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 & U. S. Army
Medical Research & Nutrition Laboratory
Denver, Colorado 80240

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

Same as 1 (a) and
Fort Sam Houston, Texas and Summit of
Pikes Peak, Colorado

2. DEPARTMENT TO USE BYPRODUCT MATERIAL

Bioenergetics Division
U. S. Army Medical Research & Nutrition
Laboratory

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

Present application is for amendment to
License No. 05-00046-13

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 and approved by the Radio-
isotope Committee, FGH and USAMRNL

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.)*

Same as 4

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

A. Carbon-14

(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. Glucose — A. 0.1 millicurie

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 Form AEC 313 a Attached

09853

(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 their approval by the Radioisotope Committee, Fitzsimons Gen Hosp and U.S.Army Medical Rsch & Nutr Lab.		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 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/cm^2)	USE (Monitoring, surveying, measuring)
As described in application for renewal of Byproduct Material License No. 05-00046-13 dated 25 August 1968. See also attached protocol.					

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

See application for renewal of Byproduct Material License No. 05-00046-13 dated 25 August 1968.

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

See application for renewal of Byproduct Material License No. 05-00046-13 dated 25 August 1968.

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 application for renewal of Byproduct Material License No. 05-00046-13 dtd 25 Aug 68 and attached protocol.

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 application for renewal of Byproduct Material License No. 05-00046-13 dtd 25 Aug 68 and attached protocol.

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. See appl. for renewal of Byproduct Material License No. 05-00046-13 dtd 25 Aug 68 and attached protocol.

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.

Dept of the Army, Fitzsimons GH &
Applicant named in item 1 USAMRNL, Denver, Colo. 80240

By: [signature]
COL ROBERT C. JONES, MC
Chairman, Radioisotope Committee
Title of certifying official

Date

1969 APR 25 PM 2 00

RECEIVED

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—615943

Form AEC-313 a (10-61) 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
Dept of the Army, Fitzsimons Gen Hospital and U.S.Army Med Resch & Nutr Lab, Denver, Colorado

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

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.
As permitted by Radioisotope Committee, FGH & USAMRNL
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 permitted by Radioisotope Committee of FGH & USAMRNL. See Trng & Exp. of Dr. S.Sanbar, attached
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): For studying glucose metabolism in health and disease and under a variety of experimental conditions.

(b) CHEMICAL FORM ADMINISTERED:
C^{14} labeled glucose

(c) DESCRIBE PROCEDURES WHICH WILL BE OBSERVED TO MINIMIZE HAZARD FROM HANDLING, STORAGE, AND DISPOSAL OF THE BYPRODUCT MATERIAL:
See attached protocol

(d) DESCRIPTION AND SKETCHES OF SPECIAL DEVICES TO BE USED FOR ADMINISTERING BYPRODUCT MATERIAL TO HUMAN BEINGS ARE
(1) ATTACHED (LITERATURE REFERENCES WILL SUFFICE) CIRCLE ANSWER: YES (NO)
(2) ON FILE WITH THE ISOTOPES EXTENSION REFER TO APPLICATION NO _____ CIRCLE ANSWER: YES (NO)

5. (a) PROPOSED DOSAGE SCHEDULE.—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):
Maximum of 50.0 microcuries per subject

(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:
The isotope solution will be sterilized rendering them pyrogen free by millipore infiltration. The isotope will be kept in a sterile, multidose, stoppered vial, and will be administered to the subjects by a medical officer.

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

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

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)

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

09859

USAMRNL - Bioenergetics Division 24 January 1969
PROTOCOL - FINAL
Project No.: 3A061102B71R Research in Biomedical Science
Task No.: 05 Environmental
Work Unit No.: 080 High Altitude Bioenergetics

ST 10: Effects of Diet and Altitude on Carbohydrate Metabolism and the Metabolic Responses to Exercise (With Labelled Glucose)

I. INTRODUCTION

Recent studies in this Laboratory (1, 2, 3, 4) have dealt with metabolic derangements which develop in human subjects who are abruptly exposed to altitude. Attempts have also been made to evaluate the beneficial effects of a high carbohydrate intake and with heavy physical exercise on performance and severity of symptoms of acute mountain sickness. Of particular importance is the demonstration that a high carbohydrate diet and heavy physical activity prior to rapid exposure of men to high altitude appear to diminish the symptoms of acute mountain sickness and improve maximum physical performance. The mechanism by which high carbohydrate-low fat intakes and exercise influence the response of human subjects to high altitude remain to be elucidated. The following is a summary dealing with carbohydrate metabolism at altitude and the metabolic changes, principally of free fatty acids, during exercise.

A. Carbohydrate Metabolism at Altitude:

At altitude, the fasting blood sugar may or may not be altered depending on whether animals or human subjects are used, the level of altitude, and acclimatization. In cats, anoxemia increase the fasting blood sugar in the absence of excitement (5). In guinea pigs, reduction of barometric pressure to 340 millimeters of mercury does not alter the fasting blood sugar (6). In dogs, abrupt exposure to high altitude does not appreciably alter the blood sugar (7, 8). In man, on the other hand, variable results have been reported by various investigators. At altitudes

in the neighborhood of 15,000 feet, and in somewhat acclimatized human subjects, the fasting blood sugar is not altered significantly (9, 10, 11, 12). However, Picon-Reategui (13) showed in native residence of Morococha, at an altitude of 4540 meters (14,900 feet), the fasting blood sugar was lower than at sea level. Similar findings were reported by San Martin (14) and by Monge (15). Finally, several investigators (16, 17, 18, 19) reported increments in fasting blood sugar at high altitude. The latter subjects were appa ıtly poorly acclimatized to the altitude which was in excess of 15,000 feet.

As with fasting blood sugars, variable changes have been reported in glucose tolerance tests at high altitude. Forbes (19) performed oral glucose tolerance tests in three human subjects at an altitude of 5300 meters. In two of the men who had been at this altitude for seventeen days, glucose tolerance was increased. In the third man who had been at this altitude for only six days, glucose tolerance was lowered. Subsequent studies by Picon-Reategui (11) utilizing both oral and intravenous glucose tolerance revealed a faster rate of glucose utilization in high altitude residents as compared with those at sea level. More recently, Janoski and Anderson (20) performed both oral and intravenous glucose tolerance tests on ten normal human subjects both at sea level and during residence at 14,100 feet. Results showed that at altitude the glucose disappearance was lower than the respective values at sea level. They also showed that insulin secretion was delayed at high altitude. It should be noted; however, that their subjects were semi-starved by choice while at altitude, and the decreased glucose tolerance may be a reflection of semi-starvation rather than altitude. Most recent studies in the Bioenergetics Division of USAMRNL (21) reveal normal glucose tolerance in normal human subjects both at sea level and altitude when the intake of food remained high (in excess of 3100 Calories/day).

Turnover studies of glucose at high altitude have not been reported in the literature to date.

Picon-Reategui (11) performed insulin tolerance tests on human subjects who resided at an altitude of 4540 meters. Results showed no changes in blood glucose, pyruvate, and lactate during the tests. However, at altitude there was an increased rate of fall of plasma inorganic phosphate and a decreased rate of fall of plasma potassium during the insulin tolerance tests. Interestingly enough, insulin reactions were reported less frequent in high altitude residents compared to the high incidents in human subjects at sea level. Picon-Reategui (22) also showed a normal hyperglycemic response to epinephrine but a subnormal response to glucagon in altitude residents.

Davidson (23) has summarized the effect of chronic exposure to actual or simulated high altitude on carbohydrate metabolism in both animals and human subjects. At altitude, compared to sea level controls, there is a smaller rise after glucagon and a greater fall after tolbutamide in plasma glucose concentrations, decreased liver glycogen after administration of cortisone, pyruvate, and glutamate, and decreased venous but similar arterial concentrations during all glucose uptake or carbon dioxide production by rat diaphragms, using glucose alone or with insulin in the incubation medium. Furthermore, there was no significant difference in the insulin:glucose ratios between hypoxic and control rats. However, hypoxic animals had a fasting plasma glucose of 17 mg percent less than sea level controls.

These conflicting data about carbohydrate metabolism at altitude reflect incomplete knowledge of this area. In order to determine the metabolic effects of a high carbohydrate diet, it is necessary to elucidate the derangements in carbohydrate metabolism which result from abrupt exposure of human subjects to high altitude. One aim of this study is to delve into the problem at greater depth than has hitherto been reported.

The remainder of this introduction will deal with energy fuel for working muscle, with emphasis on FFA.

B. Metabolic Fuel for Working Muscle:

Proteins contribute little fuel for working muscle cells. In 1866, Pettenkofer and Voit (quoted by Åstrand [24]) reported that protein catabolism during heavy exercise was not higher than under resting conditions. These findings have been confirmed by others (25). In contrast with protein, both lipids and carbohydrates provide energy for working muscle.

The percent energy yield by lipids and carbohydrates during exercise varies greatly depending on the diet composition and type of exercise. In 1911, Zuntz (26) showed that in mild exercise performed after an extremely fat-rich diet, RQ values indicated an almost exclusive fat catabolism. In 1939, Christensen and Hansen (27) reported that physical capacity was markedly reduced in human subjects who were fed for one week an extremely high-fat diet with less than 5% of the total caloric intake derived from carbohydrates. In these subjects, the RQ was low during work periods, and 70 to 99% of the energy for working muscles was derived from fat combustion.

In contrast with high-fat diets, human subjects on a normal diet (27) showed that only 50 to 60% of the energy was supplied by fat during submaximal exercise. These authors showed further that subjects living on a very high-carbohydrate diet, where 90% of the total caloric intake was derived from carbohydrates, that fat contributed only 25 to 30% of the metabolic fuel for working muscle. Thus, the diet seems to effect the participation of fat versus carbohydrate in work metabolism.

In 1928, Bock and co-workers (28) emphasized the influence of the severity of work on the proportions of fat and carbohydrate contributions to metabolism. As mentioned above, human subjects on a normal diet engaged in submaximal exercise derive up to 2/3's of their energy from fat. In contrast, the closer the human subjects work to their maximum, the more important carbohydrates become and, with exhaustive exercise, all fuel may

be derived from carbohydrate (27). Interestingly enough, fat metabolism appears to play a somewhat larger role in trained human subjects in providing energy for working muscle than in untrained human subjects (29).

C. Changes in Metabolic Substrates in Plasma During Exercise:

During aerobic work, fasting human subjects exhibit alterations in plasma free fatty acid concentrations (30, 31, 32, 33, 34, 35, 36, 37, 38). During the first ten to fifteen minutes of exercise, there is a reduction in the concentration of plasma FFA. This has been termed the circulatory phase during which time the cardiovascular adjustments to exercise occur rapidly and this is associated with increase efflux of FFA from plasma. After about twenty minutes of exercise, plasma FFA concentration returns to control levels of may be higher due to enhanced influx of FFA into the plasma. This has been termed the metabolic phase. During this phase the glycerol concentration increases, indicating enhanced lipolysis of triglycerides, presumably from adipose tissue. Finally, after stopping exercise, plasma FFA concentration rapidly increases to a peak about ten minutes after the end of exercise and subsequently returns towards control level within half to one hour. The post-exercise period has been termed the recovery phase. During the latter phase, glycerol, in contrast with FFA, abruptly falls towards control values within a few minutes after stopping exercise.

During the metabolic phase, the turnover rate of plasma FFA is increased (35, 37). These authors have shown further that in trained subjects about 75% and in untrained subjects about 50% of the FFA flux through plasma was immediately oxidized. However, despite an RQ of 0.75, only 25 to 50% of the carbon dioxide in expired air could be derived from immediately metabolized plasma FFA. This indicates that lipid sources other than plasma FFA must have been used to generate energy for muscle work.

The mechanism by which exercise enhances lipid mobilization is not fully elucidated. As reviewed by Carlson, et al (38), the activity of the sympathetic nervous system appears to be increased during exercise. In adrenalectomized patients, who are subsisted on cortisone, plasma FFA increased during exercise, suggesting that lipid mobilization during exercise occurs independent of the adrenal medulla. Norepinephrine is believed to be the substance released by the sympathetic nerve endings to stimulate lipid mobilization in adipose tissue. With regard to other hormones, growth hormone increased during exercise in man (39); however, patients with hypopituitarism have a normal FFA response during exercise, suggesting that the pituitary hormones play a minor role in stimulating lipid mobilization during exercise (40).

In support of the sympathetic nervous system being the mediator for lipid mobilization during exercise, are the findings that beta-adrenergic blocking drugs, such as pronethalol, decrease the rise in plasma FFA during and after exercise (41). Similarly, nicotinic acid has been shown to greatly diminish the mobilization of FFA and the rise in plasma glycerol during exercise (42, 43). Interestingly enough, nicotinic acid had no effects on either the rate of removal or oxidation of plasma FFA, nor did it influence the efficiency of muscular work. Carlson, et al (42) also showed that glucose administration had similar effects on plasma FFA as nicotinic acid. Against the sympathetic nervous system hypothesis; however, are the findings by Carlsten, et al (44) in which he showed that administration of Arfonad (trimethaphan) or hexomethonium, which are ganglionic blocking agents, do not alter either the metabolic response of plasma FFA during exercise nor their individual FFA composition.

The changes in plasma FFA vary with the intensity and duration of exercise. Rodahl, et al (45) showed that during heavy work lasting for ten minutes, plasma FFA decreased.

During moderate work for one hour, plasma FFA remained essentially unchanged, but they increased markedly during the recovery phase. Finally, during prolonged exhaustive work in fasting subjects, plasma FFA rose steadily. Other investigators have reported similar trends (31, 40, 46, 47, 48, 49). Young, et al (50) found that during intermittent work for 24 hours, plasma FFA stabilized at about three times the resting level. Finally, Carlson and Froberg (51) reported that walking fifty kilometers a day for ten days with low caloric intake produced increments in plasma FFA and glycerol during the first six days, and a subsequent slight decrease; plasma concentrations of cholesterol and phospholipid and triglyceride were markedly decreased during this period.

With regard to plasma FFA composition, Wood, et al (49) reported a diminution in oleic:palmitic acid ratio in plasma during acute exercise, suggesting a greater fractional turnover rate for oleic acid as compared to palmitic acid. Carlsted, et al (46) reported a decrease in the percentage of plasma stearic acid and a slight increase in the percentage of linoleic acid, but no change in oleic acid. Hagenfeldt and Wahren (52) reported that during arm exercise, the net uptake of linoleic acid is smaller than that of palmitic and oleic acid. Havel, et al (37) on the other hand, reported no relative changes in palmitic, oleic or linoleic of plasma FFA during exercise.

As to the effects of exercise on plasma lipids other than FFA, there are little or no changes in concentrations of plasma cholesterol, phospholipid or triglyceride during short exercise (31, 53). Several authors (55, 56, 57, 58) reported no effect of physical training on total serum lipids or serum cholesterol concentrations. On the other hand, other investigators have reported different results. A significant fall in plasma cholesterol phospholipids, and triglycerides have been observed after eight to nine hours of skiing (54). MacDonald (59) reported decrements in plasma lipid concentrations after 27 to 55 miles of walking. Campbell (60) reported reductions in serum cholesterol in human subjects running on a treadmill three times/week for ten

weeks. Similar findings have been reported by others (61, 62, 63, 64, 65).

The effects of high altitude on serum lipid components of human subjects were investigated by Klain and Hannon (12). Eight male subjects were exposed to an altitude of 14,100 feet for fourteen days. Total serum lipids and to a lesser degree serum cholesterol were diminished. However, serum phospholipid and FFA concentrations progressively increased during the period of altitude exposure. The increase in plasma free fatty acid concentrations at altitude may be related to the reported increase in catecholamine secretion at altitude (66).

There are no studies in the literature dealing with the changes in plasma lipid components during exercise at high altitude.

At sea level or low altitude, exercise (treadmill running with a 10% grade at 100 m/min) produces no change in plasma glucose concentration; however, glucose turnover is increased slowly, reaching a maximum at the end of exercise (67). These authors have also reported an elevated increment in blood lactate and pyruvate increase with moderate or maximum exercise, but the resting levels of these two substrates diminish with acclimatization (68). There is a paucity of information on the metabolic alterations during a standard exercise workload at altitude. Two separate studies will be conducted and will be referred to as Study I and Study II.

D. Responsibility of the Welfare of the Human Subjects (Studies I and II):

The Medical Officer, CPT S. S. Sanbar, will be medically responsible for the health of the human subjects. He will be present during all phases of the study and will have the authority to terminate all phases of the study if he has probable cause that continuation will result in injury, disability, or death to the volunteer subjects.

During the one week at high altitude, CPT R. Cohn, MC, Metabolic Division, will take over the responsibility of the subjects living on the Metabolic Ward while CPT Sanbar accompanies the other subjects to altitude.

II. OBJECTIVE

The immediate aims of the proposed study are to evaluate whether glucose metabolism is impaired during abrupt exposure of human subjects to high altitude (4300 m). The following parameters will be compared to sea level control data:

a. Intermixing glucose mass, apparent distribution space of glucose, and rates of appearance (hepatic output) and disappearance (tissue uptake) of glucose, measured by the technique of measured tracer injection of glucose-U-C^{14}.

b. Influence of physiologic dose of glucagon (1 microgram/kg body weight) on concentrations of plasma glucose, insulin, growth hormone, and free fatty acids.

c. Influence of intravenous infusion of the amino acid arginine on plasma glucose, insulin, growth hormone, and free fatty acid concentrations.

d. Metabolic pattern during submaximal exercise.

III. JUSTIFICATION

Two of the major problems encountered at high terrestial environments are the "mountain sickness" syndrome after abrupt altitude exposure and the decreased ability to perform maximal physical work at altitude. These effects of altitude would seriously handicap our military personnel if they were engaged in any conflict under these conditions. This study deals specifically with this problem, in attempting to determine why a high carbohydrate diet and heavy physical exercise will decrease the clinical

symptoms of acute mountain sickness, and to investigate the derangements in carbohydrate and lipid metabolism at altitude.

IV. EXPERIMENTAL DESIGN (Study I)

Sixteen, normal male Army volunteers from Fort Sam Houston, Texas, will be studied. The criteria for acceptance of the subjects into the study will include a normal, complete physical examination, including EKG's, the absence of prior exposure to high altitude, and a history of normal or average American food intake for at least two weeks prior to the beginning of the study. A diet containing a normal distribution of nutrients, as issued at a military garrison mess hall, will be fed ad libitum and the daily intake will be measured for each subject during the study period. With the exception of the treadmill walk, these subjects will not exercise during the entire study period.

Eight subjects will be rapidly transported to Pike's Peak, Colorado (elevation 4300 meters). Each of these subjects will remain on Pike's Peak for three days, after which he will be returned to Fort Sam Houston. On the morning following arrival to Pike's Peak (Day 1), glucose turnover and response to glucagon will be determined. On Day 2, arginine infusion tests will be performed. On Day 3, a standard submaximal exercise test will be done. All tests will be performed in the morning while the subjects are in a fasting state. As mentioned above, the total daily nutrient intake for each subject will be calculated. After completion of the altitude phase at Pike's Peak, the second group of eight men will be selected to match the first eight subjects with respect to height, weight, race, and age. They will undergo the same series of tests at sea level and they will be pair fed at the same rate as the altitude group.

Glucose Turnover: The technique of measured tracer injection of glucose-U-C^{14} will be utilized to determine the intermixing glucose mass, apparent distribution space of glucose, and rates of glucose appearance into and disappearance from circulation. A single dose of 30 microcuries of high specific

activity glucose-U-C^{14} will be injected intravenously after obtaining a blood sample in the fasting state. This dose is approximately 1/7th of the maximal recommended dose of C^{14} labelled glucose. Following injection of labelled glucose, blood samples will be obtained at 15, 25, 40, 55 and 65 minutes for determination of specific activity of plasma glucose as described by Sanbar (72). Curve fittings of the results of specific activity will be performed by an IBM Computer, and calculations will be carried out as described by Wrenshal and Hetenyi (73) and Forbath and Hetenyi (74). No other radioisotope will be administered to the subjects. Since the dose of radioisotope is relatively small, no special precautions will be utilized such as isolation of the subjects. However, the blood samples obtained from the subjects will be placed in non-breakable vials, and all contaminated solutions will be sent to the Radioisotope Branch, Physiology Division for disposal. Urine and fecal disposal will be done according to AEC regulations although it is known that most of the labelled glucose (75% +) will be disposed of as $C^{14}O_2$ in the expired air (see inclosed request for an amendment permitting the use of C^{14} labelled glucose in human volunteers).

Glucagon Tests: This will be performed on the same day that glucose turnover is done. Exactly 65 minutes following administration of glucose-U-C^{14}, a single injection of glucagon (1 microgram/kilogram body weight) will be injected intravenously and blood samples will be obtained at 1, 5, 10, 15, 20 and 30 minutes following the injection. The plasma will be analyzed for glucose specific activity, which will provide information regarding release of glucose by the liver and measurements will also be made of plasma insulin, growth hormone, and free fatty acid concentrations.

Arginine Infusion Tests: This test will be performed on Day 2. Two control blood samples will be obtained at fifteen minute intervals before starting the intravenous infusion of arginine.

Arginine (.2 g/kg body weight or a maximum of 30 g) will be administered intravenously over a period of 30 minutes. During the infusion, three blood samples will be obtained at ten minute intervals. After the infusion of arginine, blood samples will be obtained at 15, 30, 60, 90, and 120 minutes. The plasma will be analyzed for glucose, free fatty acid, insulin, and growth hormone levels.

Standard Exercise Test: Standard exercise test will consist of a twenty minute walk at 3.5 mph on a treadmill with a 4% grade. For ten minutes prior to the walk, 20 minutes of the walk, and 60 minutes after exercise, continuous measurements of oxygen consumption, carbon dioxide production, ventilation rates and volumes and pulse rates will be made with the continuous respiratory gas analyzer. Blood pressures will be obtained at ten to fifteen minute intervals throughout this period.

Blood and Plasma Parameters: Prior to the treadmill test, an indwelling catheter will be placed in an arm vein for obtaining serial samples. Blood samples will be drawn at ten minutes and immediately prior to the treadmill walk, at ten and twenty minutes of the walk and post-exercise at five, ten, fifteen, thirty, forty-five, and sixty minutes. These blood samples will be used to determine blood glucose, pyruvate, lactate, hemoglobin content, and hematocrit values and a portion of the blood will be centrifuged. This plasma will be used in the determination of free fatty acids, cholesterol, triglycerides, sodium, potassium, and total proteins. All of the tests will be done in the morning after an overnight fast.

Body Composition: Body composition measurements will be done on separate days and will include body density by displacement, body water using deuterium oxide and thiocyanate space.

Blood Volumes: Total blood volumes will be determined prior to the treadmill tests by the infusion of Evans Blue T-1824 Dye immediately after obtaining the first (-10') blood and drawing additional blood just prior to exercise for the determination.

Mental Attitude and Ability (Studies I and II): (a) General High Altitude Mood Symptom Questionnaire: A check list that reflects attitude changes and symptoms of mountain sickness will be administered twice daily on each subject throughout the study. This questionnaire has proven to be valid in recent studies (69, 70) and will provide a final numerical value which may describe the degree of severity of the clinical symptoms. (b) Digit Symbol Substitution Test (Convergent Production): This test may be adversely affected by hypoxia (70, 71). It is intended to measure information processing ability when minimal pressure of constant attention is required.

Body Weight: Body weight will be taken on each man daily, immediately upon arising and after voiding.

V. ADMINISTRATIVE

1. Division Responsibility:

This will be a Bioenergetics Division study with collaboration from Metabolic Division for the purchasing and preparation of the liquid diets.

2. Project Leaders:

The project leaders will be CPT Shafeek S. Sanbar, MC; Herman L. Johnson, Ph. D.; with C. Frank Consolazio, C, Bioenergetics Division and Harry J. Krzywicki as co-investigators.

3. Cost:

a. Personnel (Salaries):

S. S. Sanbar - 90% of time for 5 weeks	$ 1,125.00
H. L. Johnson - 90% of time for 5 weeks	1,170.00
C. F. Consolazio - 10% of time for 4 weeks	172.00
H. J. Krzywicki - 30% of time for 5 weeks	412.00
Five enlisted men - 100% of time for 5 weeks	4,125.00
Four civilian technicians - 75% of time for 5 weeks	1,875.00
One EM - Combined Maintenance	224.00

b. Travel to Fort Sam Houston and Return:

2 Project Leaders (3 trips each)	$ 750.00
10 Civilians, Military Personnel (1 trip each)	1,250.00
8 Additional trips to accompany troops to Pike's Peak, Colorado	1,000.00
8 Subjects	1,000.00

c. Per diem:

Fort Sam Houston, Texas - 6 civilians and 1 officer (26 days)	4,000.00
5 Enlisted men (26 days)	500.00
Pike's Peak - 6 civilians and 1 officer and 5 enlisted men	1,500.00
Test Subjects (8)	400.00
Miscellaneous funds for research TDY	2,000.00

d. Equipment:

Practically all on hand (Miscellaneous)	2,000.00

e. Supplies:

Miscellaneous	3,500.00

f. Contract Funds:

Refrigerator - Freezers Rental	500.00
Truck Rental	1,000.00
Air Conditioning Units	1,000.00
Car Rental	2,000.00

g. Support from Fort Sam Houston, Texas:

To include building with tables, chairs, etc.	2,500.00
Total	$26,400.00

APPENDIX I TO STUDY I

A Request for an Amendment is Submitted Pertaining to the Use of C^{14} Labelled Glucose in Tracer Amounts in Volunteer Human Experimental Research Subjects.

I. INTRODUCTION

In the "Request for Approval for Human Use of Radioisotopes in Tracer Amounts in Volunteer Experimental Research Subjects", submitted in 1968 by the U. S. Army Medical Research and Nutrition Laboratory, Denver, Colorado, for reconsideration and renewal of radioisotope license by the Atomic Energy Commission and the Office of The Surgeon General, detailed reference is made for use of C^{14} labelled carbohydrates.

The general health physics for Carbon-14 was described on page 6 of the request:

With regard to the proposed usage of labelled carbohydrates, the following statement was made on page 19, paragraph 3, of the request:

> "Studies on the interrelationship of various types of carbohydrates and other dietary components on serum triglyceride and cholesterol in the human 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 C^{14} labelled carbohydrates were included in the previous license".

The "previous license" included the following statement (page 14 of formerly approved request).

> "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)."

II. SPECIFIC AMENDMENT REQUEST

The following amendment of the inclosed request is being sought:

That, in addition to what has been stated in the "Request for Approval for Human Use of Radioisotopes in Tracer Amounts in Volunteer Experimental Research Subjects", pertaining to C^{14} labelled carbohydrates, approval be granted for intravenous administration of a maximum of 50 microcuries of C^{14} labelled glucose per adult for purpose of studying glucose metabolism in health and disease and under a variety of experimental conditions.

A sterile and pyrogen-free solution of C^{14} labelled glucose will be injected intravenously in its pure form. All other safety standards will be followed as detailed in the inclosed 1968 request.

III. DOSAGE CONSIDERATIONS AND CALCULATIONS:

a. The maximum dose of 50 microcuries of C^{14} labelled glucose to be administered per adult is well within the officially approved dosage (maximum permitted is 300 microcuries) for study of metabolic processes in man as noted in reference 75, 76.

b. Since 1954, C^{14} labelled glucose has been used in human subjects to study glucose metabolism, and the following references are only 3 of a voluminous and well-known literature on this subject (77, 78, 79).

c. According to Baker, et al (77) and Bolinger, et al (78), about 60% of C^{14} labelled glucose, injected intravenously, was expired in the first 24 hours as $C^{14}O_2$. About 90% of the label will appear in $C^{14}O_2$ in expired air within 72 hours after injection. Thus, the majority of injected glucose is oxidized to CO_2 and H_2O, mostly via breakdown to active acetate and entry into the Krebs cycle. In that regard, following the intravenous administration in man of 100 μc of acetate-1-C^{14}, Gould, et al (80) reported that approximately 56% of radiocarbon was eliminated during the first 24 hours. On this basis, they assumed that less than 25 μc of C^{14} is "retained" in the "fat compartments" of the body following a single 100 μc dose. Inasmuch as the maximum permissible dose of C^{14} compounds retained in the body is estimated to be 250 μc (Handbook 52 of the National Bureau of Standards [81]), Gould and his co-workers felt justified in administering, over a period of several months, a maximum of five 100 μc doses of C^{14} acetate to human subjects without regard to their life expectancy. Radioautograph studies by Hellman and co-workers (82) of tissues obtained at postmortem examination from patients who had received 200 μc of C^{14} acetate also failed to disclose areas of concentration of the isotope.

d. The dose of radiation from 50 microcuries of C^{14} glucose is calculated, based on above information in the literature and using the formulae described elsewhere (83). If one assumes that all 50 microcuries of C^{14} glucose are retained in the body indefinitely

(which of course is not the case), then -

$$d\beta = 51.2 \times C \times \bar{E}\beta \text{ rads (per day)}$$

where,

C = microcuries per gram body weight

= 50 μc/70,000 g for an average-size adult

$E\beta$ = average beta ray energy per disintigration in Mev

= 0.050 Mev for C^{14}

thus,

$$d\beta = 51.2 \times \frac{50}{70,000} \times 0.05 = 1.83 \times 10^{-3} \text{ r/day}$$

(or 0.013 r/week)

Therefore, even if one assumes all 50 μc of C^{14} glucose are retained in the body, the radiation received from them will be within the permissible dose of 0.1 rem per week (84).

It must be emphasized; however, that the majority of the injected dose is excreted as $C^{14}O_2$ in expired air, as referenced earlier. Thus, each subject will receive a considerably less radiation dose from the 50 μc of C^{14} glucose than 1.83×10^{-3} r/day.

Assuming that 90% (45 μc) of C^{14} is excreted from the body and 10% (5 μc) retained indefinitely, each subject will receive a total life time dose of 2.35×10^{-3} rad from the 45 μc, and 1.83×10^{-4} r/day from the 5 μc retained in the body.

IV. USE OF GLUCOSE C^{14}:

Intravenous administration of C^{14} labelled glucose will be used to study:

a. Intermixing glucose mass.

b. Apparent distribution space of glucose, and

c. Rates of appearance (mostly hepatic output) and disappearance (tissue uptake) of glucose in human subjects exposed

to altitude and various dietary and drug regimens, and in patients with metabolic disorders.

Each volunteer will sign a Voluntary Consent Statement.

ST L. The Effects of High Carbohydrate Diets on Physical Performance

I. OBJECTIVE

The primary objective of this study is to evaluate the effects of a high carbohydrate-low fat diet on physical work performance at sea level and at high altitude. This study should elucidate some of the discrepancies by various investigators.

II. JUSTIFICATION

One of the major problems in the military has been: (a) to find a means of increasing endurance and delaying the onset of fatigue of troops in combat; and (b) to relieve or reduce the severity of clinical symptomatology in troops abruptly exposed to high altitudes. Recent investigations suggest that high carbohydrate diets may be beneficial. Field commanders would need this information in order to accurately estimate their personnel requirements and operational capabilities. This study will attempt to delineate further the mechanisms by which high carbohydrate diets increase work performance.

III. EXPERIMENTAL DESIGN

Eight, young, normal male subjects (conscientious objectors) will be randomly assigned to two groups of four each, the first to consume a diet of normal American nutrient composition and the second group a high carbohydrate-low fat diet (approximately 70% of the calories from carbohydrate) Table I. All of the men will be given approximately 3600 Calories/day, with 1800 Calories from normal foods and 1800 Calories from a liquid supplement. The differences in the diet will be in the liquid portion. It is anticipated that the subjects will completely consume the daily ration during the entire study. After consultation with Major C. Miller, the diets will be prepared and fed by the Metabolic Ward personnel.

The subjects will be required for ten weeks between the period 21 April to 30 June 1969 and with the exception of week seven at Pike's Peak, the study will be conducted on the Metabolic Ward, U.S. Army Medical Research and Nutrition Laboratory.

The two groups will begin to eat their designated ration on Day 1 with the following schedule:

Weeks 1 - 2	No Exercise	Denver
Weeks 3 - 7	Heavy Physical Exercise	Denver
Week 8	Heavy Physical Activity	Pike's Peak
Weeks 9 - 10	Heavy Physical Activity (all to consume a normal diet)	Denver

All of the physiological measurements will be done in the Bioenergetics Division during the Denver phase. During the Pike's Peak phase, two men, one from each group, will be transported to the top of Pike's Peak daily for the various measurements. The weekly schedules will be as follows:

Day	Submaximal Work and Blood Parameters	I.V.G.T.T.	Maximal Work
Mon - AM	A B		
PM			
Tues - AM	C D	A B	
PM			A B
Wed - AM	E F	C D	
PM			C D
Thurs - AM	G H	E F	
PM			E F
Fri - AM		G H	
PM			G H

Work Unit 080; ST 10 24 January 1969

SCHEDULE - WEEKLY

	0 1 2	3 4 5 6 7	8 9 10
Diet - All Normal Control	Experimental (2 Groups)	Experimental (Normal and High Carbohydrate)	All (Normal Control)
Activity	None ———	Heavy at 3600 Calorie Level	
Altitude	1600 m	4300 m	1600 m ———

Measurements

Sub Max (bloods, etc.)	X			X			X			X		X	X		X
Sub Max (no bloods)					X			X				X			
Max Work	X			X			X			X		X	X		X
IV GTT Body Com-position	X			X			X			X		X	X		X

Physical Activity: After the second week, all of the subjects will maintain a daily energy expenditure of 3600 Calories for the remainder of the study.

a. Standardized submaximal physical activity will be performed on the treadmill once each week. This will consist of walking at 3.5 mph on a 4% grade for twenty minutes. For ten minutes prior to the steady state exercise, twenty minutes of exercise and sixty minutes of recovery after exercise, continuous measurements of oxygen consumption, carbon dioxide production, ventilation rates and volumes and pulse rates will be made with the continuous respiratory gas analyzer. Blood pressures will be obtained at ten to fifteen minute intervals throughout the period.

b. A maximal work performance will also be done each week on each subject and will be based on the Balke test, in which the grade will be increased 1%/minute until the man stops due to exhaustion. Only oxygen uptakes and pulse rates will be measured during this performance.

Blood Parameters: The blood and plasma parameters will be done six times on each man during the entire study, twice during the non-exercise period, twice during the heavy exercise period (weeks 2 and 4), once at high altitude and once during the final phase. All of the tests will be done in the morning after an overnight fast.

Prior to the exercise test, an indwelling catheter will be placed in an arm vein for obtaining serial samples. Blood samples will be drawn at ten minutes and immediately prior to the treadmill walk, at ten and twenty minutes of the walk and post exercise at 5, 10, 15, 30, 45, and 60 minutes. These blood samples will be used to determine blood glucose, pyruvate, lactate, hemoglobin content, and hematocrit values and a portion of the blood will be centrifuged. This plasma will be used in the determination of free fatty acids, cholesterol, triglycerides, sodium, potassium and total proteins and lipoprotein electrophoresis.

Intravenous glucose tolerance tests will be done six times during the same periods, but on a different day. On these days,

body composition measurements will be done. They include body density by displacement, body water using deuterium oxide and thiocyanate space. Total blood volumes will be determined prior to the treadmill tests by the infusion of Evans Blue T-1824 Dye immediately after obtaining the first (-10') blood and drawing additional blood just prior to exercise for the determination.

Body weights will be taken on each man daily, immediately after arising and voiding.

Balance Studies: Complete collections of urine and feces will be taken during the entire study. The fecal samples will be pooled for 7 day periods. Urines will be collected for 24 hour periods, the volume measured, and then the pH adjusted to 2.0. Water, nitrogen and mineral balances will be computed.

Water: Additional water will be available to all men ad libitum but measured. This will be kept refrigerated in plastic bottles.

Two to three weeks prior to the beginning of the study, the subjects will be supplemented daily with iron (ferrous sulfate). This therapy will continue during the entire study. Since iron therapy may have some effects at altitude, it will be eliminated during week 7. The control and high carbohydrate diets will be supplemented with vitamins and minerals so as to contain the daily NRC Allowances.

IV. ADMINISTRATIVE

1. Division Responsibility:

This will be a Bioenergetics Division and Metabolic Division study with the Metabolic Division being responsible for housing and the feeding of the test subjects.

2. Project Leaders:

The co-project leaders will be CPT Shafeek S. Sanbar, MC; Herman L. Johnson, Ph.D.; C. Frank Consolazio, C,

Bioenergetics Division; and Harry J. Krzywicki, Bioenergetics Division. Major C. Miller of the Metabolic Division will also be a co-investigator.

3. Personnel (Salaries):

S. S. Sanbar - 100% of time	$ 2,250.00
H. L. Johnson - 90% of time	2,340.00
C. F. Consolazio - 40% of time	1,720.00
H. J. Krzywicki - 50% of time	1,300.00
Five enlisted men - 100% of time	8,250.00
Four civilian technicians - 75% of time	3,750.00
One EM, Combined Maintenance	224.00
Total Salaries	$19,834.00

4. Cost:

a. Travel to and from Pike's Peak (subjects and test personnel)	1,000.00
b. Per diem - Pike's Peak phase (10 days); 3 civilians, 1 officer, 4 enlisted men, and 8 subjects	1,730.00
c. Equipment	2,000.00
d. Supplies	3,000.00
e. Foods (Dietary Components)	2,000.00
f. Contractural Rentals	
4 Vehicles	1,000.00
Truck	400.00
Total	$11,130.00

C Frank Consolazio

C. FRANK CONSOLAZIO
Chief, Bioenergetics Division

Herman L. Johnson for

SHAFEEK S. SANBAR
CPT, MC
Bioenergetics Division

STUDY II

Composition of Diets

	Groups			
	Normal	% of Total	High Carbohydrate	% Total
Liquid Portion				
Calories	1821		1823	
Protein, gm	70.1	15.2	70.0	15.2
Fat, gm	73.4	35.9	1.0	0.5
Carbohydrate, gm	224.4	48.8	388.8	84.3
Solid Portions (same for both groups)*				
Calories	1788			
Protein, gm	73.1	16.1		
Fat, gm	72.3	35.9		
Carbohydrate, gm	217.3	48.0		
Totals (All Diets)				
Calories	3609		3611	
Protein, gm	143.2	15.7	143.1	15.6
Fat, gm	145.9	35.9	73.3	18.1
Carbohydrate, gm	441.7	48.4	606.1	66.3

* The solid portion of the diets will contain TV dinners, canned meats, fruits, fruit juices, milk, dry cereal, etc.

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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 disease, 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

CURRICULUM VITAE

Shafeek S. Sanbar, CPT, MC — 10 December 1968

PERSONAL

Born: [redacted]
Height:
Marital Status:
Children:

EDUCATIONAL INSTITUTIONS ATTENDED

American University of Beirut, Beirut, Lebanon
University of Oklahoma, Norman and Oklahoma City, Oklahoma

EDUCATIONAL BACKGROUND

June 1952	High School Diploma, Tripoli Boys' School, Tripoli, Lebanon
June 1956	B. Sc., American University of Beirut, Beirut, Lebanon
June 1960	M. D. (after Internship), American University of Beirut, Beirut, Lebanon
August 1963	Ph. D. (in Biochemistry), University of Oklahoma, Norman, Oklahoma
1959-1960	Rotating Internship, University Hospital, American University of Beirut, Beirut, Lebanon
1960-1961	Resident, Department of Internal Medicine, University of Oklahoma Medical Center, Oklahoma City, Oklahoma
1963-1964	Resident, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan

Curriculum Vitae - Shafeek S. Sanbar, CPT, MC

RESEARCH BACKGROUND

1961-1963	Cardiovascular Research Trainee (supported by U. S. Public Health Service Grant HTS-5403) Oklahoma Medical Research Institute, Oklahoma City, Oklahoma
1964-1965	Post-doctoral Fellow (supported by Ontario Heart Foundation, Canada), Cardiovascular Unit, Department of Internal Medicine, University of Toronto, Ontario, Canada.
1965-1966	Research Associate (supported by Ontario Heart Foundation, Canada), and Clinical Assistant, Department of Internal Medicine, University of Toronto, Ontario, Canada
1966-1967	Fellowship support by Michigan Heart Association and Instructor, Hypertension Unit, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan
1967-1968	Advanced Research Fellow of the American Heart Association and Assistant Professor, Hypertension Unit, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan
1968-	Bioenergetics Division, USAMRNL, Fitzsimons General Hospital, Denver, Colorado

ADMINISTRATIVE BACKGROUND

1967-1968	Junior Staff Outpatient Committee, University Hospital, Ann Arbor, Michigan

PROFESSIONAL AND SCIENTIFIC SOCIETIES

Member, American Federation for Clinical Research
Member, American Diabetes Association
Fellow, Council on Atherosclerosis of the A. H. A.

Curriculum Vitae - Shafeek S. Sanbar, CPT, MC

REFERENCES

William D. Robinson, M. D., Chairman and Professor, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48104

Robert H. Furman, M. D., Head, Cardiovascular Section, Oklahoma Medical Research Institute, Oklahoma City, Oklahoma

James Conway, M. D., Head, Clinical Physiology Unit, University Hospital, Ann Arbor, Michigan 48104

PUBLIC RELATIONS EXPERIENCE

1955-1959	Chairman, Social Committee, Medical Student's Society, American University of Beirut, Lebanon
1967-1968	President, American Arab Committee, Ann Arbor, Michigan

HONORS AND FELLOWSHIPS RECEIVED

1956	B. Sc. granted ' WITH DISTINCTION"
1960	M. D. granted "WITH DISTINCTION"
1961-1963	Cardiovascular Traineeship by U. S. Public Health Service, Grant HTS-5403
1964-1965	Fellowship by Ontario Heart Foundation, Canada
1965	The Francis Hutchinson Award, University of Toronto, Ontario, Canada
1965-1966	Research Association by Ontario Heart Foundation, Canada
1966-1967	Fellowship by Michigan Heart Foundation
1967-1968	Advanced Fellowship by American Heart Association

Curriculum Vitae - Shafeck S. Sanbar, CPT, MC

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7. Evans, J. R., Jacobs, M. H., and Sanbar, S. S.: Factors Influencing Incorporation of Exogenous Fatty Acids into Myocardial Lipids. Canad. Med. Ass. J. 92: 352, 1965.

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14. Walfish, P. J. and Sanbar, S. S.: Insulin-I^{131} Disappearance Rates Following Diazoxide, Epinephrine and Glucose Administration. Clin. Res. 14: 443, 1966.

15. Sanbar, S. S. and Reynolds, C.: Effects of Epinephrine (E) and Glucagon (G) on Glucose Turnover. Clin. Res. 14: 442, 1966.

16. Walfish, P. J. and Sanbar, S. S.: Insulin-I^{131} Disappearance Rates Following Diazoxide, Adrenalin and Glucose Administration. Canad. Med. Ass. J. 96: 366, 1967.

17. Zweifler, A. J., Sanbar, S. S., and Conway, J.: Familial Hyperlipoproteinemia Type II (FH-II): Serum Lipids During GTT and After Therapy. Clin. Res. 15: 333, 1967.

18. Sanbar, S. S., Conway, F. J., Zweifler, A. J., and Smet, G.: Diabetogenic Effect of Dilantin (Diphenylhydantoin). Diabetes, 16: 533, 1967.

19. Sanbar, S. S., Smet, G., and Zweifler, A. J.: Decrease in Serum Lipids and Platelet Adhesiveness (PA) following Polyvinylpyrrolidone (PVP) Administration in Patients with Familial Hyperlipoproteinemia (FH). Circulation, 36: II-36, 1967.

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21. Knopf, R. K., Floyd, J. C., Jr., Sanbar, S. S., Fajans, S. S., Ped, S., and Conn, J. W.: Plasma Insulin Response to Arginine in Patients with Familial Hyperlipoproteinemia. J. Lab. Clin. Med., 70: 1025, 1967.

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25. Sannerstedt, R., Sanbar, S. S., and Conway, J.: Metabolic Effects of Exercise in Hypertriglyceridemic Patients. Circulation, 38: VI-171, 1968.

26. Sanbar, S. S. and West, K. M.: Rectal Absorption of Radioactive 6-alpha-methyl Prednisolone in Ulcerative Colitis. J. Med. Liban, 14: 380, 1961.

27. Sanbar, S. S. and Alaupovic, P.: Effect of Urea on the Behavior of the Protein Moiety of Human Serum Alpha-Lipoprotiens in Solution. Biochem. Biophys. Acta, 71: 235, 1963.

28. Furman, R. H., Sanbar, S. S., Alaupovic, P., Bradford, R. H., and Howard, R. P.: Studies of the Metabolism of Radioiodinated Human Serum Alpha-Lipoprotein in Normal and Hyperlipidemic Subjects. J. Lab. Clin. Med., 63: 193, 1964.

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30. Alaupovic, P., Sanbar, S. S., Furman, R. H., Sullivan, M. L., and Walraven, S. L.: Studies of the Composition and Structure of Serum Lipoproteins: Isolation and Characterization of Very High Density Lipoproteins of Human Serum. Biochem., 5: 4044, 1966.

31. Sanbar, S. S., Conway, F. J., Zweifler, A. J., Julius, S., and Hoobler, S. W.: Familial Hyperlipoproteinemic (Hyperlipidemic) Syndromes. Univ. Mich. Med. Center J., 32: 277, 1966.

32. Sanbar, S. S.: Effect of L-Leucine on Glucose Turnover in Dogs. Metabolism, 15: 557, 1966.

33. Sanbar, S. S., Evans, J. R., Lin, B., and Hetenyi, G., Jr.: Studies on the Effect of Octanoate on Glucose Metabolism in Dogs. Canad. J. Physiol. and Pharmacol., 45: 29, 1967.

34. Sanbar, S. S., Martin, J.: Stimulation by Octanoate of Insulin Secretion by Isolated Rat Pancreas. Metabolism 16: 482, 1967.

35. Sanbar, S. S.: Metabolism of Plasma Glucose and Lipids Following Diazoxide Administration in Dogs. Metabolism, 16: 259, 1967.

36. Bacon, G. E. and Sanbar, S. S.: Serum Lipids and Lipoproteins of Insulin-Treated Diabetic Children. Univ. Mich. Med. Center J., 34: 84, 1968.

37. Sanbar, S. S.: Alterations in Glucose Turnover Following Single Intravenous Injections of Epinephrine and Glucagon in Dogs. Metabolism, 17: 631, 1968.

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38. Sanbar, S. S., Zweifler, A. J., and Smet, G.: In-Vivo and In-Vitro Effects of Polyvinylpyrrolidone on Platelet Adhesiveness in Human Blood. Lancet 2: 917, 1967.

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47. Sannerstedt, R., Sanbar, S. S., and Conway, J.: Metabolic Effects of Exercise in Hypertriglyceridemic Patients (in preparation).

Curriculum Vitae - Shafeek S. Sanbar, CPT, MC

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49. Sanbar, S. S.: Diabetogenic Effect of Dilantin (Diphenylhydantoin). (in preparation).

50. DISSERTATION: Sanbar, S. S.: Structure and Metabolism of Serum High Density Lipoproteins. Oklahoma City. The University of Oklahoma, 1963, 80 pages.

51. BOOK: Sanbar, S. S.: Hyperlipoproteinemia and Hyperlipidemia. Little, Brown and Company, Boston, Massachusetts, 1969 (June).

DISPOSITION FORM

(AR 340-15)

REFERENCE OR OFFICE SYMBOL	SUBJECT
MEDEN-RI	Radioisotope User Certification

TO Recorder, Radioisotope Committee of FGH and USAMRNL

FROM Radioisotope Section USAMRNL

DATE 6 Jan 69

CMT 1

MAJ Liddle/26111

1. Request that Shafeek S. Sanbar, CPT, MC, be certified as a user of radioisotopes in human subjects under the conditions outlined in "Request for Approval for Human Use of Radioisotopes in Tracer Amounts in Volunteer Experimental Research Subjects."

2. Also request that CPT Sanbar be certified for non-human use of the following radioisotopes:

Carbon - 14	1 millicurie
Hydrogen - 3	5 millicuries
Selenium - 75	1 millicuries
Iodine - 131	1 millicurie
Iodine - 125	1 millicurie

Charles G. Liddle

CHARLES G. LIDDLE
MAJ, VC
Chief, Radioisotope Section
USAMRNL

Form AEC-313 a (3-56)
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))
Shafeek S. Sanbar	

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	100	(1) (2) (3) (4)
	Treatment of hyperthyroidism	2	(1) 2 (3) (4)
	Treatment of thyroid cancer		1 2 3 4
	Treatment of cardiac conditions		1 2 3 4
	Brain tumor localization	5	(1) 2 3 (4)
	Blood determinations	100	(1) (2) (3) (4)
	Others:	100	(1) 2 3 (4)
			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	50	(1) (2) (3) (4)
	Others:		1 2 3 4
			1 2 3 4
Other Isotopes	Fe-59-Hgb synthesis and RBC survival	10	(1) (2) (3) (4)
	Co-60 Vit. B12	10	(1) 2 3 (4)
	Hg-197-Kidney scans and renograms	50	(1) 2 3 (4)
	Se-75-Methionine for parathyroid scan	2	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 100+ hours

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

Professors of Dept. of Biochemistry (Name of physician (preceptor)), AT Unic. of Okla, Medical School (Institution)

Shafeek S. Sanbar, Cpt. MC (Signature)

TRAINING AND EXPE... ICE OF EACH INDIVIDUAL NAMED IN ITEM 4 (... 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	WHO course in Radioisotopes Course(3 credit hrs) for Ph. D.	1 Mo 3 Mos	(Yes) No	(Yes) No
b. Radioactivity measurement standardization and monitoring techniques and instruments	On the job experience at Univ. of Oklahoma Same as above	3 Yrs	(Yes) No	(Yes) No
c. Mathematics and calculations basic to the use and measurement of radioactivity	Same as above	3 Yrs	(Yes) No	(Yes) No
d. Biological effects of radiation	Same as above	3 Yrs	(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
C-14	2 μc	Amer. Univ. of Beirut, Lebanon	1 yr	Metabolic Studies
H-3	10 μc	Univ. of Okla. Med. School	3 yrs	" "
Se-75	0.5μc	Oklahoma City	3 yrs	" "
I-131	5 μc	Univ. of Toronto, Toronto	2 yrs	" "
I-125	5 μc	Univ. of Mich., AnnArbor	3 yrs	" "

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

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.

Shafeek S. Sanbar, CPT, MC
Applicant named in Item 1

Date _____

By: _____
COL ROBERT C. JONES, MC
Chairman, Radioisotope Committee
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: 1960 O - 568572

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

Section I. General Introduction

Section II. General Health Physics for Requested Isotopes*

* (for references consult Appendix I.)

Section III. Proposed Nutrition and and Metabolism Tracer Studies

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. Radioisotopes in tracer amounts as used in metabolic and physiological experiments are not nuclear agents (in the context and intent of AR 70-25 these words undoubtedly mean nuclear warfare agents). Furthermore, tracer quantities of radioisotopes as licensed by AEC and used by competent medical research scientists constitute a health hazard so minimal as to permit debate re the applicability of AR 70-25.

d. Nevertheless, to comply with the administrative technicality imposed by 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 human 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.

(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

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 urins 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 do $C^{14}O_2$, 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 health, 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 oscorbic 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).

(2) Baker, E. M., H. E. Sauberlich, S. J. Wolfskill, W. T. Wallace and E. E. Dean, Proc. Soc. Exp. Biol. and Med. 109: 737, 1962 (reprint attached, Appendix IV).

(3) Hellman, L. and J. J. Burns, J. Biol. Chem. 230: 923, 1958.

(4) Abt, A. F., S. Von Schuching and I. Enns, Am. J. Clin. Nutrition 12: 21, 1963.

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

(6) Archer, H. E., A. E. Dormer, E. F. Scowen and R. W. E. Watts. Clin. Sci. 16: 405, 1957.

(7) Schaffert, R. R. and G. R. Kingsley, J. Biol. Chem. 212: 59, 1955.

(8) Jackel, S. S., E. H. Mosbach and C. G. King. Arch. Biochem. and Biophys. 31: 442, 1951.

(9) Allen, T. H., H. J. Krzywicki, W. S. Worth and R. M. Nims, U. S. Army Med. Rsch. Nutrition Lab. Rpt. No. 250, 24 Sept. 1960.

(10) A study of the military applicability of research on ascorbic acid. Life Sciences Research Office, Federation of American Societies for Experimental Biology, Washington, D. C., August 1963.

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

Maj. E. M. Baker, Ph.D., MSC, Project Leader
H. E. Sauberlich, Ph.D. (PL-313) Co-Project Leader
G. A. Leveille, Ph.D.
Lt. Col. M. E. McDowell, M.D., MC (Serving also as attending physician)
Lt. Col. J. E. Canham, M.D., MC (Serving also as attending physician)

f. References

(1) Crawhall, J. C., R. R. Mowbray, E. F. Scowen and R. W. E. Watts, Conversion of glycine to oxalate in a normal subject. Lancet, Nov. 14, 810-11, 1959.

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(3) Berlin, N. I., B. M. Tolbert and J. H. Lawrence. Studies in glycine-2-C^{14} metabolism in man. I. The pulmonary excretion of $C^{14}O_2$. J. Clin. Investigation 30: 73-76, 1951.

(4) Hillman, L., J. J. Burns. Metabolism of L-ascorbic acid-1-C^{14} in man. J. Biol. Chem. 230: 923-930, 1958.

(5) Baker, E. M., H. E. Sauberlich and S. J. Wolfskill. Metabolism of D-glucuronolactone-6-C^{14} and D-glucuronic acid-6-C^{14} in man. Fed. Proc. 20: 85, 1961.

(6) Baker, E. M., H. E. Sauberlich, S. J. Wolfskill, W. T. Wallace and E. E. Dean. Proc. Soc. Exp. Biol. and Med. 109: 737, 1962.

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 unsaturated 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 I, 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).

(3) Personnel

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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 fat 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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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

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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 geogrophical 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 tracer):

(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 labeling 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.

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