FEASIBILITY OF DETERMINING TOTAL-BODY CALCIUM IN ANIMALS AND HUMANS BY MEASURING ^{37}Ar IN EXPIRED AIR AFTER NEUTRON IRRADIATION

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The feasibility of determining total-body calcium in rats and dogs by measuring the ^{37}Ar released after fast-neutron irradiation has been shown. This method is nondestructive, reproducible, and requires a low radiation dose. It appears that this method may be feasible for determining total-body calcium in humans if the ^{37}Ar excretion rate from the bone is as rapid as its excretion from dogs.

When irradiated by fast neutrons, some of the calcium in bone is converted to ^{37}Ar, which leaves the bone and is excreted from the body through the lungs in the expired air. This radio-argon may be separated from the bulk of the expired air and subsequently counted internally in a proportional counter. In animals, the amount of ^{37}Ar released has been found to be proportional to the calcium content of the body. This report describes the procedure, experimental results, and advantages of this method over existing methods of in vivo neutron activation analysis for assessing the calcium content of the body and describes the potential application of the method for use with human subjects.

METHOD

Production and decay of radioactive argon. Both ^{37}Ar and ^{41}Ar are produced during fast-neutron irradiation of calcium. The nuclear reaction, stable calcium isotopic abundance, and modes of decay for the two product isotopes are shown in Fig. 1. A 14-MeV neutron generator was used in these studies, and the cross sections for these reactions for 14-MeV neutrons are approximately 110 millibarns (mb) for ^{40}Ca (*1*) and 30 mb for ^{44}Ca (*2*). At neutron energies below 14 MeV, the cross section is quite variable for the (n,α) reaction on ^{40}Ca and is 430 mb at 6 MeV whereas the thermal cross section is only 2.5 mb. 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. The cross section for the reaction on ^{44}Ca has an energy threshold of 2.8 MeV and is only about 1 mb at 8 MeV.

Our studies have shown that for the same neutron dose much more ^{37}Ar than ^{41}Ar is produced in animals. 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 exhaled ^{37}Ar as a calcium measurement, except ^{41}Ar must be allowed to decay away before counting. When comparing ^{37}Ar and ^{41}Ar for use in total-body calcium measurements in regards to the lower radiation dose, interferences, and counting sensitivity—^{37}Ar appears to be by far the better 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% car-

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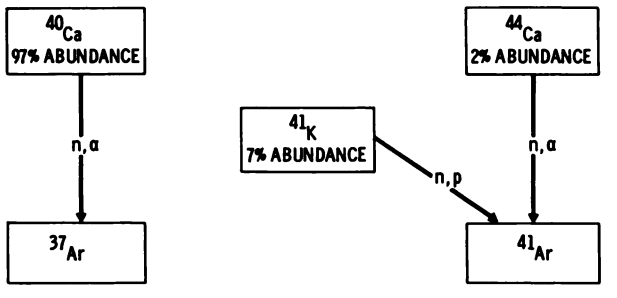

FIG. 1. Production and decay characteristics of radioactive argon.

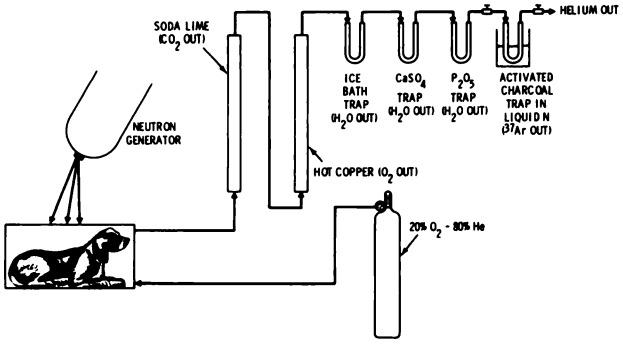

FIG. 2. Apparatus for collecting and purifying ^{37}Ar from expired air of animals.

bon dioxide, and a 6% fraction consisting of small quantities 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. The actual volume of ^{37}Ar gas that 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 500 liters/hr from a human.

By substituting for inspired air a gas mixture of 80% helium and 20% oxygen, as 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 that are adsorbed in the body tissue or that exist as a contaminate in the gas mixture. The amount of adsorbed argon in the human body is negligible and that fraction of the 800–900 cc of adsorbed nitrogen (*4*), 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 in animal studies is shown in Fig. 2. The animal such as a rat or a dog is placed inside an air-tight container. The helium-oxygen mixture flows through the container at a rate of 2 liters/min for an adult beagle dog and about 200 cc/min 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 icebath trap, $CaSO_4$ and P_2O_5 to remove water vapor; and through a trap containing activated charcoal at liquid nitrogen temperature that quantitatively collects all the ^{37}Ar and allows the helium to pass through. Except for the nitrogen that 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 that 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.

All traps and absorbers were designed for a maximum flow of 2 liters per min, which is adequate for animal experiments but would have to be scaled up in size to handle human breathing rates. An alternative would be to compress the expired air into tanks as it leaves the body and then slowly flow it through the present apparatus.

The copper used to remove oxygen was in the form of short lengths of wire less than ¼-in. long and 0.025-in. diam, 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 re-use. 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 coconut charcoal, 6–14 mesh, which is outgassed under vacuum or a flow of helium for about 5 min at a temperature of about 250–300°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%; therefore, in 93.5% of the disintegrations, the energy will be emitted as Auger electrons having an energy of 2.62 keV. The Auger electrons may be absorbed within a very small counter with essentially 100% efficiency. A range of less than 0.2 mm for a 2.6-keV electron in argon gas at atmospheric pressure can be estimated from its range in water without correction for the difference in scattering in argon (*5*). In addition to the 2.62 keV from the Auger electrons, an additional ionization energy is added within the counter by

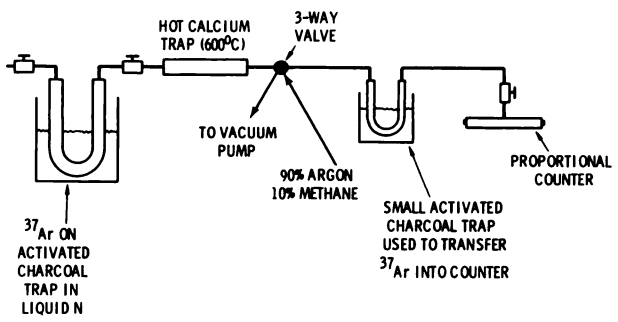

FIG. 3. Apparatus for transferring purified ^{37}Ar into proportional counter.

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/disintegration.

The counter used in these studies was made from a 2.5-cm diam and 23-cm-long aluminum tube with an 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 a ^{55}Fe x-ray source. The background of this counter is 2 cpm under the 2.82-keV Auger electron peak when placed between two large scintillation detectors that act as anticoincidence shields, and this whole assembly is surrounded by 4 in. of lead. A proportional counter is presently under construction in which the background should be as low as 0.1 cpm. 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 that is collected along with the ^{37}Ar as a result of argon that was dissolved in body tissues and fluids and that which exists as an impurity in the helium-oxygen gas mixture. A 2-hr collection of expired air from a 14-kg dog produces about 40 cc of stable argon, of which approximately 30 cc is from the helium-oxygen gas and 10 cc from that absorbed within the dog's body. The 113-cc volume proportional counter described above allows an additional 73 cc of gas to be used to flush all the ^{37}Ar into the counter. Because the average human weighs five times as much as this dog, the volume of stable argon obtained in human studies may be as much as 200–300 cc, and a correspondingly larger counter would be required.

The energy spectrum from ^{37}Ar in the proportional counter is shown in Fig. 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.

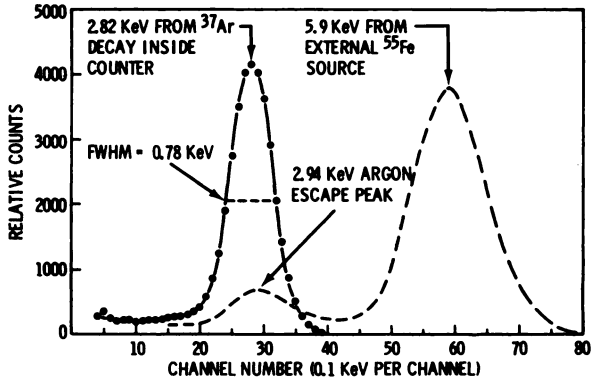

FIG. 4. Typical ^{37}Ar proportional counter energy spectrum with ^{55}Fe spectrum used for energy calibration.

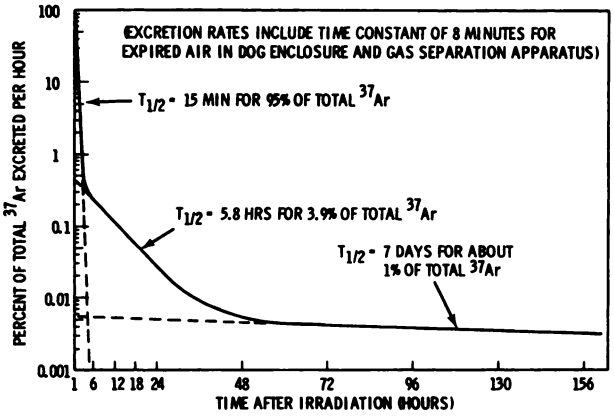

FIG. 5. Excretion of ^{37}Ar in expired air of dog after fast neutron irradiation.

RESULTS

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

In adult beagle dogs, about 88% of the ^{37}Ar is excreted within 60 min after irradiation and 95% within 120 min. Of the remaining 5%, about 4% is excreted with a half-time of 5.8 hr, and the remaining 1% leaves the bone with a half-time of about 7 days. In Fig. 5, the excretion rate per hour is shown for a period of 7 days. The excretion rate was actually followed through the 25th day, at which time the amount of ^{37}Ar in the expired air could no longer be detected with our present proportional counter. The curve in Fig. 5 was obtained from ^{37}Ar measurements of 1-hr-long collection periods from a dog that was irradiated with a high neutron dose (50 rads) to measure the excretion rate several days later—at which time the excretion rate is only 0.0001–0.00001 of the rate during the first hour. The actual half-time for excretion of the initial fast component is probably less than 15 min because this half-time includes the mean residence time of the expired gas in the dog container and gas purification apparatus and the buildup of ^{37}Ar during the irradiation period.

If a high-resolution Ge(Li) detector is used, the gamma-emitting ^{41}Ar can be measured in animals

that have been irradiated to high-dose levels. Experiments were conducted in which both live and dead animals 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. The use of a dead animal was necessary because of the rapid removal of ^{41}Ar from the live animal during and after irradiation. Whole- and partial-body counts were made 1 hr 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 that expired within 1 hr; but the counts did show that more than 85% of the ^{41}Ar produced from calcium was removed from the body of both rats and dogs within the first hour. A better estimate was not possible because the amount of ^{41}Ar produced from potassium could not be accurately determined.

The 5% of the ^{37}Ar formed in the dog, which is excreted with long half-times, is probably some of that formed in the very compact cortical bone. The ^{37}Ar was analyzed in two types of bone from a dog that had been sacrificed 2 hr after irradiation. The ^{37}Ar in the bone was determined by dissolving the bone in nitric acid and collecting and purifying the gases that were removed in a stream of helium that bubbled through the solution. The ^{37}Ar activity per gram in the femur (compact bone) was six times that of a piece of pelvic bone that was composed of both spongy and compact bone.

Reproducibility of ^{37}Ar measurements. Repeated measurements have been made on a single rat and dog. The measurements were made during an 8-hr period for the rat and during a 2-day period for the dog to insure that the calcium content of the animal did not significantly change between measurements. The animals were 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 min from the rat and 120 min from the dog after the start of a 10-min irradiation. The ^{37}Ar was counted the following day, by which time all the ^{41}Ar had decayed away. The $Fe(NO_3)_3$ standard was counted at least 2 hr after irradiation to allow for decay of the ^{13}N that was produced. Both counts were decay corrected back to the start of irradiation and the ratio of the counts are shown in Tables 1 and 2. The precision of the method appears to be within ±2%, which is adequate for many studies of changes in total-body calcium. However, some improvement is expected as more experience with the technique is gained.

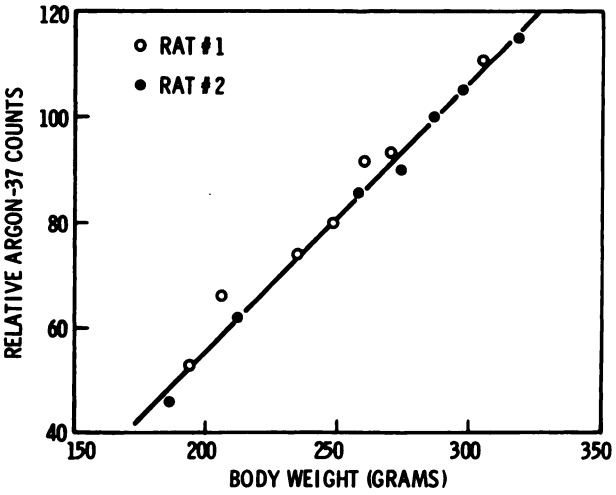

FIG. 6. Argon-37 production by neutron activation versus growth in two rats.

Comparison of ^{37}Ar production with bone growth. Periodic ^{37}Ar measurements were made on 2 rats as they increased from about 190 to 320 gm. 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 $Fe(NO_3)_3$ standard counts. The results are shown in Fig. 6. The scatter of the points around the line is at least partly due to variations in weighing the rats that depend on the time interval since the occurrence 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%. Because the bone mass of rats increases quite proportionally with body weight (*6*), 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 in which the cross section for the n,α reaction is higher than that for 14-MeV neutrons, which results in a higher ^{37}Ar production. This assumption was tested and found to be true by irradiating a solution of the $Ca(NO_3)_2$ with and without a surrounding blanket of 150 gm of paraffin wax. The paraffin wax increased ^{37}Ar production by 40%. This error can be eliminated by irradiating the animal within a large volume of moderating material such as water so that increasing body size displaces moderator material and therefore allows the total neutron moderation to remain constant. Rotation of the rat within the block of moderator may be desirable

during the irradiation to assure uniformity of activation. With these modifications to the procedure, the calcium activation in the body of the rat should be uniform regardless of the size or shape of the rat.

Radiation dose required for ^{37}Ar measurement. The neutron generator used in these studies had a total neutron output of about 3×10^{10} neutrons/sec. The doses from this output as measured by a 100-cc tissue-equivalent ionization chamber were 5.6, 1.4, and 0.14 rads per 5 min at 10, 25, and 80 in. from the neutron source. The animals were irradiated for a period of 5–10 min depending on the distance from the neutron source.

In the measurements of ^{37}Ar produced in a rat, data for which are shown in Table 1, the radiation dose received from the 14-MeV neutron generator was about 11 rads for each measurement. This dose was determined with a 100-cc tissue-equivalent ionization chamber. The rat weighed approximately 500 gm and therefore contained about 4 gm of calcium (*6*). From these data, 5.7 cpm of ^{37}Ar are obtained per gram of calcium per rad of radiation dose. For the 12-kg dog used in measurements described in Table 2, which contained approximately 100 gm of calcium (*7*), 3.9 cpm were obtained per gram of calcium per rad of dose. If a counting time of 1,000 min is used to obtain a total net count of 10,000 or more counts of ^{37}Ar, a dose of 0.44 rads would be required for the rat and 0.026 rads would be needed for the dog. If we extrapolate the dog results directly to a man containing 1,000 gm of calcium, an estimate of 0.0026 rads is needed to produce 10,000 counts in 1,000 min. This almost negligible dose for man could be reduced even further to about 0.0003 rads if the ^{37}Ar counting time of 1 week is used. The dose measurement includes that from both neutrons and gamma rays from the neutron generator.

TABLE 1. RESULTS FROM THREE REPETITIVE DETERMINATIONS OF ^{37}Ar EXPIRED FROM THE SAME RAT

Run no.	Irradiation time (min)	Standard counts	^{37}Ar counts (per 100 min)	Ratio	$\frac{^{37}\text{Ar counts}}{\text{standard counts}}$
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 ±1.2%

TABLE 2. RESULTS FROM REPETITIVE DETERMINATIONS OF ^{37}Ar EXPIRED FROM THE SAME DOG

Run no.	Irradiation time (min)	Standard counts	^{37}Ar counts (per 100 min)	Ratio	$\frac{^{37}\text{Ar counts}}{\text{standard counts}}$
1	10	401,213	12,867		0.321
2	10	398,562	12,373		0.312
3	10	417,378	13,507		0.324
				Mean	0.319
				Range	4.0% or ±2%

DISCUSSION

In the studies described above only 14-MeV neutrons were used. Because the cross section for the reaction ^{40}Ca(n,α)^{37}Ar at some lower neutron energies appears to be higher than at 14 MeV, other types of sources can and have been used. 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), which is a fission source. Argon-37 has been produced and measured in rats, using both of these sources by the author. For small animal studies of calcium content, the lower energy sources appear to be as good as or better than 14-MeV neutrons especially when comparing cost and convenience. For total-body calcium studies in humans, the 14-MeV neutrons should provide the best uniformity of calcium activation throughout the length and thickness of the body, and therefore this source would be preferred. If maximum uniformity is not essential or if only part of the body, such as a leg or arm, is being measured for relative sequential studies, the α,n or ^{252}Cf sources may be suitable.

The reproducibility of the production and release and the almost complete removal of ^{37}Ar after neutron irradiation has been shown in animals, and should the need arise, the absolute amount or changes in calcium content could be determined in these animals. Because an immediate need for determining calcium in animals did not exist, these studies were done primarily to establish the feasibility of the method for determining the calcium content and changes of calcium content in humans. Present efforts are devoted to the application of this method to humans in cooperation with physicians at the University of Washington, School of Medicine. While measurements on humans have not yet been made, if the ^{37}Ar is expired from humans in a reasonable length of time, it should provide an excellent measure of total-body calcium.

The advantages of this method for total-body calcium over existing methods using neutron activation

(*8–10*) include: (A) a much lower irradiation dose; (B) fewer and less expensive facilities and equipment; and (C) possible improved accuracy. The neutron radiation dose to a human should be only one-hundredth of that currently required in present methods. The ^{37}Ar method does not require an expensive whole-body counter, and the neutrons can be supplied from relatively inexpensive sources such as neutron generators, α,n type, or ^{252}Cf sources. Because ^{37}Ar has a half-life of 35.1 days, the precision timing required for the 8.8-min half-life ^{49}Ca method is not necessary, and fluctuations in beam current during irradiation do not affect the accuracy of the method. The accuracy of this ^{37}Ar method is better than the whole-body counting methods because there are no radioactive interferences in the separated ^{37}Ar once the short-lived ^{41}Ar has decayed away, and counting errors due to geometry and self-absorption due to various body sizes are eliminated. Studies of the uniformity of a bilateral irradiation of calcium sources in a tank of water indicate that the uniformity of the reaction ^{40}Ca(n,α)^{37}Ar throughout the body should be within $\pm 3\%$ in adult human patients. Such uniformity is better than that which is presently being obtained in total-body calcium methods.

In humans, this method could be very useful in studying and assessing bone disease and in studying the apparent calcium loss in astronauts during space travel. Even if some of the ^{37}Ar is found to release more slowly from humans than animals, the method may still be of value because any rapidly released ^{37}Ar will probably represent the more metabolically active bone that is likely to be of major interest.

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