HEADQUARTERS
DEPARTMENT OF THE ARMY
OFFICE OF THE SURGEON GENERAL
WASHINGTON 25, D.C.

IN REPLY REFER TO
MEDPS-PO

27 March 1964

Isotopes Branch
Division of Licensing and Regulation
U. S. Atomic Energy Commission
Washington, D. C. 20545

Gentlemen:

Recommend approval of the inclosed application for amendment to AEC Byproduct Material License No. 5-46-13 (A66) for Fitzsimons General Hospital and the U. S. Army Medical Research and Nutrition Laboratory, Denver, Colorado.

Your attention is invited to condition 10 of AEC Byproduct Material License No. 5-46-13 dated 3 February 1964. It is requested that this condition be amended so that use of radioisotopes in tracer amounts for human volunteers be authorized for use at those places which have been approved as a part of an authorized study by the Radioisotope Committee of Fitzsimons General Hospital and the U. S. Army Medical Research and Nutrition Laboratory, The Surgeon General, and the Secretary of the Army in accordance with statements of application dated 12 March 1964.

Sincerely yours,

Ros. G. Daniels

ROSWELL G. DANIELS
Lt Colonel, MC
Preventive Medicine Division

1 Incl
AEC-313 (in trip)

A/10

58538

8 copies

MEDDH-RM (19 Dec 63) 1st Ind
SUBJECT: Request for Approval to Use Radioisotope Tracers in Volunteers

HQ, US Army Medical R&D Command, DA, OTSG, Washington, D.C. 20315 MAR 2 1964

TO: Commanding Officer, U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, ATTN: MEDEN-CO, Denver, Colorado 80240

Subject request is granted approval per the attached memo from the Secretary of the Army.

Richard R. Taylor for
ROBERT E. BLOUNT
Brigadier General, MC
Commanding

1 Incl
wd 1 incl
Added 1 incl

Incl #1

58538

DEPARTMENT OF THE ARMY
WASHINGTON 25, D.C.

IN REPLY REFER TO:

FEB 22 1964

MEMORANDUM FOR: CHIEF OF STAFF, UNITED STATES ARMY

SUBJECT: Request for Approval to Use Radioisotope Tracers in Volunteers

Approval is granted for the use of radioactive isotopes as tracers in volunteer human subjects of research in conformance with the protocol submitted to The Surgeon General, by letter MEDRS-CO, U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado, subject as above, dated 19 December 1963.

Stephen Ailes

STEPHEN AILES
Secretary of the Army

58638

U. S. ARMY MEDICAL RESEARCH AND NUTRITION LABORATORY
FITZSIMONS GENERAL HOSPITAL
DENVER 80, COLORADO 80240

IN REPLY REFER TO:
MEDEN-CO

19 December 1963

SUBJECT: Request for Approval to Use Radioisotope Tracers in Volunteers

TO: Commanding General
U. S. Army Medical Research
and Development Command
ATTN: Chief, Medical Research Branch
Office of The Surgeon General
Department of the Army
Washington, D. C. 20315

1. Enclosed are copies of a Request for Approval for Human Use of Radioisotopes in Tracer Amounts in Volunteer Experimental Research Subjects.

2. Eight copies of the request are furnished to facilitate early processing, however, only three copies are provided with Appendix IV, "Reprints Supporting Vitamin C Studies," due to a short supply.

Marion E. McDowell

MARION E. McDOWELL
Lt Colonel, MC
Commanding

1 Incl
a/s (8 copies)

58638

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

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

Installations named in 1 (a) and, for Carbon-14, those locations named in Paragraph 6, d(3) pages 19 & 20(and subject to conditions therein specified)of approved Incl #2, "Request for Approval

(cont'd Supp ment

2. DEPARTMENT TO USE BYPRODUCT MATERIAL

Radiology Service, FGH,and USA Med Rsch & Nutr Lab

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

License No. 5-46-13 (A66) with expiratio date January 31, 1966

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.)*

As specified in License No. 5-46-13(A66) condition 12

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 specified in application(dtd Dec 6, '63) for License No. 5-46-13(A66)

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. Carbon-14	A. Vitamins	A. 10 m
B. Carbon-14	B. Amino Acids	B. 10 m
C. Carbon-14	C. Lipids (as glycerides ,cholesterol & free fatty acids)	C. 10 m
D. Carbon-14	D. Acetate	D. 10 m
E. Carbon-14	E. Carbohydrates	E. 10 m
F. Carbon-14	F. Mevalonic acid	F. 10 m
G. Carbon-14	G. Bicarbonate, CO_2	G. 10 m
H. Hydrogen-3	H. Vitamins	H. 50 m
I. Magnesium-28	I. MgO, Mg Cl_2, Mg citrate	I. 10 m
J. Calcium-47	J. Ca Cl_2	J. 10 m
K. Calcium-45	K. Ca Cl_2	K. 10 m

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.)*

See attached copies:"Request for Approval for Human Use of Radioisotopes in Tracer Amounts in Volunteer Experimental Research Subjects" submitted thru channels to Secretary of the Army 19 Dec 63 by U.S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado, and Memorandum of Approval by Secretary of the Army dated 22 Feb 64. (Incls 1&2)

58638

(Continued on reverse side)

Form AEC-313 a (3-56)
PAGE 1

UNITED STATES ATOMIC ENERGY COMMISSION

APPLICATION FOR BYPRODUCT MATERIAL LICENSE

SUPPLEMENT A—HUMAN USE

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

If byproduct material is for "human use" (internal administration of byproduct material, or the radiation therefrom to human beings), complete this supplement and attach to the application for byproduct material license.

1. (a) USING PHYSICIAN'S NAME
Department of the Army, Fitzsimons Gen Hospital and USA Med Rsch & Nutr Lab

(b) NAME AND ADDRESS OF APPLICANT (If different from 1(a))
Same

2. THE USING PHYSICIAN INDICATED ABOVE IS LICENSED TO DISPENSE DRUGS IN THE PRACTICE OF MEDICINE BY A STATE OR TERRITORY OF THE UNITED STATES, THE DISTRICT OF COLUMBIA, OR THE COMMONWEALTH OF PUERTO RICO.
NA
CIRCLE ANSWER — YES — NO

3. A STATEMENT OF USING PHYSICIAN'S CLINICAL RADIOISOTOPE EXPERIENCE (PAGE 3 OF THIS SUPPLEMENT) IS SUBMITTED IN SUPPORT OF THIS APPLICATION. IF ANSWER IS NO, USE PAGE 2 OF THIS SUPPLEMENT TO EXPLAIN OR REFER TO OTHER APPLICATION OR RELATED DOCUMENTS ON WHICH THIS INFORMATION APPEARS. As specified in Application(dtd 6Dec63) For License No. 5-46-13 (A66)
CIRCLE ANSWER — YES — NO

PROPOSED DIAGNOSIS OR TREATMENT

4. (a) DESCRIBE PURPOSE FOR WHICH BYPRODUCT MATERIAL WILL BE USED INCLUDING SPECIFIC CONDITIONS OR DISEASES TO BE DIAGNOSED OR TREATED (Use page 2 if necessary): See attached copies:"Request for Approval for Human Use of Radioisotopes in Tracer Amounts in Volunteer Experimental Research Subjects"submitted thru channels to Sec'y of the Army 19Dec63 by USA Med Rsch & Nutr Lab, FGH, Denver, Colo & Memo of Approval by Sec'y of the Army dtd 22 Feb 64(Incls 1 & 2)

(b) CHEMICAL FORM ADMINISTERED

(c) DESCRIBE PROCEDURES WHICH WILL BE OBSERVED TO MINIMIZE HAZARD FROM HANDLING, STORAGE, AND DISPOSAL OF THE BYPRODUCT MATERIAL:
As specified in Application (dtd 6Dec63)For License No. 5-46-13 (A66)

(d) DESCRIPTION AND SKETCHES OF SPECIAL DEVICES TO BE USED FOR ADMINISTERING BYPRODUCT MATERIAL TO HUMAN BEINGS ARE
(1) ATTACHED (LITERATURE REFERENCES WILL SUFFICE) As specified in Application
CIRCLE ANSWER — YES — (NO)
(2) ON FILE WITH THE ISOTOPES EXTENSION REFER TO APPLICATION NO (dtd 6Dec63) For License No. 5-46-13(A66)
CIRCLE ANSWER — (YES) — NO

5. PROPOSED DOSAGE SCHEDULE
(a) In millicuries for internally administered byproduct material other than discrete fixed sources; and in roentgens or rads, as appropriate, for internal or external irradiation from discrete fixed sources (gold seeds, cobalt needles, etc.) state separately for each condition or disease (use page 2 if necessary):
See attached copies:"Request for Approval for Human Use of Radioisotopes in Tracer Amounts in Volunteer Experimental Research Subjects"submitted thru channels to Secretary of the Army 19Dec63 by U.S.A. Med Rsch & Nutr Lab, FGH, Denver, Colo and Memo of Approval by Secretary of the Army dtd 22Feb64 (Incls 1&2)

(b) INVESTIGATIVE PROPOSAL FOR EXPERIMENTAL, NEW OR UNUSUAL HUMAN USES IS ATTACHED. (Attachment should include outline of conditions to be evaluated, including data from animal studies and/or abstract of literature reference if any, number and type of patients (i. e. age group, moribund, etc.))
CIRCLE ANSWER — (YES) — NO

6. IF BYPRODUCT MATERIAL WILL NOT BE OBTAINED IN PRECALIBRATED FORM FOR ORAL ADMINISTRATION OR IN PRECALIBRATED AND STERILIZED FORM FOR PARENTERAL ADMINISTRATION, DESCRIBE IDENTIFICATION, PROCESSING, AND STANDARDIZATION PROCEDURES:
By-product material will be obtained in pre-calibrated form for oral administration or in pre-calibrated and sterilized form for parenteral administration

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7. THE PROPOSED USE OF BYPRODUCT MATERIAL HAS BEEN, OR WILL BE, APPROVED BY THE MEDICAL ISOTOPE COMMITTEE.
CIRCLE ANSWER — (YES) — NO

HOSPITAL FACILITIES FOR INDIVIDUAL PRACTICE USE ONLY NA

8. (a) THE APPLICANT HAS COMPLETED ARRANGEMENTS FOR A HOSPITAL TO ADMIT RADIOACTIVE PATIENTS WHENEVER ADVISABLE.
CIRCLE ANSWER — YES — NO

(b) A COPY OF INSTRUCTIONS TO BE FURNISHED TO THE HOSPITAL AS TO RADIOLOGICAL SAFETY PRECAUTIONS TO BE TAKEN AND AVAILABLE RADIATION INSTRUMENTATION IS ATTACHED.
CIRCLE ANSWER — YES — NO

19 DEC 1963

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

M. E. McDowell, M.D., Lt. Col., MC, Commanding Officer and Director
J. E. Canham, M.D., Lt. Col., MC; Chief, Metabolic Division
J. E. Hansen, M.D., Lt. Col., MC; Chief, Physiology Division
E. M. Baker, Ph.D., Maj., MSC; Assistant Chief, Chemistry Division and Chief, Carbohydrate Branch
K. E. Kinnamon, DVM, Capt., VC; Chief, Radioisotope Branch, Physiology Division
J. R. Handy, M.D., Capt., MC; Chief, Cellular Physiology Section, Physiology Division
H. E. Sauberlich, Ph.D., (PL-313), Chief, Chemistry Division
G. A. Leveille, Ph.D., (GS-13), Chief, Lipid and Protein Chemistry Branch, Chemistry Division
B. M. Tolbert, Ph.D., Professor of Chemistry, University of Colorado and Consultant to USAMRNL

and

Fitzsimons General Hospital
Denver, Colorado

C. A. Moore, M.D., Lt. Col., MC, Chief, Urology Service
O. G. Stonington, M.D., Professor of Urology, University of Colorado School of Medicine and Consultant to Fitzsimons General Hospital

Add #2

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 quantities are used.

b. Therefore, this request seeks approval only for use of the specified radioisotopes, 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 will present the health physics aspects of the radioisotope tracers required (Section II); will present in detail the research methods and plans for C-14 and H-3 usage in vitamin C studies, and outline other

proposed studies referring to the vitamin C studies as a model (Section III, Par. 11); and will describe other metabolic and nutritional studies requiring other radioisotope tracers in addition to C-14 and H-3 (Section III, Pars. 12-15).

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.

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.

4. History of USAMRNL isotope usage

a. This laboratory has employed radioactive labeled compounds in studies with human subjects under AEC License Number 5-46-6 since 17 December 1957. Authorization was given initially to use Iodine ^{131} labeled

human serum albumin to measure the turnover rate of albumin of 10 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 which includes authorization for use of the following:

(1) Iodine^{131} for diagnosis of thyroid function and thyroid scanning.

(2) Iodine^{131} labeled human serum for the determination of blood volumes and plasma volumes.

(3) Iodine^{131} labeled Rose Bengal dye for determination of liver function and liver scans.

(4) Iodine^{131} labeled fats and fatty acids for determination of fat absorption.

(5) Iodine^{131} labeled renal function compounds.

(6) Phosphorus^{32} for the treatment of polycythemia vera, leukemia and bone metastasis.

(7) Chromium^{51} for the determination of red cell volume and red cell survival time.

(8) Cobalt^{60} labeled vitamin B_{12} for the diagnosis of pernicious anemia.

(9) Iron^{59} for iron metabolism studies.

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

f. Current status of AEC radioisotope licenses of USAMRNL and Fitzsimons General Hospital:

(1) This laboratory is currently licensed by AEC (License No. 5-46-12 (L 63) for human use of the isotopes listed above in Par. 4c and d; the specifically authorized study in Par. 4e having been completed. All human usage not explicitly covered by Par. 4c of AR 70-25 has been discontinued pending authorizations (requested by this document) required by AR 40-37. This AEC license will expire 31 December 1963.

(2) This laboratory also currently operates under the general (animal usage) AEC radioisotope License No. 5-46-11 (H 63). This was originally scheduled to expire 31 August 1963, but has been extended indefinitely by AEC (who are holding our renewal application dated 21 May 1963) pending consolidation of the separate FGH and USAMRNL licenses into one broad license for the entire post (a joint FGH and USAMRNL license).

(3) Consolidation of the heretofore separate licenses of FGH and USAMRNL into one broad license has been recommended by the Preventive Medicine Division of Office of The Surgeon General, and sanctioned by AEC because of the favorable record of radioisotope handling by both FGH and USAMRNL. Application for the new joint license (omitting the radioisotopes requested herein for volunteer research use) will be forwarded to AEC (through The Surgeon General) by FGH-USAMRNL within approximately 10 days.

11 March 1964 — This action now achieved with the granting of AEC License No. 5-46-13 (A'')

(4) Upon approval of the radioisotopes requested herein per Par. 3b(3) AR 40-37, application will be made to AEC (through The Surgeon General) for addition to the joint AEC license by amendment.

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
	Amino acids
	Lipids (as glycerides, cholesterol and free fatty acids)
	Carbohydrates
	Acetate
	Mevalonic acid
	Bicarbonate or CO_2

58638

By-product Material	Chemical and/or Physical Form
Hydrogen-3	Vitamins
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.

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 μc. Constants for calculating maximum permissible internal concentration of radioisotopes assumes 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. 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 Uses of Atomic Energy, Vol. 24, p. 148, 1958; The Role of Magnesium in Biological Process, J. K. Aikawa, 1963, C. C. Thomas, Publishers, Springfield, Ill.)

9. Calcium-47

This relatively recently available isotoped 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 studies outlined would not exceed 15 μc.

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

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

2. 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."

3. 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.

4. 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.

5. 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 Nutrition and Metabolism Tracer Studies

11. Vitamins: Investigations on the vitamin requirement of the human with the use of Carbon-14 or Hydrogen-3 labeled vitamins or related compounds (References cited in the paragraph (11) are listed in subparagraph 11h)

a. Background for vitamin C studies

Past studies (1) indicated that D-glucuronolactone caused increased blood ascorbic acid levels as well as increased urinary excretion of ascorbic acid in men, whereas D-glucuronic acid did not do this. To check the possible conversion of D-glucuronolactone to ascorbic acid, it was decided to study the metabolism of the lactone in two ways. One was to give the D-glucuronolactone-6-C^{14} orally and then isolate urinary ascorbic acid to determine if any of the labeled lactone had been converted to L-ascorbic acid. The other was first to label the total body ascorbic acid pool with L-ascorbic-1-C^{14} acid and then test with various loads of D-glucuronolactone to see if any changes would take place in the specific activity and rate of excretion of ascorbic acid. Further, an attempt was made to see if total body ascorbic acid and its rate of utilization were related to the fat-free body weight.

Results of studies (2) with healthy men revealed that close to one-fourth of D-glucuronolactone-6-C^{14} was converted to L-ascorbic acid whereas, on the other hand, no activity could be detected in the ascorbate derivative isolated from the urine of subjects receiving D-glucuronic-6-C^{14} acid. In addition, it was found that one-half of the urinary oxalate arises from the breakdown of ascorbic acid and is excreted at a constant rate. Further, in 6 men

of diverse body weight and degree of fatness, it was found that ascorbate utilization, as expressed in terms of C^{14} oxalate excretion, occurred at a rate of 0.207 mg per day per kilogram of fat-free body weight.

One of the more interesting findings of these experiments was that no $C^{14}O_2$ activity could be detected in the expired air of the subjects receiving L-ascorbic-1-C^{14} acid even with the use of a 15-liter ionization chamber for greater sensitivity. A fraction of 1% oxidation to CO_2 during the first 8 hours could have been easily detected by this technique. This finding was in agreement with the earlier work of Hellman and Burns (3). Recently, Abt et al. (4) reported that man excretes approximately 25% of the total activity of L-ascorbic-1-C^{14} acid via the lung. Because doubt had been caused as to whether or not man could decarboxylate C^{14} labeled ascorbic acid to $C^{14}O_2$, a series of experiments were performed in this laboratory which resulted in a publication (5) showing: 1. Chromatographic and radioautographic evidence was presented showing that progressive degradative changes occur in L-ascorbic acid dissolved in water and kept at 25° C. for a 72-hour period; 2. When a human subject received 20 μc of freshly dissolved L-ascorbic-1-C^{14} acid solution, little or no C^{14} appears in his respiratory CO_2; 3. Men who were given similar samples of L-ascorbic-1-C^{14} acid aged for 36 and 72 hours, respectively, excreted 30.6% of the ingested C^{14} as respiratory CO_2. The true nature of the compounds undergoing decarboxylation in man in these studies cannot be defined from the work presented here except that they are not reduced ascorbic acid.

b. Request for use of Carbon-14 to label vitamin C and related compounds

Therefore, because of demonstrated usefulness and necessity of using tracer techniques to study metabolic pathways, the proposal is being made that tracer amounts of Carbon-14, as glucose-6-C^{14}, glucuronolactone-6-C^{14}, glucuronic-6-C^{14} acid and ascorbic-1-C^{14} acid be administered by mouth to humans in further studies for the purpose of measuring the pool size and the rate of utilization of body ascorbic acid under varying conditions. The subjects to be used will be military personnel (volunteering for the specific study) or laboratory personnel, both male and female, or Fitzsimons General Hospital personnel (as well as Fitzsimons General Hospital patients who volunteer). The possible hazards of the experiments will be explained in advance to all subjects. Although multiple experiments may be performed on individuals, in no case will the total body radiation dose from this experiment, other experiments, or from x-rays, exceed the maximum permissible limits for normals of 5 rem per year (lower below age 25).

c. Experimental methods (using labeled vitamin C and related compounds

The L-ascorbic-1-C^{14} acid will be obtained from the California Corporation for Biochemical Research, Los Angeles. All C^{14} labeled compounds will be checked for purity prior to use by melting point measurement and by paper chromatography. The activity of all C^{14} labeled compounds will be checked by radioassay. The L-ascorbic-1-C^{14} acid will be freshly dissolved in

distilled water and immediately swallowed by the experimental subject. No cold carrier will be given to these subjects.

Total daily urine will be collected and measured from all subjects; these samples will be refrigerated and 2.0 ml of each will be taken for radioassay.

Immediately after receiving the tracer quantity of L-ascorbic acid, the subjects will be made to expire directly through a $CaCl_2$ drying train into a 5-liter Cary-Tolbert ionization chamber connected to a vibrating reed electrometer. The $C^{14}O_2$ activity, total CO_2, and the volume of flow is recorded automatically on a 6-channel recorder.

The total activity of each urine sample is determined by use of a liquid scintillation counter using P-dioxane-toluene. Oxalate in selected samples is isolated as calcium oxalate, recrystallized 4 times and dissolved in 1 N hydrochloric acid for counting in the liquid scintillation counter. Quantitative determination of the total oxalate is done by the Archer method (6). Efficiencies for all liquid scintillation counting of samples are determined individually by use of added standard C^{14} samples.

Urinary ascorbic acid levels are chemically determined by the Schaffert method (7). Urinary ascorbic acid is then isolated by the method described by Jackel et al. (8). After the dinitrophenylhydrazone (DNPH) derivatives are recrystallized, they are dissolved in P-dioxane and applied to weighed planchets and counted in a gas flow counter. All DNPH derivatives are recrystallized to constant activity which usually requires 4 to 6 recrystallizations.

Total body tissue volume (V) is estimated in duplicate tests using a body volumeter based on displacement of water (9). From body weight (M) and V, fat (F) in kg is calculated according to an equation developed in this laboratory: $F = 4.834\ V - V.336\ M$.

d. Experimental plan; studies on factors that may influence the vitamin C metabolism and requirements in man:

(1) Recapitulation of the method

Studies of body composition and the use of C^{14} isotopes have resulted in a method for stating the actual utilization of ascorbic acid by healthy men.

In human subjects who ingest 20 μc of L-ascorbic-1-C^{14} acid, the daily urinary oxalate arising from metabolism of the labeled ascorbate is subsequently excreted as a constant proportion of total C^{14} activity remaining in the body. Thus, it can be inferred that the portion of the daily oxalate which arises from metabolism of ascorbate is formed and excreted at a constant rate.

Ingestion of a single, comparatively large 0.5 gm quantity of unlabeled ascorbic acid or its precursors by subjects whose body ascorbic acid pools had been previously labeled, as described above, results in increased excretion of C^{14} ascorbate of lowered specific activity. These effects are transitory in that within 2 days total ascorbate excretion returns to previous levels and ascorbate specific activity is lower than it was prior to dilution of the body ascorbate pool.

Simultaneously, the total activity and the specific activity of the oxalate decrease, but the proportionality of total oxalate activity to specific activity of the ascorbate remains the same. From these effects, it can be inferred that the utilization breakdown of ascorbic acid in the body occurs at a constant rate irrespective of an increased rate of supply of ascorbate to the body.

Further, in 8 men of diverse body weight and degree of fatness, it was found that ascorbate utilization, as expressed in terms of C^{14} oxalate excretion, occurred at a rate of 0.207 mg per day per kg of fat-free body weight. Rarely, if ever, do adult males exceed 90 kg in lean body mass. Therefore, 28 mg per day intake would match the greatest quantity of ascorbate metabolized by the largest healthy man. Further, it is of interest to note that despite repeated reports in the literature of loss of ascorbic acid in sweat, when one of the subjects discussed above was sweated for a 6-hour period in a hot room after being labeled with 20 μc of ascorbic-1-C^{14} acid, no C^{14} activity could be detected in the collected total body sweat. The chemical analysis of the sweat indicated the presence of a small amount of ascorbate. However, when the sweat was lyophilized to dryness and then applied to a chromatographic sheet and run in a standard solvent system, no reduced ascorbic acid could be demonstrated. These results are not surprising in view of the fact that it is well known that all the chemical determinations for ascorbic acid are not absolutely specific for ascorbic acid in biological fluids.

The method as employed consists of giving an individual a single oral dose of 20-50 μc of L-ascorbic-1-C^{14} acid and then collecting a single 24-hour urine sample. The ascorbic acid contained in the urine is isolated as the dinitrophenylhydrazine derivative and counted to obtain specific activity as μc/mg of ascorbate excreted. The oxalate that is derived from the labeled accorbate and excreted is also isolated and counted. Then, by simply dividing the specific activity of the excreted ascorbate by the total C^{14} activity of the formed and excreted oxalate, one can obtain an estimate of the number of milligrams of ascorbate utilized during the 24-hour period.

This method could be used in human studies to determine whether or not there is an increased utilization or an increased need for ascorbic acid in the following conditions:

Cold
Heat
Acclimatization to heat, cold, stress and altitude
Stress
Trauma and burn patients
Infections

Moreover, this method could be used in human studies to determine whether or not adaptation occurs in people who have been on a chronic low dietary intake of vitamin C.

(2) Need for more data on vitamin C metabolism

According to the text "World Review of Nutrition and Dietetics" (Vol. III, 1963) published by G. H. Bourne, p. 187, the following conclusions regarding vitamin C are stated:

"1. The most frequently quoted recommended allowance of vitamin C for adult man under the prevailing conditions of civilization and climate varies around 80 mg L-ascorbic acid/day.

"2. High doses, though not toxic are not recommended and may, according to some findings, lead even to a negative adaption of the organism.

"3. Medium doses (up to 200 mg) are probably reasonable under some special conditions-certain types of work, rehabilitation and therapeutic allowances."

At a recent meeting held by the Federation of American Societies for Experimental Biology in Washington, D.C. on 14-15 March 1963, the Ad Hoc Committee on Military Applicability of Research on Ascorbic Acid made the following recommendation: That this laboratory, i.e. USAMRNL, attempt to study the utilization of vitamin C in humans in the following conditions:

Cold
Heat
Acclimatization
Stress
Interrelationship of vitamin C with other vitamins
Wounds and burns

(3) Extension of the methods to problems stated in preceding paragraph

In view of the above recommendations and the lack of information on the above problems, it is requested that vitamin C utilization studies in the above-named conditions, using the method previously described, in human volunteer subjects be considered for authorization.

To accomplish this request, it would require that a team of 2 or more investigators from this laboratory be sent to several geographical areas

with differing climatic conditions. The areas under consideration are (1) Camp Hale, Leadville, Climax or Mt. Evans areas in Colorado for studies on ascorbic acid in acclimatization to cold, and (3) a tropical area within the Caribbean Command for studies on acclimatization to tropical conditions. In addition to this general approval for use of isotopes, the approval and concurrence of the local commander or appropriate local health authorities would be obtained for each location and experiment. At each location, comparisons would be made between subjects who had recently arrived and those who had resided at the location for an extended period of time. These results would in turn be compared with findings obtained at this laboratory on subjects residing in Denver, Colorado. At each location, not more than 10 subjects would be studied. Normal, healthy volunteers, preferably military, would be selected to receive the C^{14} labeled ascorbic acid as previously outlined; body composition data would be obtained by skinfold or other appropriate measurements. With the data obtained from these studies, one could then assign what would be the ideal vitamin C pool size and utilization under these climatic conditions in comparison with the data previously obtained at this laboratory on normal healthy subjects.

Upon evaluation of the data, considerations would be made as to recommended allowances for vitamin C under conditions of cold, heat, altitude and acclimatization.

Studies on the interrelationship of vitamin C with other vitamins would be performed at this laboratory with normal volunteers, employing C^{14}

labeled ascorbic acid in the amounts and manner as previously outlined. Evidence of a relationship between vitamin C and vitamin B_6 in the human has been recently obtained at this laboratory in non-isotopic studies. The use of C^{14} ascorbic acid in these investigations would permit a better understanding of the apparent interrelationship. The possible increased needs for vitamin C in situations of stress (surgery or radiation therapy, as examples), wounds or burns would be performed in conjunction with Fitzsimons General Hospital should suitable patients become available.

e. Proposal to use C-14 and Hydrogen-3 labeled vitamins other than vitamin C, using the outlined vitamin C studies as a general model

Other Carbon-14 labeled vitamins would be studied in essentially the same manner and employing the same techniques and procedures as those indicated for ascorbic acid. Excretion rates, pool size, turnover rates, absorption and metabolic products will be measured for each. The influence of various nutritional states on the above parameters will be investigated in an attempt to evaluate dietary requirements for vitamins. The body pool size of ascorbic acid is considered greater than that of any other vitamin and the turnover rate is as slow or slower than other vitamins; therefore, the amount of radioactive label used for the other vitamins will be less, and with the greater turnover rate, will produce less of a body burden than the vitamin C.

When Carbon-14 labeled vitamins are not available, the above studies will be performed with the use of tritium labeled vitamins. The most commonly

employed tritiated vitamins are pyridoxine and folacin. These two vitamins have been employed at various laboratories in malabsorption studies with humans. The same parameters and procedures as outlined for Carbon-14 labeled vitamin C and the above will be employed, except that electrometer measurements of expired air will be omitted. The dosage employed will in no case exceed 100 μc of tritium labeled vitamin. This dosage of tritium represents only 10% of that routinely employed in total body water measurements and is indicative of the low radiation dose received.

f. Health physics

The dose of any of the C^{14} labeled vitamins will not exceed 50 microcuries. The following maximal radiation dosages are calculated with the aid of the ICRP Handbook (Appendix I, reference 1). The physical half time of C^{14} is considered infinite. The following is an approximation of the biologic half time. All the vitamins to be used are highly reactive; however, for ascorbic acid the turnover rate is probably slower, possibly much slower than for the other vitamins. From nutritional data, the total body ascorbic acid is almost certainly less than 6 gm, and the daily turnover greater than 10 mg. The half time of body vitamin C under these conditions would be 400 days. This estimate is obviously too long, perhaps by as much as an order of magnitude. The only data in the literature on man (L. Hellman and J. J. Burns, J. Biol. Chem. 230: 923, 1958) (E. M. Baker, H. E. Sauberlich, S. J. Wolfskill, W. T. Wallace and E. E. Dean, Proc. Soc. Exp. Biol. and Med. 109: 737, 1962) shows a pool size

of about 1.4 gm in a 70 kg man, with a half time of about 16 days. With the maximum estimate of a 400-day half time, a dose of 50 microcuries of C^{14} ascorbic acid evenly distributed in a 70-kg man will give a total radiation dose of only 1.13 rem (0.164 rem the first 13 weeks). With a half time of 16 days, the same dose will give a total radiation dose of 0.045 rem (0.044 rem the first 13 weeks). In the case of tritiated vitamins, the dosage will in no case exceed 100 μc of tritium. This dosage of tritium represents only 10% of that routinely employed in total body water measurements; consequently, the body burden is very low.

g. Personnel

For each specific phase of the studies, one of the following (other than the consultant) will be designated as project leader, and one of the Medical Officers named below will be designated as attending physician per Par. 6, AR 70-25.

Maj. E. M. Baker, Ph.D., MSC
H. E. Sauberlich, Ph.D. (PL-313)
Lt. Col. M. E. McDowell, M.D., MC
Lt. Col. J. E. Canham, M.D., MC
Lt. Col. J. E. Hansen, M.D., MC
Capt. J. R. Handy, M.D., MC
B. M. Tolbert, Ph.D. (Consultant, University of Colorado)

h. References

(1) Baker, E. M., E. L. Bierman, I. C. Plough. Metabolism 9: 478, 1960 (reprint attached, Appendix IV).

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(5) Baker, E. M., N. G. Levandoski and H. E. Sauberlich. Proc. Soc. Exp. Biol. and Med. 113: 379, 1963 (reprint attached, Appendix IV).

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12. Amino Acids: Investigations on the metabolism of amino acids in the human with the use of Carbon-14 labeled compounds (references cited in this paragraph are listed in Par. 12f)

a. Background

The recent work of Crawhall et al. (1) using $\left[1\text{-}C^{13}\right]$-glycine has demonstrated that this isotope was diluted about 2½ times during the conversion of glycine from the first metabolic pool (i.e. the pool of glycine with which a dose of glycine mixes immediately after absorption and distribution, and which can be sampled by means of the uncombined urinary glycine (2)) to oxalate, indicating that about 40% of the urinary oxalate was derived from glycine during this period.

Berlin et al. (3) did not measure the C^{14} activity in the urinary oxalate in their glycine-2-C^{14} studies. However, they did show with the use of the methyl labeled glycine that 90% of the C^{14} activity was accounted for in the expired $C^{14}O_2$ and that only 5% of the C^{14} activity was excreted in the urine. This would tend to indicate that the catabolism of glycine-2-C^{14}, insofar as oxalate formation is concerned, is far different than that of the carboxyl labeled glycine-1-C^{14}.

When C^{14} labeled ascorbic acid was orally administered to humans, Hillman and Burns (4) reported that an average of 44% of the total radiocarbon excreted in urine was recovered as oxalate. It was demonstrated in this laboratory that 50% of the urinary oxalate was derived from L-ascorbic acid-1-C^{14} and was excreted at a constant rate per day (5, 6). Therefore, it is of interest to study both glycine-1-C^{14} and glycine-2-C^{14} metabolism in humans to determine (1)

whether or not the glycine C^{14} is partially converted to and excreted as oxalate at a constant rate per day as well as (2) the amount per day excreted as urinary oxalate.

Further, it would be desirable to measure the expired $C^{14}O_2$ in a vibrating reed electrometer to determine the amount and extent of decarboxylation of the C^{14} labeled glycine in man. It should be noted that this has been done in humans using glycine-2-C^{14} (3), but not with the glycine-1-C^{14}.

b. Experimental plan (Part 1)

A total of no more than 10 human subjects would be involved in these experiments. Further, the subjects would be staff members of this laboratory, 20-46-year-old males. There would be no dietary restriction placed on these subjects.

Each subject would have to receive orally 20 μc of glycine-1-C^{14} as well as a further 20 μc of glycine-2-C^{14} at a much later date (40-80 day interval). No cold carrier glycine will be given to the subject at the time the labeled material is administered. Immediately after taking the C^{14} glycine, the subject will be made to breathe through a drying train directly into the ionization chamber of the vibrating reed electrometer. The subject will continue to breathe at intervals through the system until he reaches his background trace signal. This is done by having the subject breathe into the system for 20-30 minutes, then allowing a 30-minute rest. This process is continued until the electrometer tracing returns to background signal.

Further, 24-hour urine collections will commence with the ingestion of the C^{14} glycine label and will continue on every other 3rd day for a period of 2 weeks. The urine samples will then be analyzed for the total urinary oxalate content. The oxalate of each sample will then be isolated and counted in the liquid scintillator. Also, certain selected samples of the urine will be analyzed on the amino acid analyzer which has a flow-through scintillation detector attached. This will enable us to obtain both the specific and total activity of the urinary free glycine of other radioactive metabolites. Each urine sample will be counted for total activity. Thus, if one knows the total dose given as glycine C^{14} as well as the dose remaining in the body at any given time, as well as the specific activity of the excreted urinary glycine, one should be able to approximate the total body pool size and turnover rate of the free glycine pool.

c. Health physics pertaining to Par. 12b

The normal A.E.C. procedures shall be adhered to insofar as the administration, handling of the isotope and the disposal of the urine samples obtained from the subjects.

The dose of the C^{14} glycine will not exceed 20 μc for either the glycine-1-C^{14} or the glycine-2-C^{14}. The following maximal radiation dosages are calculated with the aid of the ICRP Handbook (Appendix I, reference 1).

The physical half life of C^{14} is considered infinite. The following is an approximation of the biologic half time of glycine-2-C^{14}. According to N. I. Berlin, B. M. Tolbert and C. Lotz (J. Clin. Investigation 31, No. 3:

335-337, 1952), the longest "half time of glycine-2-C^{14} elimination from the tissues of man is approximately 50 days." With the estimate of a 50-day half time, a dose of 20 μc of glycine-2-C^{14} evenly distributed in a 70 kg man will give a total radiation dose of only 0.056 rem (0.0478 rem the first 13 weeks). Another body compartment of considerably less importance and a half time of about 100 days was described at a later date by Berlin et al. (Proc. Soc. Exp. Biol. and Med. 88: 386, 1955). However, since the majority of the dose is not retained, but lost within the first 24-hour period as expired CO_2 as urinary excretory products, the body irradiation burden is considerably less than this value.

In the case of glycine-1-C^{14}, we have only the data of R. W. E. Watts and J. C. Crawhall (Biochem. J. 73: 277-86, 1959) using the stable C^{13} isotope to estimate the glycine metabolic pool in man. According to these authors, the pool size of glycine in a 70 kg man is 406 gm or 5.8 gm/kg. Further, they state that the turnover rate in a 70 kg man is 3.2 gm/hr. or 76.8 gm/day. Thus, $\frac{76.8}{406} = 0.189$ or 18.9% turnover. The biological $t\,\frac{1}{2}$ would then be equal to $\frac{0.693}{0.189} = 3.7$ days. Assuming then a 4-day half life for the glycine-1-C^{14}, a dose of 20 μc evenly distributed in a 70 kg man will give a total radiation dose of only 0.005 rem (0.005 rem the first 13 weeks).

d. Experimental plan (Part II)

Other Carbon-14 labeled amino acids would be studied in essentially the same manner as that employed with glycine. Similarly, pool size, turnover rates and metabolites would be measured in an attempt to study the protein and

amino acid requirements of the human. These studies should also provide additional knowledge as to the metabolic pathways and interrelationships of amino acids in the human. It is anticipated that not more than 3 subjects will be required for each amino acid investigated. The dosage of Carbon-14 employed would not exceed that indicated for Carbon-14 glycine, and with the half time estimated not to exceed that for glycine. The radiation burden, therefore, would be low and would not in any instance approach the maximum permissible dose.

e. Personnel

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G. A. Leveille, Ph.D.
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Lt. Col. J. E. Canham, M.D., MC (Serving also as attending physician)

f. References

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13. Lipids: Studies on lipid metabolism in the human with the use of Carbon-14 labeled compounds (references cited in this paragraph are listed in Par. 12c)

a. Experiment 1: Carbon-14 tracer studies on cholesterol metabolism in the human

(1) Background and procedures

Tracer amounts of C^{14}, as cholesterol-4-C^{14}, will be administered orally to study the influence of neomycin and various fats on cholesterol absorption in order to determine whether the hypocholesteremic effect of these materials is the result of an impaired absorption. In order to ascertain whether these materials influence cholesterol synthesis, acetate-1-2-C^{14} and mevalonic acid-2-C^{14} will be administered intravenously.

The work of Samuel and Steiner (Proc. Soc. Exp. Biol. Med. 100: 193, 1959) has demonstrated a hypocholesteremic effect for neomycin. Unsaturated fat has also been shown to have a cholesterol lowering effect. The mechanisms by which neomycin or unsaturated fat depress plasma cholesterol remain obscure. Unpublished data from this laboratory indicate that neomycin functions by interfering with cholesterol and/or bile acid absorption. The effect of unsaiurated fats appears to be twofold: a) an interference with cholesterol absorption mediated by the sterol fraction in vegetable fats and b) a particular systemic effect (Bronte-Stewart, Fed. Proc. 20: No. 1, Part III, p. 127, 1961).

In order to further elucidate the mechanism of action of these compounds, human volunteers fed neomycin, different fats or a control diet

will be given an oral dose of cholesterol-4-C^{14} (20-50 μc) and its absorption determined. In other subjects, similarly treated, acetate-1-2-C^{14} or mevalonic acid-2-C^{14} will be administered intravenously (50 μc) and incorporation into cholesterol will be ascertained by determining the specific activity of plasma cholesterol.

(2) Health physics

The maximum dosage to be employed is 50 μc for acetate, mevalonic acid and cholesterol. The maximal radiation dosage, calculated with the aid of the ICRP Handbook (Appendix I, reference 1) for these levels of administered radioactivity, will not exceed the permissible limits for normal subjects of 5 rem/yr. with no more than 3 rem in any 13 consecutive week period (above age 18). The half life of cholesterol is approximately 20 days (Cook, R. P.: Cholesterol, 1958, Academic Press, N. Y.) and if that of other compounds synthesized from acetate or mevalonic acid is assumed to be similar and, further, the physical half life of C^{14} is considered infinite, a dose of 50 μc of C^{14} labeled cholesterol, mevalonic acid or acetate evenly distributed in a 70 kg individual will give a total radiation dose of 0.056 rem (0.054 rem the first 13 weeks).

(B) Personnel

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Lt. Col. J. E. Canham, M.D., MC (Serving also as attending physician)

b. Experiment 2: Suppressibility of cholesterol synthesis by exogenous cholesterol loading in man

(1) Background

The relative stability of serum cholesterol levels, despite marked variation in dietary intake of cholesterol, has been attributed to compensatory changes in hepatic synthesis of cholesterol (1-5). Recently, Sipperstein and Guest have suggested, on the basis of in vitro studies, that the mechanism of this homeostatic effect is a sensitive negative feedback system whereby cholesterol inhibits the conversion of β-hydroxy-β methyl glutaryl Co A to mevalonic acid (6). These authors speculate that insensitivity of this feedback might be involved in disorders of cholesterol metabolism.

In order to test this hypothesis, it is planned to ascertain quantitatively the response of cholesterol synthesis to an exogenous cholesterol load. After data on normal subjects have been obtained, these will be compared with groups demonstrating abnormalities of cholesterol metabolism, i.e. idiopathic hypercholesterolemia-proven atherosclerosis, diabetes, hypo- and hyperthyroidosis, nephrotic syndrome.

(2) Method

Patients will be given 100 microcuries 1-C^{14} acetate intravenously or orally. Timed serum samples will be analyzed for total and C^{14} cholesterol. In certain patients, C^{14} of other serum lipids and C^{14} as $C^{14}O_2$ will also be measured.

These procedures will then be repeated after a standard cholesterol load sufficient to elevate serum cholesterol in normal subjects (7). Differences in total and specific activity of serum cholesterol before and after cholesterol loading will be used as an index of the sensitivity of the hepatic response to exogenous cholesterol. Methods of analysis will be similar to those described by Gould et al. (8).

(3) Health physics

With regard to radiation safety, reference is made to the work of Gould et al. (8):

> "The dose of 100 μc was chosen so that repeated doses could be given to the same subject without exceeding accepted values for the maximum permissible dose for man. Our studies of $C^{14}O_2$ in expired air after the administration of 1-C^{14} acetate demonstrated that approximately 56 per cent of the radiocarbon was eliminated during the first 24 hours.* On this basis, we have made the assumption that a single 100 μc dose will result in the 'retention' of not more than 25 μc of C^{14} in the slowly exchanging 'fat compartments' of the body. The maximum permissible dose for C^{14} compounds retained in the body fats is estimated to be 250 μc, according to calculations in Handbook 52 of the National Bureau of Standards.[7] Thus, we believe we are justified in administering, over a period of several months, a maximum of five such doses to human subjects without regard to their life expectancy.
>
> "Ref. 7. Maximum Permissible Amounts of Radioisotopes in the Human Body, etc., Nat. Bur. Standards Handbook, 52, pp. 12 and 18, G.P.O., Washington, D.C., March 20, 1953.

"*Shreeve also reported that 56 per cent of the C^{14} in acetate was eliminated as $C^{14}O_2$ by man at the end of 24 hours. Hellman reported that 60 per cent of the radiocarbon was retained at the end of 24 hours, and 35 per cent at the end of the first week after administration of acetate. It should be noted that he used the methyl-labeled acetate (2-C^{14}-acetate)."

In the present study, only 2 doses of 100 microcuries each will be given instead of 5 doses of 100 microcuries as above. The dose will therefore be well below the maximal permissible dose quoted.

(4) Personnel

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Lt. Col. J. E. Canham, M.D., MC (Serving also as attending physician)

c. References

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14. Carbohydrates: Investigations on the digestibility and metabolism of carbohydrates in the human with the use of Carbon-14 labeled compounds (references cited in this paragraph are listed in Par. 14e)

a. Background

Experiments have been in progress for some time at this laboratory investigating the digestibility of Carbon-14 labeled cellulose, hemicellulose and various uncommon sugars in laboratory animals as the rat, hamster and guinea pig (1, 2). Balance studies employing a vibrating reed electrometer to measure the expired carbon dioxide, together with urine and fecal measurements, have demonstrated that the rat may digest as much as 25% of the ingested cellulose (1).

In the case of the human, it is considered that cellulose passes through the digestive tract without being attacked by any of the digestive enzymes, though some bacterial decomposition probably takes place in the large intestine. Whether or not the bacterial actions are of value to the human are unclear. Various studies at this laboratory as well as elsewhere would indicate that at times cellulose is digested to a limited extent by the human (3-5). However, it must be recognized that in human balance studies, the methods for the measurement of cellulose and hemicellulose are less than satisfactory for critical evaluation. Furthermore, the possibility of bacterial decomposition of cellulose or hemicellulose in the lower intestinal tract may give rise to an "apparent" digestibility without any "true" digestibility in terms of nutrient benefit to the human. The use of Carbon-14 labeled cellulose with human subjects would give more definitive results with regard to this problem. Very exacting balance studies could be conducted.

Furthermore, the presence or lack of presence of radioactivity in the expired carbon dioxide, or in the urine and blood, would be rather conclusive evidence, which could be quantitated, that cellulose is or is not utilized. If utilization is indicated, the question of whether or not it is mediated through the intestinal flora could be readily investigated with the use of oral antibiotics (3, 4).

b. Procedures

The procedures employed would be very similar to those previously described for use with Carbon-14 labeled vitamin C. Normal, healthy volunteer subjects (minimum number) would receive orally specially prepared Carbon-14 labeled cellulose in an amount not to exceed 100 μc. The subjects will have previously received controlled levels of cellulose in the diet to investigate the influence of this dietary component on the digestibility of the C^{14} cellulose. Balance studies will be conducted with the aid of markers for the stools. The expired air will be monitored with the aid of a 5 or 15-liter chamber with a Cary-Tolbert vibrating reed electrometer and automatic carbon dioxide measurements. The expired air will be monitored until no radioactivity is detectable. Urine collections will also be made throughout the period and radioactivity measurements performed with a scintillation counter. If significant amounts of radioactivity are found to be present, attempts will be made to determine the nature of the radioactive compounds.

If evidence of cellulose digestion is noted, additional subjects will receive prior to receipt of the C^{14} cellulose oral supplements of antibiotics in

an effort to study the possible role of the intestinal flora in the digestion process. The antibiotics, neomycin and bacitracin would be employed, using the amounts and procedure previously employed in recent studies by the Metabolic Division of this laboratory to reduce or eliminate the intestinal flora of human subjects (3, 4). If C^{14} labeled hemicellulose or pectins can be made available, similar digestibility studies would be conducted with these materials.

c. Health physics

As indicated above, the dosage of the Carbon-14 labeled cellulose, hemicellulose or pectin would not exceed 100 μc. Although the digestibility of these compounds is not fully known, it is exceedingly doubtful that any are completely absorbed. If cellulose is digested to any extent, it would be most likely converted to glucose which the body readily metabolizes with a major portion being removed quickly through the lungs as carbon dioxide. Even if it is assumed that 100% digestion and assimilation takes place, this dosage of radioactivity would be considerably less than the maximum permissible dose. Glucose, the unit component of cellulose, is metabolized in large quantities each day by the human body. The radioactivity would, therefore, be readily diluted throughout the body and not concentrated or localized in a small amount of tissue. The eventual critical organ for what Carbon-14 that would be retained would be fat; but in consideration of the amount of Carbon-14 retained and deposited in the fat and with a biological half life of 35 days for Carbon-14 in fat, the radiation body burden produced by 100 μc of cellulose-C^{14} would be considerably less than the maximum permissible dose.

With the aid of the ICRP Handbook (Appendix I, reference 1), the calculated total dose would be 0.690 rem (0.576 rem the first 13 weeks). Assuming no absorption. the greatest dose received by the intestinal tract would be 0.691 rem.

d. Personnel

H. E. Sauberlich, Ph.D. (PL-313), Project Leader
Lt. Col. M. E. McDowell, M.D. (MC) (Also attending physician)
Lt. Col. J. E. Canham, M.D. (MC) (Also attending physician)
Maj. E. M. Baker, Ph.D. (MSC), Co-Project Leader
B. M. Tolbert, Ph.D. (Consultant, University of Colorado)

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15. Minerals: Studies on mineral metabolism and interactions in the human with the use of radioisotopes (references cited in the paragraph are listed in Par. 15f)

Initial studies would seek the role of magnesium and calcium in human kidney stone disease:

a. Background

Urinary calculi are among the most ancient afflictions of man. This painful and often fatal disease is known to have occurred as long as 8,000 years ago (1), and no race or geographical area has been entirely free of a calculus problem. There appear to be "stone belts" of high incidence in regions such as southern China, northern Thailand, the Punjab district of India, Arabia and Iraq. In addition, there have been reports of "stone waves," one such occurring in Europe during this century (2).

Despite the antiquity and frequency of this disease, the basic mechanisms of calculus formation remain unknown, and fully 85% of all patients who form urinary calculi have no recognized local or systemic disease (3). Most authorities agree that a nutritional deficiency or imbalance is a probable factor, but few reliable studies have been conducted to relate specific nutrients to calculus formation in human populations (4).

Studies at this laboratory (5) and elsewhere have established that nephrocalcinosis and urolithiasis (principally phosphates and carbonates of calcium) are frequently associated with magnesium deficiency in rats and other species. Of particular interest is the observation that about 20% of apparently otherwise

normal rats consuming a semipurified diet containing 400 ppm magnesium (minimum requirement for growth is 120-150 ppm) will develop uroliths similar to those found in the markedly deficient rat and that elevation of the dietary Mg to 4,000 ppm will prevent this occurrence.

Furthermore, Selye has shown that intraperitoneal administration of magnesium will prevent the formation of uroliths which normally follows experimental hyperparathyroidism in the rat. An increase in dietary magnesium will also markedly lessen the accumulation of calcium in the kidney which results from a high phosphorus intake. Despite these indications of an important role for magnesium in calculus formation and calcium metabolism, practically no published information exists on the metabolism of this nutrient in human urinary calculi disease (4).

Preliminary studies at this laboratory (6) have indicated that some populations in areas with a reported high incidence of stone formation (e.g., Burma investigations) may, indeed, consume relatively low amounts of magnesium. In addition, magnesium supplements have brought at least a temporary (6 months) halt to the formation of phosphatic type stones in a patient with no demonstrable infection or metabolic disorder and a previous rate of stone formation of 2 per month for a period of 3 years (7).

Based on this evidence, it is felt that considerable justification exists for the study of the role of magnesium as well as other factors in human renal lithiasis. Certainly, a primary objective is a determination of the value

of magnesium supplements in a large number of patients and a study of any changes in urinary constituents associated with a favorable response to this therapy.

b. Basic experimental plan (regardless of use of radioisotope tracers)

(1) Patients will be obtained through the Departments of Urology at Colorado General Hospital and Fitzsimons General Hospital. Only those subjects will be chosen who form stones at a relatively rapid rate (at least one every 2 months) and who are free of renal infections.

A subject so chosen will be kept on a metabolic ward for a 2-week period so that 2 complete 3-day fecal and urine collections can be made. Total Ca, P, Mg and vitamin B_6 intake during this period can be estimated from tables of composition or by actual analysis. The total fecal collection for 3 days will be pooled, homogenized and ashed for a determination of its content of calcium, phosphorus and magnesium. A routine urinalysis (pH, sp. gravity, crystals, etc.) will be performed on each 24-hour urine collection and at least 500 ml will be saved for subsequent analysis. Proposed urinary constituents to be analyzed for are magnesium, calcium, phosphorus, oxalate, citrate, uromucoid, vitamin B_6 and xanthurenic acid. After the specimens are received from the 2 balance periods, 420 mg of MgO (250 mg Mg) will be given daily in a single capsule to be taken after supper. Therapy should continue for at least 6 months, during which time the patient's rate of stone formation will be noted. A 24-hour collection of urine will be made every 30 days and the above-mentioned tests will be performed. Patients may be asked to repeat the balance study after 6 months to ascertain

any changes in balance or retention of calcium, magnesium or phosphorus as a result of this treatment.

(2) Progress to date

To date, one patient has been studied completely in terms outlined above, that is, this man has been on magnesium supplements as treatment for his recurrent urolithiasis. He has been on these supplements for 6 months without a recurrence in stone formation; he was then brought back into the hospital and denied the supplements for a period of a month. During both periods, his urinary excretion of calcium, phosphorus, magnesium, oxalate and mucoprotein was determined.

A second patient has been given magnesium for 6 months and has shown a favorable response in that he has not formed stones during this period. He recently returned to the hospital for follow-up studies. The findings on magnesium therapy with these two stone-forming patients will be submitted as a manuscript to the Journal of Urology.

Additional patients are understudy with the cooperation of Lt. Col. C. A. Moore, M.D. (MC) of Fitzsimons General Hospital, Dr. O. G. Stonington of Colorado General Hospital, and Lt. Col. J. E. Canham, M.D. (MC) of the Metabolic Division of this laboratory. As additional cooperative patients become available, expanded clinical trials as to the effectiveness of this treatment for chronic lithiasis will be undertaken.

c. Experimental plan incorporating use of radioactive tracers (Par. 12b(2))

The mineral balance studies thus far conducted on the above subjects appear to indicate abnormalities in calcium and magnesium absorption and excretion. However, the balance techniques leave much to be desired from the standpoint of a precise and exacting procedure to give the definitive information necessary for an unequivocal evaluation of small changes that may occur in absorption or excretion. In addition, the method gives little information on turnover rates or retention of the dietary calcium and magnesium and of the magnesium supplements.

In order to obtain the desired information that may permit a better understanding of the cause of uroliths in humans and the effect of magnesium in their treatment, the use of Magnesium-28 and Calcium-45 or 47 is proposed. Accurate information on the absorption and turnover of the elements in the stone-forming subject could be readily obtained with the use of these isotopes. The information could be obtained on the patient both before treatment and after a period of magnesium treatment to determine changes or interactions that may have occurred in calcium or magnesium metabolism. Such data may give an insight into the mechanism of action involved. Of equal importance, comparative studies with the use of several salts or oxides of Magnesium-28 could be readily performed in an attempt to explain the reason for the apparent success obtained with MgO at this laboratory, while other salts of magnesium have been of no value (4, 8). Similar studies in a minimum number of normal volunteer subjects would be carried out as necessary to evaluate the findings in the patients.

It is hoped that a better understanding of the problem would result from the isotope studies which would lead to a screening test that would identify which stone-forming patients that could be expected to receive beneficial effects from magnesium therapy. The performance of the isotopic studies indicated appear highly necessary before recommendations or large scale treatment with magnesium be initiated with stone-forming subjects.

d. Procedures and health physics

The patients would be handled in a manner similar to that employed at present for non-radioactive mineral balance studies of calcium, magnesium and phosphorus. Selected stone-forming patients or normal volunteers would be placed on the Metabolic Ward at this laboratory. The subjects would receive a controlled diet without magnesium therapy. After a period of 7-10 days on these diets, with balance studies conducted, the patients would receive a tracer dose of either Mg-28 (not exceeding 20 μc) or Calcium-47 (not exceeding 5 μc) or Calcium-45 (9) (not exceeding 15 μc) orally. Markers would be employed to assist in the stool collections. Urine, stools and blood samples would be collected and analyzed until essentially no activity could be detected. It is hoped that dosages of radioisotope may be reduced further to one-half the amounts indicated and still permit satisfactory measurements. This would then permit double labeling of selected patients or repeat labelling of a patient or normal volunteer following 6 months of magnesium therapy without approaching the maximum permissible body burden of radiation. If the dosage cannot be reduced sufficiently, then other

patients or subjects would receive the second isotope or the isotope after 6 months of magnesium therapy. Balance studies with known diets and intakes and maintenance on the Metabolic Ward for periods of 5-6 days (i.e. before and after the 6-months' therapy period--not maintenance on the Metabolic Ward throughout the 6-months' period) would be associated with all subjects. If the oral studies indicate further evaluation of the retention and turnover of magnesium or calcium in the body, a limited number of select volunteer patients or normal volunteers would receive intravenously administered isotopes. In all instances, the intravenous dose would not exceed 35 μc. For comparative purposes, a limited number (3-5) of normal, healthy volunteer subjects would be placed on the same diets and balance studies performed with the use of the same isotopes in the same dosage as employed with the volunteer patients.

The healthy physics of Mg-28, Ca-47 and Ca-45 has been considered briefly before. It should be emphasized that the maximum dosages obtained with the amount of isotopes used in the proposed studies will at no time equal the maximum permissible dosage.

With the aid of the ICRP Handbook (Appendix I, reference 1), the calculations below were made. In each case "1" is the infinite dose received by the critical organ and "2" is the dose received during the first 13 weeks by the critical organ. The critical organ is given in parentheses.

A. 5 μc of Ca-47 administered orally (bone)
   1. 0.359 rem
   2. 0.359 rem

B. 2.5 μc of Ca-47 administered intravenously (bone)

1. 0.150 rem
2. 0.150 rem

C. 15 μc of Ca-45 administered orally (bone)

1. 5.883 rem
2. 1.897 rem

D. 7.5 μc of Ca-45 administered intravenously (bone)

1. 0.245 rem
2. 0.079 rem

*E. 20 μc Mg-28 administered orally (bone)

1. 0.465 rem
2. 0.465 rem

*F. 35 μc of Mg-28 administered intravenously (bone)

1. 1.992 rem
2. 1.992 rem

*G. 35 μc of Mg-28 administered intravenously (whole body)

1. 0.044 rem
2. 0.044 rem

*H. 20 μc of Mg-28 administered orally (stomach)

1. 0.116 rem
2. 0.116 rem (i.e. residence time of 1 hour)

*I. 20 μc of Mg-28 administered orally (small intestine)

1. 0.079 rem
2. Same (i.e. residence time = 4 hours)

*J. 20 μc of Mg-28 administered orally (upper large intestine)

1. 1.284 rem
2. Same (i.e. residence = 8 hours)

*K. 20 μc of Mg-28 administered orally (lower large intestine)

1. 1.720 rem
2. Same (i.e. residence time = 18 hours)

*Radiation burden for Mg-28 were obtained by calculations and the use of:

1. ICRP Handbook (Appendix I, reference 1).

2. Peaceful Uses of Atomic Energy, Vol. 24, Part 1, "Isotopes in Biochemistry and Physiology," 1958, United Nations Publication.

3. Radioactive Isotopes in Medicine and Biology: Medicine, S. Silver, 1962, Lea and Febiger, Publishers.

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6. Radiological Health Handbook, PB 121784R, U. S. Dept. of Health, Education and Welfare, Public Health Service, U. S. Dept. of Commerce, 1960.

According to information supplied from the above sources, absorption of magnesium from the G.I. tract is very low. However, for the purpose of these calculations, 60% absorption was assumed when the isotope was administered orally and the critical organ considered bone. However, when segments of the G.I. tract were considered the critical organ, no absorption was assumed. As previously stated, elimination after absorption is very rapid. Again, however, in order not to underestimate the dosage, an intake and retention of 90% of the isotope was assumed to be removed from the blood by the critical organ (bone).

All collected excreta would be disposed of in an acceptable manner under the supervision of the Radioisotope Branch of this laboratory.

e. Personnel

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Lt. Col. C. A. Moore, M.D. (MC), Fitzsimons General Hospital, Project Leader (Also attending physician)
Lt. Col. J. E. Canham, M.D. (MC), USAMRNL (Also attending physician)
Dr. O. G. Stonington, M.D., Colorado General Hospital (Also attending physician)
Capt. G. E. Bunce, Ph.D. (Consultant, Tripler General Hospital, Hawaii)
Lt. Col. M. E. McDowell, M.D. (MC), USAMRNL (Also attending physician)

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2. Radiological Health Handbook, U. S. Department of Health, Education and Welfare, Sept. 1960.

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5. Use of Radioisotopes in Animal Biology and the Medical Sciences, Vol. 1 and 2, 1962, Academic Press.

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.

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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

58638