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LOW DOSE HUMAN CALCIUM ASSAY IN VIVO VIA THE $^{40}\text{Ca}(n,\alpha)$ REACTION*¹

by

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ABSTRACT

A compact medical cyclotron has been investigated to elucidate its merit as a neutron source for both qualitative and quantitative activation analysis human studies in vivo of calcium and other elements within the human body and at reasonable radiation dose levels. Emphasis is given to those properties necessary for carrying out quantitative whole body calcium measurements using the $^{40}\text{Ca}(n,\alpha)^{37}\text{Ar}$ reaction, which because of the low radiation dose and relatively modest equipment requirements, give this method potential for wide application in diagnostic studies of calcium metabolism.

Interpretation of measurements of rates of patient calcium bone accretion, resorption, exchangeable pool size, etc. are hampered by lack of information concerning the size of the total body pool of calcium. Over the past few years a method of making this measurement with fair accuracy and good precision has been developed (1,2,3). The basis of this technique is thermal neutron capture in the 0.18% ^{48}Ca isotope ($^{48}\text{Ca}(n,\gamma)^{49}\text{Ca}$). The daughter nuclide ^{49}Ca subsequently emits a 3.1 MeV gamma ray with a half-life decay time of 8.9 minutes. Respective dose levels to the patient utilized at the Birmingham (U.K.), Seattle and Brookhaven laboratories are 150, 200 and 28 mrad (4,5). These levels are achieved using very expensive whole body counters such as the one at Brookhaven (6). Such dose levels can at present only be justified in a small category of research studies in patients with limited life expectancy.

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With another technique on-line, i.e. during exposure, measurement of the prompt gamma rays produced by thermal neutron capture has been reported (7,8) for human activation analysis in vivo. It was shown that when prompt detection is employed at a local site of neutron irradiation the relative calcium, sodium and chlorine mass can be measured using a Ge(Li) detector. Fremlin (9) has employed a prompt gamma technique using a pulsed neutron beam to quantitate nitrogen in the body. A short pulse of fast neutrons lasting about 20 μ sec. is delivered to the patient whose body moderates these fast neutrons to thermal energies for a further period of a few tens of μ seconds. The slow neutron flux becomes uniform about 250 μ sec. after the pulse has ended. The detector is then triggered to start counting the prompt γ -rays for an appropriate short time. A dose of 6 mrad was required to analyze the 10.8 MeV prompt peak arising from nitrogen.

Recently a study was carried out by H.E. Palmer (10) at Battelle Pacific Northwest Laboratories wherein he showed the feasibility of measuring whole body calcium in vivo in rats and dogs utilizing the $^{40}\text{Ca}(n,\alpha)^{37}\text{Ar}$ reaction (isotopic abundance 96.97%). In collaboration with Brookhaven National Laboratory we have carried out studies to determine the feasibility of this method in humans (11,12).

The ^{37}Ar ($T_{1/2} = 35.1$ days) technique has two major advantages; neutron exposure requirements are considerably reduced and the whole body counter assay system is replaced with a small and relatively inexpensive low-level proportional counter. The extreme sensitivity of this technique is achieved by collecting patient respiratory product following irradiation, separating out the argon component and counting each disintegration of ^{37}Ar with approximately 70% total system efficiency in a small (10 c.c.) proportional counter.

Experimental Techniques

Gas collection and storage

The respiratory gas collection and storage apparatus is mounted on a portable cart. This allows easy control over subject gas supply and collection prior to, during and post irradiation. The subject can be moved to various convenient locations during these periods. Fig.(1) shows the basic components

Fig. 1. The apparatus used to store patient expired respiratory gas.

of the system. The 20% oxygen-80% helium tank provides the breathing gas used through the entire procedure. Helium is substituted for the usual nitrogen component in air because it is more readily removed than nitrogen. This supply also eliminates the nearly 1% of stable argon in the atmosphere. The only stable argon that will be found in the exhaled gas will come from that which is washed out of the subjects tissues and a small trace in the breathing gas. A leak tight face mask with inlet and outlet one-way valves is provided to control intake of breathing gas and exit of exhaled gas. The exhaled gas passes through indicator NaOH, for CO₂ removal, and indicator silica gel, for water vapor removal. These are removed at this stage because there is no hazard associated with placing them near the subject and the devices do not interfere with exhaling. The collected gas volume is reduced and moisture condensing in various parts of the system is avoided. A 30 liter Douglas bag provides a buffer volume. This variable system volume is necessary because expiratory air rates vary, making intermittent operation of the high pressure pump convenient. The pump is rated to 1500 PSI at 11.3 liters/minute, which compares to a patient ventilatory response in the resting basal state of 5.8-10.3 liters/min. The pump serves to fill a high pressure cylinder with patient expired gas. This allows the gas to be stored while awaiting further processing.

Gas Separation and Counting

The very low dose required for this experiment depended primarily upon reducing background in the ³⁷Ar counting apparatus to on the order of 5 counts per day. A pure argon fraction must be obtained to keep the gas proportional counter volume as small as possible and to eliminate trace radioactive gases such as ⁸⁵Kr, ²²²Ra and tritium. The counter must be made of radiochemically pure materials (Quartz envelope and zone refined metals). A volume must be provided for the counters that is surrounded by an anticoincidence ring of gas proportional counters, which in turn is surrounded by at least eight inches of lead shielding. Data from each counter is stored in a 256 channel portion of a multichannel analyzer.

Fig. (2) illustrates the first steps in the gas separation procedure. The high pressure cylinder is attached to the system and allowed to leak in

Fig. 2. Gas processing instrumentation used to separate inert gases from exhaled gas.

through the copper tube furnace (450°C) to remove oxygen. A buffer tank is provided to keep the system from reaching too high an internal pressure. The gas is then passed through a titanium gettering furnace (950°C), where nitrogen and other non-inert gases are removed. An activated charcoal trap, held at liquid nitrogen temperature, collects all remaining gases except helium, which passes through and is vented from the system. The trap is warmed to release the inert gases allowing them to be transferred into a titanium gettering cleanup bulb and then into a transfer bulb.

The gas transfer bulb is attached to the system shown in Fig. (3) where inert gas separation and transfer of the argon sample into a proportional counter occurs. A column gas chromatograph, consisting of a charcoal column and a thermal conductivity cell, is used to separate the Kr, Xe, and Ar components. Separate traps are provided for each fraction as they come off the column. A toepler pump transfers the argon from its trap into a final titanium furnace. The calibrated stem allows volumetric determination. The sample is then transferred into a proportional counter for quantitative ^{37}Ar radioactivity assay.

Fig. 3. Instrumentation required to separate argon from other inert gases and to fill into a gas proportional counter.

Neutron Source Study

The choice of neutron source for the $^{40}\text{Ca}(n,\alpha)^{37}\text{Ar}$ reaction is based upon two considerations. First, it is desirable that the dose be delivered within a short time interval. Second, the neutron energy spectrum should be such that the most effective use is made of each source neutron. A ^{238}Pu -Be source was used in our initial study because its characteristics are well known. However, the effective cross-section for producing ^{37}Ar in a calcium tank (11 kg calcium in the form of calcium nitrate dissolved in water 2' x 3' x 4') is only 96 mb using the ^{238}Pu -Be source (13). Whereas, excitation function measurements (14,15,16,17,18) show the cross-section for

the $^{40}\text{Ca}(n,\alpha)^{37}\text{Ar}$ reaction starts out near zero at 2 MeV, rises sharply to 200 mb at 3.8 MeV and subsequently falls off very slowly reaching 120 mb at 15 MeV. Approximately 25% of the neutrons from the ^{238}Pu -Be source are below 1 MeV (19).

Two different reactions have been investigated using our CS-15 cyclotron. They are the $^9\text{Be}(^3\text{He},n)$ using 23.3 MeV ^3He ions and the $^6\text{Li}(p,n)$ reaction using 14.7 MeV protons. The neutron energy spectrum arising from the ^9Be reaction at this energy has been measured (20). In this case virtually 100% of the source neutrons are above 1 MeV and 85% are above 4 MeV, making it look like an excellent source for our purposes, with a potentially much higher effective cross-section. The Li target has not been investigated at our energy, but studies at 10.3 MeV indicate it too has strong potential (21). All measurements made concerning these reactions were repeated in an identical manner for both of them. Dosimetry for the ^9Be reaction has been carried out in this laboratory (22). The cyclotron provides ample neutron dose rates to perform the required irradiations in very short intervals (even a fraction of a second, if that were desirable). Another source possibility would be a relatively modest D-T generator (10¹¹ neutrons/sec.)

The uniformity of production of ^{37}Ar with depth in a water tank has been determined directly using the $^{40}\text{Ca}(n,\alpha)^{37}\text{Ar}$ reaction. This information was needed to verify that the $^{27}\text{Al}(n,\alpha)^{24}\text{Na}$ reaction can be used to map the ^{37}Ar production distribution in more realistic phantoms of the human body. Absolute quantitative calcium measurements obtained from in vivo human exposures depend upon our knowledge of this production efficiency throughout the body. Aluminum activation foils 1/2" in diameter weighing 0.25 gm represent a minor perturbation in the neutron field and are convenient to assay in a NaI(Tl) well crystal. Calcium nitrate powder samples were sealed in pyrex bulbs. Eight samples weighing approximately 2 gm each were suspended at 5.7 cm depth intervals in a 30 x 30 x 35 cm water tank. The ^{37}Ar , produced by reaction of the ^{40}Ca with neutrons from 23 MeV helium-3 bombardment of beryllium, was quantitatively removed by evacuation and flushing with helium. Collection was done on a liquid nitrogen cooled charcoal trap. The samples were then transferred to a gas proportional counter for assay.

Quantitative Whole Body Excretion Study In Vivo

A quantitative experiment with a normal human male subject was carried out using fast neutrons produced in the $^9\text{Be}(^3\text{He},n)$ reaction. Low background counting techniques were applied to assay breath samples containing as little as 0.01 dpm corrected to end of irradiation (EOB). Six samples were taken in the 12.5 hr period following two one minute bilateral fast neutron exposures. Total neutron absorbed dose in this irradiation was limited to less than 2 mrad.

The total ^{37}Ar produced in the body was estimated as described below. The ratio of the total activity expired to the total activity produced is the fraction of ^{37}Ar removed from the body by way of expiration. The absolute production of ^{37}Ar per gram of calcium was determined by irradiating calcium nitrate in water solution. This solution was sealed in a pyrex bulb and placed within a water moderated calf phantom. The phantom was then exposed in a position equivalent to that of the calf location. An Alderson phantom containing a human skeleton, with access holes where aluminum activation foils could be placed, was then used to measure the average fast neutron flux relative to the tibia in a total of 22 bone subgroups. The phantom's outer plastic case allowed its body space, representing soft tissue, to be filled with tissue equivalent neutron moderator. The percent bone tissue in each of these bone compartments was determined by analyzing the bone tissue

weights measured and tabulated by Mechanik in 1926 (23) for six total male human skeletons.

Weighting the percent bone tissue in each compartment by the relative neutron flux measured therein and summing the results was done to reduce flux non-uniformity errors.

Estimation of the subject's total body calcium mass was made from two independently determined algorithms for normal males published by Cohn et al. (24) and Nelp et al. (25). Multiplication of this calcium mass by the measured absolute ^{37}Ar production per gram calcium at the tibia position and by the weighted sum yielded the absolute production of ^{37}Ar resulting from this exposure.

Bone Tissue Analysis In Vitro

Rowland et al. (26) showed that the radon produced in dog bones from internally deposited radium burdens exhibited identical fractional radon release in vivo and in vitro. Marinelli, L.D. et al. (27) showed fractional radon release from humans carrying long term radium burdens to exhibit a small standard deviation between individuals. Rowland et al. measured in vitro the fractional release from one of these bones and found virtually identical results to Marinelli's in vivo results, i.e. essentially permanent trapping in bone crystal was responsible for radon trapping. Since there is evidence from electron micrographs (28) that senile bone exhibit larger average crystals than those in infant bones, an age correction will be required for crystal trapping of inert gases because this is crystal size dependent. This should not be large, however, especially in adults, because radon fractional release changes by only about 20% in the extreme case of a mean crystal size represented by a newly forming bone region (at close to time of radium injection) and that years later when crystal size in the radium containing regions are more representative of the mean in the individual.

A section of normal human cortical bone was taken from the right fibula of a 18 year old man and studied to determine fractional argon release. Argon and radon are both inert gases. Two portions, each about 5 grams, were fixed in acetone and dried at room temperature. One portion was not processed further. The other was ashed in a muffle furnace for 5 hours at 650°C. Both portions were placed in pyrex containers. The containers were evacuated and flushed three times with helium and then sealed in a helium atmosphere at 600 mm of mercury. Irradiation was carried out using neutrons produced by the $^9\text{Be}(^3\text{He},n)$ reaction. Activity in the container was removed by flowing helium through it until no more ^{37}Ar could be extracted, then admitting 1 molar HCl to dissolve the bone crystal and finally bubbling helium through the resulting solution until all ^{37}Ar that had been crystal trapped was removed. The sum of these two quantities is the total ^{37}Ar produced and the ratio of the first fraction to the total equals the released fraction.

RESULTS

The results of the in vivo exhalation study are shown in Fig. (4). Displayed is a plot of the absolute ^{37}Ar activity in disintegrations per minute per exhaled minute versus time following irradiation extended to 12.5 hr. The vertical bars are 69% confidence limits on each measurement. The horizontal bars correspond to the time period over which each breath sample was collected. A least square, two-exponential component fit to the data satisfies the following relation:

$$A = 0.0468 \exp(-0.0177t) + 0.00197 \exp(-0.0039t),$$

Fig. 4. The absolute ^{37}Ar activity rate of release from a human subject's body by exhalation in disintegrations per minute versus elapsed time following irradiation.

where A is the activity in dpm per minute exhaled and t is the time in minutes following irradiation. The 40 and 200 minutes half-time components account, respectively, for 96 and 4% of the total exhaled ^{37}Ar activity. Integration of these results amounts to $3.2 \pm 0.5 \text{ dpm}_{\text{EOB}}$.

The estimated total activity produced (R) is the product of the production sensitivity in the tibia (S_{Tibia}), the weighting factor and the calcium mass (M_{Ca}). The weighting factor is the sum of the products of percent bone (b_i) in each bone subgroup with the neutron flux in that subgroup relative to the tibia neutron flux ($\phi_i/\phi_{\text{Tibia}}$).

$$R = S_{\text{Tibia}} \sum_{i=\text{bone subgroups}} \left(b_i \frac{\phi_i}{\phi_{\text{Tibia}}} \right) M_{\text{Ca}} (\text{gm})$$

$$= 3.8 \times 10^{-3} (\text{dpm}_{\text{EOB}}/\text{gm Ca}) (1.27) (1069) (\text{gm Ca})$$

$$= 5.2 \pm 10\% \text{ dpm } ^{37}\text{Ar}$$

The ratio of total activity expired to total produced gives a fractional release of 0.6.

The *in vitro* bone study gave 0.67 for the fractional release of argon from the acetone fixed bone. On the other hand the ashed bone fractional release was only 0.17. This is further confirmation of the work of Rowland et al. (26) on radon that the inert gas fraction remaining in bone can be accounted for by essentially permanent trapping in individual hydroxyapatite

crystals. Ashing causes the individual crystals to grow in size which would be expected to reduce the fraction of gas released, if crystal trapping is the mechanism responsible. The agreement between the in vivo and the in vitro results in both studies is excellent.

As can be seen in Fig. (5), the calcium bulb study of neutron fluence as a function of depth in a H₂O tank was shown to agree as expected with the results of the aluminum and indium activation foil measurements. It is clear from this measurement that relative neutron flux results obtained from aluminum foil mappings in phantoms will not differ significantly from those obtained using calcium.

Fig. 5. Neutron flux measurements with depth in a water tank comparing aluminum, calcium and indium assay materials. Neutrons are derived from the $^9\text{Be}(^3\text{He},n)$ reaction.

CONCLUSIONS

A single quantitative human whole-body calcium measurement in vivo has been carried out which has significantly added to our understanding of the mechanisms involved in the exhalation of ^{37}Ar produced in bone. Our results demonstrate an extremely low dose requirement for this assay. For example, a one hour exhaled gas collection and overnight activity assay would require a dose of 7 mrad with a 1% statistical counting error. If collection were extended to four hours and one week analysis, the dose required would be lowered to 0.6 mrad.

The total fractional release behavior of radon and argon are remarkably similar. The radon data taken by itself indicates high confidence that fractional release in vivo of an inert gas is stable and reproducible. The comparative total gas release figures in vivo and in vitro give us an accounting of all the activity produced. Any dependence due to changes in mean bone crystal size in total fractional release in humans, such as those related to growth or disease processes, can be evaluated in vitro with human

bone samples obtained from autopsy cases. Algorithms can be derived in this manner for corrections to in vivo results found necessary.

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