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Research Report

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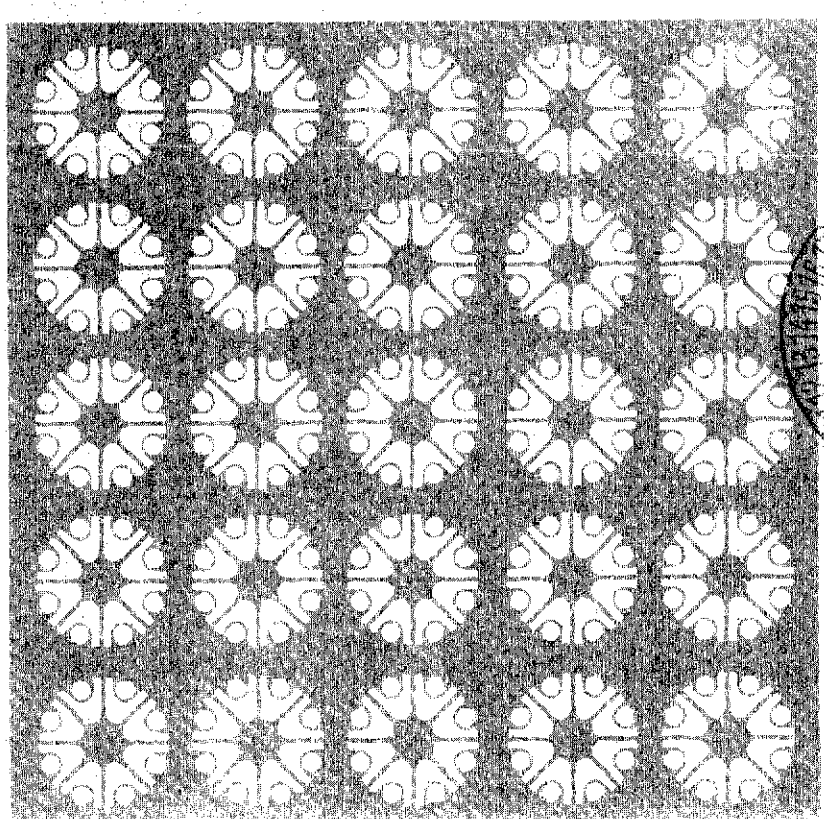

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Final Report of Work Completed on

STUDY OF BONE MINERAL METABOLISM

Under Contract NAS-9-14248
July 1, 1974 to December 31, 1974
to the
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Lyndon B. Johnson Space Center
Houston, Texas

by

H. E. Palmer
Physics and Instrumentation Department

January 13, 1975

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STUDY OF BONE MINERAL METABOLISM

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ABSTRACT

The major portion of this work was devoted to a study of the use of ^{85}Sr as an indicator of the skeletal location and relative amount of bone demineralization which occurs during immobilization of the body or body parts, bed-rest or space flight. In this study the bone mineral replacement which occurs after immobilization is measured rather than measuring the bone loss which occurs during immobilization. In a study with two adult beagle dogs, the ^{85}Sr uptake in a leg which had been immobilized for two months was 400 percent higher than the uptake in the legs in regular use. This greatly increased uptake probably resulted from only a few percent loss in bone mineral and indicates that losses less than one percent can be easily detected and located. The sensitivity, simplicity and the low radiation dose associated with the use of this method indicates that it should receive strong consideration for use on humans in bed-rest and space flight studies.

Other work under this contract was devoted to the development of methods for measuring changes in total body nitrogen and in assisting the Johnson Space Center in calibrating a whole body counter for total body potassium measurements.

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PART I

THE USE OF ^{85}Sr IN THE MEASUREMENT OF BONE MINERAL REPLACEMENT AFTER IMMOBILIZATION OF A FRONT LEG OF A DOG

Similarity of Sr and Ca Metabolism

The hazard associated with the long-lived radionuclide ^{90}Sr has resulted in extensive research on the metabolism of strontium and how this metabolism compares to that of calcium. Dolphin and Eve[1] have proposed a model for Sr metabolism and have compared this with the calcium model as presented in Table 1. Although there are differences in

TABLE 1. Comparison of Calcium and Strontium Metabolism
[From Dolphin and Eve[1]]

	Calcium	Strontium
Daily absorption from gut	0.450 g/day	0.3 mg/day
Exchangeable pool	5.0 g	1.2 mg
Total in plasma	0.35 g	0.08 mg
Accretion rate in bone	0.5 g	0.12 mg
Skeletal content	1040 g	250 mg
Half-life in exchangeable pool	3.7 days	2.8 days
Half-life in bone	2770 days	2000 days
Fractional transfer		
Diet to exchangeable pool	0.38	0.19
Exchangeable pool to bone	0.53	0.29
Diet to bone	0.20	0.055

the fractional transfer from gut to plasma and plasma to bone, once the strontium becomes firmly bound in bone it acts similarly to calcium and remains there with a biological half-life of several years.

From the data in Table 1, a procedure was developed in theory and presented in a previous report[2] which indicated that small amounts of bone mineral loss could be detected by measuring the uptake of ^{85}Sr during a period of bone mineral replacement. A summary of these theoretical calculations indicated that bone mineral losses of 10, 2 and 1 percent

would result in ^{85}Sr uptake during bone replacement of 1400, 390 and 240 percent, respectively, over ^{85}Sr uptake due to normal bone turnover. These calculations indicate that bone mineral losses less than one percent could be easily detected and the relative loss and the location of the loss could be determined by simple collimated scanning of the skeleton.

Experimental Procedure

To produce bone mineral losses by mechanisms similar to that resulting from space flight, the left front legs of two beagle dogs were immobilized by placing them in casts for a period of two months. The dogs were 7 and 4 years old and in normal physical condition. The dogs were fed the normal diet which is fed to the Battelle beagle dog colony. At the end of the two-month period, the left front legs were released from the casts and allowed to resume normal use. Intravenous injections of 1 μCi of ^{85}Sr were given each dog at the time the casts were removed and again one week later. Starting at one week after the last injection, the count rate of ^{85}Sr in the lower part of the right and left legs were measured and similar counts were made at one week intervals on each dog until five weeks after the last ^{85}Sr injection. At this time the older dog was sacrificed and the bones of the front legs were removed to allow detailed scanning of the ^{85}Sr deposition from the end of the scapula to the end of the toes. Since the ^{85}Sr uptake in the younger dog appeared to be about the same as that of the older dog and also had a slightly interferring burden of ^{22}Na from a previous NASA sponsored experiment, he was transferred into other useful studies rather than sacrificed for detailed measurements. The leg bones of the older dog were scanned with a 6"x4" diameter Na(Tl) detector covered by a 1" wide slit collimator. The connected leg bones were moved past the collimator and counted at 1" intervals.

Results

A comparison of the counts on the lower parts of the left and right front legs of the live dogs showed 2.6 and 3.0 times more in the left leg, which had been immobilized, than in the right leg for the 7 and 4

year old dogs, respectively. This ratio remained constant for the 5-week period up to the time of sacrifice of the 7-year old dog. The amount in the legs remained constant within the error of measurement on the live dogs with the exception of 30 percent loss due to radioactive decay.

A detailed scan over the length of the leg bones of the 7-year old dog is shown in Figure 1. The ^{85}Sr distribution has been matched to positions on a photograph of the front leg bones. The ^{85}Sr deposition near the bone joint between the radius and foot was four times higher in the left leg than in the right one. The left and right leg differences in ^{85}Sr deposition are smaller at the other two joints presumably due to less difference in the stress applied to the left leg joints during and after immobilization. With the leg in the cast, some stress could still be put on the joints between the scapula and humerus and the humerus and radius by pushing against the cast. However, very little stress could be put on the radius-foot joint.

Assuming a bone mineral loss of a few percent occurred in the lower joint area, this loss would be difficult to observe by other methods, such as x-ray radiography, x-ray absorption, and neutron activation. With this ^{85}Sr uptake method we have a very striking 400 percent difference for only a few percent loss, which also means that losses lower than 1 percent could be easily detected by this method. The method is not quantitative in its present state but only gives relative rates at which bone mineral is being replaced at sites where losses occurred and it would appear that the initial rate of ^{85}Sr uptake is somewhat proportional to the extent of original loss.

The method could be easily applied to human use and would involve giving one or possibly two low-level injections of ^{85}Sr immediately after discontinuance of a bed-rest study or space flight. Two weeks later the skeleton can be scanned with a detector and slit collimator. Individual arm and leg scans can be made in addition to scans along the vertebral column and lateral scans over the pelvis and shoulder regions. For the best results, a preflight experiment should be made on the subject to determine the normal ^{85}Sr uptake in all parts of the skeleton due to

normal bone mineral turnover rates. The preflight scan would then be compared to the post-flight scans to determine the location and the relative amount of any bone mineral loss. This method should be especially helpful in measuring the bone mineral loss during space flight in the vertebra which are very difficult to measure by other techniques due to the thickness of the body.

Radiation Dose and Limitations

The use of ^{85}Sr as a tracer for measuring bone mienral replacement after disuse can only be made in the absence of other radionuclides in the body which have gamma ray energies higher than 0.4 MeV. The presence of ^{59}Fe commonly used in hematology studies during space flight and bed-rest studies would completely overshadow the radiation from the ^{85}Sr and, therefore, ^{55}Fe would need to be substituted for ^{59}Fe when ^{85}Sr experiments are planned.

The radiation dose to an adult human from an injection of ^{85}Sr would be about 44 millirem to the skeleton and 23 millirem to the total body as calculated by the conservative methods suggested by the International Commission on Radiological Protection (ICRP).[3] More recent and probably realistic calculations according to the Medical Internal Radiation Dose Committee (MIRD)[4] indicate doses of only 3.9 and 3.0 millirads would be given to the skeleton and total body, respectively, from a 1 μCi injection. Since those parts of the skeleton which have undergone demineralization may take up as much as 10 times as much ^{85}Sr as normal bone, the more conservative ICRP dose estimates are the most appropriate because of this increased nonuniformity of ^{85}Sr deposition. A 1 μCi injection would be adequate to conduct the ^{85}Sr study within a reasonable length of time.

Other Studies Using ^{85}Sr

The use of ^{85}Sr was investigated for use in measuring accelerated bone formation in rats. Two groups of three rats each were used in the experiment. At six weeks of age one group of rats was placed on a

commercial synthetic calcium deficient diet while the other group was placed on a totally adequate synthetic diet. The calcium deficient diet was maintained for three weeks with the one group of rats which retarded their total body calcium content by 30 percent when compared to the group on the calcium adequate diet. The calcium content was determined by the method of measuring ^{37}Ar expired after neutron irradiation which was developed under a previous contract.[2] At the end of the three week period, all rats were placed on the same synthetic diet which contained double the amount of calcium which was present in the regular calcium adequate diet. Also starting at that time the rats were injected intra-peritoneally twice each week for three weeks with ^{85}Sr. At the end of three weeks the ^{85}Sr was allowed to clear the soft tissues and the ^{85}Sr content of the rats was measured the following week. During this three week period of calcium enriched diet and ^{85}Sr injections the rate of increase of total body calcium in the group which had been on the calcium deficient diet was about two times that of the control group. It was expected that the ^{85}Sr deposition in the calcium deficient rats would be double that in the control group. Whole body counts on both groups of rats showed that the ^{85}Sr deposition in the bone was about the same in both groups and possibly slightly higher in the control group. These results are difficult to interpret, but it is obvious that the bone mineral kinetics of diet induced bone mineral deficiency in growing rats is greatly different than that in adult beagle bone due to disuse. This suggests that future studies directed at bone mineral loss in space should be done only with animals which have a mature skeleton and induced demineralization should be done only by disuse rather than chemical or dietary methods.

Summary and Recommendations for Future Use of ^{85}Sr to Measure Bone Mineral

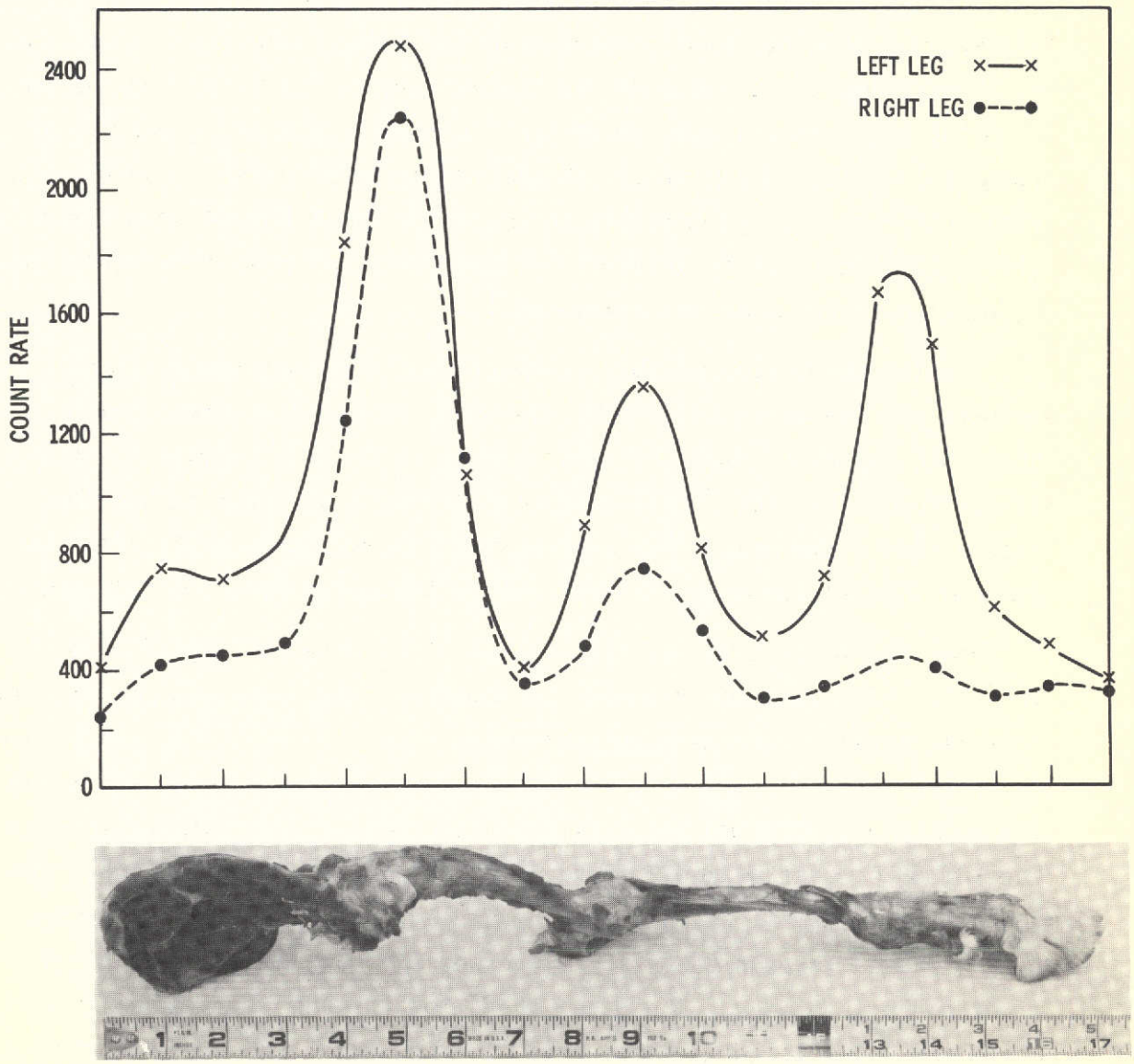
From the dog experiments, it appears that the ^{85}Sr tracer method will allow a sensitive determination of the location and relative amount of bone mineral loss which has occurred during space flight by measuring the amount of ^{85}Sr deposited in the bone mineral replacement which occurs during a short period after the end of space flight. The current methods

for detecting and measuring changes in bone mineral content are limited to the extremities and even then cannot reliably see anything less than a 2 percent change. The ^{85}Sr tracer method can be used equally well on any part of the skeleton and it appears that bone mineral losses as low as 0.1 percent could be detected under a carefully controlled experiment.

Because of the apparent simplicity and sensitivity of this method, it is recommended that it be tried with subjects participating in the next bed-rest studies and with the astronauts who will be on the next space flight mission. Further research and development should be aimed at providing standard scanning radiation detecting equipment which can be used at a variety of locations and conducting some ^{85}Sr uptake experiments on a few normal people to establish some average deposition patterns for all parts of the skeleton.

FIGURE 1

COMPARISON OF ^{85}Sr UPTAKE IN FRONT LEGS OF A DOG AFTER LEFT LEG HAS BEEN IMMOBILIZED FOR 2 MONTHS



PART II

A STUDY OF THE USE OF ^{11}C IN EXPIRED AIR AFTER NEUTRON IRRADIATION AS A METHOD FOR MEASURING TOTAL BODY NITROGEN

Observed changes in lean body mass in astronauts during space flight have resulted in the need for a method for a more accurate determination of these changes. Because of the proportionality of total body nitrogen to total body protein and, therefore, to lean body mass, a measure of changes in total body nitrogen should result in a measure of changes in lean body mass. It is feasible to determine total body nitrogen by in-vivo neutron activation analysis followed by total body counting of the ^{13}N but there are some interferences.(5)

During the period of this contract, while evaluating exisitng methods for measuring total body nitrogen, it was discovered that ^{11}C occurs in the expired air of rats exposed to neutron irradiation and it was found that the ^{11}C is produced only by a p,α reaction on the stable ^{14}N in the body. The protons for this reaction result from fast neutron interaction with the hydrogen in the body producing fast neutrons.

The two major forms of the expired ^{11}C have been identified as ^{11}CO and $^{11}CO_2$. Preliminary attempts to identify $^{11}CH_4$ were unsuccessful but low concentrations are probably present. The ^{11}CO and $^{11}CO_2$ are easily separated from the expired air and measured. Figure 2 shows a diagram of the neutron irradiation and ^{11}C collection apparatus. Either a gas mixture of 80 percent He and 20 percent O_2 or pure O_2 flows through a tube containing the small animal. During and after irradiation the expired air is passed through $CaSO_4$ to remove water vapor and small quantities of nitrogen-13 oxides. The gases then flow through a soda lime trap where the $^{11}CO_2$ is collected. The ^{11}CO and other gases then flow through hot copper oxide which converts CO to CO_2 and the $^{11}CO_2$ formed there from the ^{11}CO is collected on a second soda lime trap. The ^{11}C in the soda lime traps is then measured between two large NaI(Tl) detectors operated in coincidence so that essentially only the 0.51 MeV annihilation photons

are counted. The soda lime traps are constructed in a circular configuration so that the ^{11}C is counted with the same geometry regardless of where it is deposited within the circular tube.

Most of the ^{11}C is expired as ^{11}CO from the rat. The ratio of ^{11}CO to $^{11}CO_2$ is about two and appears to be constant over a variety of environmental conditions. The $^{11}CO_2$ is expired about twice as rapidly as the ^{11}CO when the rat breathes a gas mixture containing 20 percent O_2. The slower excretion of the ^{11}CO is due to its strong attachment to the hemoglobin of the blood. Since most of the ^{11}C is in the form of ^{11}CO and because the decay half life is only 20.4 minutes, it is important that the ^{11}CO be rapidly excreted from the body before it decays away. Several methods were tried in an attempt to increase the excretion rate of the ^{11}CO. Both exercise and cold environment were used to increase the metabolic activity of the animal and both of these stresses resulted in at least a doubling of the excretion rate. However, the most significant increase occurred when the rat breathed pure O_2 which resulted in an excretion rate 4.5 times that obtained with 20 percent O_2, no exercise and room temperature. The results of these studies are shown in Figure 3 and indicate that essentially all the ^{11}CO is expired in time for it to be measured before it decays. The use of pure O_2 as the breathing gas does not change the amount nor the excretion rate of the $^{11}CO_2$. Its only effect seems to be that of enhancing the release of the ^{11}CO from the hemoglobin so that it can be released through the lungs. This is indicated by the fact that the oxygen tension of the blood is increased by a factor of five when changing from 20 percent O_2 to 100 percent O_2. This results in a change of a factor of 4.5 in the excretion rate of ^{11}CO.

The interest in ^{11}C measurements in expired air is in its possible use in determining the total body nitrogen which is directly related to the total body protein content. This measurement could become important in nutritional studies and in the study of certain disease . The ^{11}C is produced only from the nitrogen in the body, but it has not been directly compared to the nitrogen content by making the corresponding chemical

analysis of total body nitrogen. The ^{11}CO extracted from various concentrations of nitrogen solutions in water has been found to be exactly proportional to the nitrogen content. Future studies will compare the expired ^{11}CO from small animals with the actual total body nitrogen content and will determine if this technique can be extended to humans. Although it is expected that the ^{11}C excretion may be somewhat independent of the respiration rate, the lower respiration rates per unit of body surface area of the human compared to that of the rat may limit the use of this technique with humans due to a possible much slower excretion rate of the ^{11}CO and $^{11}CO_2$.

FIGURE 2

DIAGRAM OF NEUTRON IRRADIATION AND ^{11}C COLLECTION FACILITIES

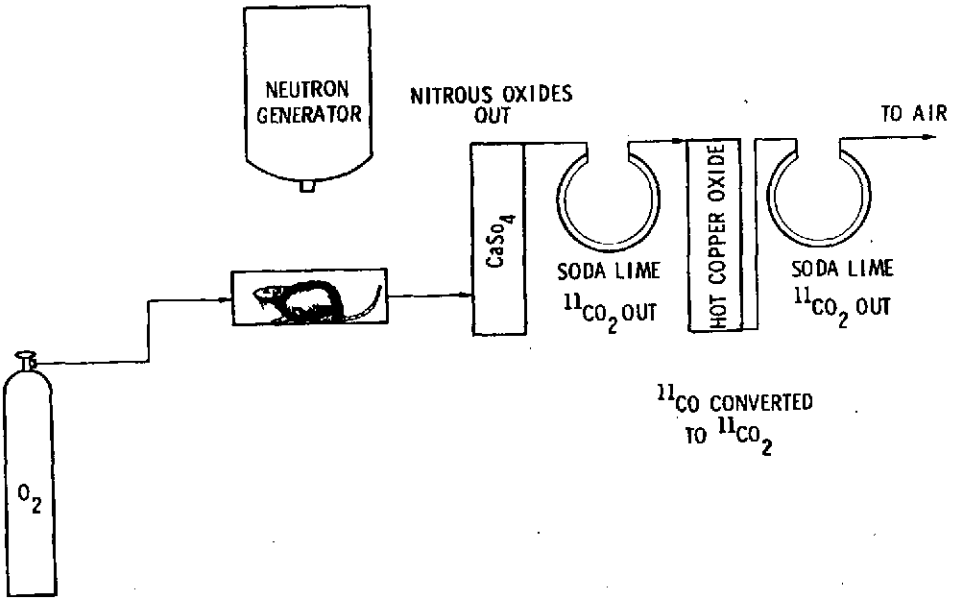
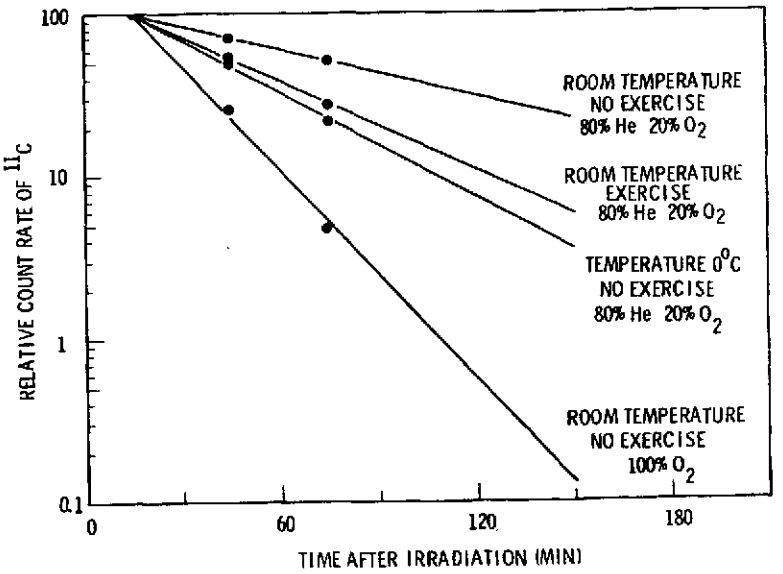


FIGURE 3

^{11}CO EXCRETION IN EXPIRED AIR FROM A RAT AFTER 14 MeV NEUTRON IRRADIATION (30 MINUTE SAMPLE COLLECTIONS)



PART III

OTHER ACTIVITIES

In other work under this contract, consultation was provided to the Food and Nutrition Branch on calibration of the Johnson Space Center whole body counter for the measurement of total body potassium in adult humans. The counting equipment and instrumentation was determined to be adequate for this purpose and plans were developed for a precise calibration of the counter by counting several individuals at the Battelle Northwest whole body counting facilities and at the Johnson Space Center facilities. The individuals used for this purpose will have their total body potassium accurately determined by a combination of a ^{42}K exchange method and whole body counting at Battelle facilities.

Additional consultation was given to the NASA sponsored work at the University of Washington which is being conducted by Dr. T. K. Lewellan and Dr. W. B. Nelp. This consultation was in the form of suggestions, ideas and evaluation of methods for determining total body calcium in humans by measuring expired ^{37}Ar after neutron irradiation. This method was developed and successfully demonstrated on animals at Battelle under previous NASA contract.

REFERENCES

1. Dolphin, G. W. and Eve, I. S., Phys. Med. Biol, 8(2):193 (1963).

2. Palmer, H. E., Continued Investigation of Kinetic Aspects of Bone Mineral Metabolism, Battelle-Northwest Final Report to the National Aeronautics and Space Administration, Johnson Space Center, Houston, Texas, for period February 1, 1973 to February 28, 1974 under Contract NAS-9-13235, February 1974.

3. International Commission on Radiological Protection: Report of Committee II on Permissible Dose for Internal Radiation, Pergamon Press, London (1959).

4. Loevinger, R. and Berman, M.,"A Schema for Absorbed-Dose Calculations for Biologically Distributed Radionuclides, J. of Nucl. Med., Supplement Number 1, Pamphlet No. 1: 9-14 (1968).

5. Palmer, H. E., and Nelp, W. B., "Estimation of Total Body Phosphorous and Nitrogen In Vivo, In Vivo Neutron Activation Analysis (T. Nagai, Ed), IAEA Press, Vienna, Austria, p. 127 (1973).

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