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Final Report

Investigate Methods for Measuring Muscle and Bone Mass Changes in Astronauts and Animals which Occur During Space Flight

December 1, 1976 to August 31, 1977
NAS 9-14248

to
National Aeronautics and Space Administration
Lyndon B. Johnson Space Center
Houston, Texas

by
H. E. Palmer

August 31, 1977

Battelle
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ABSTRACT

Sodium-22 is being studied as a tracer for bone mineral metabolism studies. Dogs are being grown from puppies to adulthood on a diet containing a constant level of ^{22}Na in order to uniformly tag the entire skeleton with a long-lived radionuclide. This study is still in progress and the dogs are still growing. Potassium-40 measurements were made on people who are replacing muscle mass lost due to leg injuries. It appears that ^{40}K measurements do provide an accurate and convenient method for determining relative changes in the muscle content of the leg. The results of a third study show that ^{11}CO, which is formed from nitrogen in the body of a dog during fast neutron irradiation, is excreted too slowly to be used as a measure of total body nitrogen.

INTRODUCTION

This report describes further work in the development of methods for determining changes in bone mineral and muscle mass which may occur in astronauts and animals during space flight. Most of the funding of this period of study was devoted to raising beagle dogs from puppy age to adulthood. The time required to daily mix the radioactivity with the food has increased the animal care charges to about three times normal. This higher charge rate will continue for seven more months, at which time the ^{22}Na diet will be discontinued. Since this study is still in progress and all the measurements will be made during the latter part of the study there are no results available for this report.

The studies of ^{40}K and ^{11}C measurements for determining total body nitrogen or muscle mass have been completed during this reporting period.

THE USE OF ^{22}NA AS A TRACER FOR LONG-TERM BONE MINERAL LOSS AND TURNOVER STUDIES

Background

Recent studies[1] have shown that sodium is a small but definite and constant component of bone mineral in both cortical and trabecular types of bone (see Appendix 1). The ratio of sodium to calcium or phosphorus in bone is constant and therefore a study of the metabolism of sodium in bone should provide information on total bone mineral kinetics. Long-term radioactive isotope tracer studies of bone mineral loss or turnover is not possible using calcium or phosphorus because the radioactive half-lives of isotopes of these two elements are too short and also because these two bone minerals are partially recycled into new bone during bone resorption. Certain isotopes of strontium are useful as bone mineral tracers but strontium also is partially recycled during bone resorption and the half-life of the gamma ray emitting nuclide ^{85}Sr is only 65 days.

Sodium-22 has a decay half-life of 2.6 years and when it is released from bone during resorption it is greatly diluted by the large amount of stable sodium in soft tissues and fluids and only an insignificant amount is recycled into newly forming bone. Because of these characteristics it appears that ^{22}Na can be uniformly deposited into the entire skeleton of an adult animal by feeding the animal a diet containing a constant amount of ^{22}Na from birth to adulthood. Just prior to space flight, the ^{22}Na diet is stopped and the ^{22}Na is rapidly removed from the soft tissues leaving only that ^{22}Na deposited in the bone (see Appendix 1). The release rate of the ^{22}Na from the whole skeleton or any part of the skeleton can be monitored in flight with a simple NaI(Tℓ) scintillation detector. The release rate of the ^{22}Na is proportional to the bone resorption rate and the changes with time can be compared to measurements made on similar animals kept on Earth. The results will provide relative comparisons of bone loss among different parts of the skeleton and with further development should provide actual grams of bone which have been lost or turned over. Excellent results should be obtained from parts of the skeleton such as the vertebra which are very difficult to obtain by other methods.

If bone sodium can be used to study bone mineral loss and turnover rates the use of ^{22}Na as a tracer will allow measurements to be made which have not been done before. Recent experience indicates that it is difficult for investigators in bone mineral kinetics to consider changes in bone sodium as a valid indication of bone mineral changes. Our experience indicates that the use of bone sodium is as valid as calcium or phosphorus and actually provides more information because the sodium is not recycled into new bone. Other information in Appendix 1 shows that ^{22}Na does stay in the bone of an adult human for very long periods of time and that the bone mineral turnover rates of the various types of bone in the body vary from a few weeks to many years as one would expect. The curve on ^{22}Na retention in human bone does not provide information on the size of the bone pool at each turnover rate since the bone was not uniformly tagged with ^{22}Na. By uniformly tagging the bone with ^{22}Na from birth both the turnover rate and the fraction of bone involved at that rate could be determined on the whole skeleton or any region. This type of information has never been obtained before and in addition to providing valuable information on space flight effects it would provide new important information on basic bone mineral kinetics for use in medical applications.

Growing Dogs on ^{22}Na Diet

Seven female beagle puppies were selected for the study and at ten to twelve weeks of age six of the puppies were started on the special diet containing ^{22}Na. The other puppy was not started on the ^{22}Na diet until her weight reached 8 kg at twenty weeks of age. This was done to determine if the ^{22}Na would completely equilibrate in the skeleton during only the last half of her growth.

The food consisted of a low sodium dietary food for dogs which contained only 0.01% Na. At feeding time a solution of Na Cℓ containing a fixed amount of ^{22}Na was thoroughly mixed into each pound of food which brought the Na content of the food up to 0.12%.

The ^{22}Na content in the food was 0.5 μCi per gram of Na or 0.28 μCi per pound of food. Assuming that a 15 kg beagle dog will have about 4 grams of non-exchangeable Na in the skeleton and allowing the decay of the ^{22}Na

during growth, the skeletal burden at eighteen months should be 1.6 μCi.

At the end of this reporting period, August 31, 1977, the dogs will be eleven months old. The ^{22}Na diet will continue until the dogs are about eighteen months old and have attained a mature and non-growing skeleton. At that time, the ^{22}Na will be removed from the diet and the rapid removal of ^{22}Na in the soft tissues will be caused by feeding a high Na Cℓ content diet for about one week. The ^{22}Na in the bone will be measured periodically in the dogs. Two of the dogs will be fed a diet of unsupplemented meat and liver which will induce nutritional secondary hyperparathyroidism[2] which will produde a 20 percent loss of bone mineral in the vertabrae and a 13 percent loss in the long bones over a twelve-month period. Two other dogs will have a front leg placed in a cast which will induce osteoporosis of the leg bones due to disuse. The other three dogs will be used as controls and will have a normal diet and activity. The measurements of ^{22}Na in the dogs will include both total body and individual bone content. Changes in total body calcium will alsc be made by in vivo neutron activation analysis. The time schedule for completing this study is eighteen to twenty months after the end of this reporting period, or about February 1979.

At the beginning of this study it was anticipated that two of the dogs would remain on the ^{22}Na diet for an indefinite period so that they might be used in Spacelab Life Sciences experiments. However, since the first dedicated life sciences pressurized experiment is not scheduled to start until 1981, it now seems more reasonable to raise another set of dogs for this purpose if the ^{22}Na proves to be a successful tracer for bone mineral loss.

THE MEASUREMENT OF THE ^{40}K CONTENT IN THE LEG WITH INCREASING MUSCLE MASS

In a previous report on this study[3] the measurements on the injured legs of people which had been placed in casts for six weeks or longer were described. The measurements were made for a period of six months or more after the casts were removed. Leg muscle lost during the period of inactivity was replaced as the leg regained its normal use. This study was completed on four people and the results are shown in Figures 1, 2, 3, and 4. The error bars on the curves in these figures are the standard deviations of the net counts and do not represent errors due to repositioning.

In Figure 1, the person had a very normal recovery and a 25 percent increase in ^{40}K was measured. This is equivalent to an approximate 20 percent loss of original muscle mass. In Figure 2, the very large increase can be explained by the fact that the person was young and very slender at the time he fractured his lower leg. After healing, his muscle developed far beyond its original size as he became more mature and began working at a job requiring physical labor. In Figures 3 and 4, the people had complications in the healing of their injured legs and completely normal use of the leg had not been attained at the end of their measurements. The ^{40}K counts in Figures 3 and 4 show a slower and smaller increase than that found in the measurements shown in Figures 1 and 2. For the first three weeks after the leg was removed from the cast the ^{40}K content was the same or slightly less in most cases. This is due to soreness of the leg during this period and sustained use of the leg is not sufficient to rebuild, and in some cases, maintain leg muscle content.

Although these relative measurements do not provide quantitative results on the muscle mass change, they do provide a percent change which appears to follow the actual muscle content of the upper leg. It appears that this method could be used to measure the relative muscle content in the legs of astronauts before and after space flight.

The large NaI(Tℓ) detectors used for these measurements were satisfactory but were not of optimum shape. A large annular shaped detector in which the leg or arm can be placed through the center would provide greater precision

and sensitivity than that shown in Figures 1 through 4. If this type of detector were placed in the whole body counting facilities at the Johnson Space Center, any muscle mass changes exceeding two percent could be measured in the upper legs of adult humans and any change exceeding three percent could be measured in the arms and lower legs.

THE EXCRETION RATE OF ^{11}C FROM THE DOG AFTER FORMATION FROM BODY NITROGEN DURING FAST NEUTRON IRRADIATION

Previous studies with rats[4] have shown that the ^{11}CO produced after neutron irradiation is expired reproducibly and in sufficient time to be measured and related to the body nitrogen content. Subsequent studies on humans showed that the ^{11}CO was expired too slowly and most of the 20.4 minute half-life ^{11}CO decayed away before being expired. Since the dog may be used in future space flight experiments, a study was made to determine if the ^{11}CO is excreted from the dog as rapidly as it is from the rat.

An adult beagle dog was irradiated for five minutes with 14 MeV neutrons. The ^{11}CO was collected for three consecutive fifteen-minute intervals and measured. The rate of excretion was found to be similar to that for humans and is too slow for this method to be of any use in determining the total body content of nitrogen. The rate of ^{11}C excretion after correcting for radioactive decay was approximately the same for each of the fifteen-minute collection periods, and was much lower than expected from the extrapolation of the results obtained with rats. It appears that most of the ^{11}CO decays within the body before it can be excreted and measured. The experiment was repeated and the results were confirmed. The physiological differences between the rat and man and dog which produce the significantly different excretion rate of ^{11}CO are not understood at this time. However, there are no plans for further research of the ^{11}CO method for total body nitrogen determination.

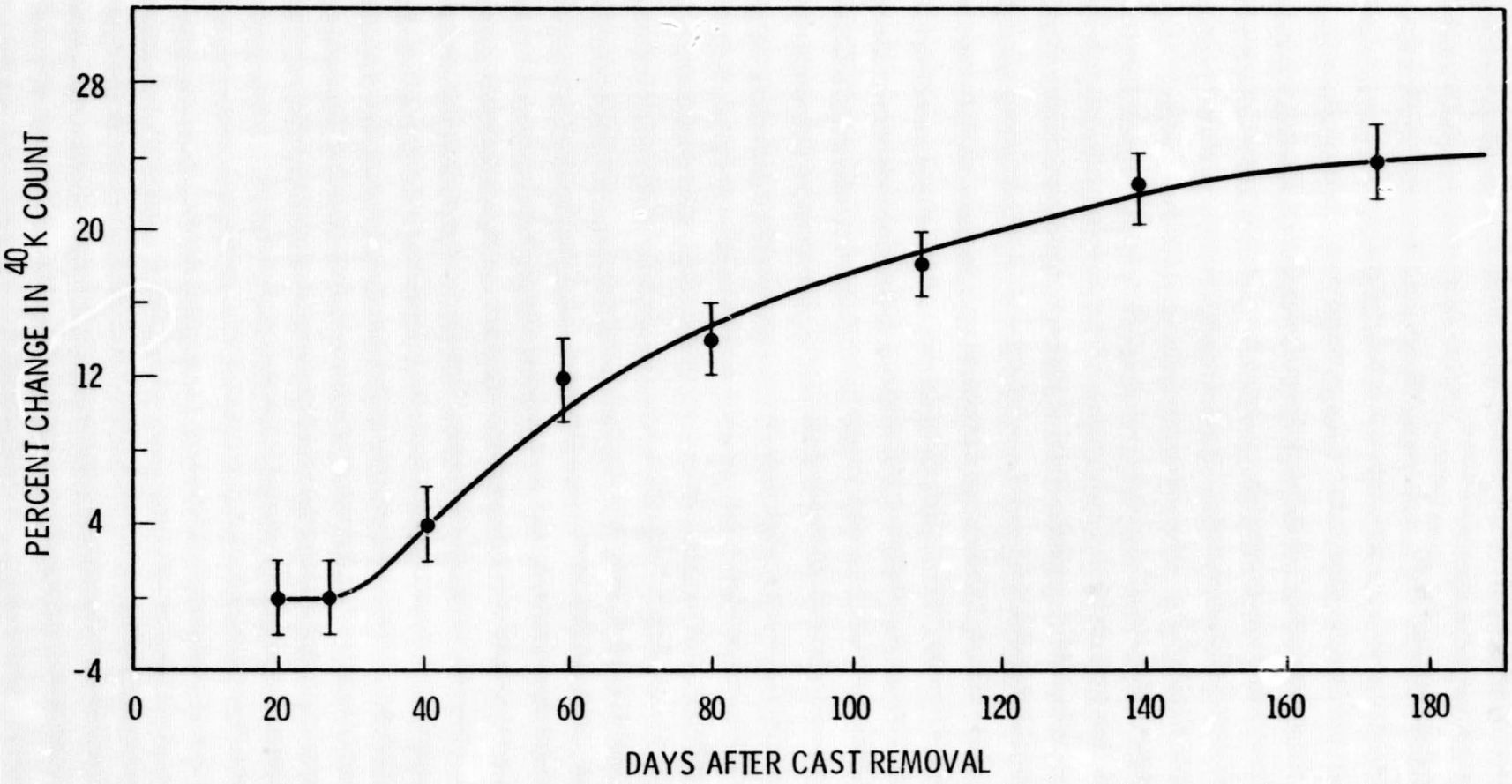

FIGURE 1. ^{40}K Content in Upper Leg of Patient #1.

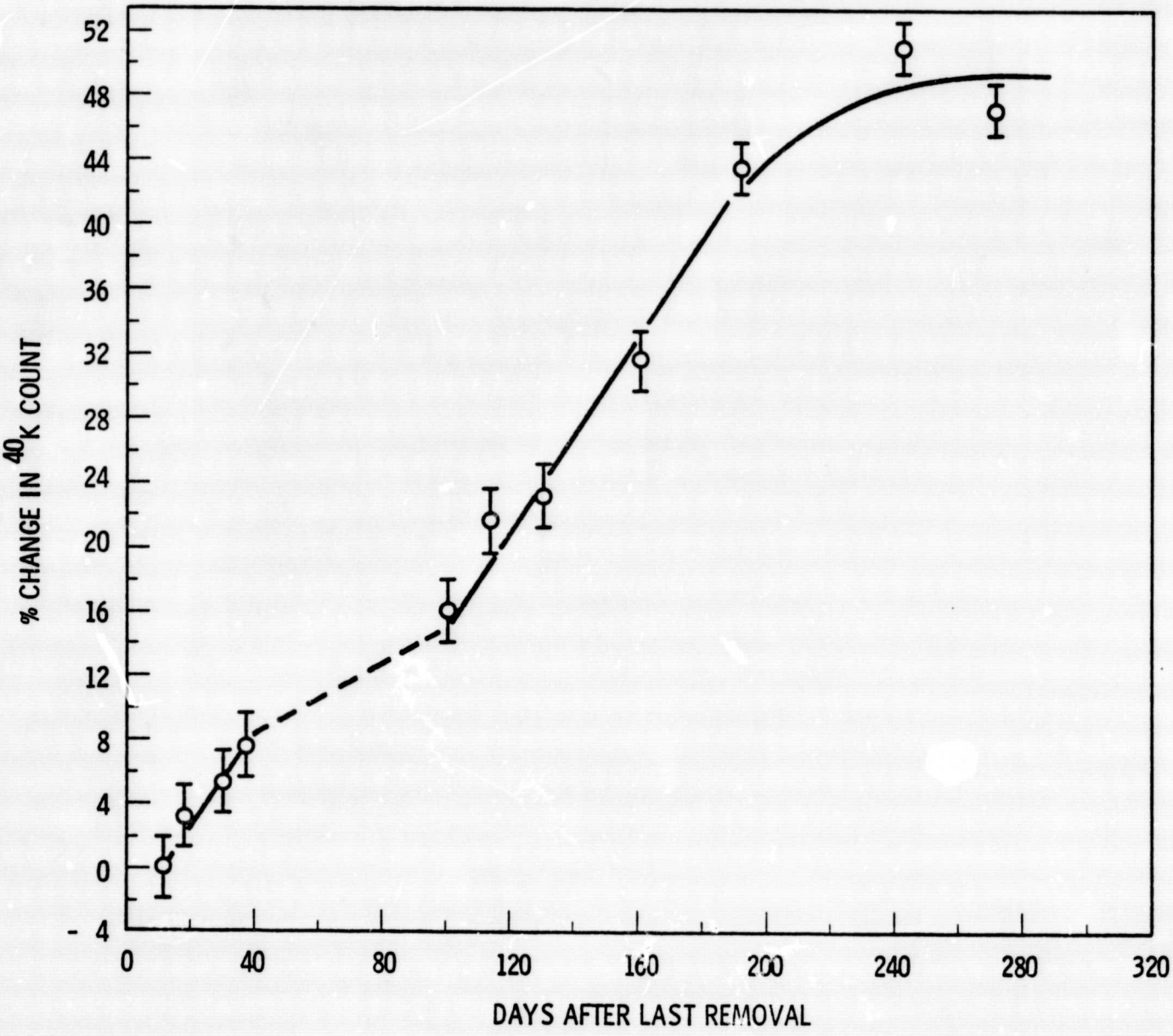

FIGURE 2. ^{40}K Content in Upper Leg of Patient #2. Patient's leg in cast for 6 weeks; 1st measurement 11 days after cast removal; leg refractured again on 37th day and was placed in a walking cast until the 98th day.

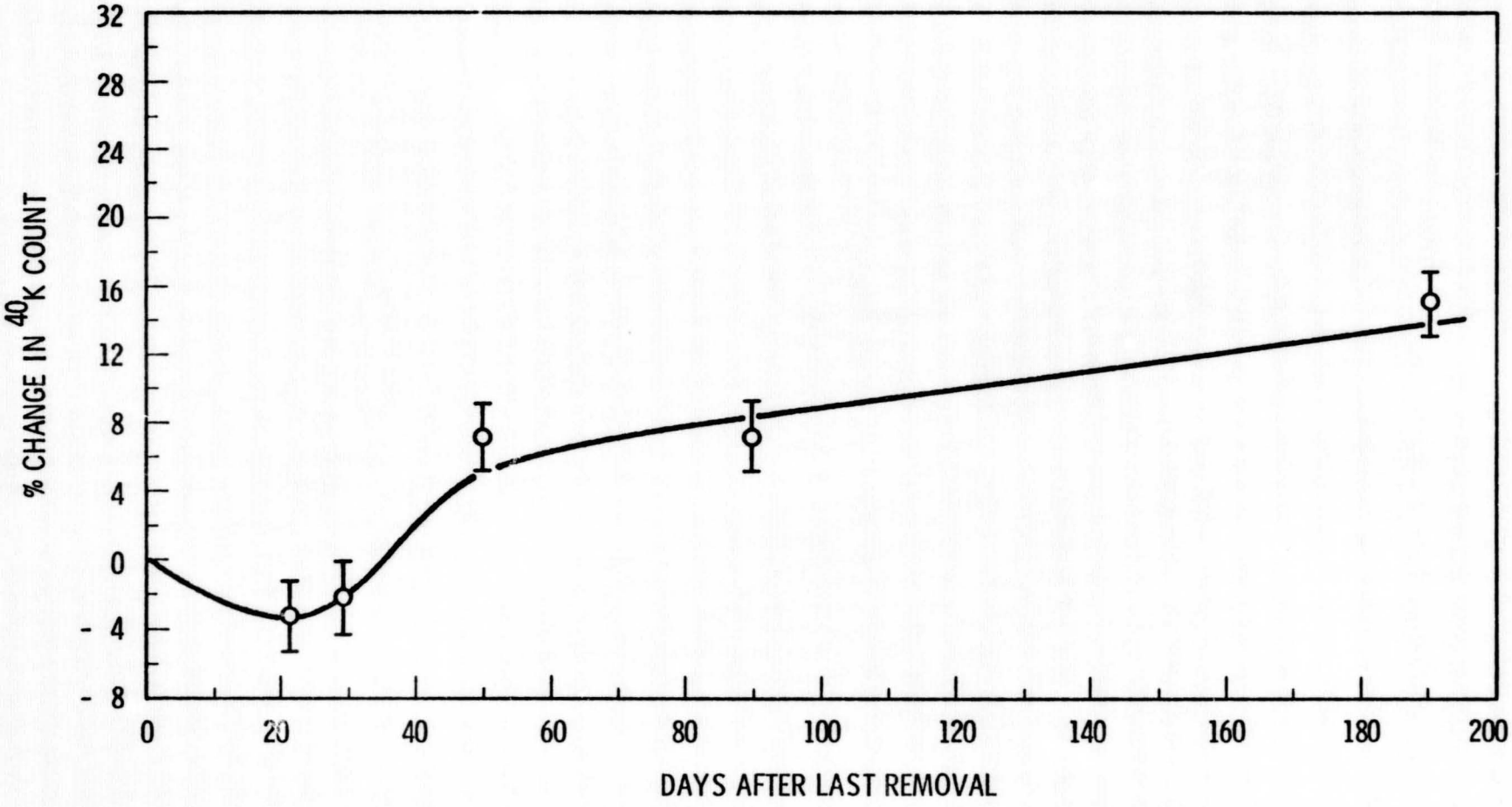

FIGURE 3. ^{40}K Content in Upper Leg of Patient #3.

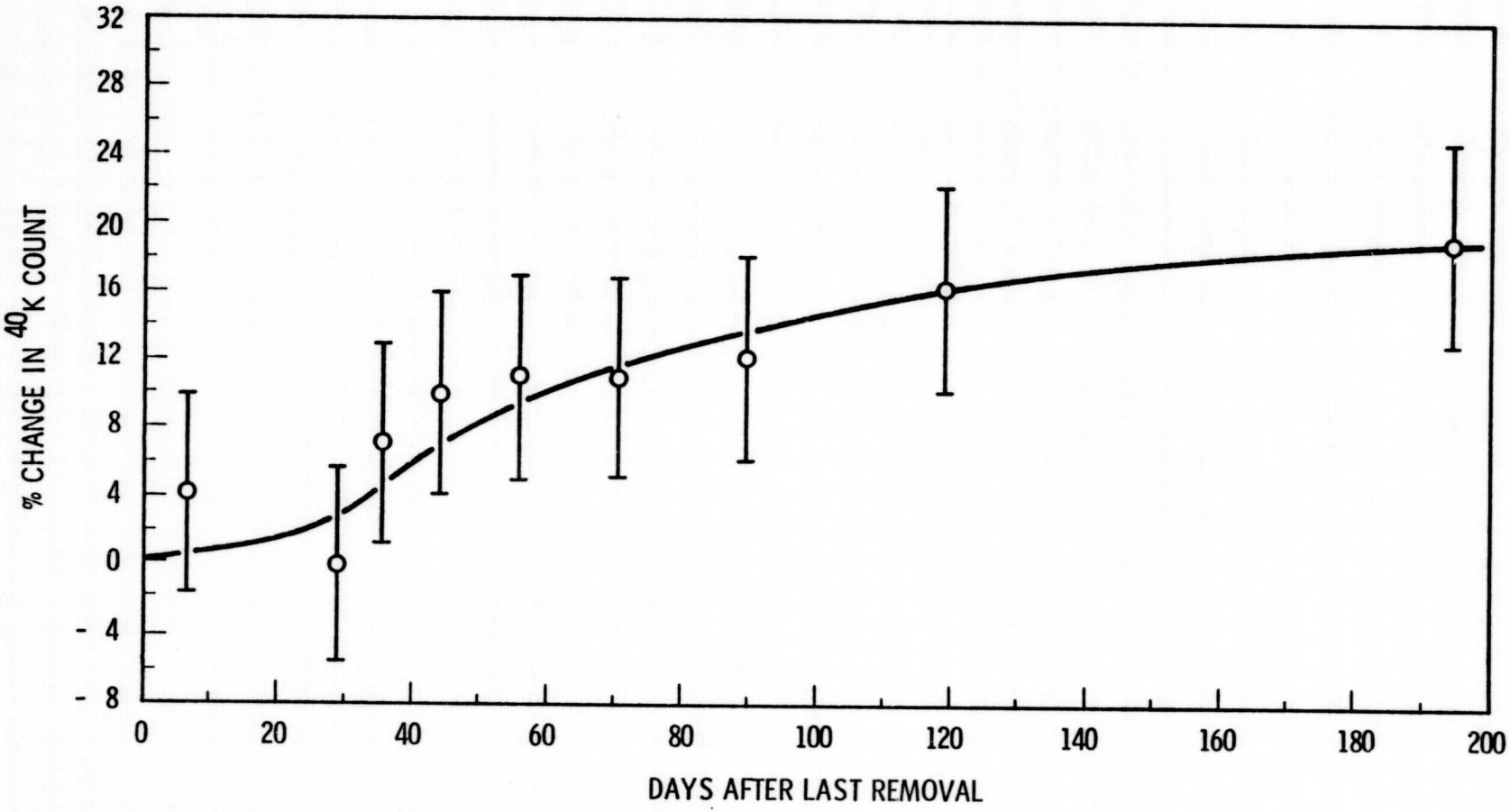

FIGURE 4. ^{40}K Content in Upper Leg of Patient #4.

APPENDIX 1

SODIUM AS A STOICHIOMETRIC COMPONENT OF BONE MINERAL

The sodium of the body is present in body fluids, cells, and in bone. About 40% of the total Na is in bone, but nearly half of this readily exchangeable. This leaves about 20% or about 20 grams of Na which is fixed in bone of an adult human. The constant ratio of sodium to calcium in bone is shown in Figure 5, which shows calcium and sodium content of dry bone from autopsy and biopsy samples from 19 people.[5] These people included both normals and those in various stages of renal failure with various bone densities. The error bars represent uncertainties in a single measurement. With the exception of a few scattered values the relationship between sodium and calcium appears to be a linear function and the resorption of bone results in the same fractional removal of both elements.

LONG-TERM RETENTION OF ^{22}NA IN ADULT HUMANS

The turnover rate of Na in bone depends on the type of bone and exhibits biological half-lives varying from several days to many years. The uptake in bone has been observed by whole body counting after the administration of 50 μCi of ^{22}Na.[6] The retention in the body was measured for six years after administration. Figure 6 shows the retention in bone after ^{22}Na has left the soft tissues. Biological half-lives of ^{22}Na in some parts of bone up to 3.5 years are evident and much longer half-lives are indicated. Since the ^{22}Na remained in the soft tissues and fluids only a few weeks the amount of deposition into the bone having a lower turnover rate was very low and the size of these low turnover rate bone pools cannot be determined.

If the ^{22}Na is grown into the bone of animals from birth to adulthood both the turnover rate and size of these various bone pools could be determined which would provide completely new information about long-term bone mineral kinetics. This method would also allow the effects of weightlessness on bone mineral metabolism to be determined.

THE USE OF ^{22}NA TO MEASURE BONE RESORPTION RATES IN GROWING RATS

It is known that bone resorption rates in young rats will increase up to 200% when the rat is placed on a calcium deficient diet. The greatest resorption rates occur in very young rats. Two groups of rats were grown from gestation to 13 weeks of age on a diet containing a constant amount of ^{22}Na. At 13 weeks the ^{22}Na was eliminated from the diet and one of the group was placed on a calcium deficient diet to induce increased bone resorption. After the ^{22}Na had cleared the soft tissues from dilution by the

THE USE OF ^{22}NA TO MEASURE BONE RESORPTION RATES IN GROWING RATS (contd)

normal stable sodium in the diet, the amount remaining in the bones of the two groups is shown in Figure 7. The ^{22}Na in the calcium deficient rats was only about 40% of that in the control group which indicates significant increase in bone resorption had occurred. The high slope of the curves up to the ninth week after the end of the ^{22}Na diet is due to the removal of ^{22}Na still in the soft tissues. Recently developed methods which are described in the following section would have allowed the ^{22}Na to be removed from the soft tissues within five days rather than nine weeks and this would have allowed more definitive data to be obtained on the correlation of ^{22}Na loss with bone resorption. For more details of this study see Reference 1.

THE RAPID REMOVAL OF ^{22}NA FROM THE SOFT TISSUES OF ANIMALS

The ^{22}Na can be rapidly removed from the soft tissues and fluids by feeding animals excess amounts of stable sodium in the form of sodium chloride or sodium bicarbonate. Figure 8 shows that the biological half-life of the ^{22}Na in soft tissue can be reduced from six days for rats on a normal diet to 0.7 day for rats on a diet containing at least 4.5% Na. This high sodium diet caused a tremendous increase in the water intake by the rats but no adverse effects were observed. The feeding of the high sodium diet will allow an animal to be grown to maturity on a ^{22}Na diet to uniformly tag all bone and then in five days eliminate all of the ^{22}Na in the body except for that in the bone. Any measurements made of ^{22}Na in the body or body parts will be of ^{22}Na in the bone. Figure 8 shows that the body burden of ^{22}Na actually leveled out before the end of the high sodium diet. This leveling out is due to that ^{22}Na which had been deposited in bone and was being released at the normal bone turnover rate. For more details of this study see Reference 7.

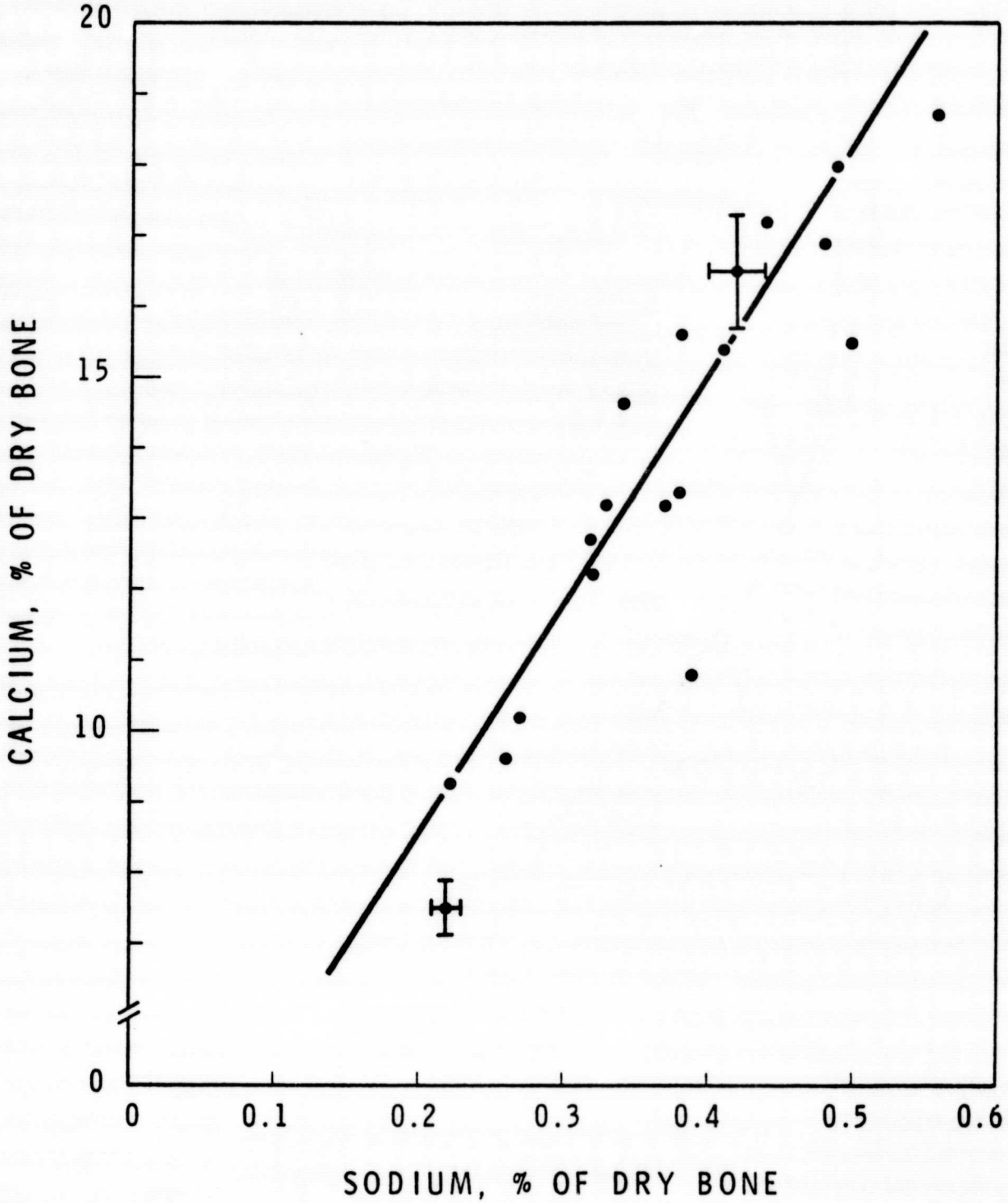

FIGURE 5. Calcium Versus Sodium Content of Human Bone.

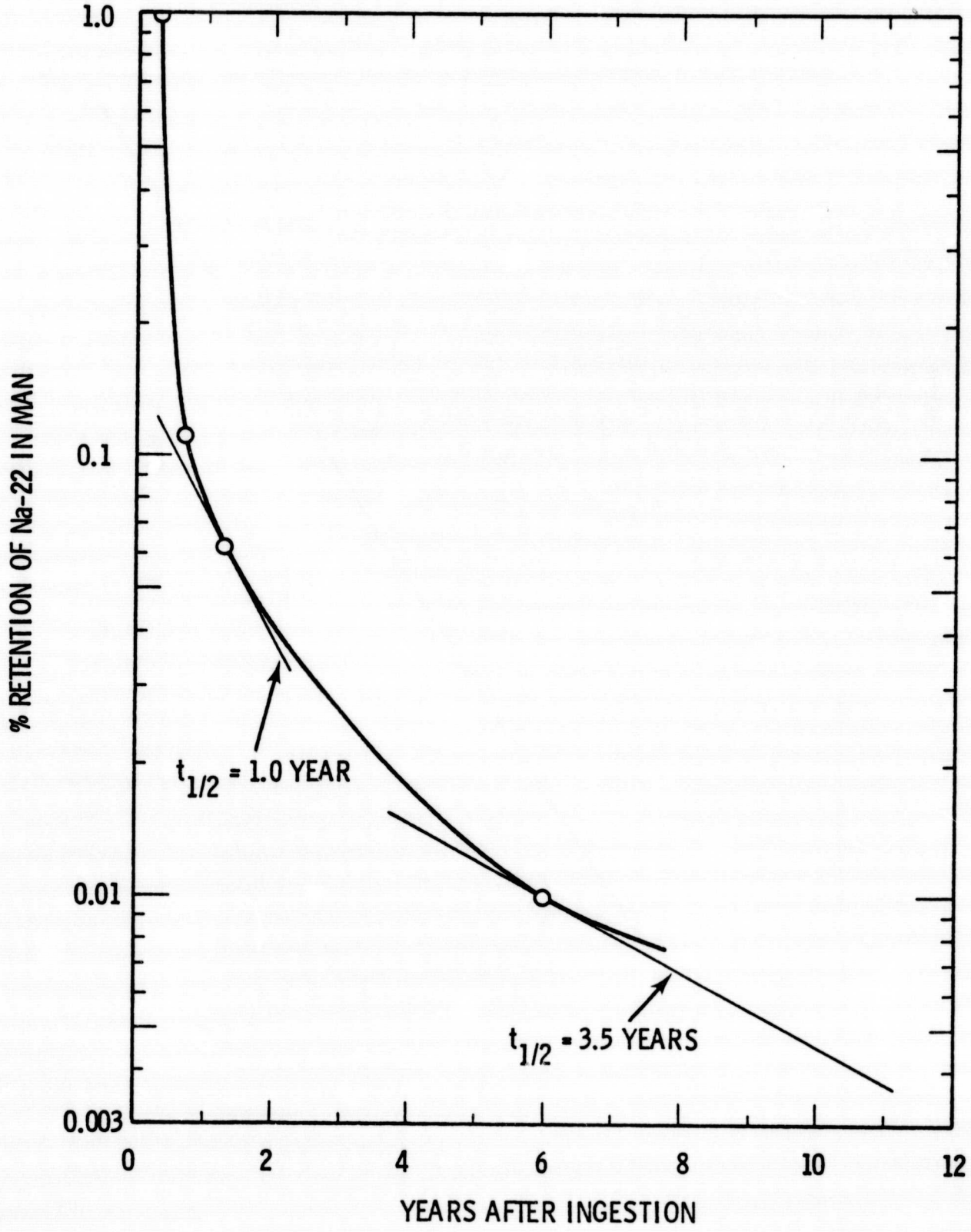

FIGURE 6. Retention of Na-22 in Adult Human Starting 100 Days After Administration of 50 μCi (Data Corrected For Radioactive Decay).

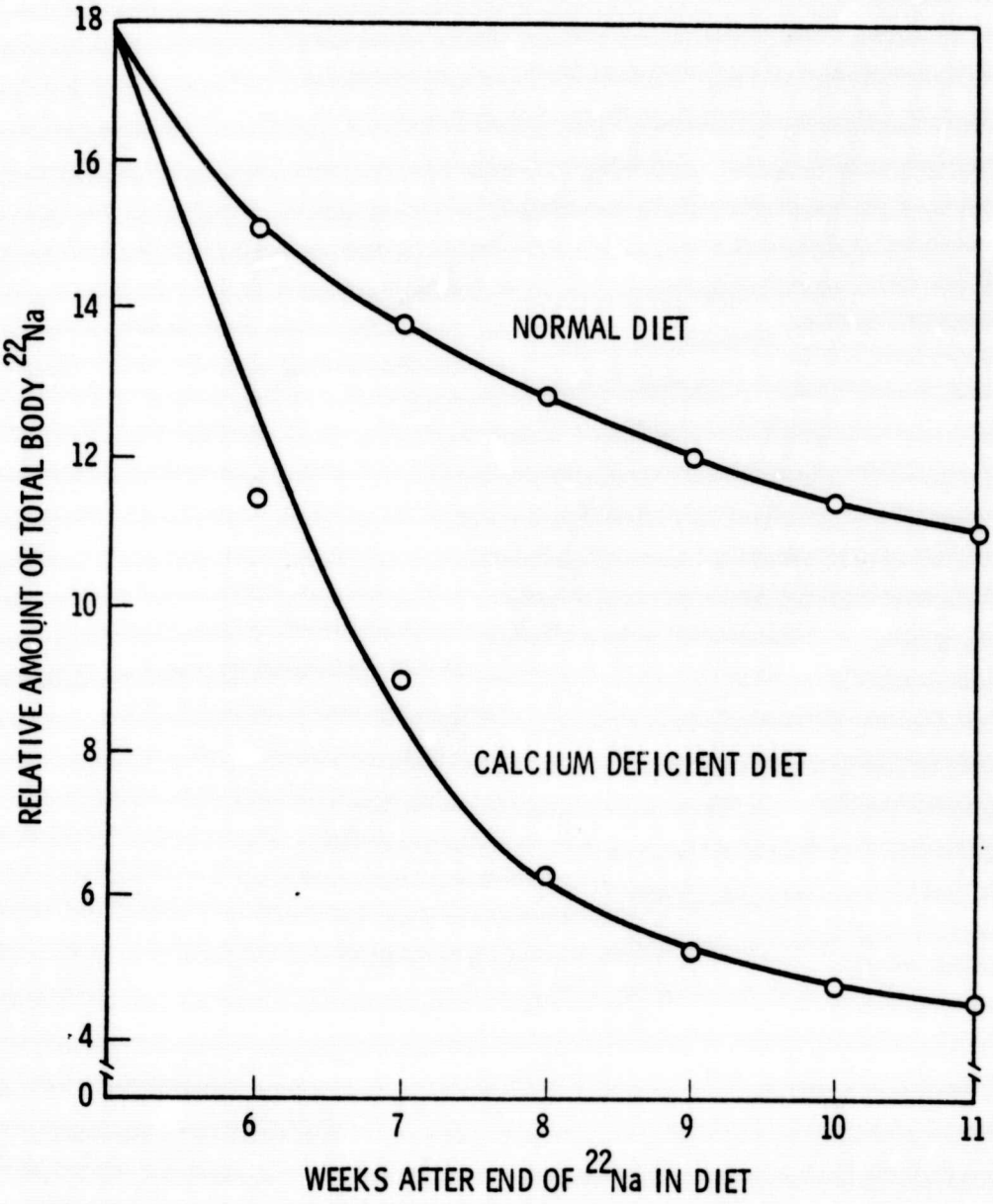

FIGURE 7. Comparison of ^{22}Na Levels in Calcium Deficient and Normal Rats Starting With 18 Week Old Rats.

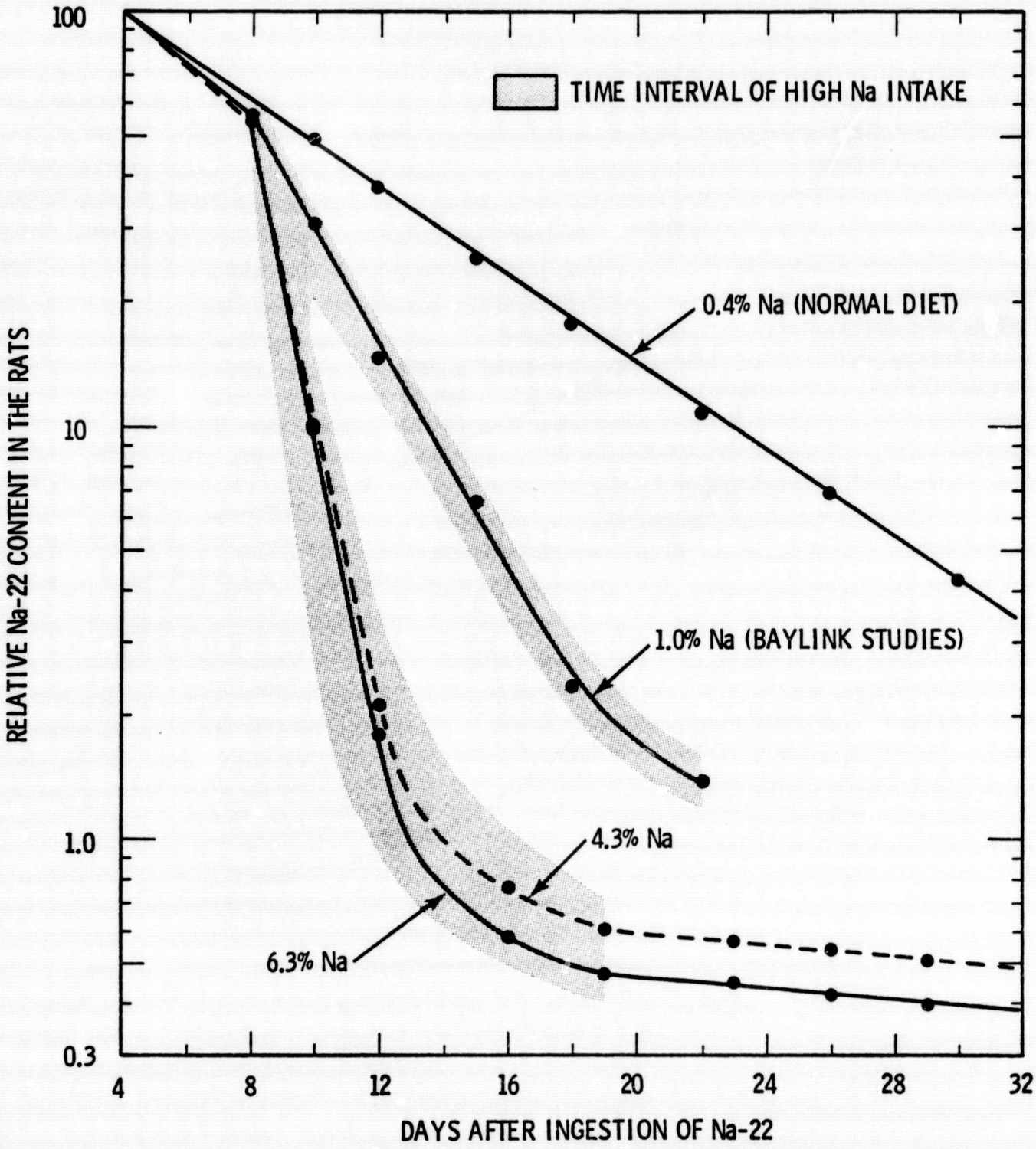

FIGURE 8. Retention of Na-22 in Rats Eating Food Containing High NaCl.

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