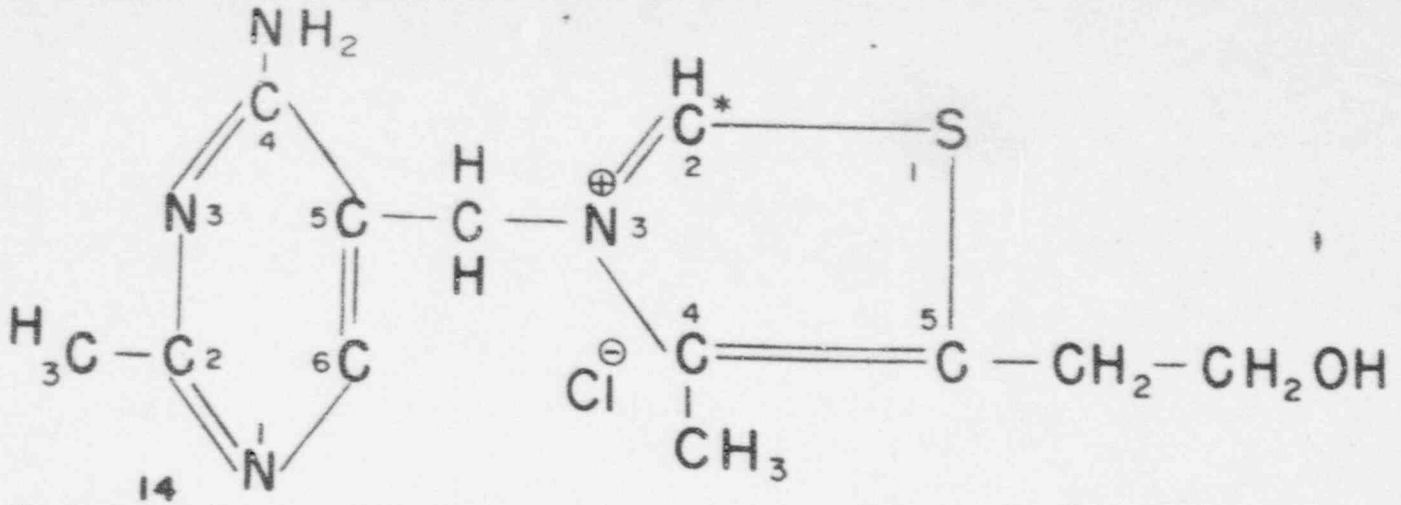
TABLE VII

The amino acid composition of Metabolite III-5 which was isolated from the urine of human subjects who had received ^{14}C-labeled thiamine.

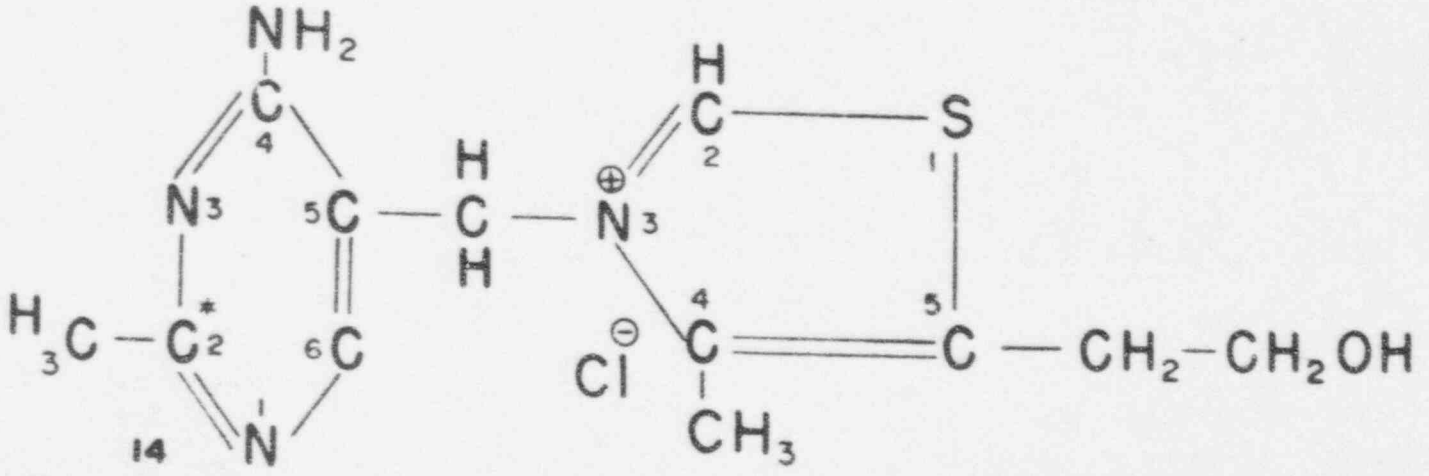
Amino Acid	% Composition	Number of amino acid residues in Molecule[a]
Alanine	1.5	4
Arginine	1.4	2
Aspartic acid	2.2	4
Cysteine	-	-
Cystine	-	-
Glutamic acid	1.4	3
Glycine	14.4	48
Histidine	43.7	70
Isoleucine	4.4	8
Leucine	4.8	9
Lysine	10.4	18
Methionine	-	-
Phenylalanine	3.2	5
Proline	2.1	4
Serine	4.4	10
Threonine	1.0	2
Tryptophan[b]	-	-
Valine	2.6	6

a. In the calculation of the number of amino acid residues estimated to be present in the protein moiety of Metabolite III-5, the following assumptions were made: (a) the molecular weight was assumed to be 25,000 and (b) no allowance was made in the molecular weight for the presence of the thiamine derivative.

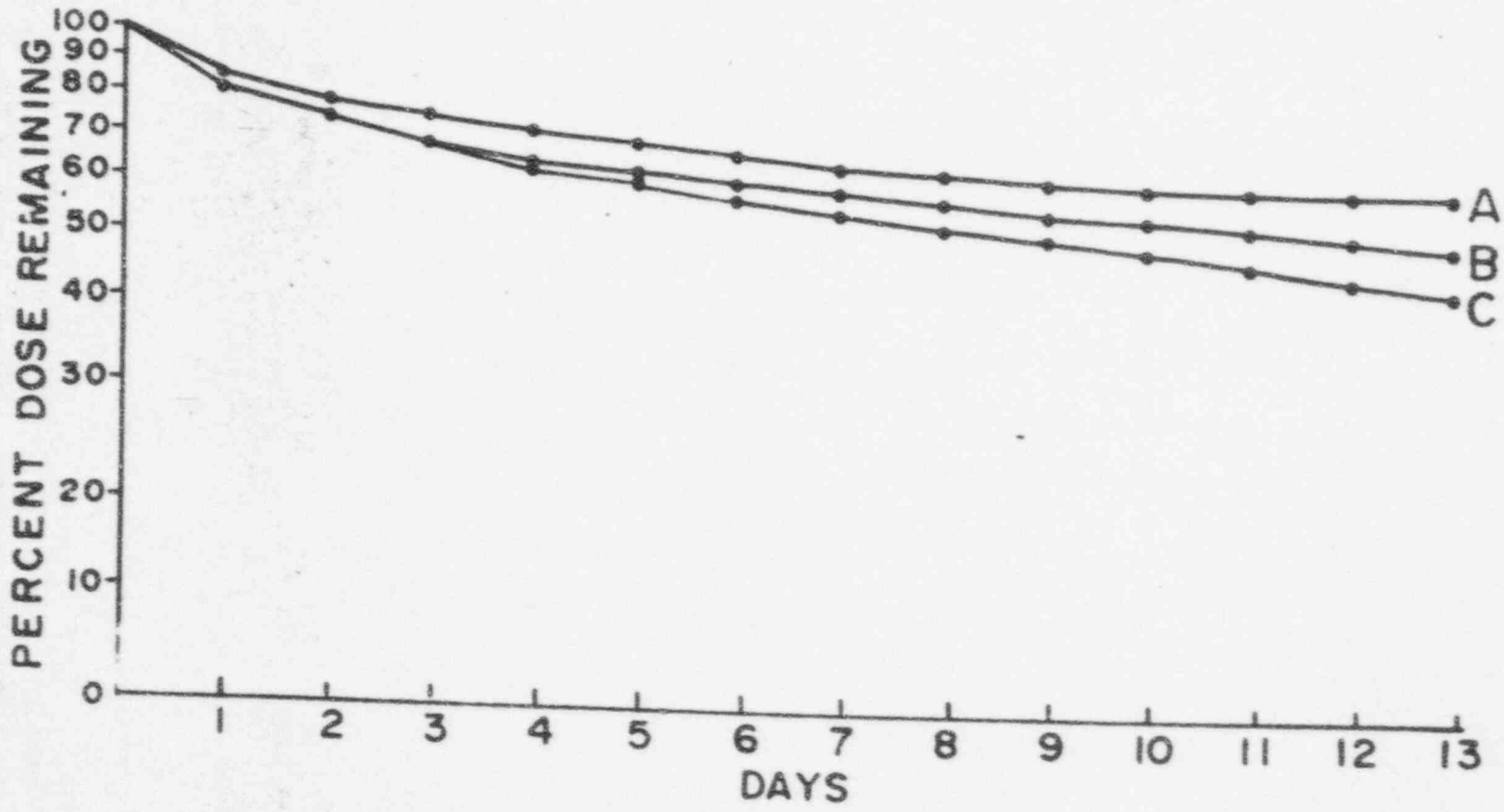
b. Not determined.



2-^{14}C-THIAZOLE LABELED THIAMINE



2-C-PYRIMIDINE LABELED THIAMINE


PERCENT DOSE REMAINING
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DAYS
A
B
C

APPENDIX VIII Part B-4

METABOLISM OF ASCORBIC-1-^{14}C ACID IN EXPERIMENTAL HUMAN SCURVY[1,2]

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INTRODUCTION

Previously, there have been no published reports of the use of isotopic techniques in studying the metabolism of ascorbic acid in men depleted of vitamin C. There have, however, been numerous studies on the metabolism of this vitamin in man, and there have been many other studies on the metabolism of labeled ascorbic acid in animals (1-6). Accordingly, the objective of this study was to observe the metabolism of L-ascorbic-1-^{14}C acid in a group of volunteers who had been depleted of vitamin C for a prolonged period of time.

A joint study between the U.S. Army Medical Research and Nutrition Laboratory and the University Hospitals at Iowa City was recently completed. In this study, the objectives were (a) to induce a deficiency of ascorbic acid in healthy men; (b) to label their ascorbic acid body pool with L-ascorbic-1-^{14}C acid in order to study total pool size, rate of depletion and minimal requirements for the vitamin; and (c) to observe the relationships between body pool size, clinical evidence of scurvy and physiological functions. In addition, it was hoped to ascertain the minimal amount of ascorbic acid necessary to alleviate clinical signs and symptoms of scurvy and to replete the body pool size. Finally, efforts were made to study the metabolic end-products of ascorbic acid excreted in the urine. This study will be reported in two sections. Details of management, symptomatology, diet and response to repletion will be published separately in the clinical report (7). Information relating to body pool size, rate of depletion, minimal requirements for vitamin C, rate of repletion and metabolic end-products of L-ascorbic acid are presented in this paper.

EXPERIMENTAL PROCEDURES

General Plan

Initially, six healthy prison volunteers were selected for participation in the study. They were fed a synthetic liquid diet deficient in vitamin C but adequate

in all other nutrients (7). Isotopic labeling was accomplished through the administration of L-ascorbic-1-^{14}C acid on the 23rd day of the deficiency. Repletion, which commenced on the 100th day, was accomplished through the daily administration of controlled amounts of the labeled vitamin. The initial labeling dose of radioactive vitamin was given orally and consisted of 0.86 mg of L-ascorbic-1-^{14}C acid with a total activity of 23.90 μCi. This label was not given on the first day of the depletion phase because of an unavoidable delay in obtaining permission to administer the isotope. The deficient diet was fed to the men for 99 days, at which time clinical signs and symptoms suggestive of mild scurvy became apparent. At this point, the body pool of ascorbic acid had been largely depleted, judging from the cumulative radioassay data. This calculation was based on the cumulative loss of radioactive material in the urine. Losses in feces and in expired CO_2 accounted for less than 3% of the initial radioactive dose.

Repletion of the subjects with L-ascorbic acid was begun on the 100th day, utilizing L-ascorbic-1-^{14}C acid administered in controlled doses ranging from 4 to 64 mg daily. The specific activity of this material was 0.05 μCi/mg. After two weeks of repletion, the diet was changed from a liquid-type to a solid-type, which entailed a dietary increase of L-ascorbic acid intake from 0 to that of 2.5 mg/day. Thus, the total ascorbic acid intake of the men during the repletion phase ranged from 6.5 to 66.5 mg. Supplementation with ^{14}C-labeled ascorbic acid was continued in the two subjects receiving 32 and 64 mg daily until ascorbic acid appeared in the urine. These men were then given the same dose of unlabeled ascorbic acid (32 and 64 mg daily) in order to determine whether or not the daily urinary ^{14}C-excretion curve while on a high intake of ascorbic acid was identical to that obtained on a zero intake of the vitamin. The other two subjects received daily for a period of 98 days 4 or 16 mg of radioactively labeled ascorbic acid. At the end of that time,

resupplementation of all subjects was increased to 600 mg daily of unlabeled ascorbic acid for an additional 13 days to ensure saturation of body stores.

Radioisotope Methods

The L-ascorbic-1-^{14}C acid employed in this experiment was obtained from New England Nuclear Corporation[7] and had a specific activity of 5.1 μCi/μM. Prior to administration of the labeled ascorbic acid to the subjects, it was treated with sodium hydrosulfite in a ratio of 2 moles hydrosulfite to 1 mole of ascorbic acid, in order to accomplish reduction of any dehydroascorbic acid present (3). The isotopically-labeled vitamin was chromatographed and radioautographed prior to administration in order to assure purity. Aliquots of the ascorbic acid employed, including the initial dose and the supplemental vitamin, were assayed for radioactivity. Duplicate aliquots of each man's daily 24-hour urinary excretion of carbon-14 were counted in a liquid scintillation spectrometer. All scintillation counting was performed employing Colosolve CS1 and CS2 solubilizers[8]. This reduced problems of chemoluminescence and allowed the dissolution of 1 ml of raw urine in the toluene-based scintillation system.

Thin-layer chromatography (TLC) (8), using silica gel plates, was employed to separate ascorbic acid and other organic ^{14}C-labeled components present in the urine. Areas on the plates corresponding to the labeled organic products were scraped off and eluted with water or trichloroacetic acid (TCA). Aliquots were counted for ^{14}C activity and colorimetrically analyzed for the presence of ascorbic acid (9).

Measurement of urinary oxalate excretion was determined enzymatically by the oxalate decarboxylase method (10). A slight modification of this method was made in that a nitrogen gas flow system was added to trap evolved $^{14}CO_2$ into monoethanolamine:methyl cellosolve (1:2) solution for scintillation counting.

Fecal samples were counted for radioactivity by first subjecting them to wet combustion and then flushing the evolved $^{14}CO_2$ with nitrogen gas into monoethanolamine:methyl cellosolve (1:2) solution, followed by counting in a liquid scintillation spectrometer.

Evolved $^{14}CO_2$ from the breath was estimated by having the subjects exhale directly through a solution of monoethanolamine:methyl cellosolve, utilizing a Kofrangi apparatus. Collection of expired air was performed for a period of 20 minutes immediately following the administration of the ^{14}C-labeled ascorbic acid, and aliquots of the trapping solution were counted in a liquid scintillation spectrometer.

RESULTS

Urinary excretion of ^{14}C-labeled radioactive material is shown in Fig. 1 which plots the microcuries per day (5-day average) excreted by each of the four subjects who completed the study. The period of time between the 23rd and 99th day represents the ascorbic acid depletion phase. The ascorbic acid repletion phase is represented by the period of days 100 to 198 of the study. Following day 198, the response of the uniformly-labeled ascorbic acid body pool to the daily intake of 600 mg of unlabeled ascorbic acid can be seen. Three changes in the supplementation phase should be noted. On day 156, subject N was increased from his previous intake of 8 mg/day of ascorbic acid to a dose of 64 mg/day containing 0.05 μCi/mg of radioactivity. On day 176, subject N began receiving 64 mg/day of unlabeled ascorbic acid. On day 155, subject S was changed from an intake of 32 mg/day of labeled ascorbic acid to 32 mg/day of unlabeled ascorbic acid. The dotted lines seen in Fig. 1 indicate the time at which the level of supplementation was changed. The departure of the curve from linearity shown in Fig. 1 during the first 5 days can be attributed to a "flushing" phenomenon. In fact, the half-time of this initial flushing action was 2 days.

Fig. 2 should be considered in two parts. Part A (days 1-99), curve I, is the plot of a semilog equation calculated by a least squares fit of the urinary excretion data from all subjects. Curve II (mg/day) and curve III (body pool size in g) are derived mathematically from the fitted curve I, and thus are models only[9]. Part B, curve IV, is a 5-day average plot of actual μCi/day excreted by subject S (who received 32 mg/day of labeled ascorbic acid) and may be considered as a representative subject. Curve V (μCi/day) shows the response of subject S's labeled pool to a change from 32 mg/day of labeled ascorbic acid to 32 mg/day of unlabeled ascorbic acid. The slope of curve V is statistically the same as that obtained for curve I (part A). Curve VI (part B) is the calculated "μCi equivalent gram" of ascorbic acid retained by subject S. Detectable ascorbic acid first appeared in subject S's urine when his μCi equivalent gram pool was repleted to a level of 1.5 g of ascorbic acid. This occurred on the 155th day of repletion (Fig. 2; part B; curve VI).

Depletion Phase

By plotting the total average microcuries of ^{14}C excreted in the urine during the depletion phase (Fig. 2; part A; curve I), one can see that depletion of the body stores of ascorbic acid takes place as a first order exponential process occurring at a constant rate of 2.6% per day of the total existing pool. If one assumes that extrapolation back to day 1 gives a true representation of the reduction of the body ascorbic acid pool, then depletion by day 99 has resulted in a 92% reduction of the presumed original ascorbic acid body pool size. The radiometric data, however, showed that the average subject's pool had been reduced to 9% in only 76 days, suggesting that there was a departure from the linear log curve during the last part of the depletion phase. Some support for this hypothesis may be found by examining the data shown in Fig. 1 for the last 15 days of depletion. Here it may be seen, in a 5-day individual data average, that there was a change in the slopes of the μCi/day excretion curves for each of

the subjects on day 84. Fig. 2 (part A; curve III) shows that the body pool size of ascorbic acid had been reduced by day 84 to about 20% of its extrapolated original size. At the close of the deficiency phase, there were convincing signs and symptoms of mild scurvy in all four of the subjects who completed the study (7). It should be noted that the daily urinary-^{14}C excretion by each subject during the depletion phase did not differ greatly from one to another. The correlation coefficient for the regression line of the average man was $r = 0.98$ (Fig. 2; part A; curve I). Slopes for the excretion curves obtained during the depletion phase for each man ranged from -0.0096 to -0.012, with an average of -0.011.

Repletion Phase

Fig. 1 also shows the daily urinary-^{14}C excretion by each subject during the repletion phase. Although all four subjects were repleted at differing controlled levels of ascorbic acid, their supplements all contained a specific activity of 0.05 μCi/mg during the initial part of the repletion phase. Subjects S and N, however, who were given larger repleting doses of the ascorbic acid, did not receive the radioisotope during the latter portion of the repletion phase but, instead, were given nonlabeled L-ascorbic acid.

The repletion curves shown in Fig. 2 (part B; curves IV and VI) indicate that a fraction of the ^{14}C-labeled ascorbic acid dose retained in the body pool tended to decrease with time as the total ascorbic acid body pool increased with size.

Fig. 3 shows, on a linear scale, the microcuries of ^{14}C excreted daily, divided by the dose administered during the repletion phase, in order to equate the data for varying radioisotope intakes. Again, it must be emphasized that subject N was changed from a dose of 8 mg/day of labeled ascorbic acid to one of 64 mg/day on day 156. On day 176, the administration of labeled ascorbic acid to subject N was discontinued, and 64 mg of unlabeled ascorbic acid was fed. The administration of labeled ascorbic acid to subject S was discontinued

on day 155, and 32 mg of unlabeled ascorbic acid was fed. On day 199, the intake of unlabeled ascorbic acid supplement was increased to 600 mg/day for all subjects. Data for subject N indicate that there is little difference in the urinary isotopic excretion pattern for a change of 0 to 8 mg/day of labeled ascorbic acid, as compared with a later change of 8 to 64 mg/day intake of labeled ascorbic acid.

Urine from the subjects who were repleted with 32 or 64 mg/day of labeled ascorbic acid contained no detectable free ascorbic acid, as measured by TLC separation, followed by radioassay and colorimetric assay, until the cumulative μCi equivalent gram body pool had been repleted with ascorbic acid to an estimated level of 1.5 g. At that time, reduced ascorbic acid was found in the urine of both subjects. Following this, the repletion of these two subjects was changed from labeled to unlabeled ascorbic acid. This was done in order to determine the slope of excretion of radioactivity in subjects with a known pool size of labeled material (Fig. 2; part B; curve V). The slope of this curve (-0.0084) did not differ significantly from the average slope observed during the depletion phase of the study. This was also true for the subject who was given 64 mg daily of unlabeled ascorbic acid after the time free ascorbic acid appeared in his urine. His daily urinary-^{14}C excretion curve's slope was -0.011.

During the final phase of the study, when all of the subjects were receiving 600 mg of unlabeled ascorbic acid daily, the slope of urinary excretion was four times greater than that observed with intakes of 34.5 or 66.5 mg daily. This is seen in Fig. 4, which is a plot of the 2-day average μCi/day excreted during the last 13 days of the study. The cumulative urinary radioassay data obtained from all subjects would indicate that the daily per cent of isotopically-labeled ascorbate catabolized is independent of ascorbic acid intake between the ranges of 10.5 and 66.5 mg/day. It should be noted that this occurred in subjects whose body ascorbic acid pools were not fully repleted.

Although the results of the daily urinary-^{14}C excretion of labeled material were the same for the depletion phase of subject L as all other subjects, special attention should be called to the repletion phase of this subject. Here it should be noted that on an ascorbic acid intake of 6.5 mg daily for a period of 98 days, the per cent of the total dose retained (Fig. 5; curve L), as well as the daily urinary-^{14}C excretion (Fig. 3; curve L), seemed to indicate that a greater per cent of the ingested supplement was catabolized. Thus, perhaps the low level of supplementation with L-ascorbic acid was marginal for this subject's needs. In fact, during the period between days 120 and 126, this subject excreted more radioactivity than he ingested, thus indicating a net loss of ascorbic acid (mg/day equivalent), and suggesting an increased requirement for ascorbic acid in excess of his intake of 6.5 mg/day. During the same period of time, this subject was exposed to severe emotional stresses and when these stresses were relieved, his excretion of radioactively-labeled material in the urine decreased so that the slope of his daily urinary-^{14}C excretion curve became positive (Fig. 6). By the 97th day of resupplementation, he had retained 186 mg of ascorbic acid (or almost 2 mg/day, based on accumulated radioactivity). It should also be noted that all other clinical parameters measured became normal when the intake of ascorbic acid was only 6.5 mg/day (7). Of course, the blood levels and the urinary excretion of ascorbic acid remained essentially nil, commensurate with his low level of intake.

Urinary Metabolites of L-Ascorbic-1-^{14}C Acid

Attempts were made to isolate the ^{14}C-labeled metabolites excreted in the urine by means of thin-layer chromatographic, column chromatographic and electrophoretic procedures. The urine appeared to contain two major and two minor ^{14}C-labeled components, none of which were L-ascorbic acid. These components proved to be extremely chemically stable. Furthermore, these metabolites were extremely soluble in 90% ethanol. Although exact chemical

identification of these compounds has not yet been accomplished, further studies are in progress on this aspect, employing gas chromatographic procedures and other techniques.

Other Studies

Urinary excretion of ^{14}C as oxalic acid, derived from the labeled ascorbic acid, occurred at a rate of approximately 10%/day of the total ^{14}C radioactivity present in the urine. The $^{14}CO_2$ expired in the breath of the subjects following administration of the ^{14}C-labeled L-ascorbic acid indicated that less than 2% of the ingested radioactivity was excreted by this route. Fecal excretion of ^{14}C-labeled material indicated that less than 1% of the total daily loss of ^{14}C occurred by this route.

The rates of urinary excretion of nitrogen and creatinine were measured routinely. The plot of the daily urinary nitrogen, creatinine and nitrogen/creatinine ratio for the average man is presented in Fig. 7. It can be seen that during the depletion phase the slope of the plot of the nitrogen/creatinine ratio was +0.015, suggesting a negative nitrogen balance, while during the repletion phase, the slope was reversed to -0.045, suggesting a positive nitrogen balance. The diets fed during both the depletion and repletion phases supplied a constant intake of 90 g of protein daily. The data suggest that impaired protein utilization occurred during the depletion phase, which upon repletion with ascorbic acid, appeared to be reversed. The significance of these findings requires further investigation.

DISCUSSION

One of the remarkable aspects of this study was that labeling of the body ascorbic acid pool during the depletion phase resulted in no detectable urinary excretion of ^{14}C-labeled reduced or dehydroascorbic acid. All of the radioactivity excreted daily was as stable organic forms that did not migrate in the TLC

acetonitrile-butyronitrile solvent system (9). The radioactive material, however, was separated by the use of other TLC solvent systems, column chromatography, or through the use of preparative electrophoretic procedures.

It should be noted from the cumulative carbon-14 excretion during the depletion phase that none of the four subjects had an excretion curve that differed from the average man greater than ±3%, despite marked differences in body weight and age (7). Furthermore, from the data obtained during the repletion phase, it was possible to calculate a "μCi equivalent mg" of ascorbic acid retained. Although the subjects were resupplemented with ascorbic acid at different levels of intake, the supplemental material had a constant specific activity of 0.05 μCi/mg. Consequently, one could, by using the daily radioisotopic excretion and retention data, calculate the so-called "μCi equivalent mg." That is, each microcurie of ^{14}C radioactivity retained or excreted represents an equivalent quantity of ascorbic acid in accordance with the specific activity of the ascorbic acid ingested. Since the specific activity of the ascorbic acid administered during the repletion phase of this study was 0.05 μCi/mg, then each 0.05 μCi retained or excreted would be equal to 1 mg of ascorbic acid.

The subject who received 32 mg of ascorbic acid per day excreted in the urine carbon-14 labeled reduced and dehydroascorbic acid at the point in time when his body pool of ascorbic acid had been resupplemented to 1.5 g. The subject who received 64 mg of ascorbic acid daily excreted reduced ascorbic acid in his urine at the point in time when his body pool of ascorbic acid had been resupplemented to 1.46 g. The urinary excretion of reduced and dehydroascorbic acid was not investigated on the subject receiving only 6.5 mg/day of ascorbic acid, since it was not anticipated that his body pool of ascorbic acid would be repleted during the repletion phase. Examination of Fig. 4, curve L, revealed a lag of 3 to 4 days before an increased daily urinary excretion of ^{14}C occurred. This would indicate that the labeled pool had to be

fully resupplemented before an increased excretion of the radioisotope would ensue. Furthermore, the peak daily urinary-^{14}C excretion was also delayed by 3 days when compared to the subjects on the higher intakes of ascorbic acid. The decline of the slope of the daily urinary-^{14}C excretion curve for subject L, after peak radioactivity excretion had occurred, was the same as that noted for the other subjects. It is of interest to note that during the repletion phase of the study, the subjects appeared to adapt to the level of ascorbic acid intake within the range of 10.5 to 66.5 mg per day. The subject who received only 6.5 mg/day of ascorbic acid demonstrated greater μCi/day excretion and less μCi/day retention during this same period, indicating that this level of ascorbic acid intake was probably close to the minimal requirement of the subject. This is supported by the observation that this low level of ascorbic acid intake not only cured scurvy, but maintained all other clinical and biochemical parameters evaluated in a normal state during the repletion phase (7).

It is of further interest to note that when the subjects were fed 600 mg of unlabeled ascorbic acid daily for the last 13 days of the study, only a 4-fold increase in the cumulative daily μCi excretion occurred, even though the level of ascorbic acid intake had been increased as much as 90-fold. Thus, it would appear that on a high intake of ascorbic acid, only a limited quantity of the ingested vitamin is equilibrated with the body ascorbate pool.

The statement of what might be an optimal daily ascorbic acid requirement for the adult human cannot be made from the data obtained from this study. Nevertheless, an intake of 6.5 mg per day of ascorbic acid was sufficient to cure deficiency symptoms in one subject. This finding is in agreement with the conclusions of the Sheffield study, where an intake of greater than 5 but less than 10 mg of ascorbic acid per day was sufficient to cure scurvy in man (11).

SUMMARY

A study of the metabolism of ascorbic-1-^{14}C acid in experimental human scurvy was conducted with the following results:

1. Labeling the body ascorbic acid pool during the depletion phase resulted in no detectable urinary excretion of ^{14}C-labeled reduced or dehydroascorbic acid.

2. Urinary excretion of ^{14}C by all subjects occurred as a first order process during the depletion phase. The urinary ^{14}C excretion curves of the four subjects did not differ from the average man by more than ± 3%, despite marked differences in body weight and age.

3. First symptoms of mild scurvy appeared in the subjects when their body ascorbic acid pool had been reduced to approximately 300 mg.

4. Once the body pool of ascorbic acid was repleted to a level of 1.5 g, urinary loss of reduced ascorbic acid occurred.

5. The rate of repletion of ascorbic acid was found to be a zero order process and proportional to the level of daily ascorbic acid intake.

6. When the subjects were fed a high intake of ascorbic acid, only a limited quantity of the ingested vitamin was equilibrated with the ^{14}C-labeled ascorbate pool.

7. All of the radioactivity excreted during the depletion phase was in the form of stable organic material that did not behave as ascorbic acid. This organic material was separated into four unknown compounds.

8. This study would indicate that a daily intake of less than 6.5 mg per day of ascorbic acid is sufficient to alleviate and prevent scurvy in man.

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FOOTNOTES

[1]From the Chemistry Division, U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado, and the Department of Internal Medicine, University Hospitals, University of Iowa, Iowa City, Iowa.

[2]Supported in part by U.S. Army Medical Research and Development Command, Office of The Surgeon General, Grant #DADA 17-67-C-7111.

[3]Assistant Chief, Chemistry Division, U.S. Army Medical Research and Nutrition Laboratory.

[4]Professor, Department of Internal Medicine, University of Iowa.

[5]Instructor, Department of Internal Medicine, University of Iowa.

[6]Chief, Chemistry Division, U.S. Army Medical Research and Nutrition Laboratory.

[7]Chemist, Chemistry Division, U.S. Army Medical Research and Nutrition Laboratory.

[8]Address: 575 Albany Street, Boston, Massachusetts.

[9]The acid and neutral solubilizer was obtained from Sental Associates, Inc., Littleton, Colorado.

[10]Curve III assumes an original ascorbic acid body pool size of 2 g, based on data obtained from previous studies (5).

LEGENDS FOR FIGURES

Fig. 1: Excretion of ^{14}C-labeled material in the urine of four subjects. The points represent the average of 5 days plotted at the midpoint of the interval.

Fig. 2: A. Mathematical models of urinary excretion, pool size and catabolism based on urinary excretion data. B. Changes in pool size and ^{14}C-urinary excretion during repletion for one subject (S).

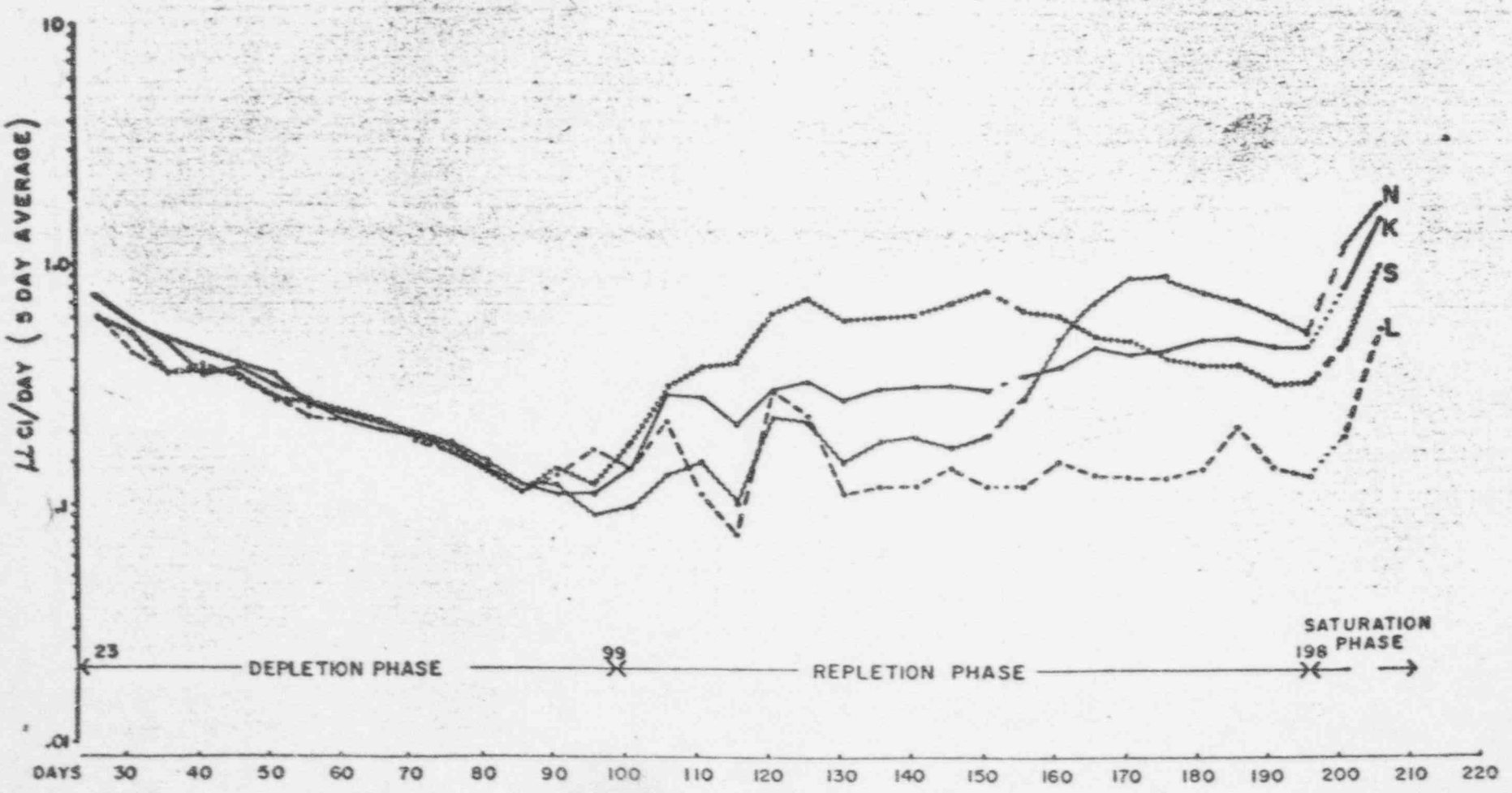
Fig. 3: Daily ^{14}C-urinary excretion of all subjects divided by the dose in microcuries each received.

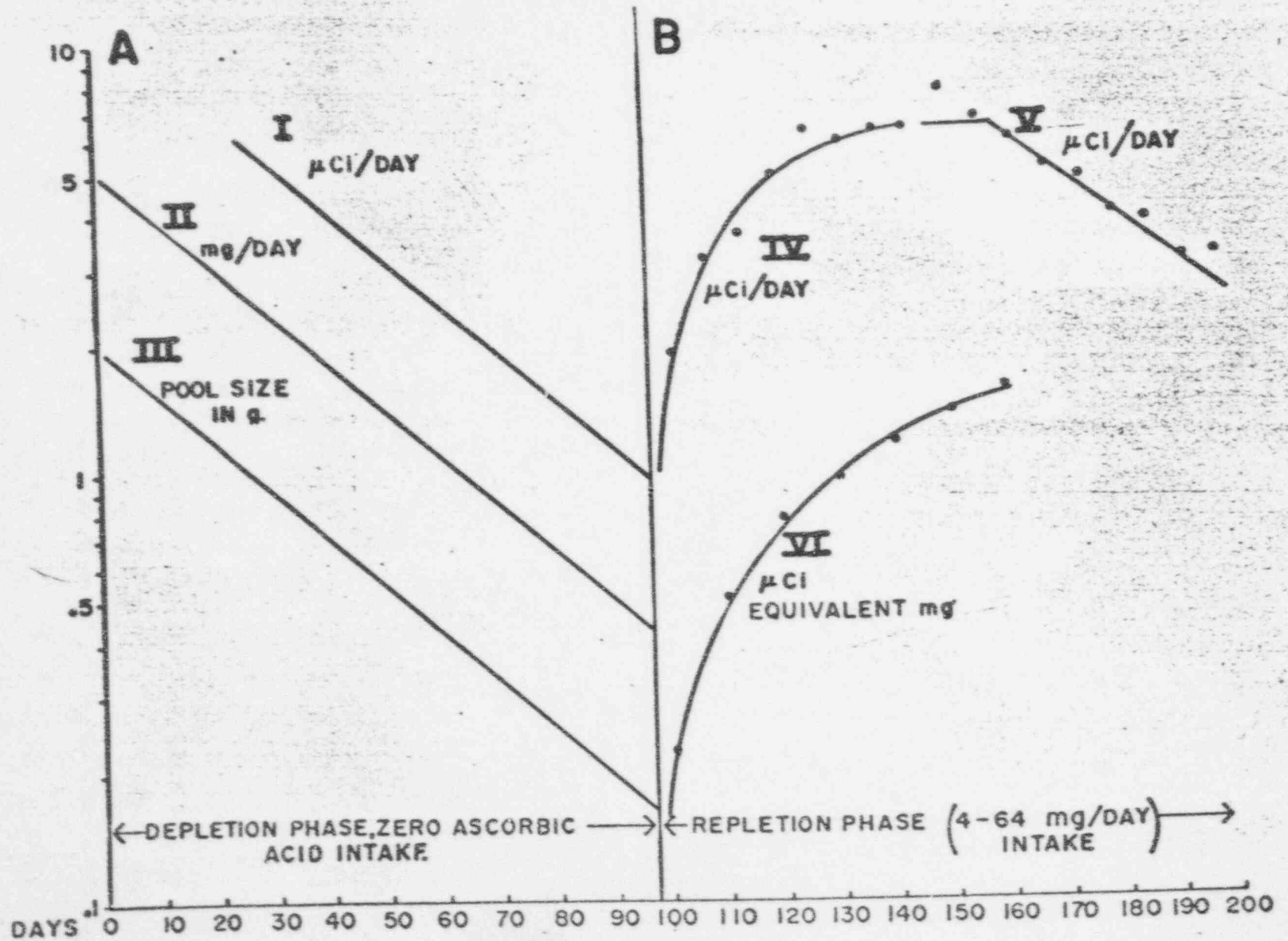
Fig. 4: Daily ^{14}C-urinary excretion during saturation phase.

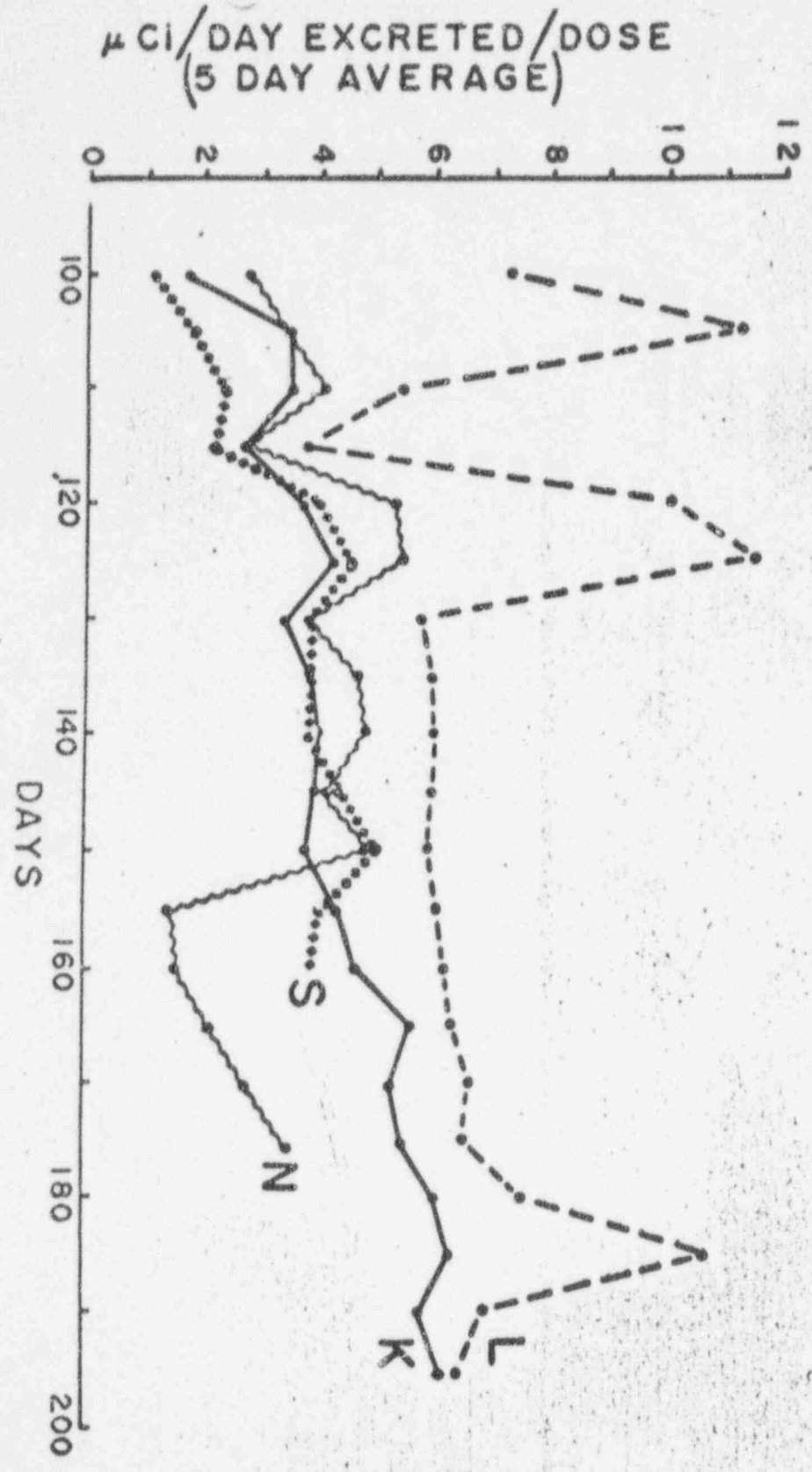
Fig. 5: Per cent of the total daily administered ^{14}C-ascorbic acid retained by each subject. This per cent was calculated by subtracting the daily ^{14}C-urinary excretion from the total daily ^{14}C intake and dividing this difference by the total ^{14}C intake.

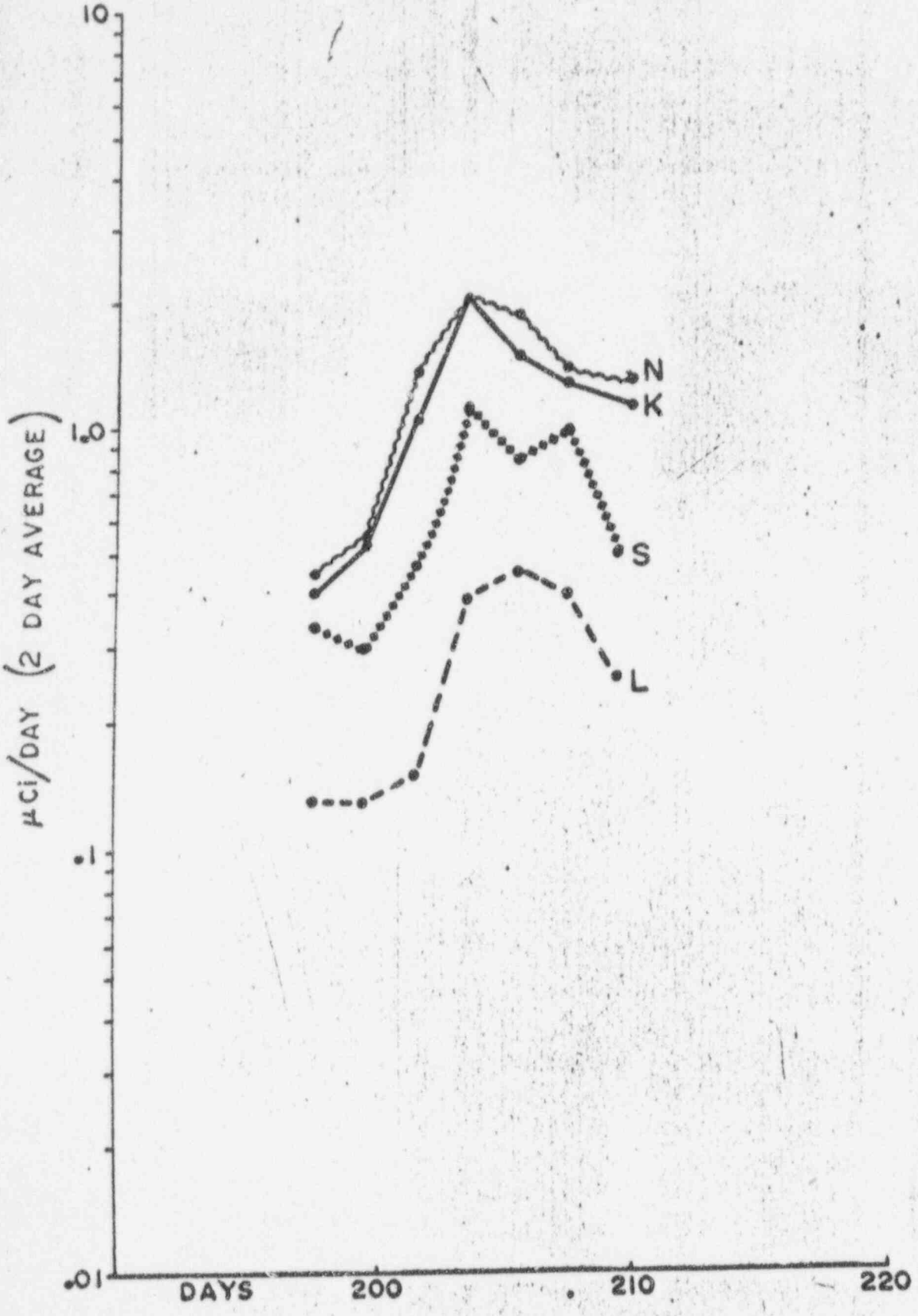
Fig. 6: Daily urinary excretion of ^{14}C of subject L during and after a stress period (days 120 to 126).

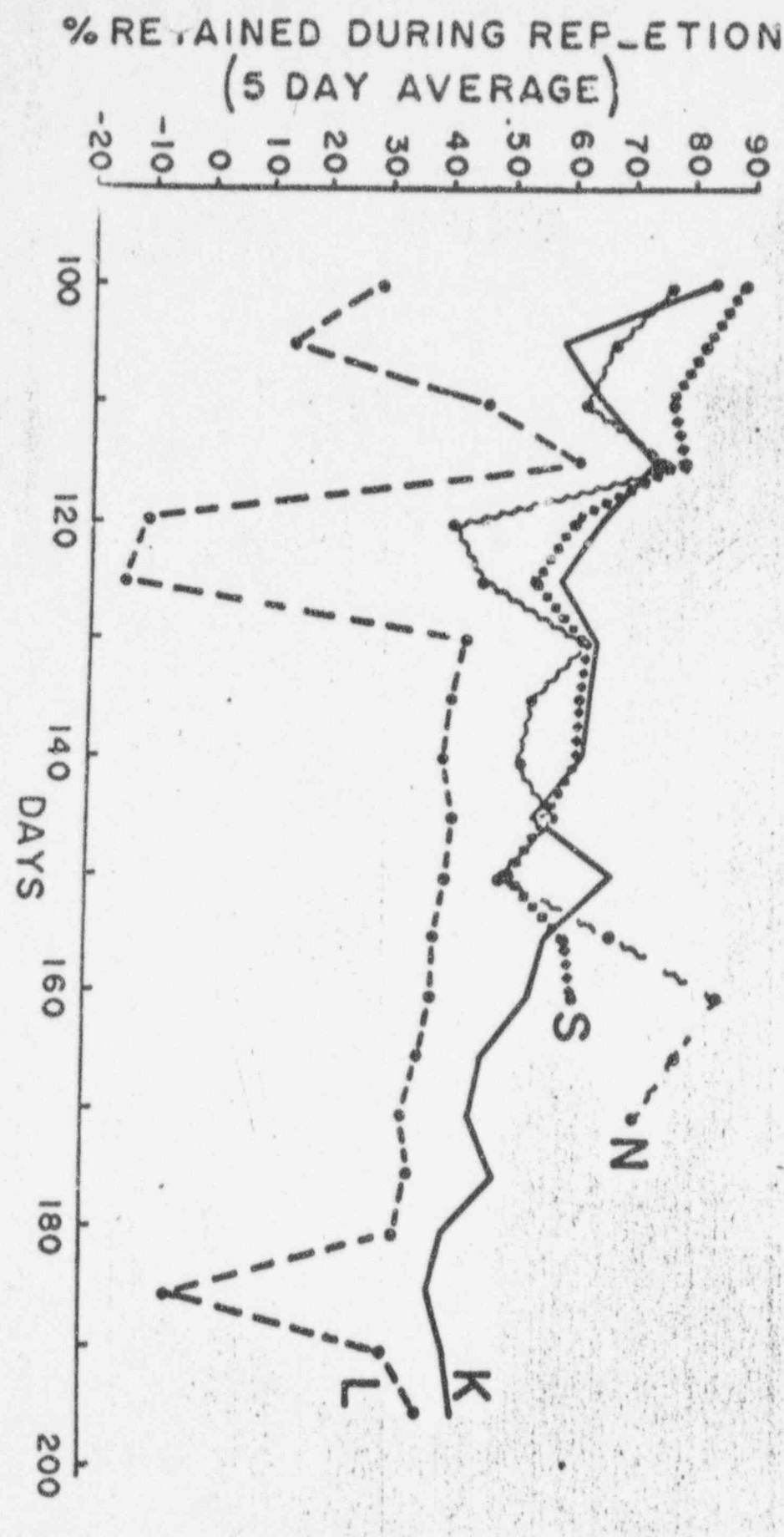
Fig. 7: Demonstrate the average 24-hour excretion of urinary nitrogen and creatinine during both depletion and repletion.

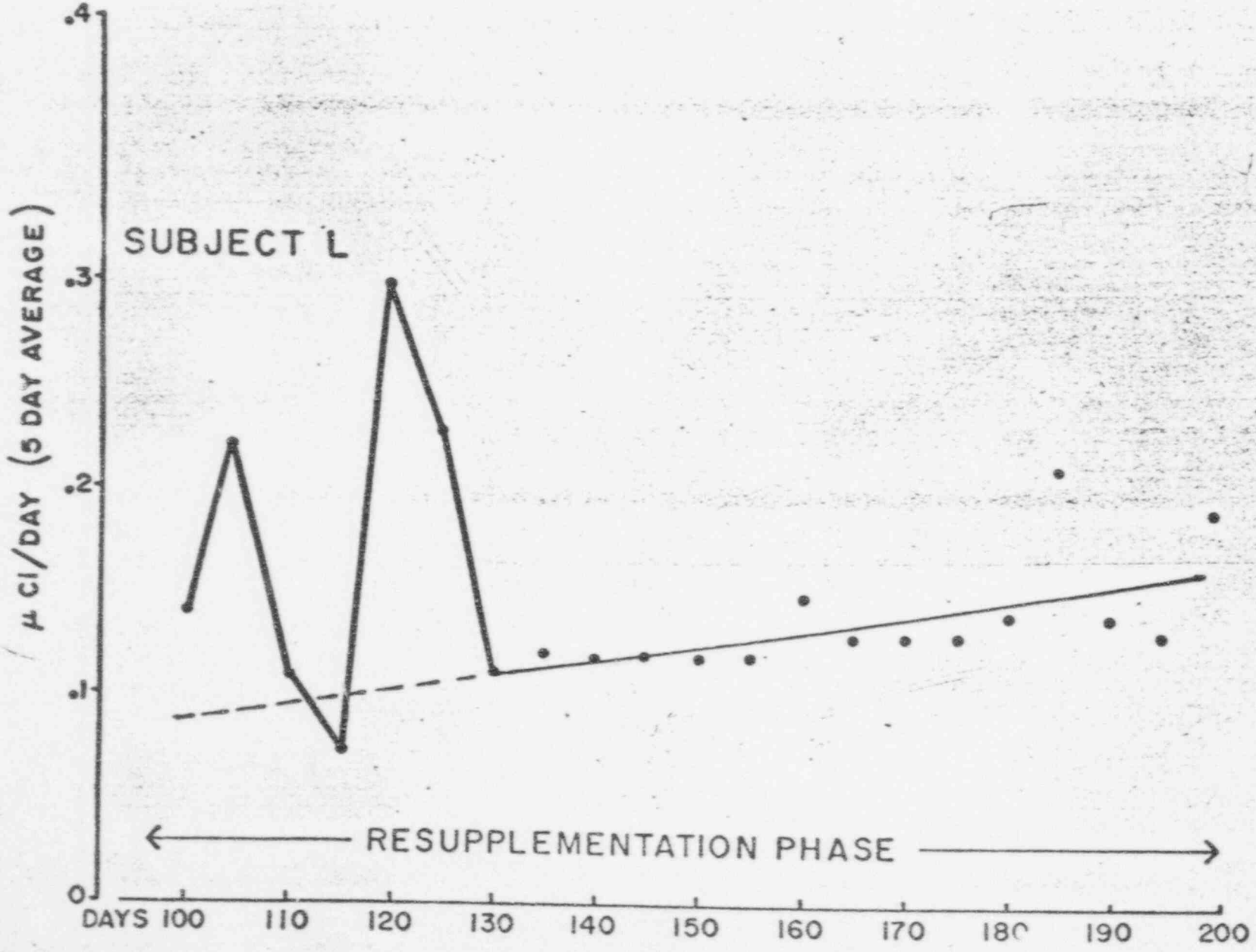

μ Ci/DAY (5 DAY AVERAGE)
10
1
.01
N
K
S
L
23
DEPLETION PHASE
99
REPLETION PHASE
198
SATURATION PHASE
DAYS
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220

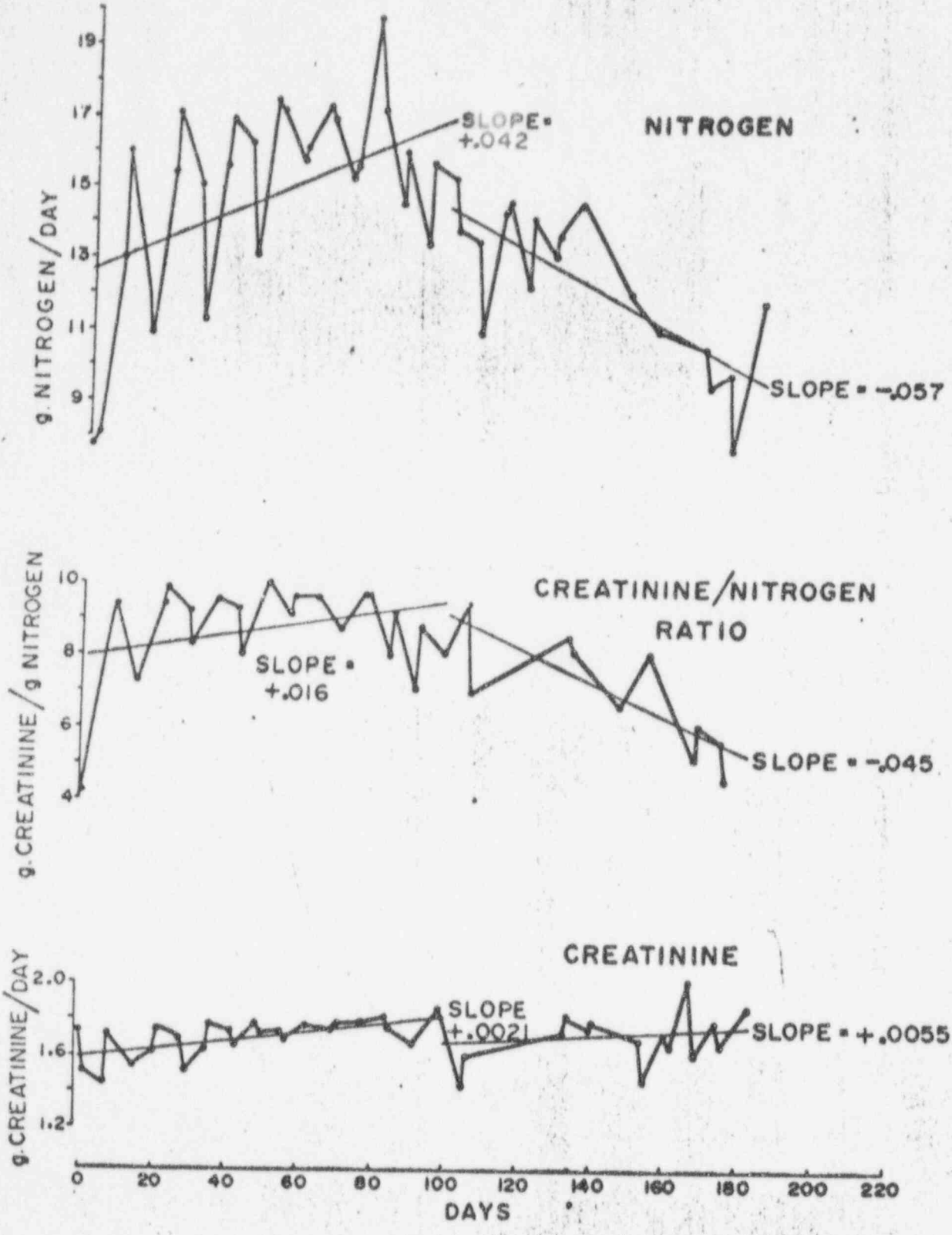

A
B
10
5
1
.5
.1
I
μCi/DAY
II
mg/DAY
III
POOL SIZE
IN g.
IV
μCi/DAY
V
μCi/DAY
VI
μCi
EQUIVALENT mg
←DEPLETION PHASE, ZERO ASCORBIC ACID INTAKE→
←REPLETION PHASE (4-64 mg/DAY INTAKE)→
DAYS 0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200


μ Ci/DAY EXCRETED/DOSE
(5 DAY AVERAGE)
0
2
4
6
8
10
12
100
120
140
160
180
200
DAYS
S
N
K
L


10
1.0
.1
.01
μCi/DAY (2 DAY AVERAGE)
N
K
S
L
DAYS
200
210
220


% RETAINED DURING REPLETION
(5 DAY AVERAGE)
-20
-10
0
10
20
30
40
50
60
70
80
90
100
120
140
160
180
200
DAYS
L
y
K
S
N


SUBJECT L
μ CI/DAY (5 DAY AVERAGE)
.4
.3
.2
.1
0
RESUPPLEMENTATION PHASE
DAYS 100
110
120
130
140
150
160
170
180
190
200


g. NITROGEN/DAY
19
17
15
13
11
9
SLOPE = +.042
NITROGEN
SLOPE = -.057
g. CREATININE/g NITROGEN
10
8
6
4
CREATININE/NITROGEN RATIO
SLOPE = +.016
SLOPE = -.045
g. CREATININE/DAY
2.0
1.6
1.2
CREATININE
SLOPE +.0021
SLOPE = +.0055
0
20
40
60
80
100
120
140
160
180
200
220
DAYS

APPENDIX VIII PART B-5

AD_____

ASCORBIC ACID, BIOLOGICAL FUNCTION AND CHEMISTRY

ANNUAL PROGRESS REPORT

by

Bert M. Tolbert, Ph.D.
(Norman F. Witt, Ph.D., Acting Director)

April 1968

(For the period April 1967 to April 1968)

Supported by

U. S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
Office of The Surgeon General, Washington, D. C. 20315

Contract No. DA-49-193-MD-2611
Department of Chemistry
University of Colorado
Boulder, Colorado 80302

Introduction. The object of this research is to increase our knowledge concerning the biochemistry and chemistry of ascorbic acid. Although ascorbic acid has been studied extensively for many years much of its chemistry and both the details and broad pattern of its biological function(s) remain to be worked out. In spite of this deficiency of knowledge about vitamin C, it is one of the most widely used supplementary vitamins.

Good progress continues to be made in these studies - for two reasons: We have made extensive use of double labeled (^{14}C and ^{3}H) ascorbic acid and secondly, we have made good use of modern physical organic techniques. The following areas have been actively worked on during the past year:

1. Chemistry of Ascorbic and Isoascorbic Acids and their Oxidation Products (E. Bell).
2. Synthesis of L-Ascorbic-6-^{14}C Acid (D. Karr, E. Bell).
3. Preparation of the Four Ascorbic Acid Isomers and Studies of Their Specificity in an Enzyme Reaction (M. Kutnik).
4. Ascorbate Metabolites in Rats (W. Scharf).
5. Subcellular Distribution of ^{14}C Following Injection of Ascorbic-1-^{14}C Acid (V. Richmond).
6. Ascorbic Acid in Microbial Systems; Development of an Ascorbate Requiring Mutant (L. Ray).

Personnel Associated with Study.

Norman F. Witt, Professor, Department of Chemistry, University of Colorado, Acting Director in Dr. Tolbert's absence.

Bert M. Tolbert, Professor, Department of Chemistry, University of Colorado, Boulder, Director (on leave August, 1967 to August 1968).

Eugene M. Baker, Ph.D., Lt. Col. U.S. Army, MRNL Fitzsimons General Hospital, Assistant Director.

Howerde E. Sauberlich, Ph. D., Chief, Chem. Div., MRNL, Fitzsimons General Hospital, Assistant Director.

Virginia Richmond, Ph.D., Assoc. Research Biochemist.

Dale B. Karr, M.S., Research Associate, Biochemist.

Ellen M. Bell, M.S., Research Associate, Chemist (Resigned Aug. 31, 1967).

Lee Ray, B.S., Research Assistant, Ph.D. candidate.

Walter Scharf, Ph.D., Visiting Professor, Post Doctoral, Spring and Summer, 1967.

Mark Kutnink, Undergraduate, part-time Research Assistant.

Publications:

During the past year the following papers and abstracts were published, and papers presented:

1. NMR Studies of Ascorbic and Dehydroascorbic Acid. by E.M. Bell, B.M. Tolbert, J.V. Mengenhauser and E.M. Baker, Accepted for publication, *J. Phys. Chem.*

2. Guinea Pig Urinary Metabolites from Ascorbic Acid, by D.B. Karr, E.M. Baker and B.M. Tolbert, *Fed. Proc.* 27, No. 2, 256 (1968). (Abstract)

3. Subcellular Distribution of Label from Ascorbate, by W. Scharf, S.C. March, E.M. Baker and B.M. Tolbert, *Fed. Proc.* 27, No. 2, 256 (1968). (Abstract)

4. Urinary Metabolites from Ascorbate in the Guinea Pig, by Dale B. Karr, E.M. Baker and Bert M. Tolbert. Manuscript in preparation.

1. NMR Studies of Ascorbic Acid and Dehydroascorbic Acid

Ellen M. Bell

The first phase of this study has been completed, a manuscript prepared and accepted for publication in *The Journal of Physical Chemistry*. The paper's abstract is as follows:

NMR studies are reported for L-xyloascorbic acid, D-araboascorbic acid, L-xyloascorbic-4-D_1 acid, L-araboascorbic-4-D_1 acid, L-dehydroxyloascorbic acid and D-dehydroaraboascorbic acid. The ascorbic acids show ABC_2 spectra and there is no indication of restricted rotation of the C-6 group by hydrogen bonding at any pH. The spectral assignments were confirmed by deuterium labeling. Coupling constants were computer calculated and confirm spectral assignments.

L-dehydroxyloascorbic acid has an ABCD type spectrum which is ascribed to hemiketal binding of the C-6 oxygen to the C-3 carbon. L-dehydroarabo-ascorbic acid shows all the correct chemical properties for a monomeric hemi-ketal, but complex NMR spectra are obtained that could not be analyzed.

2. Identification of Urinary Excretion Products from Labeled Ascorbic Acid

By Dale B. Karr

Studies on Guinea Pig Urine.

The study on the radioactive metabolites of ascorbic acid found in guinea pig was continued. The materials isolated from the urine either as a lead precipitate at pH 8 (Pb material) or as non-precipitating material at this pH, (S material), have now been chromatographed in several systems and subjected to acid hydrolysis.

1. ^{3}H Metabolites:

The compounds present in the "Pb" material and containing only tritium are most likely threonate, xylonate and lyxonate. Evidence supporting this was obtained from electrophoresis at pH 5.6 in 0.2M ammonium acetate. The mobility of threonic acid and glucuronic acid with respect to picric acid are 1.5 and 1.3 respectively: The 5-carbon acids similar in structure and charge would move in between the above acids.

The tritium labeled metabolites had mobilities (with respect to picric) of 1.5 and 1.4. The material at 1.4 also contained double ^{14}C, ^{3}H labeled material. Additional evidence was obtained by descending chromatography in ethylacetate:pyridine:water, (8:2:1) and methyl ethyl ketone:acetic acid: saturated borate solution (9:1:1). In the first the R_f's of threonolactone and glucuronolactone are 0.75 and 0.60 respectfully and the urinary ^{3}H metabolites are .75 and .68.

In the second system the R_f's are: threonolactone; 0.65, xylonolactone; 0.3; and lyxonolactone, 0.48. The threonolactone we used as a standard had an R_f of only 0.56. Likewise the other standards we compared had lower mobilities. The ^{3}H metabolites had mobitites of 0.56 (threonolactone), 0.45 and 0.18.

2. ^{14}C Metabolites: Electrophoresis of Pb material separated a small amount of material containing mostly ^{14}C. Its mobility with respect to picric was 1.85 and is presumably oxalate.

3. ^{14}C, ^{3}H Metabolites: The compounds containing both ^{14}C and ^{3}H consist of acids, glucuronides, (or larger conjugates of ascorbate), and a 6-carbon lactone.

Electrophoresis in 0.2 M ammonium acetate, pH 5.6, gave evidence that there are at least two acidic compounds R_f of 0.91 and 1.4 with respect to picric acid. The R_f of the 6-carbon uronic acid, glucuronic acid was 1.26.

Chromatography in ethylacetate:pyridine:water (8:2:1) separated ^{14}C, ^{3}H com-

pounds into 3 bands R_f 0.03, 0.09 and 0.56 corresponding to acids, disaccharides or glucuronides and 6-carbon lactone of uronic acid, respectively. The 6-carbon lactone is presumably in equilibrium with the 6-carbon acid. Chromatography in methylethyl ketone:glacial acetic acid:borate-saturated water: 9:1:1 verified the existence of at least 4 compounds containing both ^{14}C and ^{3}H label.

^{14}C, ^{3}H Metabolites in the "S" material.

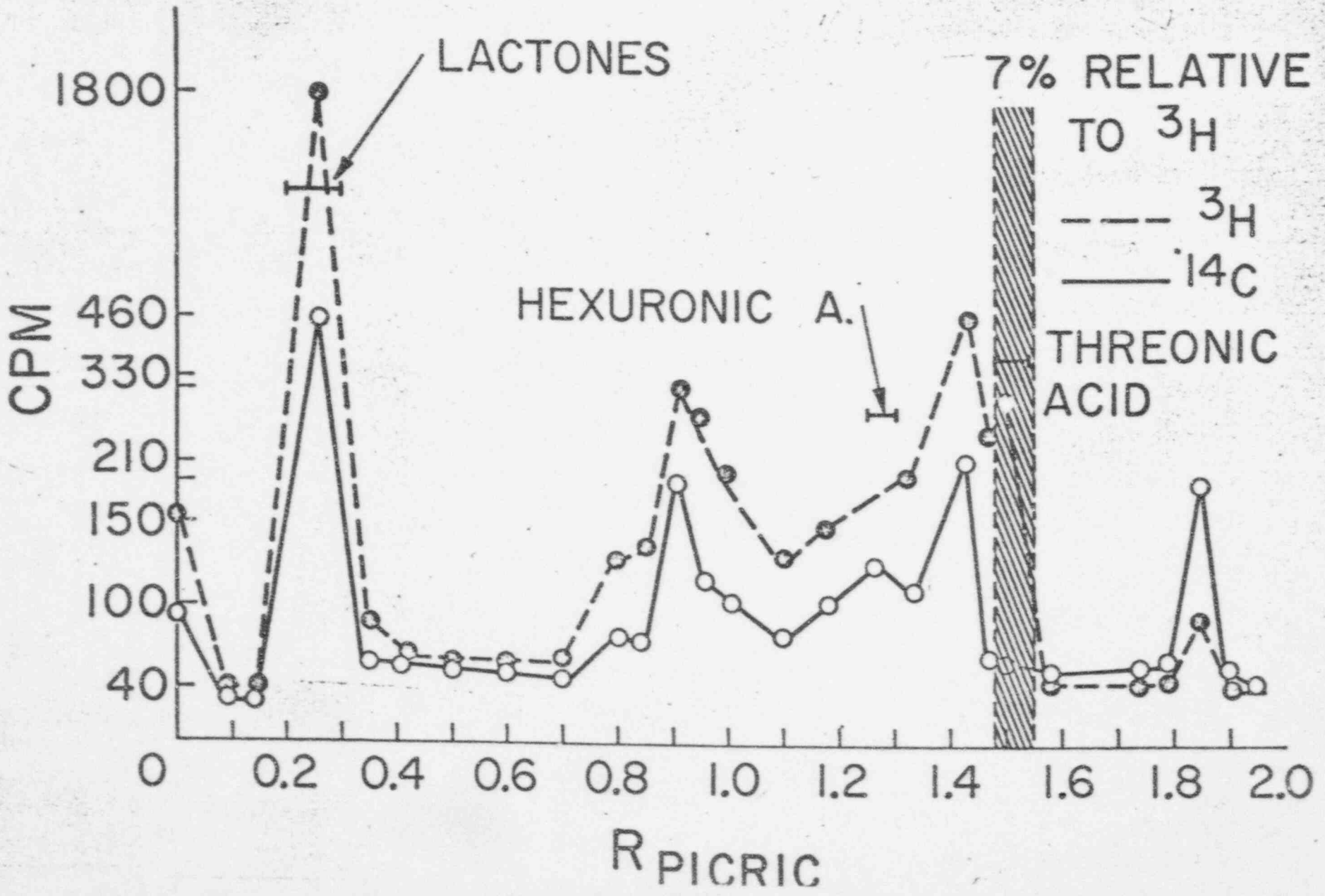
Two metabolites both containing ^{14}C and ^{3}H, can be separated by chromotography on DE-81 anion exchange paper in 0.13 M sodium acetate, pH 4.6, and ethanol (1:4) and on Whatman paper in methyl ethyl ketone:glacial acetic acid; saturated borate solution (9:1:1). The mobilities of these compounds are very similar to materials in the "Pb" fraction, thus the possibility exists that the Pb precipitation was incomplete with respect to these metabolites, although their mobilities are not alike on Sephadex G-10.

In general all the ^{14}C, ^{3}H material in both the "Pb" and "S" material are susceptible to decarboxylation when heated to 121° alone or in the presence of 2 N TFA.

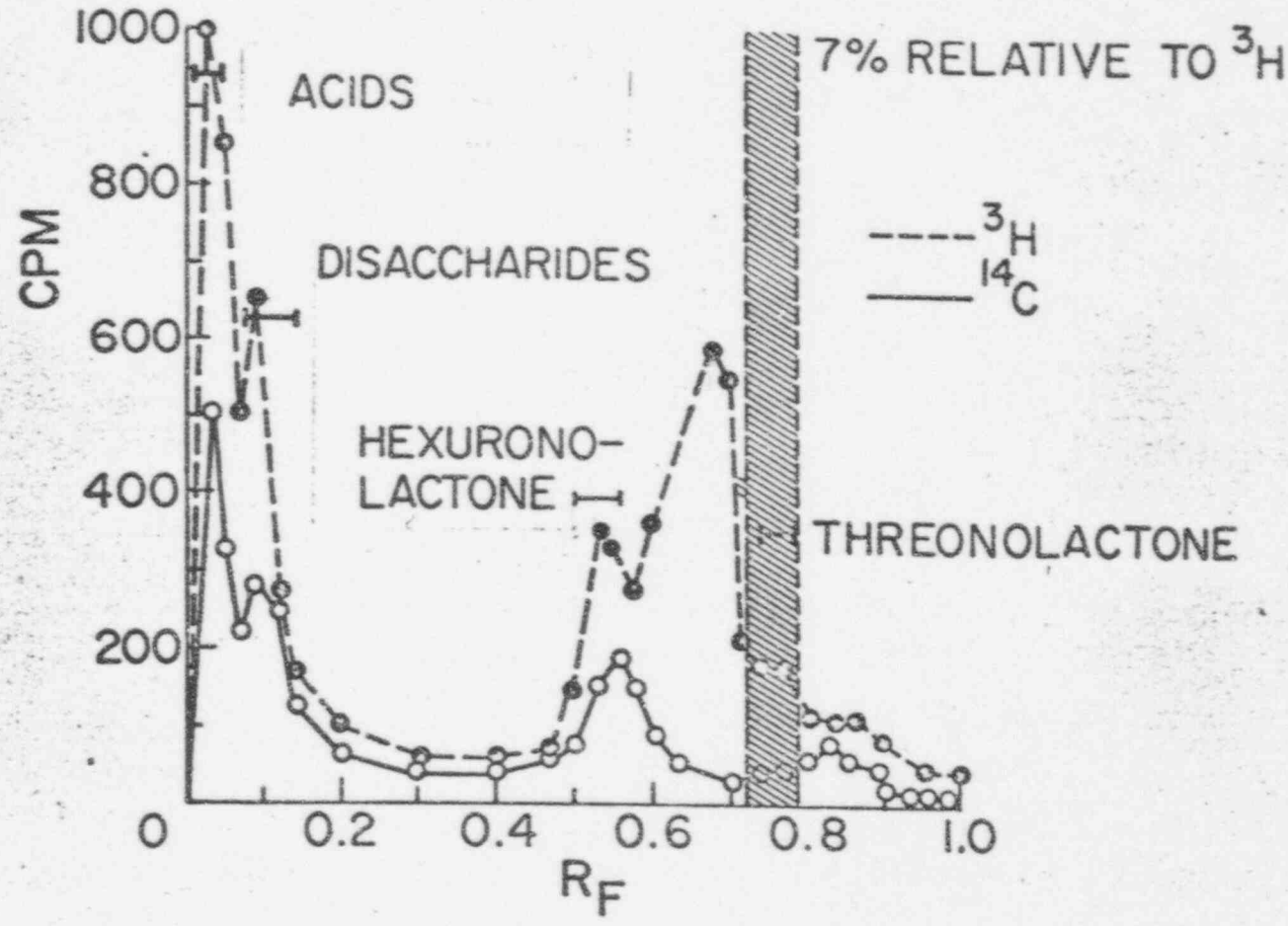
Their molecular weights could not be determined on Sephadex G-10 because of secondary interactions. The effect of the enzymes, pepsin and B-glucuronidase, on the labeled metabolites in both "Pb" and "S" material was investigated. Results were inconclusive, because no changes were noted in the mobilities of the metabolites on DE-81 anion exchange paper or on Sephadex G-10. Further investigation of the ^{14}C, ^{3}H metabolites, is needed to establish their identity and reinvestigation of the effects of enzymes using one of the other chromatography systems, electrophoresis, or a different size of Sephadex. Following are figures of the chromatographic separations of the "Pb" and "S" materials.

ELECTROPHORESIS OF PD MATERIAL IN

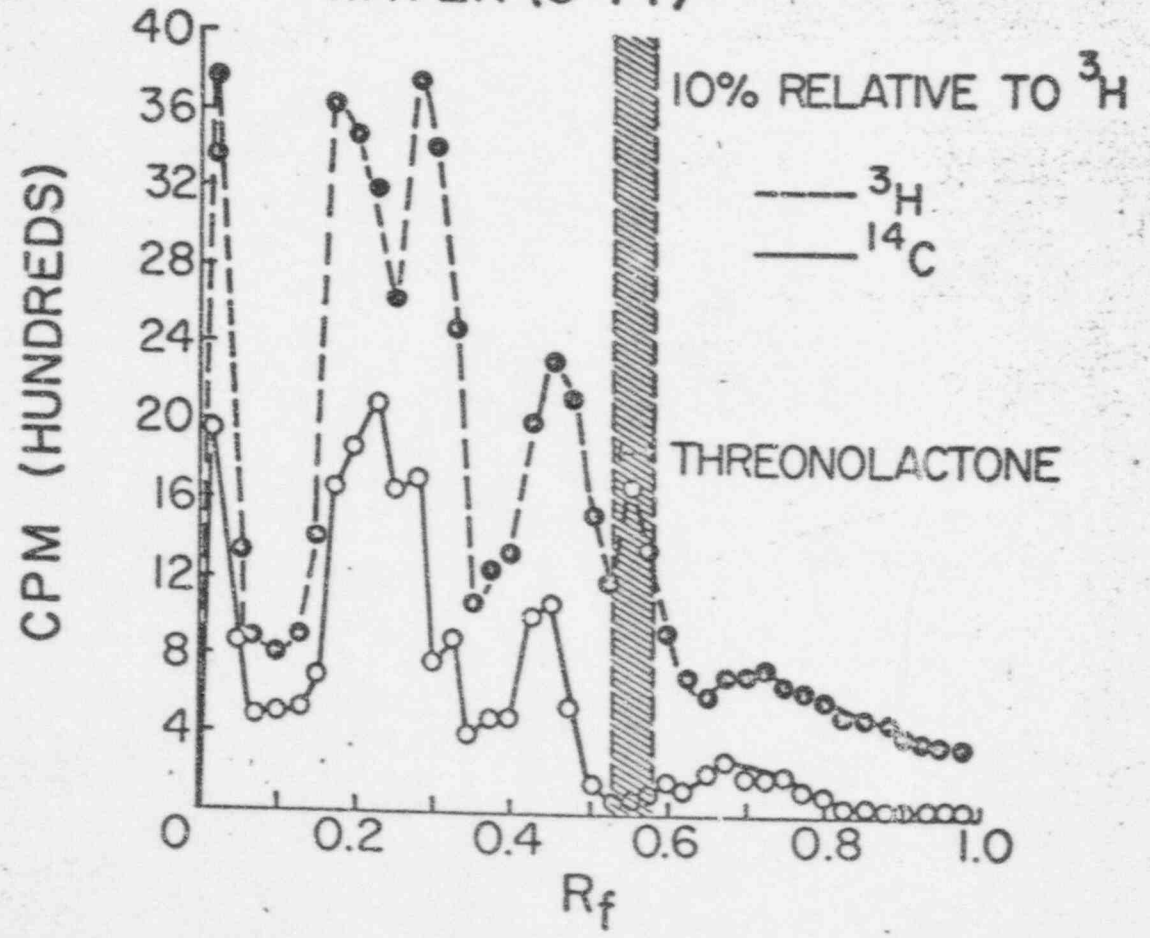
0.2M AMMONIUM ACETATE pH 5.6



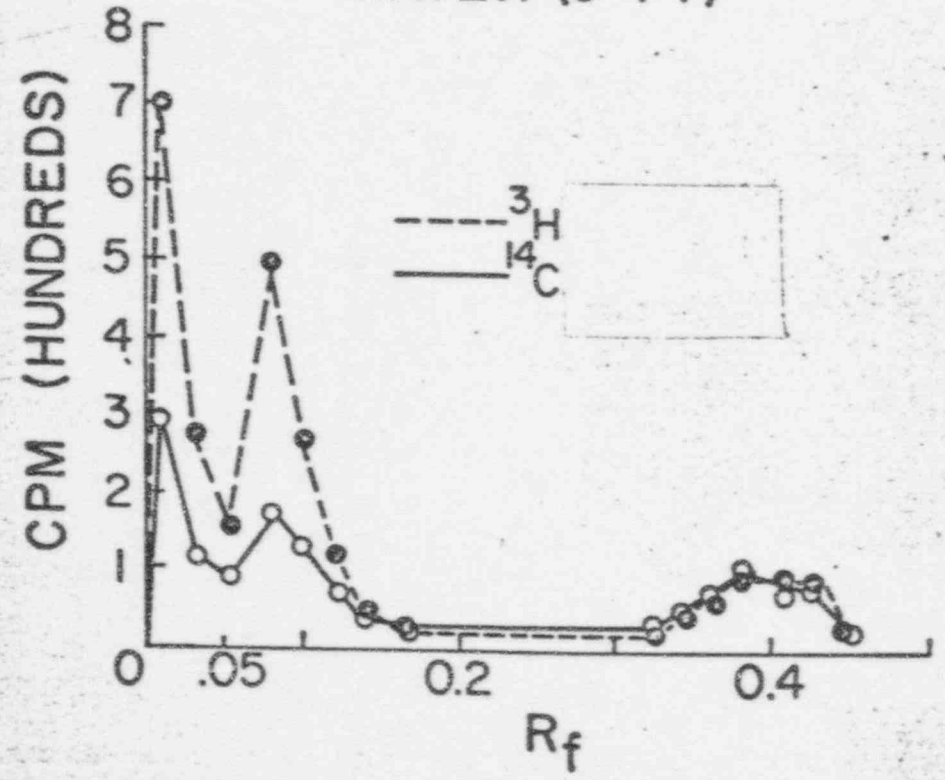
CHROMATOGRAPHY OF Pb MATERIAL IN ETHYLACETATE: PYRIDINE: WATER (8:2:1)



CHROMATOGRAPHY OF Pb MATERIAL IN METHYLETHYLKETONE: GLACIAL ACETIC ACID: BORATE SATURATED WATER (9:1:1)



CHROMATOGRAPHY OF S MATERIAL IN METHYLETHYLKETONE: GLACIAL ACETIC ACID: BORATE SATURATED WATER (9:1:1)



Synthesis of L-Ascorbic-6-^{14}C Acid

By Dale B. Karr and Ellen M. Bell

The synthesis of L-ascorbic-6-^{14}C acid from D-glucose-1-^{14}C was started by Bevill, continued by Bell and is being furtner studied. Bell carried out the reduction of the D-glucose-1-^{14}C (10 mC/230 mg) to D-sorbitol-6-^{14}C, and one conversion of unlabeled sorbose to ascorbate following Bevill's procedure through the synthesis of 2-keto-L-gulonic acid. Ascorbic acid was formed by dissolving 2-keto-L-gulonic acid and 0.1 g ascorbate in 1 N HCl-50% ethanol at 45° swept with carbon dioxide. After five hours the solution was lyophilized and ascorbate acid recrystallized from acetonitrile. The success of this method was unclear because of the addition of carrier ascorbic acid in the final step. Ascorbic acid formation was determined by TLC in AcCN, BuCN, H_2O.

The project was turned over to Dale Karr when Ellen Bell left. Since then two attempts to convert unlabeled sorbose to ascorbic acid have been unsuccessful. Modifications were introduced both in the conversion of K^+(2-ketogulonate)$^-$ to 2-keto gulonic acid and in the formation of ascorbate from 2-ketogulonic acid.

The synthesis of labeled ascorbic acid is a difficult procedure and no adequate directions exist in the literature for a small scale procedure. Previous investigators who have tried to prepare ascorbate-6-^{14}C have ended up with negligible or questionable products. The most difficult step is the final one, the conversion of 2-keto-L-gulonate to ascorbic acid.

The current method for the synthesis of ascorbate from glucose has been carried out on unlabeled glucose.

1. <u>D-sorbitol</u>

To 200 mg of sodium borohydride dissolved in 30 ml of 1 N ammonium

hydroxide cooled to 0°, is added 230 mg of D-glucose. The reaction mixture is stirred at 0°C for 30 minutes and at 20°C for 4 1/2 hours. Then two ml of glacial acetic acid is added to destroy the excess sodium borohydride. The solvents are removed on a rotoevaporator. The residue is dissolved in 30 ml of absolute methanol and the volatile methyl borate remcved on the Rotovac. The addition of methanol is repeated seven times.

2. L-Sorbose

The residual syrup and 770 mg of D-sorbitol are dissolved in 10 ml of water and inoculated with 10-20 mg of Acetobacter suboxydans 621 pelleted at 3000 RPM. The suspension is incubated at 30°C for at least 36 hours. The conversion to L-sorbose is determined by chromatography in ethylacetate: pyridine:water (8:2:1) for 18 hours.

When the conversion is complete the Acetobacter suboxydans are removed by centrifugation at 15,000 RPM. The solution is lyophilized and the resulting gum is suspended in 3 x 15 ml reagent acetone. The gum is resuspended in 50 ml spectroquality acetone overnight. The acetone is decanted and the now powdery material flushed with nitrogen.

3. Diacetone-L-sorbose[1]

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(1) Peter G. Dayton, *J. Org. Chem.*, 21, 1535 (1956).

Under nitrogen atmosphere the L-sorbose is cooled to 0°C and to it is added a cold solution of 50 ml spectroquality acetone and 2 ml of concentrated sulfuric acid. The suspension is stirred under nitrogen at 0°C for two hours warming every 1/2 hour to room temperature or until the L-sorbose is dissolved. The solution is then kept at 4°C for 18 hours.

The resulting yellow, acid solution is added in small portions to 8 g of potassium carbonate in 60 ml of water while swirling and cooling in

an ice bath. Then 150 ml of acetone, used to rinse the original flask is added to the mixture. The precipitated salts are removed by suction filtration and washed with additional acetone. The acetone is removed in vacuo from the combined washings and filtrate. The 35 ml of remaining aqueous solution is extracted 7 x 100 ml of ether. The ether extracts are concentrated to dryness on a Rotavac.

4. Potassium diacetone-2-keto-L-gulonate

To the diacetone-L-sorbose dissolved in 14 ml of cold 4 1/2% potassium hydroxide is added, dropwise, 1.4 g of potassium permanganate in 60 ml of water over a period of one hour. The mixture is then heated for fifteen minutes at 50°C. Fifty ml of absolute ethanol is added to destroy excess potassium permanganate. The precipitate is removed by suction filtration. The colorless filtrate is adjusted to pH 8 with carbon dioxide and then concentrated to dryness.

5. Diacetone-2-keto-L-gulonic acid

The potassium diacetone-2-keto-L-gulonate is dissolved in 40 ml of water, cooled to 5-10°C and brought to pH 2 with 0.5N sulfuric acid (approximately 11 ml). The solution is extracted immediately with 6 x 50 ml of ether. The combined ether extracts are washed once with 40 ml water and concentrated to dryness. The residue is flushed with nitrogen.

6. L-ascorbic acid

The diacetone-2-keto-gulonic acid, and 0.1 g ascorbic acid, under carbon dioxide atmosphere, is dissolved in 20 ml of 1 N trichloroacetic acid in 50% ethanol. The solution under carbon dioxide, is kept at 45°C for 18 hours. It was then concentrated to yellow oil. Ten ml of ethanol is added, followed by 23 ml of petroleum ether. After crystallization begins, the mixture is kept at 4°C until crystallization is completed.

One pilot run starting with D-glucose-2-^{14}C has been completed. Results are given below:

Steps in synthesis of L-ascorbic acid	Intermediary Product Percent	R_f TLC AcCN, BuCN, H_2O	Recovery Estimate Grams
1. D-sorbitol	100	0.00	1.0
2. L-sorbose	80	0.00	0.8
3. Diacetone-L-Sorbose	80	0.80	0.7
4. K^+diacetone(2-keto-L-gulonate)	100	0.72	0.7
5. Diacetone-2-keto-L-gulonic A	60	0.72	0.4
6. L-ascorbic acid			
a. 2-keto-L-gulonic acid	86	0.0	0.29
b. L-ascorbic	4	.22	0.01
c. Other contaminants DKG & deactone 2-keto-L-gulonic acid	10	.09, .72	

The remaining radioactivity in steps 3 and 5 was found in the water fraction. It was not ether extractable since in both steps, the last ether extraction contained less than 1% of the radioactivity. No sorbose was detectable in the water fraction of step 3.

Before further pilot runs with D-glucose-2-^{14}C and D-glucose-1-^{14}C are made, the conversion of 2-keto-L-gulonic acid to L-ascorbic acid will have to be improved. The following modifications will be tried: (1) the solution of 2-keto-L-gulonic acid in 1 N trichloroacetic acid and 50% ethanol will be maintained at 60° for 18 hours instead of 45°C, (2) the percent of ethanol in the solution will be increased to 90%, without changing the normality of the trichloroacetic acid, and (3) the 2-keto-L-gulonic acid will be converted to its methylester, as prescribed by Bevill, and then the latter converted to L-ascorbic acid.

The L-ascorbic-6-^{14}C and L-ascorbic-4-^{3}H (synthesized by E. Bell) will be used in animal experiments to determine if cleavage occurs between C-4 and C-6 during the metabolism of L-ascorbic acid. A change in the C/H dpm ratio of the metabolites would indicate such a cleavage. We suspect no change in this ratio will be observed.

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2. Wells, P.A., Stubbs, T.T., Lockwood, L.B. and E.T. Roe, 1937, Sorbose from Sorbitol, *Ind. Eng. Chem.*, 29, 1385.

Subcellular Fractionation of Rat Tissues Following Injection of C^{14} l Ascorbic Acid

By Virginia Richmond and G. Philip Anderson.

The existence of a "combined" form of ascorbic acid as an active complex has long defied efforts to isolate such a form. Our second attempt to elucidate such a complex has been to measure the uptake of labeled ascorbic-1-^{14}C acid in the gross subcellular fractions from several tissues in the rat. Since ascorbic acid is subject to rapid air oxidation under many conditions all of the procedures detailed here, except for the actual decapitation of the animals, were done in a nitrogen atmosphere using an Isolet such as those used in a premature nursery.

Three animals were injected with approximately 100 µC of ascorbic-1-^{14}C acid [Nuclear Chicago] intraperitoneally in one dose of 1 ml. normal saline. Approximately 24 hours after injection the animals were decapitated, placed immediately into an ice bath and put into the Isolet. The organs were excised, placed on ice, weighed and homogenized with a Teflon-glass homogenizer in enough 0.32 M sucrose, 0.001 M EDTA to give a 10% homogenate (w/v). The centrifuge tubes were capped while in the nitrogen atmosphere and were uncapped

only when again in the isolet. The first centrifugation was at 1,000 x g for 10 minutes, to bring down the nuclei and cell debris. The pellet was washed with 2 ml. of the sucrose solution again resuspended and centrifuged and the supernatant solutions combined. The pellet was the P_1 fraction.

The second centrifugation was designed to collect the mitochondria-like particles and was carried out at 18,000 x g for 30 minutes in a Sorvall R-C-2. The pellet was again washed by resuspending in fresh sucrose solution recentrifuged. This pellet constituted the P_2 fraction.

To obtain the microsomal-like fraction, P_3, the combined supernatants were centrifuged at 100,000 x g for 60 minutes in a Spinco Model L centrifuge and this fraction was washed as described above. The particulate fractions P_1, P_2, and P_3 were solubilized in cold 2 N sodium hydroxide and radioactivity and protein concentration were determined on aliquots of the solutions. Protein concentrations were estimated using the Lowry technique; these were correlated to an albumin standard and expressed as mg albumin per total sample. The averaged specific activities are given in the following table. The greatest uptake of radioactivity was in the cerebellum with the mitochondrial fraction of the midbrain and the cerebrum having only a little less activity. This is consistent with Scharf's earlier findings where oxygen protection was not observed. Lung and spleen are next in the quantity of specific activity. The highest specific activity was in the supernatant solutions for all of the tissues examined. It had been hoped that a difference in the specific activities from the various subfractions might indicate a localization of the ascorbate complex and thus indicate an enzyme system(s) which might utilize ascorbic acid. This observation was not forthcoming.

TABLE I

Specific Activity of Subcellular Fractions and Supernatant Solutions from Rat Tissues Following Injection of ^{14}C-l-Ascorbic Acid

Tissue and Fraction		Specific Activity	
		DPM per mg as albumin	DPM per gm as wet weight
Cerebrum	P_1	3,350	21,102
	P_2	10,733	64,006
	P_3	3,426	4,314
	Supernatant	26,653	405,353
Cerebellum	P_1	3,659	64,231
	P_2	8,020	50,061
	P_3	15,051	20,924
	Supernatant	39,150	461,760
Midbrain	P_1	4,760	23,089
	P_2	4,394	40,914
	P_3	10,989	27,382
	Supernatant	31,166	345,222
Heart	P_1	735	3,531
	P_2	3,903	2,860
	P_3	1,263	3,377
	Supernatant	100,731	204,623

(Table I continued)

Tissue and Fraction		Specific Activity	
		DPM per mg as albumin	DPM per gm as wet weight
Kidney	P_1	4,314	11,667
	P_2	7,513	11,260
	P_3	568	1,466
	Supernatant	62,722	224,060
Liver	P_1	2,846	13,137
	P_2	10,680	17,431
	P_3	769	3,312
	Supernatant	19,742	361,742
Lung	P_1	1,563	28,719
	P_2	24,455	30,255
	P_3	4,139	6,697
	Supernatant	28,770	446,670
Spleen	P_1	6,449	38,381
	P_2	5,645	61,010
	P_3	3,035	16,191
	Supernatant	41,031	1,259,400
Adrenal (1 animal only)	P_1	10,107	46,210
	P_2	10,247	46,600
	P_3	757	3,440
	Supernatant	293,589	114,500

The various supernatant solutions have been studied for a possible radioactive complex by their behavior on chromatographic columns and by thin layer chromatography. The chromatographic mediums employed have been: Sephadex G-200, Sephadex G-100, Sephadex G-50 and Sephadex G-10 and DEAE cellulose, DEAE Sephadex A-50. All of these columns have indicated a low molecular weight series of compounds and have separated from two to eight peaks from standard ascorbic acid samples as well as several from the tissue supernatant solutions.

These fractions are being further characterized by thin layer chromatography and by gas - liquid chromatography.

Distribution and Ubiquity of Ascorbic Acid in Biological Systems

By Lee Ray

We are interested in finding an organism capable of endogenous ascorbate synthesis in order to use it for the preparation of an ascorbate requiring mutant. Such a mutant could be used to study the function and fate of labeled ascorbate, the enzymes involved in ascorbate metabolism, and, perhaps, as a microbiological assay for ascorbate.

In a literature search some reports of ascorbate in lower organisms (algae, fungi, yeast) were found, but these results need to be verified by newer analylical procedures. There was no report of endogenous ascorbate synthesis by any microorganism. Since then we have assayed eleven bacterial species for free ascorbic acid.

The assay involves preparation of a cellular extract by fracturing the bacteria in a French pressure cell and centrifuging down the cell debris.

The extract is treated with trichloroacetic acid, to remove protein material, and is then applied to a silica gel TLC plate. The TLC plate is developed in a solvent system of acetonitrile, butyronitrile and water which produces a characteristic movement of the ascorbate. In addition, the location of the ascorbate is determined by standards run simultaneously at the edge of the plate. This process separates the ascorbate from cellular contaminants. The area which should contain the ascorbate is scrapped off the plate and subjected directly to a modified Schaefferts-Kingsley assay. This is a timed reaction which produces a dinitrophenylhydrazine derivative of the ascorbate. The amount of colored product is determined spectrophotometrically, and the amount of ascorbate present may be determined quantitatively by assaying a standard ascorbate solution and comparing their OD's.

The following strains were investigated: Salmonella typhimurium, Staphylococcus aureus, Aerobacter aerogenes, Alcaligenes faecalis, Bacillus subtilis, Serratia marcescens, Pseudomonas putida, Pseudomonas fluorescens, Escherichia coli (strains K12 and D2) and an α-hemolytic streptococcus.

Of these eleven strains two (B. subtilis and A. faecalis) seem to have higher ascorbate levels than the others. Unfortunately, even in these two the non-specific, background reactivity is of the same magnitude as the calculated ascorbate content, i.e. about 5 mg ascorbate/g of cells. In order to see if these may actually contain ascorbate, we are working to see if they can synthesize ascorbate. A radiometric assay is used. This involves growing the bacteria on glucose-^{14}C as the sole carbon source, and treatment through the TLC separation, as before. The area corresponding to the ascorbate is again scraped off, but, instead of assaying it by the previous method, liquid scintillation counting is used. If the ascorbate fraction proves to be labeled,

it will be preliminary evidence for ascorbate synthesis. Of course it will be necessary to prove that the material is indeed ascorbate and not something else which happens to be moving the same on the TLC plate. If we can find a microorganism which snythesizes ascorbate we will proceed to the preparation and isolation of an ascorbate auxotroph.

Isomeric Specificity of Ascorbic Acid

By Mark Kutnink

INTRODUCTION

Two isomers of ascorbic acid, namely L-xyloascorbic acid and D-arabo-ascorbic acid, are commercially available. Each of these can be subjected to conditions which cause it to isomerize and yield an equilibrium mixture which has a composition of approximately 50% of the original isomer and 50% of its corresponding 4-epimer. Thus, L-xyloascorbic acid, and D-xyloascorbic acid can be obtained from D-araboascorbic acid. The main objectives of this work are to prepare gram quantities of these commercially non-available isomers of ascorbic acid. This work has entailed determining the best method of separating the components of the epimeric mixtures obtained from the isomerization reactions. Part I deals with this phase of the work. Part II discusses experiments now in preparation and proposed for utilizing the group of 4 isomers of ascorbic acid, now available.

PART I

A. Investigation of methods proposed for the separation of components of epimeric mixtures of ascorbic acid.

1. Ion Exchange Column

The feasibility of this method requires that the titration curve of a xyloascorbic acid be sufficiently different from that of an araboascorbic acid to effect a separation on the column. Two titrations were carried out in which 100 ml of 1 M solutions of L-xyloascorbic acid and D-araboascorbic acid were each titrated with 10 N potassium hydroxide. Graphs plotting pH of the ascorbate solutions versus ml of base added indicate the L-xylo and D-arabo isomers of ascorbic acid to be similar enough in both pKa_1 and pKa_2 that separation on an ion exchange column is unlikely. This possibility was not further studied.

2. Thin Layer Chromatography (TLC)

Brenner, et al. (1964) report the separation of xylo and arabo ascorbic acids on plates of silica gel (0.25 mm) after developing in a solvent system of acetonitrile-butyronitrile-water (66:33:2). The work described here investigates the ability of plates 1 mm in thickness to separate large quantities of ascorbic acid. The procedure for preparing the silica gel slurry (30 g silica gel to 60 ml water in which is dissolved 1.8 g metaphosphoric acid) was a modification of that used by Brenner in that the metaphosphoric acid was dissolved in 60 ml of a 5% aqueous methanol solution. One millimeter plates were then spread and activated overnight in an oven at 110°C. Two plates were then streaked with a water solution containing 0.25 g L-xyloascorbic acid and 0.25 g D-arabo-ascorbic acid. A third plate was streaked with a water solution containing only 0.05 g of each isomer. In the first two cases, when the plates were placed in the developing solvent, the portion of silica gel between the bottom edge of the plate and the origin cracked and dislodged from the plate. The third plate did not crack as badly. Solvent rise continued, and after terminating the development, the plates were sprayed with 5% iodine in

chloroform. No definitive separation of isomers was observed. No further work was done on this method.

3. Adaptation of Silica Gel TLC to a Silica Gel Column

Silica gel sifted 60 mesh was activated according to the procedure described above for thin layer plates. It was then placed on a 2 x 27.6 cm column with a solution of acetonitrile-butyronitrile (2:1). The flow rate was 2 1/3 ml per minute. The solvent was allowed to run level with the top of the column and a solution of 0.0488 g L-xyloascorbic acid and 0.0491 g D-araboascorbic acid in 0.15 ml water was applied to the column. The addition of 3 ml of the acetonitrile-butyronitrile solution immediately precipitated the ascorbic acid from the water solution. After the liquid was allowed to run level with the column, a glass wool plug was placed on the top and elution was begun, first with 100 ml of the acetonitrile-butyronitrile solution, and then the acetonitrile-butyronitrile-water solution (66:33:2). 145 fractions were collected, each containing 7 ml solution. One-tenth ml of every fifth fraction was assayed for ascorbate content according to the colorimetric method of Fairbridge, et al. (1951). The concentration of ascorbate was determined by the amount of tetrazolium chloride reduced to formazan, which has a maximum absorbance at 485 mμ. All fractions had an optical density of zero. The column was then dissected into bands, and the assay was repeated directly on the column material. The results indicated a greater concentration of ascorbate in the bottom region of the column, with ascorbate distributed to some degree throughout the column's length. No distinct separation of the isomers into two regions was indicated.

4. Recrystallization from Acetonitrile

This method works and is discussed in the following section.

B. Methods of Isomerization of Ascorbic Acid

1. The first method of isomerization attempted was based on Bell's procedure for labeling ascorbate with tritium and was done as follows:

25 g L-xyloascorbic acid were neutralized with 15.93 g potassium hydroxide in 50 ml water. The solution was transferred to a 250 ml round-bottom flask, the neck was constricted, and nitrogen gas was bubbled through for 30 minutes. The flask was then evacuated under a water aspirator, sealed, and placed in an oven at 110°C for 24 hours. The flask was opened (contents found to be under slight pressure) and the resulting dark brown liquid was neutralized to pH 2 with Dowex 50 (H+) resin. The solution was shell-frozen and lyophilized to dryness. The material was then extracted four times with hot acetonitrile. Upon cooling, the extractions yielded a brown gummy material. TLC indicated that the first extraction contained two compounds sensitive to the iodine-chloroform spray reagent. The R_f values identified them as L-xyloascorbic acid and L-araboascorbic acid. The other three extractions showed only traces of the L-xylo isomer after TLC. The small yield of material and its gummy nature did not lend itself to collection and weighing.

2. The second method of isomerization, and that employed for the remainder of the work was essentially that of Brenner, et al. (1964):

17.6 g L-xyloascorbic acid were dissolved in 200 ml of a 50% aqueous solution of methanol, and neutralized with 11. 2 g potassium hydroxide. The solution was then refluxed at 75°C for 15 hours, after which the solution was neutralized with Dowex 50 (H+) resin to pH=2, filtered, and the filtrate concentrated to remove most of the water and methanol. The remainder of the solution was shell-frozen and lyophilized to dryness yielding a yellow solid. Four extractions were made by refluxing the material in acetonitrile. The

following modifications of the method were made:

(1) A 10% excess of potassium hydroxide was added.

(2) Nitrogen gas was bubbled through the solution during isomerization.

(3) 30 to 36 hours were taken to isomerize.

The material which precipitated from the four extractions when the acetonitrile cooled was collected and saved along with the mother liquors. TLC indicated that most of the L-araboascorbic acid was concentrated in the mother liquors of the first and second extractions, along with a small amount of the original material, L-xyloascorbic acid. Similarly, when the reaction was carried out with D-araboascorbic acid as the starting material, most of the D-xylo isomer was found to be in the precipitations obtained from the third and fourth extractions (along with some of the starting material). Recovery of ascorbic acid (including both isomers) varied, but in one case was as high as 44.6%. Reactions were then begun on a double scale, and since recrystallization from acetonitrile is of some benefit in removing contaminants, those extractions shown to have little contamination were saved. Altogether, 8.86 g D-xyloascorbic acid contaminated with D-araboascorbic acid, and 20.8 g L-araboascorbic acid contaminated with L-xyloascorbic acid were obtained.

C. Methods employed for purifying the isomers of ascorbic acid

1. Of several methods tested for purifying the 20.8 g of L-araboascorbic acid, the best one involved taking a 2.0 g sample of the material and heating it in 50 ml acetonitrile for about 15 minutes with stirring. The mixture was then filtered and the undissolved ascorbic acid washed with cold acetonitrile. TLC indicated that the filtrate contained both L-araboascorbic acid and the

contaminating L-xyloascorbic acid. This process was repeated on the sample until most of the L-xylo isomer had been removed.

2. The method employed for purifying the contaminated D-xyloascorbic was as follows:

2.0 g samples of the contaminated isomer were heated in 100 ml acetonitrile for about 15 minutes. The mixture was then filtered while hot, and the filtrate allowed to cool while under aspiration. A precipitate slowly formed in the filtrate which, when collected, was shown by TLC to be D-xyloascorbic acid remarkably purer than the starting material. This procedure was repeated until none of the original 2.0 g sample remained (about eight times). The percentage of recovery of purified D-xyloascorbic acid from the filtrates averaged about 44%. Cooling the filtrate in an ice bath did not increase the yield, but rather caused the precipitate to form smaller crystals. This method was found to yield crystals of greater purity than that employed for purifying the L-arabo isomer, but unfortunately was not adaptable to this isomer.

D. Characterization of Products

1. Melting point:

0.6 g sample L-araboascorbic acid 160-166°C

7.5 g sample L-araboascorbic acid 165-170°C

2.8 g sample D-xyloascorbic acid 188-192°C

2.4 g sample D-xyloascorbic acid 190-194°C

These compare with values of 168-170°C for commercial D-araboascorbic acid, and 190-193.5°C for commercial L-xyloascorbic acid.

2. Thin Layer Chromatography

Preparations are in progress to analyze, according to the method of

Shaefferts and Kingsley (1955), material scraped from the xylo and arabo regions of TLC plates spotted with the four samples listed above.

3. Polarimetry

Commercial L-xyloascorbic acid	$[\alpha]_D^{25} = +24°$
2.8 g sample D-xyloascorbic acid	$[\alpha]_D^{25} = -21.12°$
2.4 g sample D-xyloascorbic acid	$[\alpha]_D^{25} = -5.7°$
Commerical D-araboascorbic acid	$[\alpha]_D^{25} = 16.46°$
0.6 g sample L-araboascorbic acid	$[\alpha]_D^{25} = +2.82°$
7.5 g sample L-araboascorbic acid	$[\alpha]_D^{25} = +8.33°$

PART II

It has been shown that the hydroxylation of a polypeptidyl proline precursor of collagen is greatly enhanced by the presence of ascorbate. The exact nature of the involvement of ascorbate is unknown. If, however, it is functioning as a coenzyme, the stereospecific requirements of proline hydroxylase for ascorbate can be determined by hydroxylations in which stereo isomers of ascorbate are substituted. Such experiments are now in preparation employing the assay for collagen proline hydroxylase devised by Hutton. et al. (1966): Labeled substrate is prepared by incubating L-proline, uniformly labeled with tritium in a mixture of minced 8-day chick embryos, Krebs-Ringer buffer, and α, α' dipyridyl. Proline hydroxylase is obtained from the 105,000 g supernatant fraction of homogenized 8-day chick embryos. Since the conversion of polypeptidyl proline to hydroxyproline involves the replacement of a C-4 hydrogen atom of proline, and this atom then equilibrates with water, enzyme activity can be determined by measuring the amount of HTO distilled from the reaction mixture. After hydrolysis of the polypeptide,

proline and hydroxyproline can be separated on a column of Dowex 50(H^+) and their specific activities determined. Hydroxylations are to be carried out in which 3 isomers of ascorbic acid (D-araboascorbic acid, D-xyloascorbic acid, and L-araboascorbic acid) are substituted for L-xyloascorbic acid.

Other experiments proposed for using the isomers of ascorbate are designed to determine the specificity of the ascorbate requirement of microorganisms, and the nature of ascorbate deficiency in guinea pigs.

SUMMARY

The preparation and purification of isomers of ascorbic acid are described. These isomers are to be used in determining the nature of the ascorbate requirement of proline hydroxylase.

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APPENDIX VIII - Part D

Study Progress Report: Effects of Selective Coronary Arteriography on Myocardial Blood Flow in Man

To date eleven patients have received intracoronary injections of Xenon-133 in saline to measure myocardial blood flow before and after injection of radiopaque contrast agents. The data from two patients was not satisfactory for inclusion in the present analysis because of inadequate control measurements in one and because nitroglycerin was administered during the study in the other. Five of the remaining nine patients showed increases in myocardial blood flow ranging from 10-82% above control. Three patients had no significant change in flow and in one patient a 17% fall in flow was observed. Variability of duplicate control measurements has been small averaging $\pm$ 3.2% of the mean control value for each patient. No complications attributable directly or indirectly to this study has been observed.

Correlation of the type of response observed and the presence and severity of coronary artery disease is presently being investigated.

APPENDIX VIII SEC. E

6 April 1967

PROTOCOL: Effects of Selective Coronary Arteriography on Myocardial Blood Flow in Man

INTRODUCTION

Selective coronary arteriography has become an important addition to the list of diagnostic procedures available for the detection and evaluation of coronary artery disease. Studies of the coronary circulation utilizing this technique have materially aided our understanding of various aspects of this disease. The hemodynamic consequences of injection of radiopaque agents directly into the coronary circulation have been studied both in animals and man (1 - 3). Changes in myocardial blood flow (MBF) have been observed in opened-chest dogs (4, 5) and more recently in intact dogs, as well (6). However, we are not aware of any studies reporting the effect of coronary arteriography on MBF in man. The study to be described in this protocol is designed to fill this gap in our knowledge.

METHODS

MBF will be measured by selective intracoronary injection of Xenon-133 gas dissolved in sterile saline and calculated in ml/min/100 gm from the precordial disappearance of radioactivity. The details of the procedure to be followed have been fully described by Ross, et al. (7). In addition, one of the investigators (Captain R. P. Carson) has had a year's experience with this technique, both in animals and man, while a postdoctoral fellow at the University of Michigan. Enclosed are three figures from his studies illustrating the method of calculating MBF from the precordial radioactivity curve (fig. 1), the reliability of the method for measuring MBF (fig. 2), and the results of sublingual administration of nitroglycerin on MBF in nine patients (fig. 3).

Subjects will consist of patients with suspected or known coronary artery disease undergoing selective coronary arteriography during cardiac catheterization. Patient consent will be obtained after they are informed of the procedures to be performed and the risks involved from the study and the use of a radioisotope.

The study will be conducted in the cardiac catheterization laboratory of Fitzsimons General Hospital. Four measurements of MBF will be made on each subject, two during a control period and then 30 seconds and again 300 seconds following coronary injection of contrast agent. Each measurement will require an injection of 50 - 100 microcuries of Xenon-133, the total amount of radioactivity administered to each subject being less than 500 microcuries.

It has been estimated that 90-95% of an administered dose of Xenon-133 is expired during the first passage through the lungs. Captain Carson was able to confirm this in a dog study. Because of this and the small doses used, the systemic radiation dose to the subject is small. The organs receiving the largest exposure are the heart and lungs. Lassen (8) has calculated the exposure dose from an intra-arterial injection of 5 millicuries of a saline solution of Xenon-133 as follows: tracheal mucosa - 96.8 mrad, lung - 17.5 mrad, adipose tissue - 8.8 mrad, gonads - 1.1 mrad, and other tissues - 1.6 mrad. For the local organ being studied: brain (400 gm tissue exposed) - 86 mrad, kidney (150 gm tissue exposed) - 31 mrad. The average weight of an adult human heart is 300 gm. We will be injecting one tenth this dose of Xenon-133; thus, the exposure dose will be correspondingly reduced.

Since the injected Xenon is excreted from the body through the lungs, the expired air of the subjects will be collected in suitable containers such as meteorological balloons or other large-capacity containers and subsequently

released outside in an open area. As the total dose to be administered is low and the expired air will be collected, contamination of laboratory air will be negligible. In animal studies at the University of Michigan, no appreciable increase in room background could be detected using an ionizing chamber monitor under the conditions described.

The Xenon-133 will be obtained precalibrated in saline solution from the Sterling Forest Research Center, Union Carbide Corporation, New York. It will be stored in the Radioisotope Clinic in a hood with lead shielding of 1 cm or more thickness to keep the exposure rate below 2.5 mrad/hr. Disposable gloves will be used to handle the material. Aliquots of the solution will be drawn up through a Millipore filter into sterile airtight syringes and transported in lead shielded containers to the cardiac catheterization laboratory the day of the study. Contaminated syringes, gloves, etc., will be handled through the Radioisotope Division. Any Xenon solution remaining at the end of the study will be allowed to decay for two months (10 half-lifes) and then be disposed of by pouring down a sink drain.

The detecting and recording equipment will be mounted on a portable cart and will consist of a 2 x 3 inch sodium iodide crystal in an adjustable probe, a linear ratemeter, and a strip chart recorder. A portable instrument will be available for continuous monitoring of the room air during the study.

INVESTIGATORS

Principal investigator for this study will be: Captain Richard P. Carson, MC.

Associate investigators will be: Captain Charles Peterson, MC; Lt. Colonel Robert Jones, MC; Major David Preston, MC.

RICHARD P. CARSON
Captain, MC

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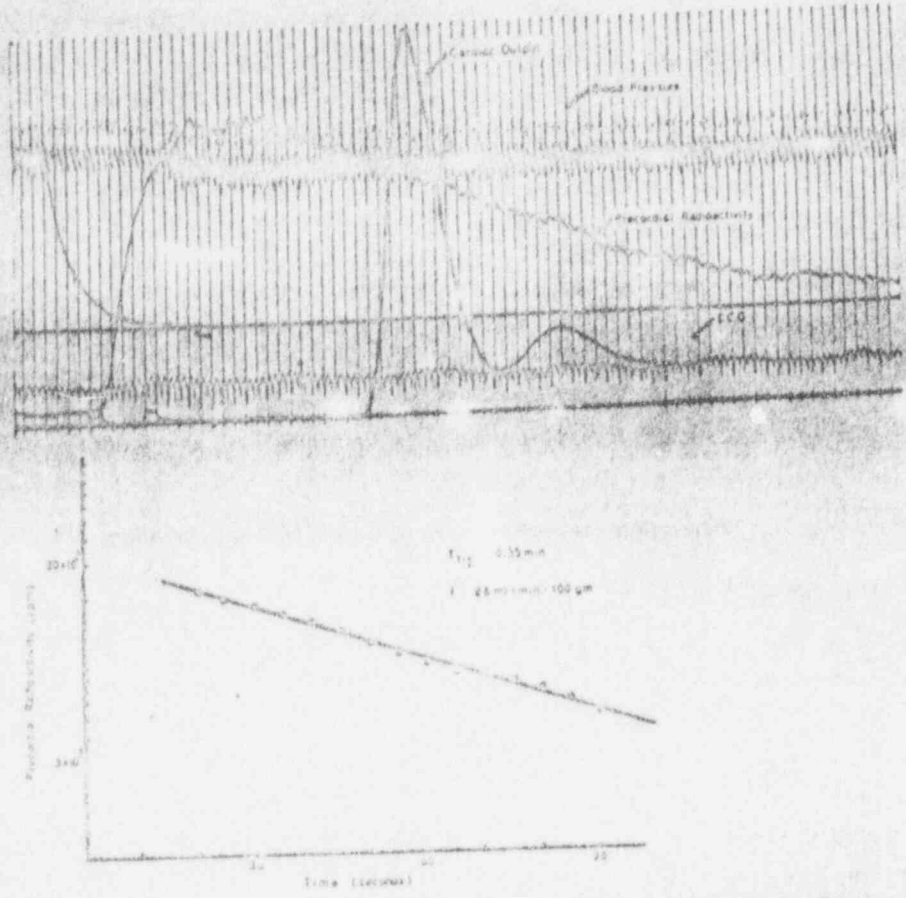

FIG. 1

Above: Portion of record from closed-chest dog experiment showing typical precordial radioactivity curve following injection of Xenon-133 in saline through a catheter positioned within the ostium of the left coronary artery. Vertical lines represent 1 second time intervals.

Below: Semilogarithmic replot of the washout portion of the curve (after subtracting background). The half time of this line is used to calculate flow from the formula

$$\text{Flow} = \frac{\frac{\log 2 \times 100}{T}}{\delta}$$

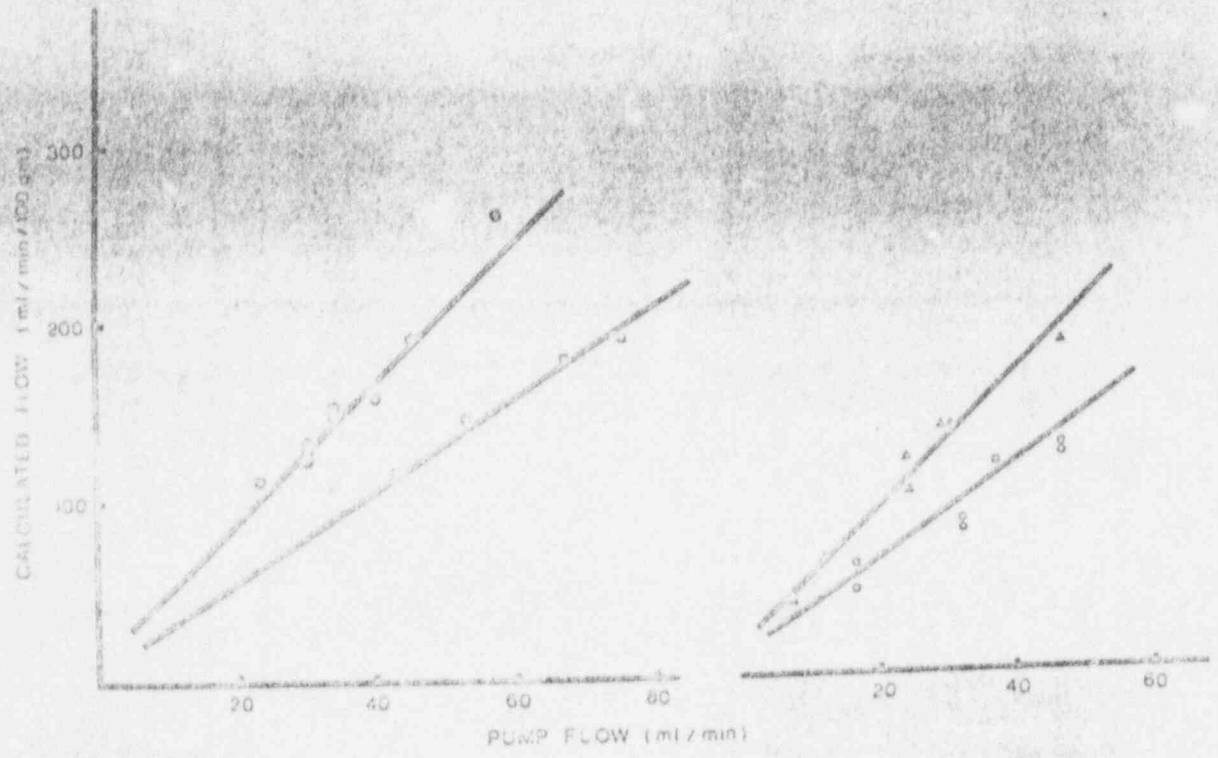

FIG. 2

Results of 4 opened-chest dog experiments in which posterior circumflex branch of left coronary artery was cannulated and blood supplied from fe artery via an externally controlled rotor pump. Xenon-133 in saline was injected through a side arm in the tubing between the pump and the coronary vessel. The scintillation probe was positioned over the thoracotomy at the level of the anterior chest wall. MBF was determined by the Xenon method at various pump speeds. Flow rates calculated from the Xenon washout curves were then plotted against the corresponding pump flow rates.

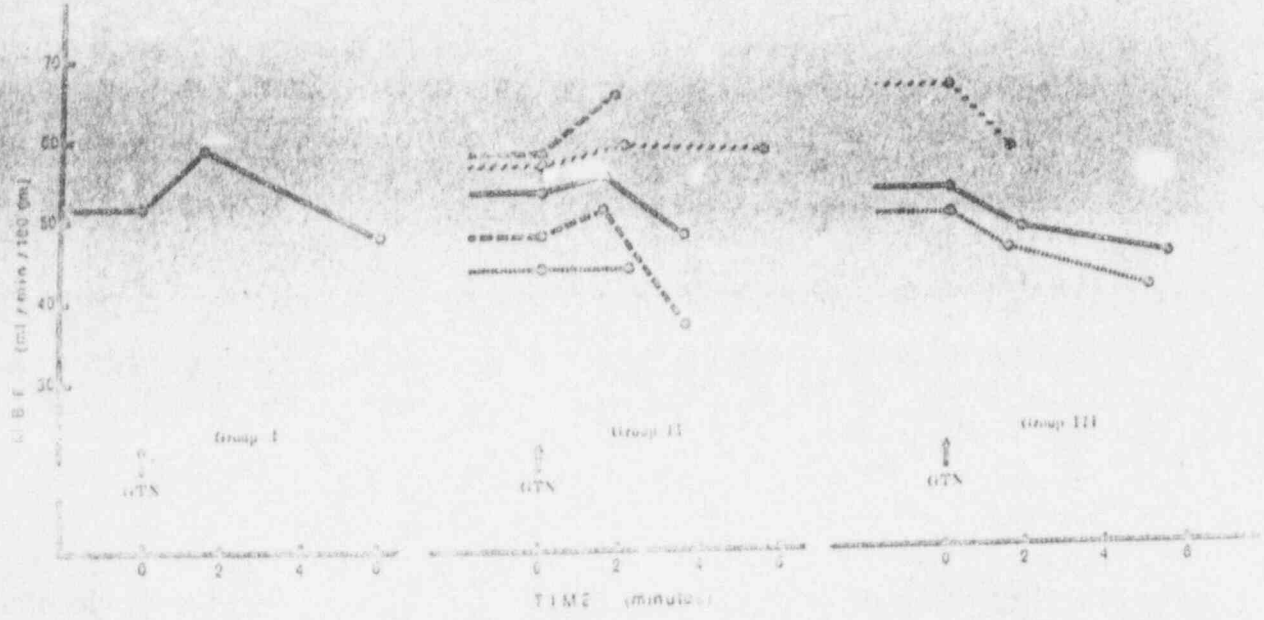

FIG. 3

Effects of sublingual administration of 0.3 mg nitroglycerin in 9 male subjects. MBF was calculated from the disappearance rate of precordial radioactivity following selective injection of Xenon-133 in saline into the right coronary artery.
Group I - One patient with no cardiac disease.
Group II - Five patients with coronary artery disease.
Group III - Three patients with myocardial hypertrophy.

APPENDIX XIII SEC. C

USAMRNI. March 1967
PHYSIOLOGY DIVISION PROTOCOL

Project No. 3A104501B71R Research in Biomedical Sciences
Task No. 05: Environmental Medicine
Work Unit No. 82: Metabolic Effect of Altitude
Study No. 4: Endocrine Effects of Altitude

I. INTRODUCTION

It has been established that acute exposure of man to high altitude causes the onset of a distressing, incapacitating syndrome, termed "mountain sickness. (1, 2, 3) This syndrome is characterized by impaired physical and psychomotor performance. The transitory effects of high altitude exposure - the most debilitating symptoms - severe headache, nausea and vomiting, extreme fatigue and anorexia usually last up to 5 - 6 days in most subjects. These symptoms are variable in intensity in different people or in the same person at different times.

Various theories have been advanced in the attempt to explain the etiology of mountain sickness. Research has been concerned primarily with the evaluation of cardiopulmonary and acid-base alterations in man. Still, all of the reported, established alterations do not explain the phenomenon of acclimatization that occurs in most subjects within 5 - 7 days. Recently, in the attempt to discover the cause of mountain sickness and the mechanisms of adaptation, investigators have focused on endocrine function, shifts in body fluids, and electrolyte balance in man at high altitude. The following sections summarize the observed alterations of endocrine function, body fluids and electrolytes in man at high altitude. There is a wealth of literature of similar observations conducted on animals which is essentially consistent with the reports on man (4 - 14).

Body Fluids and Electrolytes in Man at High Altitude

When man ascends rapidly to high terrestrial locations, there is a continuous decrease in plasma volume, a decrease in extracellular fluid volume, and a rise in intracellular fluid volume. Total body water increases (15). Total body potassium obtained from body scanning for K^{40} is noted to be increased by one research group (16) but similar measurements made by USAMRNL show that total body potassium decreases (15). Excessive urinary excretion of sodium, potassium and chloride ions has been documented (15, 17). There is a significant rise in salivary sodium to potassium ratio (18) and urinary sodium to potassium ratio (17). In addition to electrolyte changes, urine volume increases at high altitude (19).

These observed effects of altitude on man resulting in body fluid shifts and electrolyte alterations suggest diminished aldosterone secretion by the adrenal glands. Very recently it has been reported that urinary aldosterone excretion does decrease at altitude, approaching zero within three days of high altitude exposure (19). These findings appear to be consistent with the observed pattern of urinary electrolyte excretion. Additional investigation is necessary in order to establish and correlate the electrolyte changes with aldosterone secretion at high altitude by means of balance studies with human volunteers. If aldosterone excretion is indeed diminished at high altitude in the face of sodium loss, diminished plasma and extracellular fluid volumes, the mechanism is unknown and is contrary to accepted physiological controls of aldosterone secretion.

Adrenocortical Function in Man at High Altitude

Various investigators using intermittent chamber studies in man (20, 21), or actual field studies at high altitude (17, 22, 23),

have observed a rise in the 24-hour urinary excretion of 17-ketosteroids and 17-hydroxycorticosteroids during acute exposure. One research group did note a difference in the excretion pattern of 17-hydroxy-corticosteroids and 17-ketosteroids. The latter decreased initially. Adrenal function has been studied in a group of high altitude natives, and is the same as a comparable group living at sea level (24).

These studies are generally consistent with the known physiological control of pituitary adrenal function during stress. The correlation of adrenocortical function to "mountain sickness" and the magnitude of environmental adrenocortical steroid secretion and ACTH release during stress require additional investigation. The accurate measurement of these hormones during acute altitude exposure can determine the desirability of a possible therapeutic means of controlling the symptoms of high altitude stress in man.

Glucose Tolerance in Man at High Altitudes

Studies in man (25, 26, 27, 28) have established that there is a lower fasting blood glucose level and greater utilization of glucose at high altitude. Whether the cause of enhanced glucose utilization is secondary to increased insulin secretion or the effect of adreno-corticosteroids on glucose metabolism, is not known, and should be determined.

II. OBJECTIVE

The objectives of the present proposal to study human subjects at high altitude are (1) to evaluate through balance studies, the nature of electrolyte alterations; (2) to determine the magnitude of the stress response at altitude by evaluation of pituitary and adrenocortical function; (3) to measure and correlate the aldosterone secretion and excretion in relationship to electrolyte changes; and (4) to investigate the mechanism for increased glucose utilization.

The purpose of the study will be to correlate these findings with the severity of mountain sickness symptoms at 14, 100 feet.

III. JUSTIFICATION

Reports concerning the Indian Army (29) have furnished alarming evidence of incapacitating medical and personnel problems resulting from altitude sickness, when a military force attempts to function on mountain locations. An understanding of the physiological alterations during altitude acclimatization and the relationship of these to various mountain sickness symptoms might lead to successful methods of pre-selecting or pre-conditioning troops.

Knowledge of the endocrine function in man at high altitude would shed light on the adaptive changes that occur during the stress of hypoxia. Significantly patho-physiological alterations in hormone secretion can be dealt with therapeutically. Recent Army Research Office conferences (30, 31) have pointed to a military need for studies on physiology, pharmacology and performance in high terrestrial environments.

IV. EXPERIMENTAL DESIGN

A. The subjects will be ten U. S. Army volunteers (19 to 25 years old) who have signed a volunteer form indicating their knowledge of the scope of the procedures planned, including the intravenous administration of tracer quantities of radioactive steroid compounds and their willingness to serve as subjects. The radioactive concentration of these isotopes will be recorded in the subjects' Army Health Records. The subjects will be interviewed, examined and selected by a medical officer after review of their health records to exclude cardiopulmonary, renal or endocrine disorders. A medical officer is to be present during the entire study and is authorized to terminate the experiment at any time continuation would be detrimental to the health of the subject.

The study will be conducted for a total period of 21 days beginning 11 September 1967. The sea level studies will be conducted at Fort Lewis, Washington. This site meets the requirements that the sea level site be located in a temperate climate. A hot, humid climate would make electrolyte balance studies inaccurate. In addition, heat exposure alters blood volume and this would interfere with studies on aldosterone secretion.

During the sea level test period, constituting days 1 to 14, the subjects will be started on a constant metabolic diet which will require eight days of equilibration of body electrolytes prior to initiating balance studies. This diet will be continued until the end of the study on day 21. On day 15 the subjects will be flown to Colorado Springs or Denver and transferred by Army vehicle to the Army mobile laboratory on Pikes Peak (14, 100 ft). The altitude test phase will be conducted there with living quarters provided for the subjects.

B. Clinical Study

1. Plan of study

a. Sea level test period - 0900 hours day 1 to 0900 hours day 15.

b. Travel period - day 15.

c. Altitude test period - 0900 hours day 16 to 0900 hours day 21.

2. Diet

A prepared liquid diet comprising daily consumption of 2,800 calories as 55% carbohydrate, 15% protein, and 30% fat. The daily electrolyte and mineral intake will be 100 mEq. of sodium, 80 mEq. of potassium, 1600 mg of calcium, and 1200 mg of phosphorous.

The diet and water content are to be constant and analysed. During the day of travel, the subjects will be maintained on this diet. On days 5 and 19, the subjects will fast from 21:00 hours until 14:00 hours the following day (glucose tolerance test). There will be no smoking during this interval.

3. Water intake

Distilled water will be used for drinking purposes. Daily water intake will be measured and recorded. A minimum of 1500 ml of water per subject per day is to be consumed.

4. Medications

a. Glucose - 100 grams in distilled water administered orally on days 6 and 20 at 09:00 hours

b. 4-C^{14}- cortisol (1μc) and 1, 2-H^{3}- aldosterone (2μc) - administered intravenously in 10 ml sterile solution of 10% ethanol in water at 09:00 hours on days 9 and 17.

c. Sodium chloride - 500 mg in gelatin capsules in the event of diminished dietary intake to maintain constant daily sodium intake.

d. Potassium replacement elixir (5 ml contains 10 mEq. of potassium as gluconate and citrate salts) - to maintain constant daily potassium intake if intake of food diminishes when anoretic.

e. Calcium gluconate tablets - 500 mg if food intake diminishes when anoretic to maintain constant daily intake of calcium.

f. Aspirin and Darvon - administered orally at the discretion of the medical officer for headache. The dosage and time of administration are to be recorded.

g. Intravenous infusions of isotonic sodium chloride and potassium will be administered if severe vomiting occurs in order to maintain electrolyte and water balance.

5. Collections

a. Urine

Total 24-hour urine collections will be obtained from each subject starting at 09:00 hours each day. Each collection period will end on 09:00 hours the following day with the subjects voiding at this time and adding this specimen to the previous day's collection. The days of collection are 8 through 14 and 16 through 20. The urine will be stored in large plastic bottles and kept under refrigeration at all times during collection. At the end of each 24-hour collection period, all urine collected will be frozen and will be kept in this state until analysed at the USAMRNL. All specimen bottles will be labeled with name of subject, and the starting and ending dates of the 24-hour collection. Radioactivity labels will be affixed to all 24-hour urine samples collected after the administration of radioisotopes to the subjects.

b. Blood

(1) Plasma insulin and glucose

At 08:30 hours on days 6 and 20, 5 ml of venous blood will be drawn into fluoride-oxalate vacuum tubes. Following oral glucose administration, similar samples will be taken at 15, 30, 45, 60, 90, 120, 240, and 300 minutes. The tubes will be labeled and the contents frozen. The subjects are to refrain from smoking from the period of fasting until the final blood sample is drawn.

(2) Plasma ACTH and cortisol

On days 9, 10, 13, 16, 17 and 18, venous blood will be taken at 08:45 hours. Thirty ml of blood will be drawn into a syringe containing heparin and transferred to centrifuge tubes and centrifuged. The plasma will be transferred to screw-cap glass tubes, labeled "cortisol" and frozen. Forty ml

of venous blood will be collected through sterile plastic tubing directly into a large centrifuge tube containing heparin. The centrifuge tube will remain immersed in an ice-water bath during the collection, then immediately centrifuged at 12 degrees centigrade. The plasma will be transferred to screw-cap tubes labeled "ACTH" and frozen. All tubes will be labeled with name of subject, time and date.

3) Fifteen ml of venous blood will be taken at 15:00 hours on days 8, 9, 10, 13, 14, 16, 17, 18, 19, and 20. Following centrifugation, the serum will be transferred to labeled test tubes and frozen.

c. Feces

Feces will be collected in plastic bags on days 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19 and 20.

d. Vomitus

Any vomitus will be collected in plastic bags for analysis.

C. Measurements

1. Electrolytes

Concentrations of sodium and potassium in the diet, venous blood, stools and urine will be determined by AutoAnalyzer or flame photometry. Calcium will be measured by atomic absorption spectrophotometry. Chloride and creatinine will be measured by the Auto-Analyzer. The Fiske and Subbarow method will be used for phosphate concentrations.

2. Nitrogen

Urinary nitrogen content will be determined by the Auto-Analyzer. The macro-Kjeldahl technique will be used for nitrogen in the diet and in the feces.

3. Fat

Diet and stool fat will be determined by routine analytical methods.

4. Glucose and insulin

Plasma glucose will be measured by AutoAnalyzer. Plasma insulin will be determined by Captain J. Anderson, Metabolic Division of the USAMRNL employing the method of Morgan and Lazarow (32).

5. Steroids

a. Plasma cortisol by the Peterson modification (33) method of Silber and Porter (34) using the Beckman DU Spectrophotometer.

b. Total 24-hour urinary 17-ketosteroids and 17-ketogenic steroids by the Sobel (35) modification of the Norymberski method. Gas-liquid chromatography will be used to determine individual 17-ketosteroids.

c. Twenty-four hour urinary aldosterone excretion by a modification of the double isotope technique of Kliman and Peterson (36) using 1, 2-H^3-aldosterone, 1, 2-H^3-tetrahydroaldosterone, and acetic-1-C^{14} anhydride.

d. Twenty-four hour urinary cortisol excretion by the method of Erlich (37), using 1, 2-H^3-cortisol and acetic-1-C^{14} anhydride.

6. Plasma adrenocorticotropic hormone

ACTH levels in plasma will be determined by the method of Vernikos-Danellis (38) by means of bioassay in hypophysectomized rats.

7. Plasma and urine osmolality

Osmolality will be determined by means of the Fiske osmometer.

D. Radioactive Steroids as Tracers in Human Subjects

1. Purification

4-C^{14}-cortisol (specific activity SA 15-30 mc/millimole)

and 1,2-H^3-aldosterone (SA 35 curies/millimole) obtained commercially from New England Nuclear Corporation will be tested for purity by chromatography on three separate systems. The purified isotopes will be dissolved in absolute ethanol, and sterilized rendering them pyrogen-free by Millipore filtration. The isotopes will be kept as a 10% solution of ethanol in sterile water in a sterile, multi-dose, stoppered vial. They will be administered intravenously to the subjects by a medical officer.

2. Dosage considerations and calculations

It has been determined that over 90% of injected radioactively labeled cortisol is excreted by human subjects via the kidney within the first 48 hours (39, 40). Furthermore, no radioactivity can be detected in the body fluids in four days (39). The biological half-life of radioactive cortisol in the human bloodstream is 60 - 80 minutes (41). Similarly, over 90% of radioactive aldosterone injected into human subjects is excreted in the urine within 48 hours (42). The location of the tritium and carbon-14 labels in the steroid nucleus is such that the labels remain an integral part of the compound in the body and are excreted intact as steroid metabolites without degradation. (43). This factor has enabled numerous investigators to employ these isotopically labeled steroids in clinical research without the hazards of critical organ concentration, the random labeling of body water by tritium, or the expiration of carbon-14 carbon dioxide in human subjects.

Based on knowledge gained from reports in the literature, one can make the safe assumption that the effective half-life (T 1/2) of these isotopes in man is one day (an over-assumption). The total body burden would be calculated as follows: (44)

1. 4-C^{14}-cortisol. 1 μc injected into a 70 kg man assuming total body distribution with an effective T 1/2 of one day.

$$D\beta \simeq 73.8 \times C \times \bar{E}\beta \times T \text{ rads}$$

$\bar{E}\beta \approx 0.050$ Mev. for C^{14}

$C \approx 1.0/70,000$

$T \approx 1$ day

$D\beta \approx 73.8 \times 1.0/70,000 \times 0.050 \times 1$ rads

$D\beta \approx 5.27 \times 10^{-5}$ rads

2. 1, 2-H^3 - aldosterone. Two μc injected into a 70 kg man assuming total body distribution with an effective T 1/2 of one day.

$D\beta \approx 73.8 \times C \times \bar{E}\beta \times T$ rads

$\bar{E}\beta \approx 0.006$ Mev. for H^3.

$C \approx 2.0/70,000$

$T \approx 1$ day

$D\beta \approx 72.8 \times 2.0/70,000 \times 0.006 \times 1$ rads

$D\beta \approx 1.26 \times 10^{-5}$ rads

During the control period 1.0 μc of 4-C^{14}-cortisol and 2.0 μc of 1, 2-H^3-aldosterone will be injected simultaneously (6.53×10^{-5} rads). This will be repeated once at high altitude the following week. The total radiation dose received by each individual will be no more than 1.3×10^{-4} rads which is considerably below the limits generally agreed upon for an internal emitter - approximately 0.1 rem per week (5 rem per year).

3. Monitoring radioactivity

During the experimental studies, after the administration of radioisotopes all excreta will be collected until levels of radioactivity are equal to background levels. All samples containing radioactivity will be returned to the U.S. Army Medical Research and Nutrition Laboratory at Denver, Colorado for analysis. All rad active waste will be transported to Denver and disposed of by the Radioisotope Section of the USAMRNL as outlined in "Procedures for Use of Radioactive Material" (See Appendix E Application for Renewal and Amendment to AEC Byproduct Material License No. 5-46-13 (A66) dated June 1966). All areas where radioisotopes are used will be monitored by means of a PAC-3G with a beta probe (Eberline Instrument Company, Serial No. 1226). Also periodic wipes will be taken with moist gauze and returned to the U.S. Army Medical Research

and Nutrition Laboratory for counting in a Packard Model 3314 liquid scintillation counter (Serial No. A3314-05-00712).

All rules, regulations and limitations set forth by Army AEC and local authorities, including those embodied in AR 70-25; AR 40-37; Title 10, Part 20, Code of Federal Regulations "Standards for Protection Against Radiation"; and Handbook 69 of the National Bureau of Standards will be complied with.

Enclosed and attached to this protocol is the Voluntary Consent Statement relating to the intravenous administration of radioisotopically labeled steroid compounds as tracers. (See Appendix I)

4. Determination of the Secretory Rates of Cortisol and Aldosterone

a. Cortisol secretion rate

The cortisol secretion rate will be determined by the method of Roginsky, et al. (45) from the combined 48-hour urine collection following isotope injection.

b. Aldosterone secretion rate

This will be determined by the method of Kelly, et al. (46) using the double isotope derivative method.

V. ADMINISTRATION

A. The study will be the responsibility of the Physiology Division of USAMRNL.

B. The personnel and their responsibilities will be assigned as follows:

1. Captain A. H. Janoski, MC: Project leader; responsible medical officer.

2. John P. Hannon, Ph. D.: Project Co-leader.

3. J. L. Shields, Ph. D.: Project Co-leader.

4. Captain R. P. Carson, MC: Medical Officer
5. Captain B. Whitten, MSC: Administrative officer
6. George J. Klian, Ph. D.: Technical supervisor
7. Major C. G. Liddle, V. C.: Radiation officer
8. One NCOIC: Subject control and dietary supervisor
9. Four enlisted military technicians
10. Two civilian technicians

C. Continuous physician coverage during the study will be the responsibility of Captains A.H. Janoski and R. P. Carson of the U. S. Army Medical Corps.

D. Cost

1.	Equipment	800.00
2.	Chemicals, including solvents	$4,000.00
3.	Per diem for subjects	210.00
4.	Travel for subjects	1,500.00
5.	Diet for subjects	500.00
6.	Air freight (samples collected during study)	700.00
7.	Class A Funds	600.00
8.	Travel for project personnel (7 investigators, 5 enlisted men, 2 civilians). Includes one round trip to Seattle	2,100.00
9.	Rental truck for equipment (30 days)	900.00
10.	GSA vehicles (2 for 21 days)	400.00
11.	Lab rats for bioassay (250 rats)	800.00
12.	Per diem for investigators	2,400.00
13.	Per diem for enlisted men	
	a. Sea level site - 14 days (government quarters available, government mess not available).	560.00

14.	Per diem for civilian technicians (21 days)	$672.00
15.	Three investigators' per diem for three-day site survey	144.00
16.	Three investigators' travel to Seattle for site survey (3 round trips)	450.00
17.	Additional per diem for 3 investigators for four days at sea level site to set up study	192.00
18.	Additional per diem for 4 enlisted men for four days at sea level site to set up study (government quarters available; government mess not available)	128.00
19.	Miscellaneous expendable items	$1,200.00
	Total	$18,816.00

E. Miscellaneous

Since the final approval by the AEC for the use of isotopes in human studies rests on the approval of the protocol and definite site selection for sea level studies, it is requested that final action on this protocol be no later than 5 June 1967. If the protocol is approved at this time, application would then be made to the AEC for action on the license amendment. Furthermore, final action on the protocol at this date would enable the investigators to prepare the isotopes for human administration and to obtain the necessary chemical supplies for the field study.

F. Additional Information

See Appendix I and Appendix II.

A. H. JANOSKI
Captain, MC

APPENDIX I

VOLUNTARY CONSENT STATEMENT

Military _____ Military Patient _____ Civilian _____ Civilian Patient _____

I, _____, having the capacity to consent, voluntarily and without force or duress consent to participate in research involving the use of tracer amounts of radioisotopes. I have been informed of, and understand, the nature, duration, and purpose of the experiment, the method and means by which it is to be conducted, the inconveniences and hazards to be expected, and the effects upon my health and person which may possibly come from participation in the experiment.

Specifically, I agree to receive (intravenously / orally) a small quantity of _____ containing _____ microcuries of _____. I also agree to furnish urine and stool samples for the period following until no detectable radioactivity is present and submit to measurements of expired gases if Carbon-14 has been received.

I understand that I may at any time during the course of the experiment revoke my consent and withdraw from the experiment without prejudice.

I do not at this time have any physical diseases, except for the following _____ _____, or mental disease, to the best of my knowledge.

_____ _____ _____

DATE SIGNATURE

_____ _____

SIGNATURE OF WITNESS

APPROVAL

I have personally ascertained that the quality of the foregoing consent is sufficient to permit the volunteer to participate in the experiment.

_____ _____

ATTENDING PHYSICIAN PROJECT LEADER

APPENDIX II

SUBJECT STATEMENT

Date _____

I voluntarily agree to participate as a subject in the experiment to be conducted on high altitude. I am aware that I may withdraw from the experiment at any time without prejudice or penalty of any kind. It has been explained to me that constant medical supervision will be maintained and that neither the exposure to high altitude nor the experimental techniques used in this study are unduly hazardous. I realize that in some subjects the high altitude may cause any or all of the following symptoms: dryness of the mouth and nose, excitement, blurring of vision, dizziness, tiredness, tremor, lack of appetite, mild cramps, thirst, confusion, a sense of well-being, sleepiness, muscular aches, ringing in my ears, nausea, runny nose, headache, hunger, sleeplessness, coughing, rapid heart beat, chest pains, fatigue, constipation, fever, muscular stiffness, stomach ache, itching or sneezing.

The nature and purpose of the experiment have been explained to me and I sign this statement fully understanding the project, any hazards connected with it, and my rights.

_____

(Name)

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APPENDIX VIII - Part F

I. Secretion Rates of Aldosterone and Hydrocortisol

Preparation

4-C-14 labeled hydrocortisol (0.1 mc) and H-3 labeled aldosterone (0.5 mc) were separately purified by column chromatography. Following appropriate dilution with 10% absolute ethanol in 5% dextrose and water each steroid solution was filtered through 0.2 micron sterile membrane filter.

Study

Ten human volunteers were studied at sea level and at an altitude of 14,100 feet. In order to determine the secretion rates of aldosterone and hydrocortisol each subject who had been placed on mineral balance, received 2.0 uc of H-3 aldosterone and 1.0 μc C-14 labeled hydrocrotisol intravenously. Complete urine collections were carried out for 48 hours. The urine has been kept in a frozen state pending completion of other studies.

II. Excretion Studies of Aldosterone and Hydrocortisol

Methods

The 24 hour urinary excretion of aldosterone is being determined by the double isotope dilution derivative method. To an appropriate aliquot of 24 hour urine collection is added 2000 dpm of H-3 aldosterone. Following hydrolysis and purification the extract is acetylated with 200 lambda of acetic anhydride (C-14 labeled specific activity 1.0 mc/millimole). For the acetylation step it is necessary to obtain 10-12 millicuries of C-14 labeled acetic anhydride (SA 1.0 mc/mM) and dilute to various specific activities required with 20% acetic anhydride in benzene. The specific activity is then determined by acelylating desoxycorticosterone and crystallizing to constant specific activity.

To date attempts have been made to determine known quantities of aldosterone by the above method in order to verify the specificity and reliability of the technique. It has been determined that the initial solution of H-3 aldosterone in 10% ethanol and 5% dextrose and water is no longer to be used because of chemical decomposition. An additional 0.5 mc of H-3 labeled aldosterone was transferred to a versine washed amber volumetric flask and taken to volume with re-distilled benzene in 10% methanol (40,000 dpm/cc) and is kept refrigerated to prolong chemical purity.

Free urinary hydrocortisol is to be studied by the competitive binding technique of Murphy using C-14 labeled hydrocrotisol *in vitro*.