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Research Report

Final Report of Work Completed on

KINETIC ASPECTS OF BONE MINERAL METABOLISM

Under Contract NAS 9-12463
January 4, 1972 to January 3, 1973
to the
National Aeronautics and Space Administration
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ABSTRACT

Two techniques were studied for measuring changes in bone mass in rats. One technique measures the ^{37}Ar produced from calcium during neutron irradiation and the other measures the changes in the ^{22}Na content which has been incorporated within the rat bone. Both methods are performed *in vivo* and cause no significant physiological damage. The ^{37}Ar leaves the body of a rat within an hour after being produced, and it can be quantitatively collected and measured with a precision of ±2% on the same rat. With appropriate irradiation conditions it appears that the absolute quantity of calcium in any rat can be determined within ±3% regardless of animal size. The ^{22}Na, when uniformly distributed in bone, can be used to monitor bone mineral turnover and this has been demonstrated in conditions of calcium deficiency during growth and also pregnancy coupled with calcium deficiency.

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PART I

THE DETERMINATION OF TOTAL BODY CALCIUM IN THE RAT BY MEASURING ^{37}Ar IN THE EXPIRED AIR AFTER FAST NEUTRON IRRADIATION

Production of Radioactive Argon in the Body

Both ^{37}Ar and ^{41}Ar are produced during the fast neutron irradiation of calcium. The nuclear reaction, stable calcium isotopic abundance and modes of decay for the two product isotopes are shown in Figure 1. A 14 MeV neutron generator is used in these studies and the cross sections for these reactions for 14 MeV neutrons are approximately 110 millibarns for ^{40}Ca[1] and 30 millibarns for ^{44}Ca.[2] As the neutron energy decreases below 14 MeV the cross section for the (n,α) reaction increases with ^{40}Ca and decreases with ^{44}Ca. For instance, at 6 MeV the reaction cross section is 430 millibarns[3] for ^{40}Ca and less than 1 millibarn for ^{44}Ca. The cross-section information for the ^{40}Ca reaction at neutron energies between 6 and 14 MeV has not been found in the literature and possibly has not been measured. Our studies have shown that for the same neutron dose much more ^{37}Ar than ^{41}Ar is produced in animals. Another reason for using ^{37}Ar as a measure of total body calcium is that an (n,p) reaction on ^{41}K also produces ^{41}Ar and, therefore, the potassium in the body produces an interference for the calcium measurement. There are no apparent interferences in the use of ^{37}Ar as an indicator of calcium content except that ^{41}Ar must be allowed to decay away before counting. When comparing ^{37}Ar and ^{41}Ar for use in total body calcium determinations in regards to the lowest radiation dose, interferences, and counting sensitivity, the ^{37}Ar appears to be by far the best isotope to collect and measure.

Separation and Purification of ^{37}Ar From Expired Air

The components of normal expired air are approximately 75% nitrogen, 15% oxygen, 4% carbon dioxide and a 6% fraction consisting of water vapor, argon, hydrogen, etc. The boiling point and adsorption characteristics of nitrogen are so similar to those of argon that it is very difficult to separate a small quantity of ^{37}Ar from a large volume of nitrogen. In the rat,

the actual volume of ^{37}Ar gas which will be produced, collected, and purified is less than 1×10^{-13} cc (3×10^{6} atoms) whereas the normal volume of expired nitrogen would be approximately 1 to 10 liters per hour from a rat depending on its size.

By substituting for inspired air a gas mixture of 80% helium and 20% oxygen such as that used by deep sea divers, the separation of ^{37}Ar is greatly simplified. The use of this gas mixture eliminates nitrogen and stable argon except for the small quantities which are adsorbed in the body tissue and which exist as a contaminate in the gas mixture. The amount of adsorbed argon in the body is negligible and that small fraction of adsorbed nitrogen which is expired during the ^{37}Ar collection period is removed by reacting with hot calcium at 600°C.

The ^{37}Ar is easily separated from oxygen, carbon dioxide, and water vapor and the apparatus and procedure for doing this with animals is shown in Figure 2. The animals such as rats or dogs are placed inside an air-tight container. The helium-oxygen mixture flows through the container at a rate 200 cc/minute for a rat. The gas leaving the container passes through a column of soda lime particles to remove CO_2, through hot copper at 400°C to remove oxygen, through an ice bath trap, $CaSO_4$ and P_2O_5 to remove water vapor and through a trap containing activated charcoal at liquid nitrogen temperature which quantitatively collects all the ^{37}Ar and allows the helium to pass through. Except for the nitrogen which also adsorbs on the activated charcoal, the ^{37}Ar is pure enough to be transferred directly into a proportional counter for measuring the activity. Figure 3 shows the apparatus for transferring the ^{37}Ar into the counter. Hot calcium removes nitrogen and any remaining oxygen and the gas is transferred to a small activated charcoal trap which leads directly into the counter. This trap is warmed and all the ^{37}Ar is swept into the counter with a counting gas composed of 90% argon and 10% methane.

The copper used to remove oxygen was in the form of short lengths of wire less than 1/4 inch long and 0.025 inch diameter which can be purchased in the cupric oxide form and then reduced with hydrogen at 400°C. This makes a very reactive column for oxygen removal and the column can be easily regenerated from oxide to copper with hydrogen for indefinite reuse. The $CaSO_4$ is a commercial drying agent and the P_2O_5 is used as a covering on glass beads for the final

removal of water vapor. The activated charcoal is a commonly available cocoanut charcoal, 6-14 mesh, which is outgassed under vacuum or a flow of helium for about 5 minutes at a temperature of about 250 to 300°C before being used to absorb the ^{37}Ar.

Measurement of ^{37}Ar Activity

The fluorescent yield of the 2.63 keV chlorine X-rays emitted during ^{37}Ar decay is only 6.5 percent; therefore, in 93.5 percent of the disintegrations, the energy will be emitted as Auger electrons having an energy of 2.62 keV. The Auger electrons will be absorbed within a very small counter with essentially 100% efficiency. The calculated range of a 2.6 keV electron in argon gas at atmospheric pressure is less than 0.02 mm. In addition to the 2.62 keV from the Auger electrons, an additional ionization energy is added within the counter by low energy photons emitted when the L and M levels are filled so that the total energy released within the counter is 2.82 keV per disintegration.

The counter used in these studies was made from a 2.5 cm diameter and 23 cm long aluminum tube with a 0.025 mm stainless steel center anode wire. A small thin beryllium window is located midway between the ends of the counter to allow calibration with an ^{55}Fe X-ray source. The background of this counter is 2 counts per minute under the 2.82 keV photopeak when placed between two large scintillation detectors which act as anticoincidence shields and the whole apparatus shielded by 4 inches of lead. A proportional counter is presently under construction in which the background should be as low as 0.1 count per minute. The low background coupled with the near 100% counting efficiency allows a very sensitive measurement of the ^{37}Ar activity.

The size of the proportional counter is determined by the amount of stable argon which is collected along with the ^{37}Ar as a result of argon which was dissolved in body tissues and fluids and that which exists as an impurity in the helium-oxygen gas mixture. A 1 1/2 hour collection of expired air from a 500 gram rat produces about 4 cc of stable argon of which approximately 3 cc is from the helium-oxygen gas and 1 cc from that absorbed within the rat's body. The 113 cc volume proportional counter described above allows an additional 109 cc of gas to be used to flush all the ^{37}Ar into the counter.

The energy spectrum from ^{37}Ar in the proportional counter is shown in Figure 4. A multichannel analyzer is used to analyze the pulses from the proportional counter. The resolution of the proportional counter for the 2.82 keV photopeak is 0.78 keV or 28% FWHM, and counts in the energy region from 1.80 to 3.80 keV are used to quantify the ^{37}Ar present.

Excretion Rate of ^{37}Ar From Rats

The excretion rate of ^{37}Ar as a function of time has been studied in rats by analyzing half-hour and one-hour long fractions of air expired during and after neutron irradiation (see Figures 5 and 6). In adult rats about 90% of the ^{37}Ar is excreted within 30 minutes, 99% within 60 minutes, 99.9% within 90 minutes, and there does not appear to be any significant long-term excretion component.

If a high resolution Ge(Li) detector is used, the gamma emitting ^{41}Ar can be measured in animals which have been irradiated to high dose levels. Experiments were conducted in which both live and dead rats of the same size were simultaneously irradiated at the same distance and angle from a 14 MeV neutron source. It was assumed that the ^{41}Ar would remain in the dead animal and that it would be removed in the expired air from the live animals. Whole and partial body counts were made one hour after irradiation. The interference of other isotopes such as ^{24}Na and interference from ^{41}Ar produced from potassium in the body prohibited an accurate estimation of the calcium produced ^{41}Ar expired within one hour, but the counts did show that more than 85% of the ^{41}Ar produced from calcium was removed from the body of rats within the first hour. This information plus the fact that ^{37}Ar cannot be detected in the expired air of the rat after 90 minutes suggests that all of the radioactive argon has been removed from both the bone and body of the rat within this time.

Reproducibility of ^{37}Ar Measurements

Repeated measurements have been made on a single rat. The measurements were made during an 8-hour period for the rat to ensure that the calcium content did not significantly change between measurements. The rat was irradiated in a reproducible position and a solution of $Fe(NO_3)_3$ was also irradiated simultaneously for use as a neutron flux monitor. An (n,p) reaction on the

iron produces ^{56}Mn which can be easily measured. The expired air was collected for 90 minutes after the start of a 10 minute irradiation. The ^{37}Ar was counted the following day to allow all the ^{41}Ar to decay away. The $Fe(NO_3)_3$ standard was counted at least 2 hours after irradiation to allow for decay of the ^{13}N which was produced. Both counts were decay corrected back to the start of irradiation and the ratio of the counts are shown in Table 1. These values plus duplicate determinations on six other rats, which are described later, indicate that the precision of the method appears to be within $\pm$2% which is adequate for many studies of changes in total body calcium.

Comparison of ^{37}Ar Production With Bone Growth

Periodic ^{37}Ar measurements were made on two rats as they increased from about 190 to 320 grams. The rats were irradiated inside a lucite tube without any additional moderation. The ^{37}Ar counts were normalized to a constant neutron irradiation using the $Fe(NO_3)$ standard counts. The results are shown in Figure 7. The scatter of the points around the line is due to variations in weighing the rats which depends on time interval since the occurance of fecal or urinary excretion and water intake.

The ^{37}Ar production increases with body weight as expected but whereas the body weight increased 68%, the ^{37}Ar production increased by 130%. Since the bone mass of rats increases quite proportionally with body weight,[4] the greater increase in ^{37}Ar production was assumed to be caused by increased neutron moderation of the 14 MeV neutrons in the increased amount of body tissue. This reduces the energy of some of the neutrons to an energy where the cross section for the (n,α) reaction is higher than that for 14 MeV neutrons which results in higher ^{37}Ar production. This effect was tested and was confirmed by irradiating a solution of $Ca(NO_3)_2$ with and without a surrounding blanket of 150 grams of paraffin wax. The paraffin wax increased ^{37}Ar production by 40%.

Using this information an irradiation enclosure was designed and constructed for rats which provides a uniform irradiation and activation of the calcium independent of body size. This enclosure is shown in Figure 8, and uses a lucite tube inside a polyethylene block. The rat is placed inside the tube which contains two blocks of polyethylene, one fixed and one adjustable

in height. With the adjustable polyethylene block the rat is compressed into essentially a cylindrical shape with just enough room for breathing expansion. After the irradiation the adjustable block is moved to allow the rat comfortable space during the 1 1/2 hour collection of expired air. With this facility, the neutron moderation and scattering in the space occupied by the rat should be about the same as that in the polyethylene block. When the rat is irradiated equally from two sides by rotating the tube 180 degrees half way through the irradiation, the ^{37}Ar production in all parts of the bone should be constant on a per gram calcium basis. This constant value should be independent of the size of the rat.

To test this method the ^{37}Ar was measured, after irradiation, two times on each of six rats ranging in weight from 205 to 590 grams. The purified expired ^{37}Ar was counted until at least 10,000 net counts were obtained. The rats were then ashed in a furnace. The calcium content of the ash has not yet been determined but the results compared to body and ash weight are shown in Table 2.

The reproducibility of the duplicate ^{37}Ar measurements on each rat are within the range of $\pm 2\%$ which is in agreement with results shown in Table 1. If the method is independent of body size the ratio of ^{37}Ar to Ca content should be constant. From the table it can be seen that the ratio of ^{37}Ar to ash weight is constant for females and is the same for the two male rats but the ratio for male and female rats appears to be different. A relative calcium analysis was performed by activating the ash of each rat with a ^{252}Cf source and then measuring the ^{49}Ca produced. The results show that indeed the calcium content of the male rat ash is about 10% lower than that in the female rat ash. Therefore, it can be concluded that this method can measure the calcium content in a rat of any size within an error of $\pm 3\%$ or better and that sequential measurements on the same rat can be made within 2%. With further practice and improvement of techniques it should be possible to reduce these errors to the $\pm 1\%$ level.

Accurate calcium analyses of the rat ash samples are being done by our colleagues at the Verterans Administration Hospital in Seattle who have studied bone mineral metabolism in rats for many years and routinely perform this type of analysis. The results will not be available for this report but will be included in the last monthly report of this work.

Radiation Dose Required for ^{37}Ar Measurement

In the measurements of ^{37}Ar in a rat, data for which are shown in Table 1, the radiation dose received from the 14 MeV neutron generator was about 11 rad for each measurement. This dose was determined with a 100 cc tissue equivalent ionization chamber. The rat weighed approximately 500 grams and therefore contained about 4 grams of calcium.[4] From these data 5.7 counts per minute of ^{37}Ar are obtained per gram of calcium per rad of radiation dose. If a longer counting time is used the radiation dose can be significantly reduced. For instance, if 10,000 total counts are desired for a 24-hour count a radiation dose of only 0.3 rad is necessary for a 500-gram rat. This dose is much below the level for detectable physiological damage to the rat except for possibly a very, very slight change in opacity of the eye lens.[5] The dose measurement includes that from both neutrons and gamma rays from the neutron generator.

Other Types of Neutron Sources

In the studies described above only 14 MeV neutrons were used. Since the cross section for the reaction $^{40}Ca(n,\alpha)^{37}Ar$ at lower energies appears to be as high and higher than at 14 MeV, other types of sources can be and were used in some preliminary experiments. These include radioactive (α,n) sources such as ^{238}Pu, Be ($\bar{E} \sim 4.2$ MeV) and ^{252}Cf ($\bar{E} \sim 2.3$ MeV). ^{37}Ar has been produced and measured in rats using both of these sources. For small animal studies of calcium content, the lower energy sources appear to be as good or better than 14 MeV neutrons especially when comparing the cost and convenience with that of a neutron generator.

Summary and Recommendations for ^{37}Ar Method for Measuring Total Body Calcium In The Rat

The purpose of this research was to explore the possibility of measuring small changes of calcium in small animals which may be put into space in the forthcoming Skylab orbital space flights. The results of this research show that it is presently possible to measure the total body calcium in live rats of any size with a precision of $\pm 2\%$ and an absolute accuracy of $\pm 3\%$. Future improvements in the method should reduce both of these values to the $\pm 1\%$ level with 95% confidence. This prediction is based on the fact that

the irradiation facility is a first prototype and several improvements are already obvious. In addition the use of (α,n) neutron sources which have a very constant neutron output may further improve the precision and accuracy.

The best use of the accuracy and precision obtainable with this method would be on small adult animals which had reached a constant body size and weight. A total body calcium measurement on such an animal before and after space flight would then definitely determine if more than 2% of the body calcium had been lost. These measurements could be made on an individual rather than a group basis and provide very direct information. Unfortunately the rat does not reach a constant size but seems to maintain a slow growth during its entire lifetime. Therefore, the measurements on rats would probably have to be done on a group basis with the measurement on a group of rats in space being compared with measurements of a group of rats remaining on earth and any calcium loss occurring in the rats in space might appear as a retarded calcium growth when compared to the rats on earth. Since the rat is such a convenient and congenial laboratory animal it is not recommended that it be replaced for this purpose but some consideration should be given to the use of a small animal such as a small breed of dog or cat which appears to attain a rather constant skeletal size.

Before this method is applied for measuring possible calcium loss in animals sent into space, further work should be done to determine if the accuracy and precision can be improved since there is much evidence that improvement is possible. In addition, several groups of rats or other animals should be analyzed with the procedure to ensure the complete efficacy of the method.

TABLE 1. Results from Three Repetitive Determinations of ^{37}Ar Expired from the Same Rat

Run No.	Irradiation Min/Time	Standard Counts	^{37}Ar Counts (Per 100 Min)	Ratio $\frac{^{37}Ar\ Count}{Standard\ Counts}$
1	10	256,610	24,091	0.0939
2	10	261,582	25,173	0.0962
3	10	258,988	24,800	0.0958
			Mean	0.0953
			Range	2.4% or ±1.5%

TABLE 2. ^{37}Ar Produced From Deficient Sized Rats After Uniform Neutron Irradiation

Rat No.	Body Weight	Ash Weight	^{37}Ar Counts Per Hour (Duplicate Results)	Ratio $\frac{\text{Average } ^{37}Ar \text{ Count}}{\text{Ash Weight}}$
1M	590 g	19.17 g	6,912 7,083	36.5
2F	433	15.52	6,221 6,228	40.1
3F	358	13.18	5,300 5,228	40.1
4F	309	12.06	4,955 5,044	41.5
5M	281	8.20	2,900 3,011	36.0
6F	205	7.11	2,888 2,966	41.0

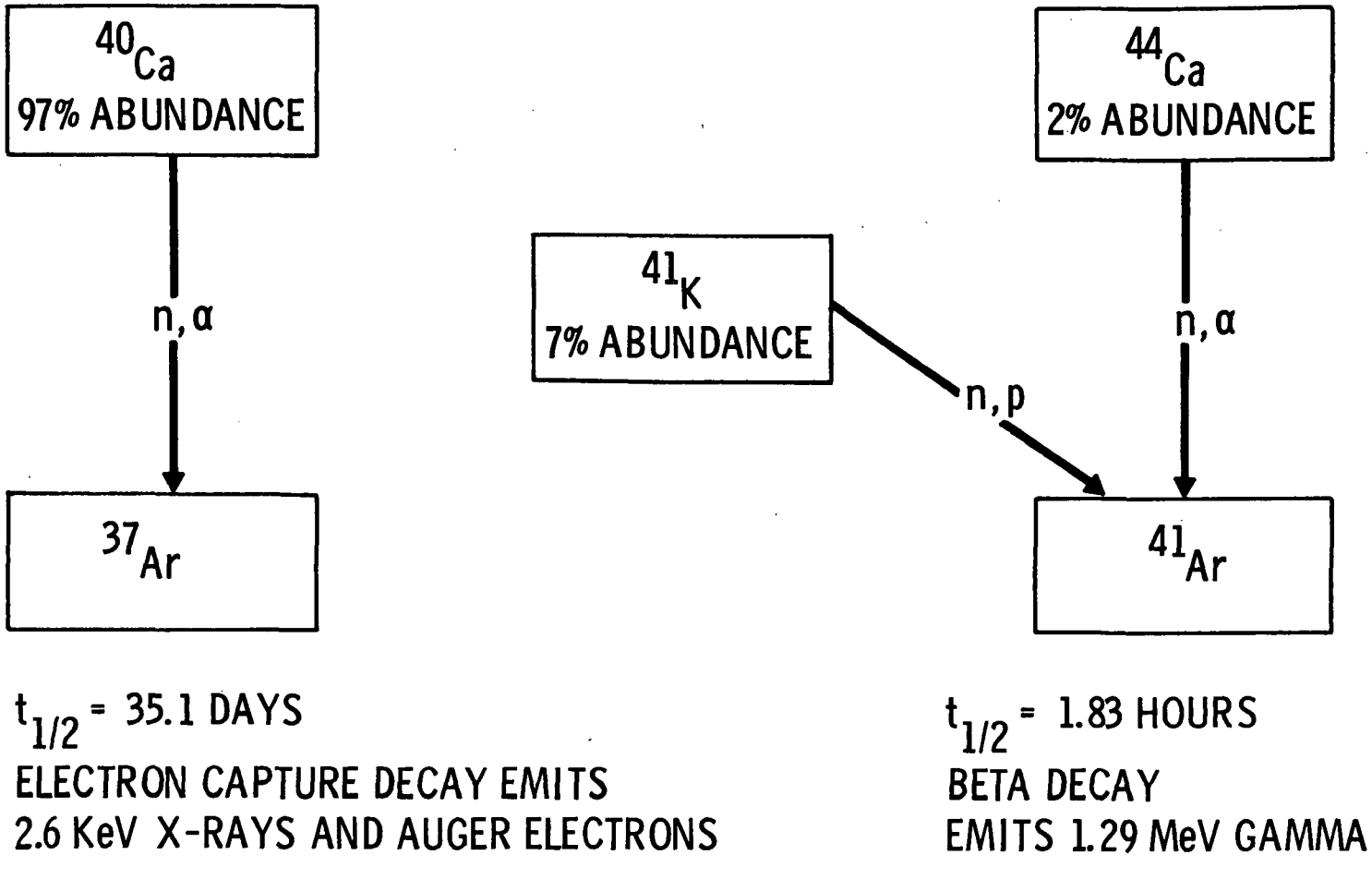

FIGURE 1. Production and Decay of Radioactive Argon

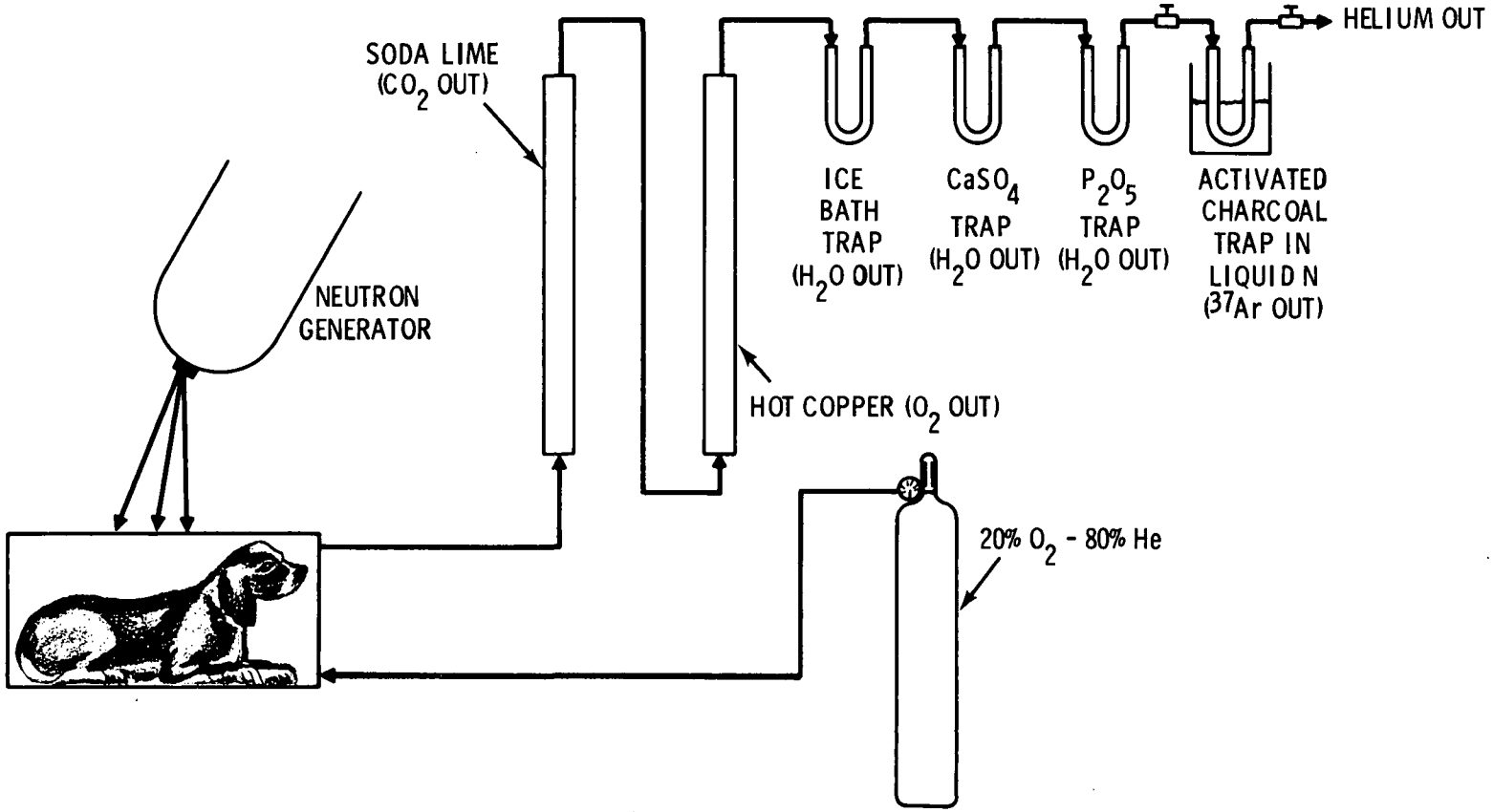

FIGURE 2. Apparatus for Collecting and Purifying ^{37}Ar From Expired Air of Animals

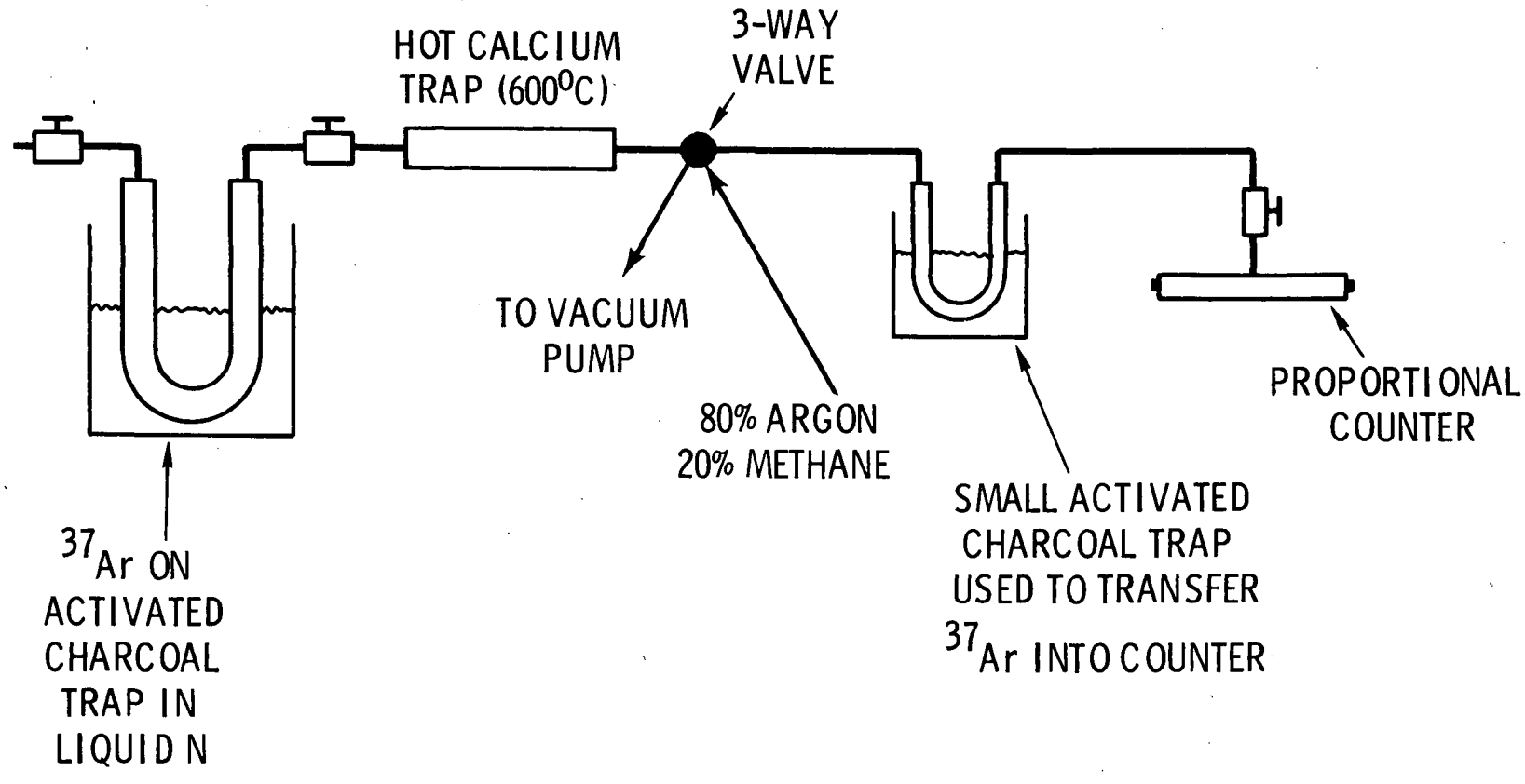

FIGURE 3. Apparatus for Transferring Purified ^{37}Ar from Activated Charcoal Trap into a Proportional Counter

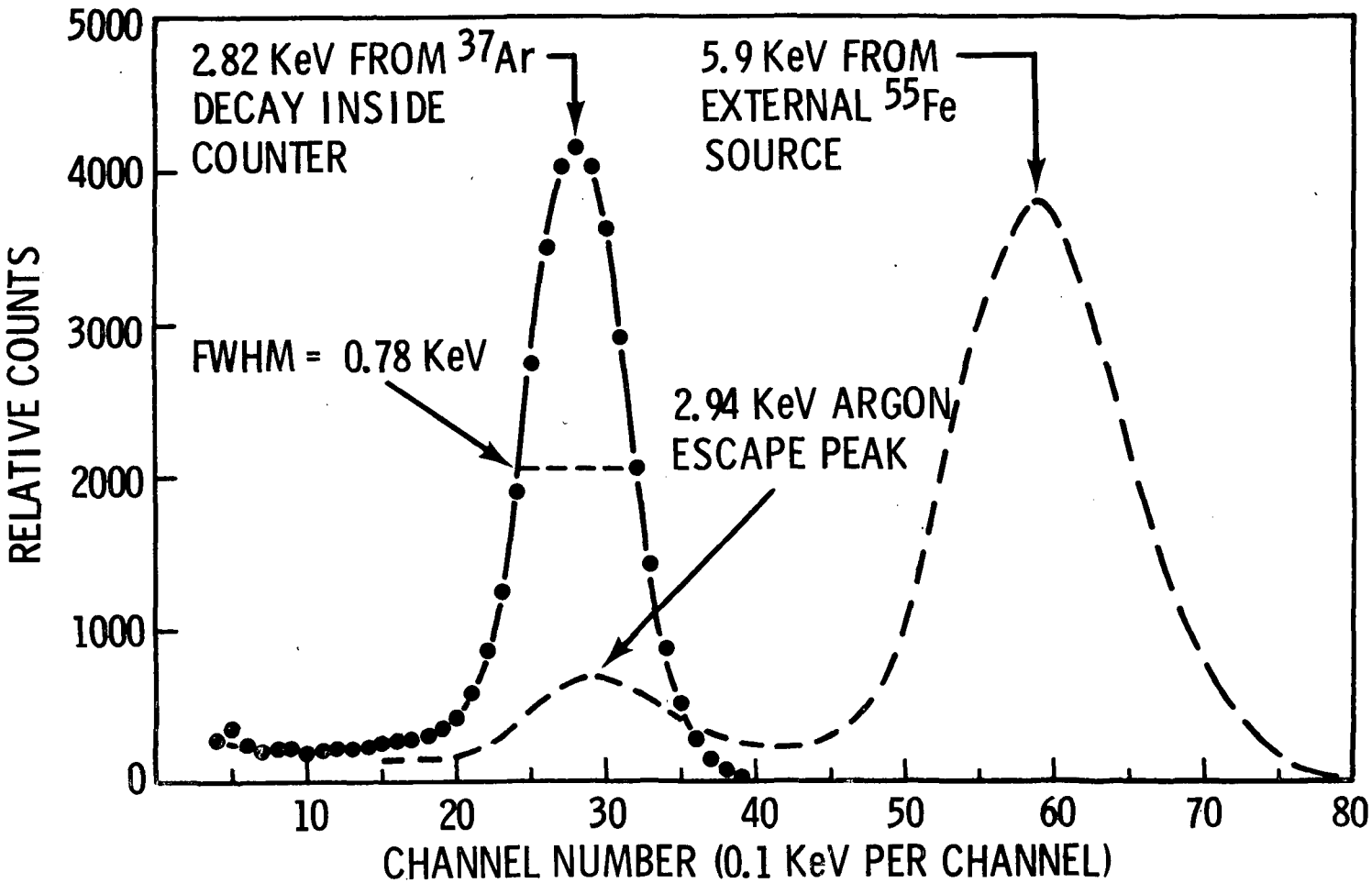

FIGURE 4. Typical ^{37}Ar Proportional Counter Energy Spectrum with ^{55}Fe Spectrum Used for Energy Calibration

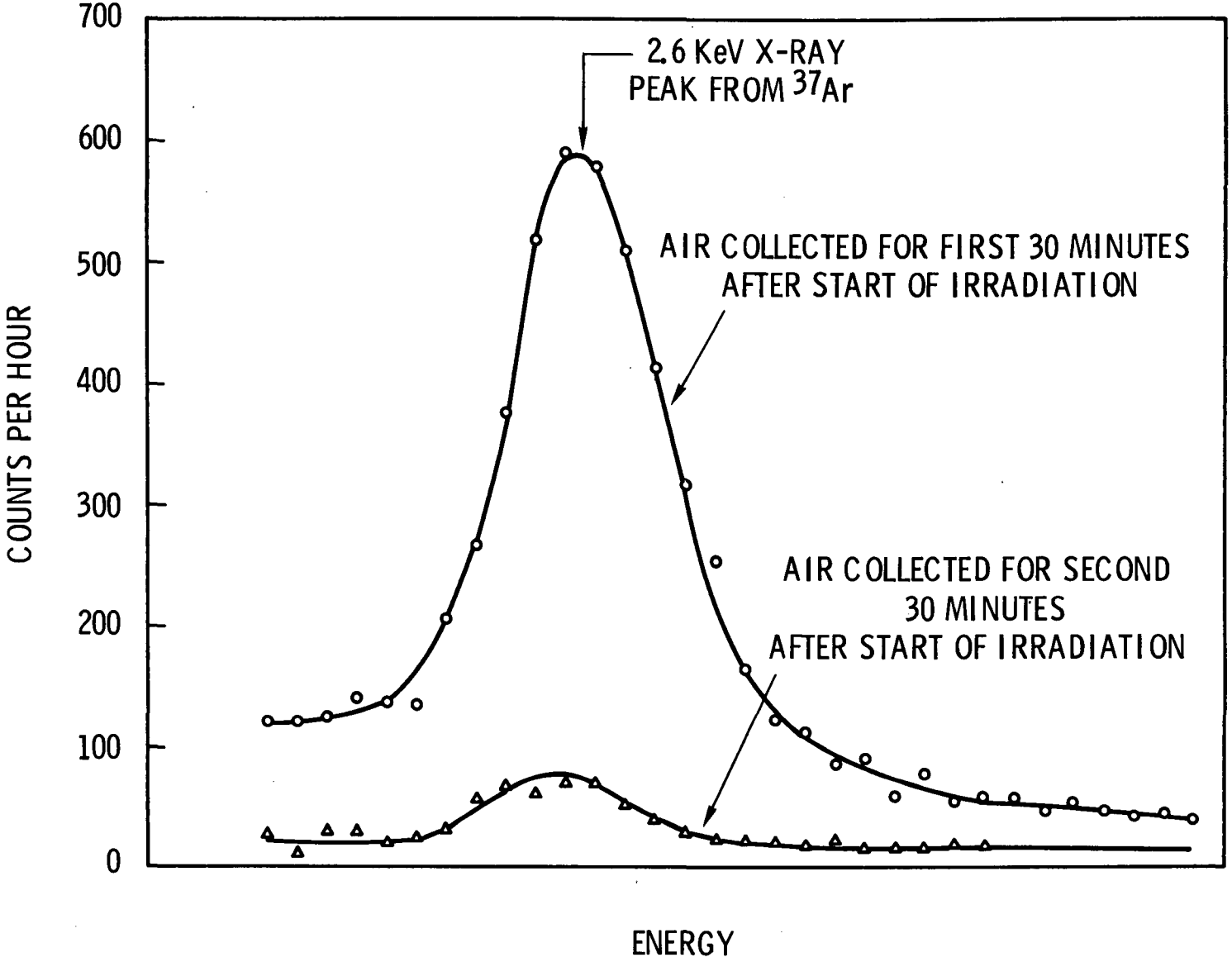

FIGURE 5. ^{37}Ar in Expired Air from a Rat Irradiated With Fast Neutrons for 5 Minutes

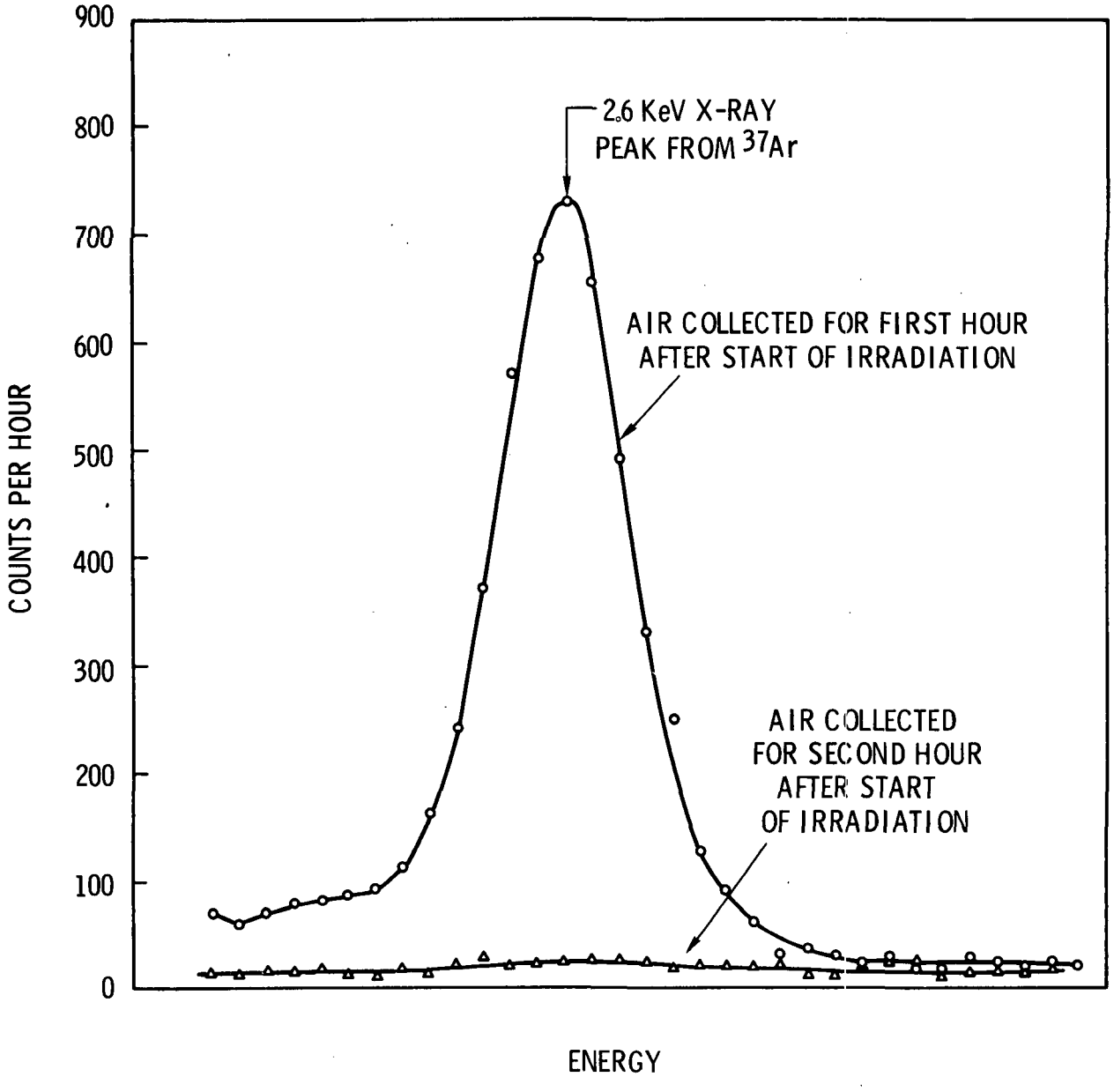

FIGURE 6. ^{37}Ar in Expired Air from a Rat Irradiated with 14 MeV Neutrons for 5 Minutes

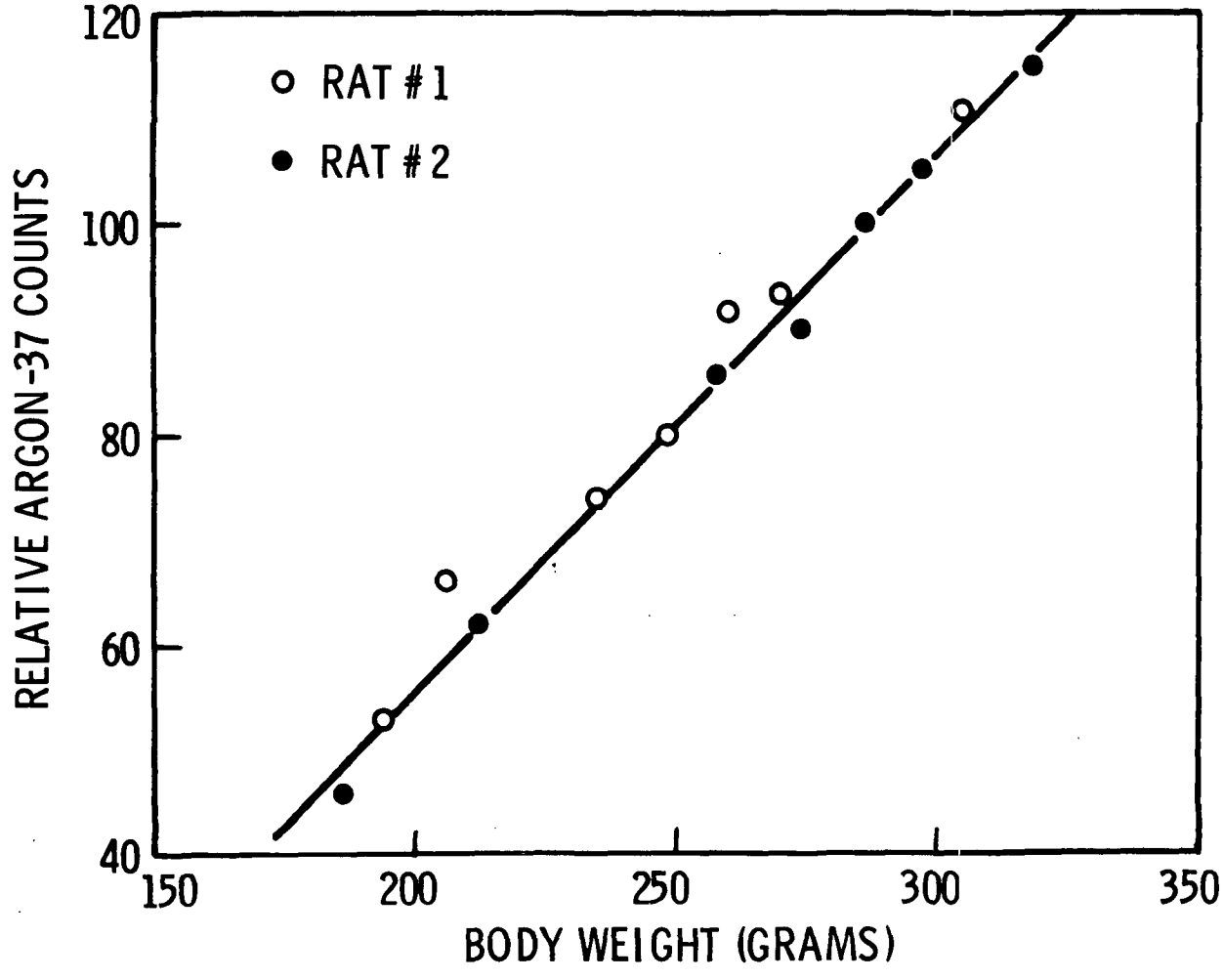

FIGURE 7. ^{37}Ar Production by Neutron Activation vs Growth in Two Rats

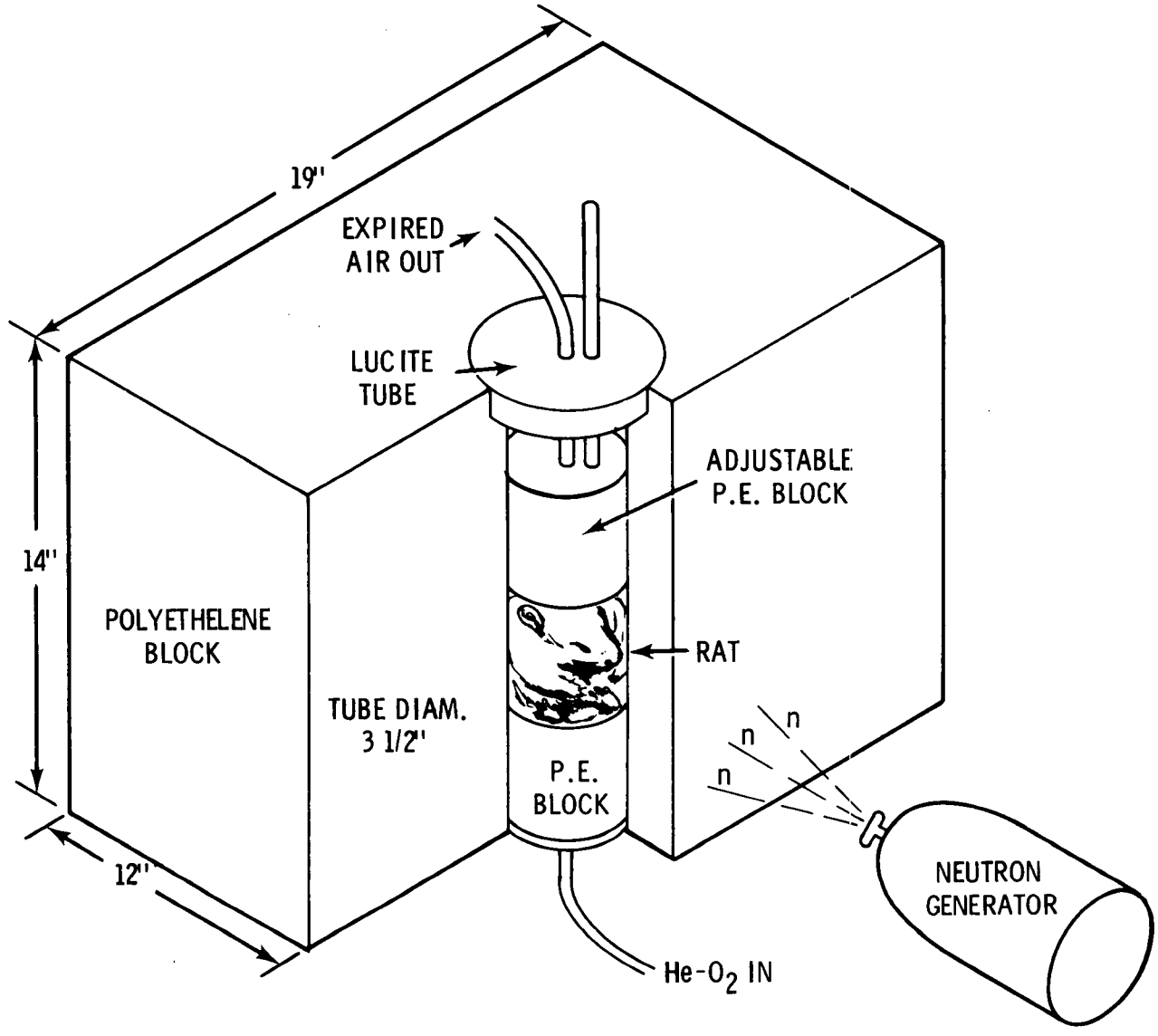

FIGURE 8. Neutron Irradiation Facility for Rats in Determining Calcium from Expired ^{37}Ar

PART II

THE USE OF ^{22}Na IN BONE AS A MEASURE OF BONE MASS CHANGES OR BONE METABOLISM

The elements of calcium, phosphorus, oxygen, hydrogen, carbon and nitrogen make up more than 99% of human bone, but none of these elements have a long lived radioactive isotope which emits gamma rays which can be measured outside the body. Therefore, it is difficult to perform long term studies of bone metabolism using an isotope of these major elements. ^{85}Sr with a 65 day half-life has been used extensively as a substitute for calcium and its metabolism in the body is very similar to that of calcium. In studying bone mineral loss or turnover, ^{85}Sr, like calcium, tends to be reabsorbed after release during bone resorption and this complicates the determination of bone mineral change or turnover.

Sodium is a small (0.06%)[6,7] but constant fraction of bone mineral. ^{22}Na has a radioactive decay half life of 2.6 years and emits 0.51 MeV anihilation photons which can be measured by detectors external to the body. Since most of the stable sodium in the body is in the soft tissues and fluids of the body, when an atom of sodium is released from bone the probability of it being reincorporated back into newly formed bone is very low because of the great dilution by non-bone sodium. Therefore, if ^{22}Na is uniformly distributed in bone and very little exists in soft tissues, the changes in bone mass may be represented by changes in the total body ^{22}Na content. The purpose of this study was to determine if ^{22}Na could be used as a monitor of bone metabolism.

Experimental Plan

Bone resorption rates can be greatly increased in young growing rats by placing them on a calcium deficient diet.[8] Resorption rates 3 to 4 times normal can be caused in younger rats but as the rat grows older only small increases in bone resorption can be produced by calcium deficiency. In this experiment, rat bone was uniformly tagged with ^{22}Na. The rat was then placed on a calcium deficient diet to cause increased bone resorption.

In order to obtain rats with ^{22}Na uniformly distributed in the bone, female rats were raised from conception to about 90 days of age on a nutritionally adequate synthetic diet tagged with ^{22}Na. For comparison, another group of rats were raised from conception to 77 days of age on a diet tagged with ^{85}Sr. The reason for doing this was that the ^{85}Sr closely follows calcium metabolism and as bone is resorbed the ^{85}Sr will be temporarily released but to some extent will be reabsorbed into new bone just as calcium. If this is true ^{85}Sr will not be a good indicator of increased bone resorption. It is expected that ^{22}Na will be released upon bone resorption but will not be significantly reabsorbed into new bone and therefore will be a very sensitive indicator of increased bone resorption.

Procedure

Three adult Wistar descendant female rats were bred and started on a diet tagged with ^{22}Na and two female rats were bred and started on a diet tagged with ^{85}Sr. From the litters 18 female rats on ^{22}Na tagged food and 10 female rats on ^{85}Sr tagged food were selected for feeding to adult size. Unfortunately the rats became stunted in their growth from what is thought to be a lack of vitamins in the commercially prepared synthetic diet. All rats were similarly effected and they began growing more rapidly when vitamins were added starting at about 45 days of age. At 90 days of age the ^{22}Na rats averaged 118 grams and the ^{85}Sr rats averaged 136 grams whereas normal female rats would be 225 grams at this age. The rats showed no other adverse symptoms and none of them died before the end of the experiment. The stunted growth probably did not effect the primary purpose and results of the experiment.

Figure 9 shows the grouping and diet schedule for the rats raised on ^{22}Na and ^{85}Sr tagged food and at a later time on calcium deficient food. After the radioactivity was removed from the diet, it was allowed to leave the fluids and soft tissues of the body before any measurements were made. This required only a few days for the ^{85}Sr but required about 9 to 10 weeks for the ^{22}Na.

Group 1 was maintained on a nutritionally adequate synthetic diet and was used for a control group for groups 2 and 3 in the ^{22}Na experiments. Group 3 was placed on a calcium deficient diet at 13 weeks of age on the same day as the radioactive food was stopped. Group 2 was maintained on an adequate diet until 23 weeks of age when they were placed on a calcium deficient diet.

Group 4 was maintained on a nutritionally adequate diet and was used as the control group for group 5 which was placed on a calcium deficient diet at 12 1/2 weeks of age and 10 days after the end of the ^{85}Sr diet.

The radioactivity tagged food contained 0.060 μCi of ^{22}Na per gram of food for the ^{22}Na studies and 0.014 μCi of ^{85}Sr per gram of food for the ^{85}Sr studies. The average amount incorporated into bone of each rat was at the end of the radioactive diet was about 0.14 μCi of ^{22}Na in groups 1, 2 and 3, and 1.68 μCi of ^{85}Sr in the bone of each rat of groups 4 and 5. This provided plenty of activity and allowed short accurate measurements.

Whole Body Counting Results

After allowing seven days for the ^{85}Sr to clear the gastrointestinal tract the rats in groups 5 and 6 were whole body counted once each week between two large 4 inch thick and 9 3/8 inch diameter NaI(Tl) scintillation detectors. They were placed inside a tube which restricted their movement and allowed reproducible counts to be made. Initial counts before and after thorough bathing of the rats showed that there was no significant external contamination and later, disection and counting of bone, soft tissue, and skin showed that all the measured ^{85}Sr was in the bone. The whole body counting results for the ^{85}Sr tagged rats were shown in Figure 10, and they indicate that even though increased bone resorbtion occurred in these growing calcium deficient rats the ^{85}Sr content remained the same as that of the calcium adequate rats apparently because the rats became very efficient in resorbing the ^{85}Sr back into newly formed bone. Therefore it appears that changes in ^{85}Sr levels in bone cannot be used as an indicator of bone resorption especially in cases of calcium deficiency.

At the end of the ^{22}Na diet the body burden of the ^{22}Na in the rats was mostly contained in the soft tissues and therefore periodic counts on rats in groups 1, 2, and 3 were not started until near the end of the soft tissue clearance which occurred with a biological half life of about 7 days. Starting at the fifth week after the end of the ^{22}Na diet one rat from each group was counted each week. Counts before and after bathing indicated there was no significant external contamination. The counts on the three rats were normalized at this first count and the results from subsequent weekly counts are shown in Figure 11. The soft tissue clearance is not complete until about the tenth week after the end of the ^{22}Na diet. After this period the

relative ^{22}Na content in the calcium deficient rat was 41% lower than the average of two rats on the calcium adequate diet. This would indicate that the bone resorption during this period in the calcium deficient rat was about 2 times that of the rats on a normal diet.

At 7 weeks after the end of the ^{22}Na diet all six rats of each group were counted each week. The results were normalized to the seventh week measurement and all shown in Figure 12. Again the ^{22}Na loss is significantly greater in the calcium deficient group but the difference is not as great as in the single rat comparison because of the increased age of the rats. The increased bone resorption due to calcium deficiency is very age dependent and varies from several hundred percent for rats a few weeks old to just a few percent for the more adult rats. This is also shown in Figure 12 where rats in group 2 were put on a calcium deficient diet at 11 weeks after the end of the ^{22}Na diet (24 week old) and the ^{22}Na loss did not increase compared to group 1. This part of the experiment was terminated at 18 weeks after the end of the ^{22}Na diet when it appeared that the rate of loss of ^{22}Na in the calcium deficient groups was no greater than that in rats on a calcium adequate diet.

^{22}Na Loss During Pregnancy and Lactation

Another experiment was performed on the group 1 rats which had been fed an adequate diet since birth. At 30 weeks of age, two of the females were bred. As soon as conception was evident these two rats were placed on a calcium deficient diet. Three other rats of group 1 were used as controls and maintained on the calcium adequate diet. The two rats were bred in attempt to cause a loss in their total body calcium as the calcium is transferred from the mother to meet the calcium requirements of the fetus and suckling rats under calcium deficient conditions. During such a transfer of calcium the ^{22}Na content of the mother rat should be decreased. One of the mother rats bore a litter of eight and the other a litter of four. The young rats were left with the mother for 5 weeks after birth.

Although ^{22}Na counts on the mother rats were made at weekly intervals, the results were rather meaningless since during the gestation period they greatly increased in weight and their counting efficiency changed. Therefore only counts obtained after birth were used. The whole body counting results

are shown in Figure 13. During the gestation and lactation period the control rats lost 10% of their ^{22}Na due to normal calcium turnover from their continued slow growth. The mother of eight rats lost 25% of her ^{22}Na and the mother of four lost 13% which indicates that there was an increased calcium loss from the mother rats and a corresponding decrease in ^{22}Na.

Summary and Recommendations for ^{22}Na Studies

From the studies described above, it is obvious that there is an increase in ^{22}Na loss from the rat bone when conditions are imposed on the rat which cause increased bone resorption. How closely this follows bone resorption is not known but it appears to be a much better indicator of bone resorption and bone loss than strontium and probably calcium isotopes.

Although this study was conducted to determine the usefulness of ^{22}Na in measuring possible bone mass decrease in animals in space flight, it may be useful in many other areas of bone mineral metabolism research. Further studies need to be done to more firmly evaluate this technique for these uses. Three suggested future studies are listed below:

1. Grow rats for a shorter period of time on a ^{22}Na diet (about 4 weeks) then cause the soft tissue ^{22}Na to be more quickly eliminated by increasing the NaCl content in the diet. This would allow the high bone resorption at early ages to be correlated with the ^{22}Na loss under calcium deficient conditions.
2. Grow rats for a longer period of time on ^{22}Na diet. Cause ^{22}Na to be more quickly eliminated as above. Breed rats and place them on calcium deficient diet. As calcium is transfered from mother to infant, compare the ^{22}Na loss with the calcium loss as measured by the ^{37}Ar techniques described in Part I of this report. The results of this study would be more applicable to animals placed in space flight than the previous suggested study.
3. The use of ^{22}Na may be applicable to measuring bone mass loss occurring in astronauts during space flight. Although an astronaut cannot be grown to adulthood on a constant ^{22}Na diet, if after space flight the astronaut was given some ^{22}Na, some of it would be deposited in bone during the next few weeks as the bone which

was lost during space flight is replaced. So instead of measuring bone loss the replacement bone increase would be measured. Since Na is laid down in bone in an exact fraction of that of other bone mineral components the amount and the exact location of the replacement bone might possibly be measured. The long half life, and the positron decay make ^{22}Na very ideal for this purpose, and replacement in such bones as the lower vertebrae could easily be detected and measured. These types of studies could initially be done on dogs by measuring the ^{22}Na taken up in broken or partially sawed bones.

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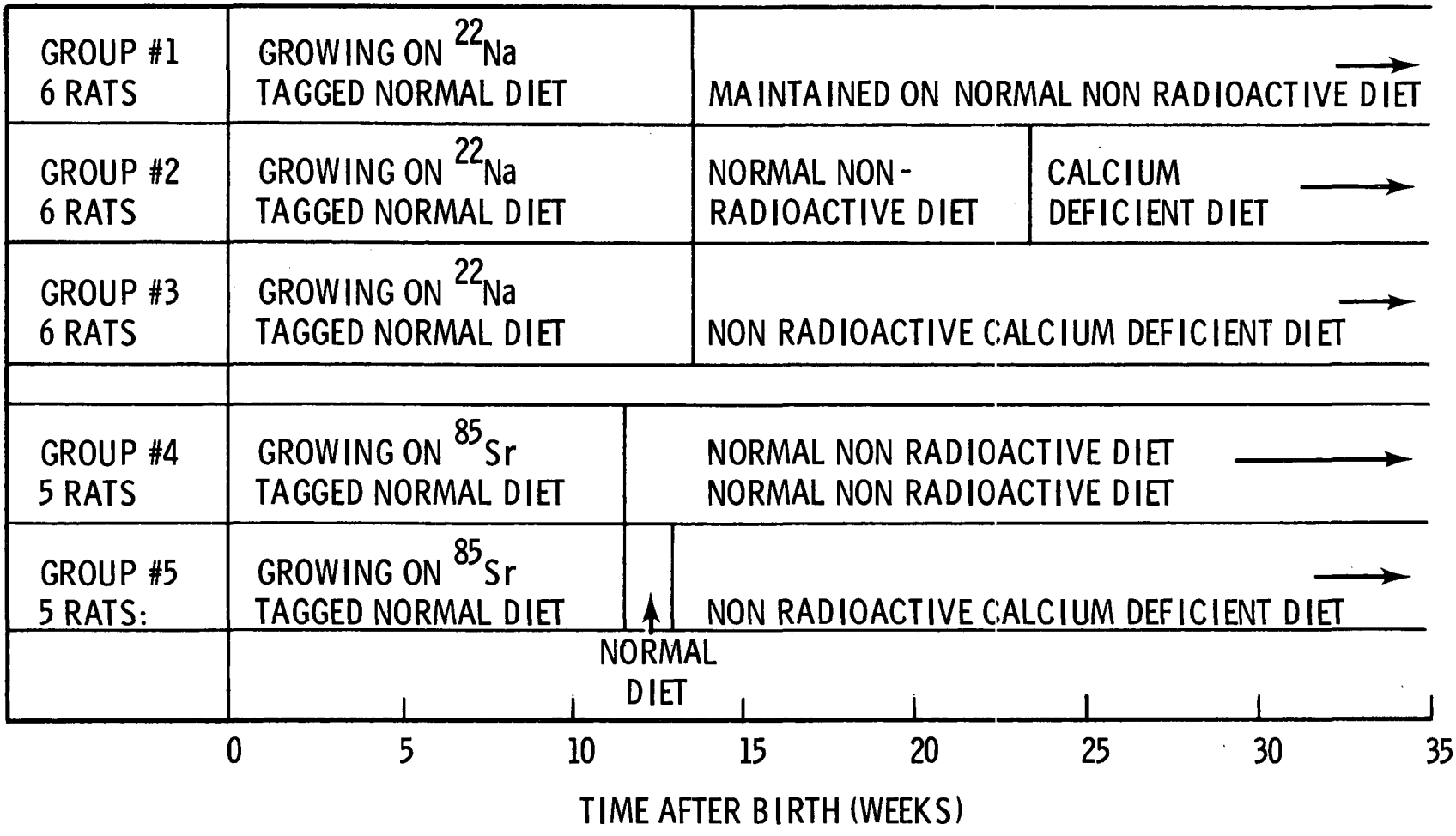

FIGURE 9. Diet Schedule for Obtaining Rats with ^{22}Na and ^{85}Sr in Bone and Producing Calcium Deficiency

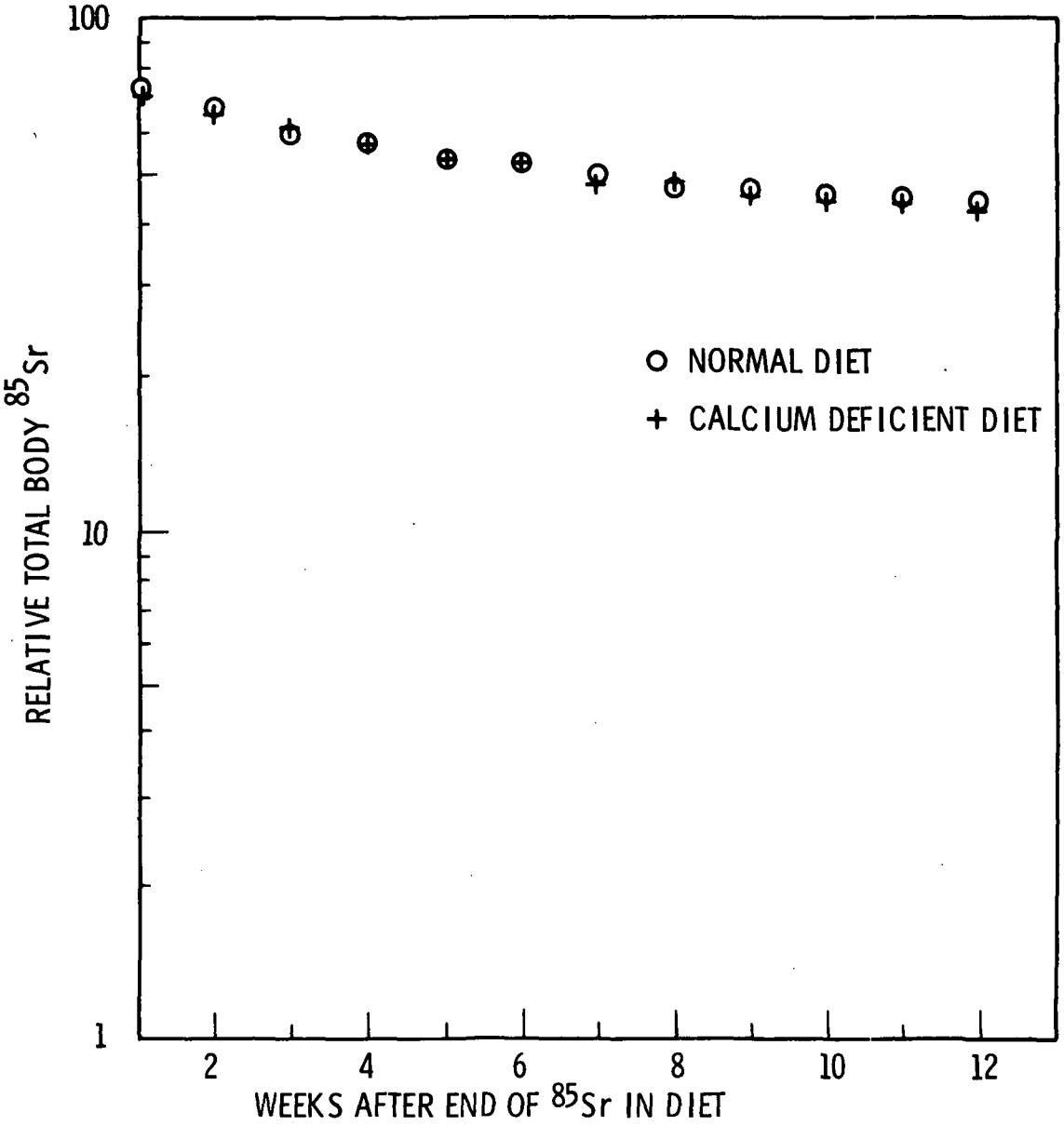

FIGURE 10. Comparison of ^{85}Sr Levels in Calcium Deficient and Normal Rats Starting With 14 Week Old Rats

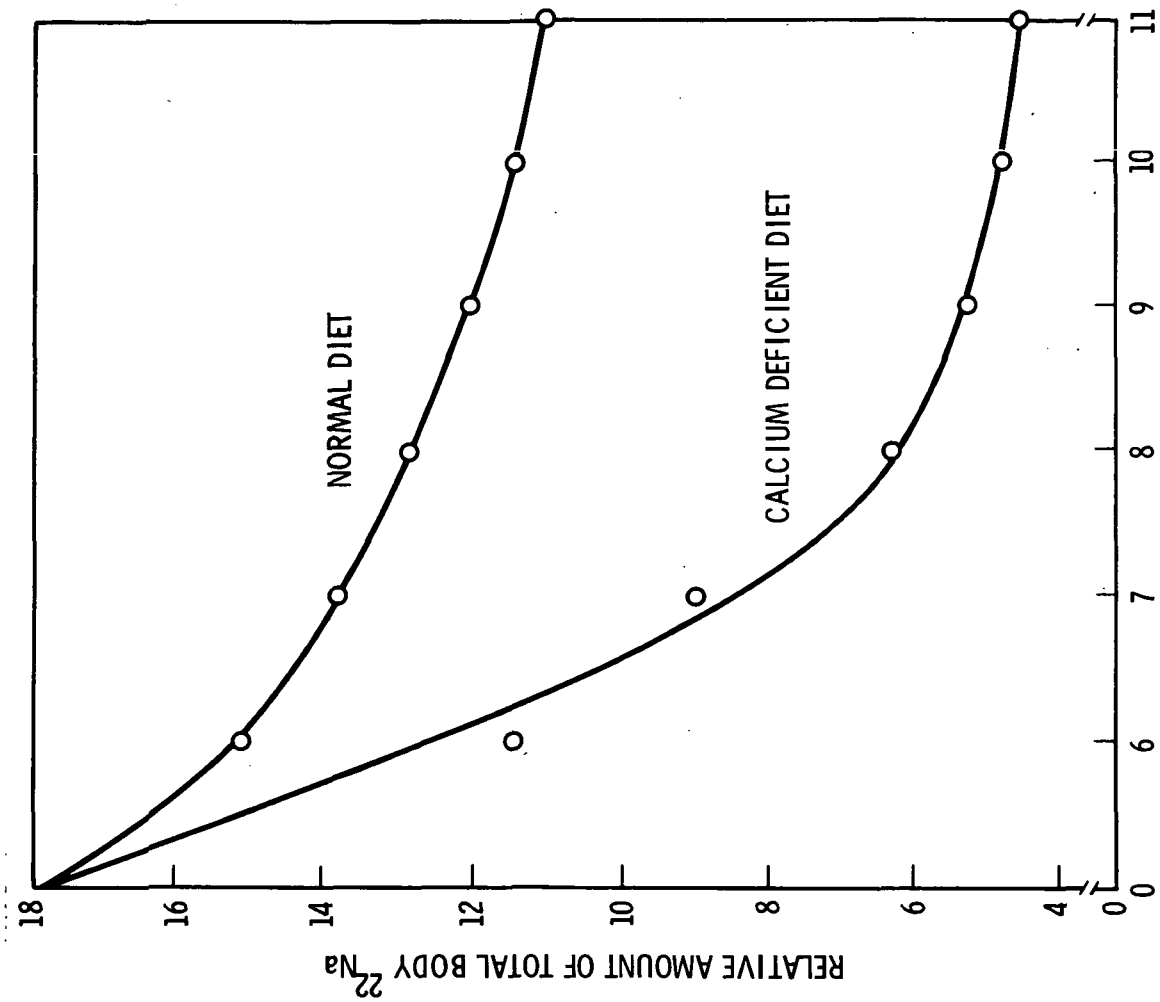

FIGURE 11. Comparison of ^{22}Na Levels in Calcium Deficient and Normal Rats Starting With 18 Week Old Rats

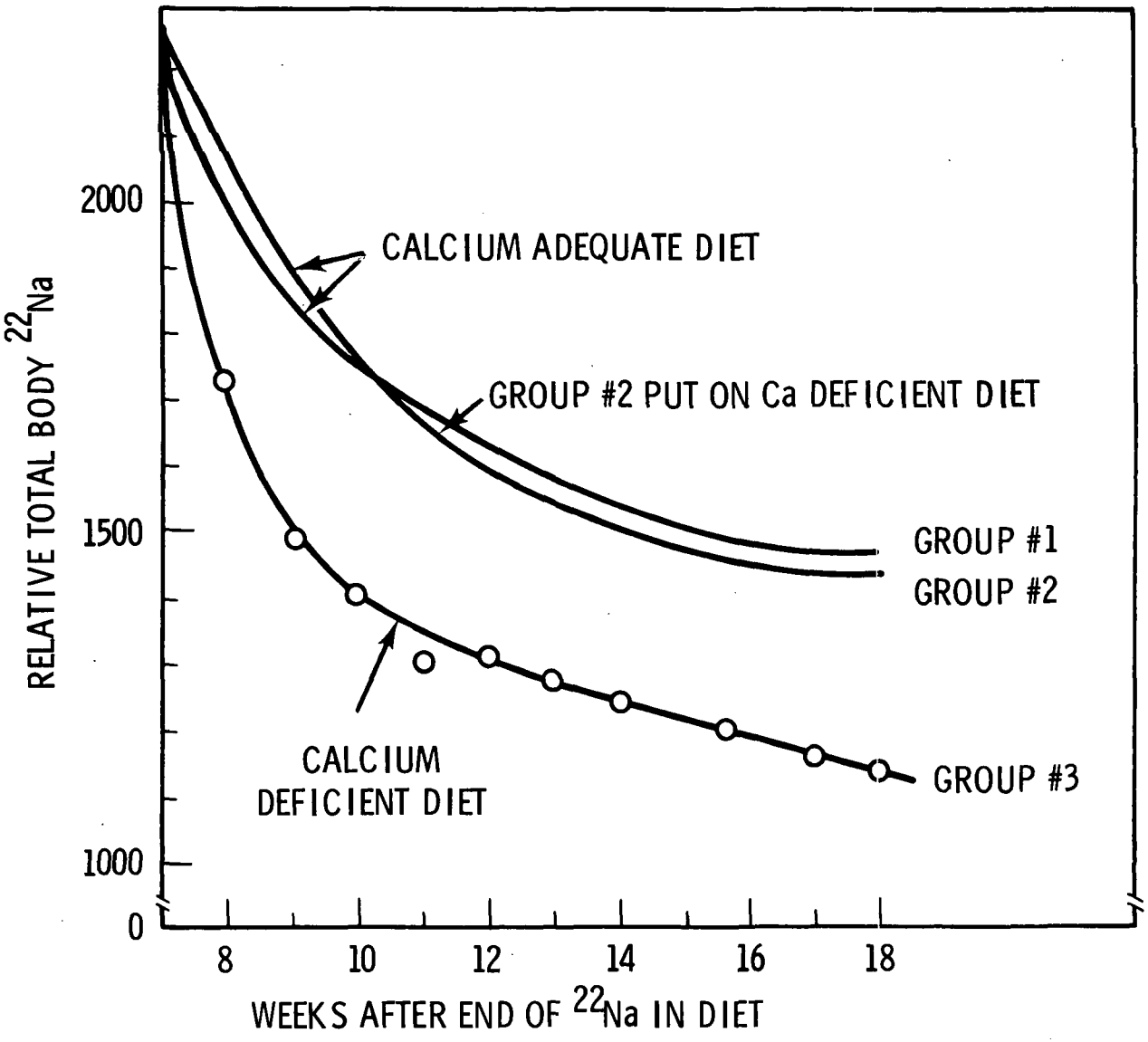

FIGURE 12. Comparison of ^{22}Na Levels in Calcium Deficient and Normal Rats After 20 Weeks of Age

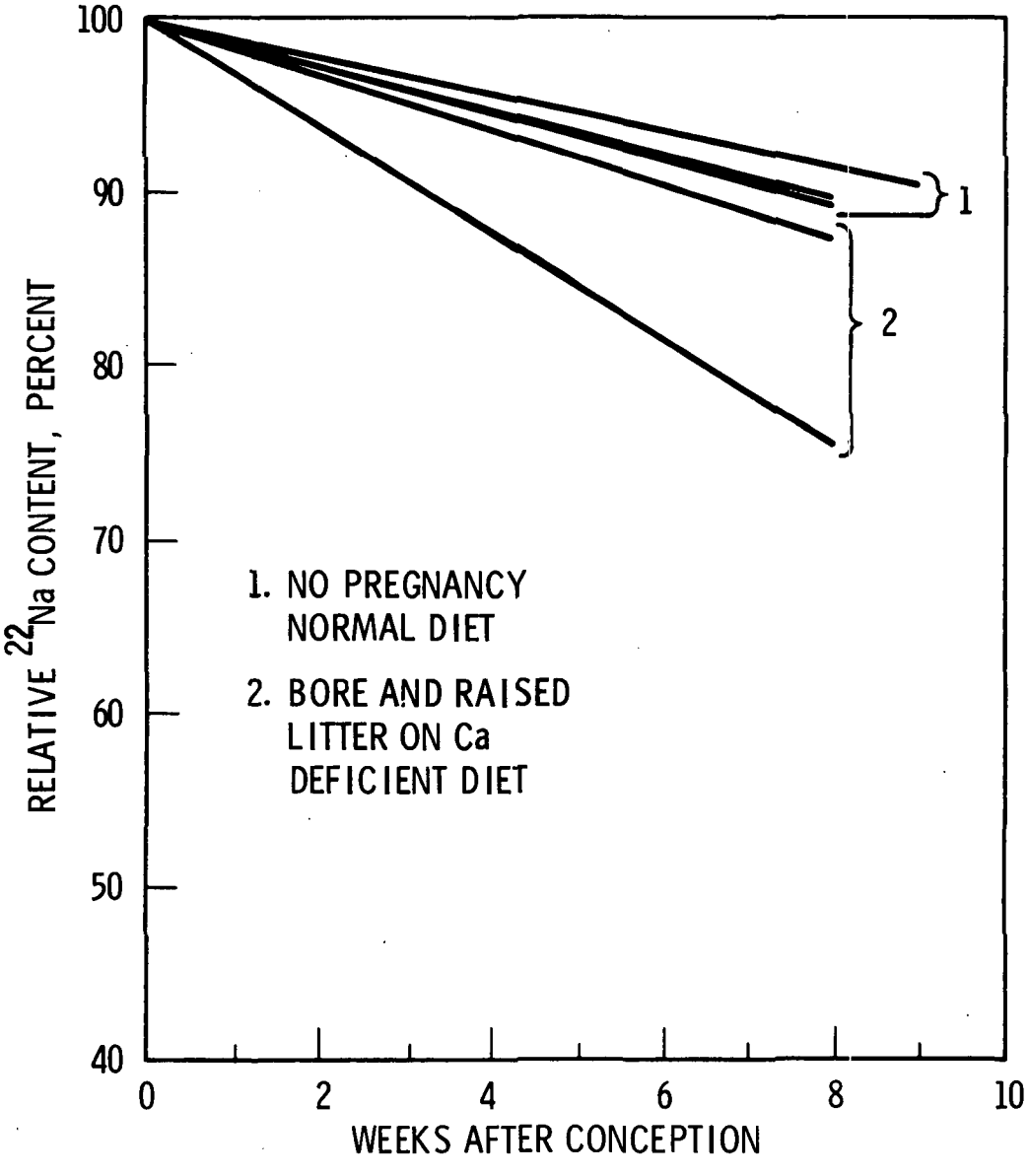

FIGURE 13. Effect of Pregnancy and Lactation on Total Body ^{22}Na Content

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