IN VIVO NEUTRON ACTIVATION ANALYSIS FOR CALCIUM IN MAN

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The calcium status of volunteers and patients has been measured by in vivo neutron activation analysis, using Pu-Be neutron sources to irradiate the trunk. The results of sequential measurements on 15 volunteers, five patients with osteoporosis, and one patient with osteomalacia are presented. The measurements with the human volunteers showed reproducibility was 6.4% (1 s.d.), slightly worse than that with a skeletal phantom (4.8%). The osteomalacic patients showed a dramatic change in calcium count (+77%) as a result of treatment; a large change is also shown by radiography. The osteoporotic subjects on calcium supplements showed a significant ($p < 0.002$) transient increase in calcium count of >10%, whereas the patient without calcium supplements showed decreases of up to 26% at 8 months.

The in vivo method of measuring body calcium by neutron activation analysis (IVNAA) has been developed over the last few years (*1–4*). This method provides a means of estimating changes in total (or of a large fraction) skeletal calcium during life with an accuracy of within a few percent. The present paper describes measurements with an IVNAA technique that preferentially examines the trunk and presents results obtained from sequential measurements of osteoporotic and osteomalacic patients. We believe this partial-body technique may have advantages over total-body IVNAA because it may show larger percentage changes in some disease states than will total-body measurement.

Changes in body composition may be diagnostic of the progression of disease or the results of therapy. In metabolic bone disease, which affects all of the bones of the body, change in one bone should to some degree reflect overall change in the skeleton. Various methods have been devised therefore to determine quantitative changes of mineral density in individual bones (although in fact these methods look at only a fraction of individual bones). Goldsmith, et al (*5*) have compared various techniques for diagnosing osteoporosis, using spinal x-rays as the definitive test. They conclude that bone density of the radius or the fifth middle phalanx by gamma or x-rays is more likely to reflect the degree of osteoporosis than bone density of the olecranon or the os calcis. Clearly metabolic bone disease does not affect all bones to the same degree. The work of the Churchill Hospital group using rabbits (*6*) further supports this by showing that turnover rates of calcium and strontium are greater in vertebrae than in long bones, e.g., the femur.

Thus, examination of a small bit of an individual bone may not reveal abnormality in the skeleton as a whole. To provide a more reliable assessment of bone mineral status, IVNAA techniques were developed to measure total skeleton calcium. But if one portion of the skeleton is more severely affected than others, measurement of the total skeletal calcium may fail to detect small changes in bone calcium whereas such changes may be clearly shown when only the more severely affected area is measured. For studying osteoporosis, a technique for examining the spine should be better than one which examines either relatively less affected areas or the skeleton as a whole.

IVNAA has been used for partial-body determinations; its greatest use so far has been in the determination of iodine in the thyroid (*7*). With respect to calcium determinations, Comar (*8*) has presented studies on irradiation of the leg bones, and our own group has done work on irradiation of the ankles (*9*). Over the last few years we have developed a technique for measuring calcium in the trunk, using

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plutonium-beryllium (Pu-Be) sources that have advantages of stability and cost compared with accelerator sources. A description of the apparatus has been given elsewhere (*10*); Cohn (*11*) has recently published an account of a Pu-Be facility for whole-body evaluation. To the present time we have made measurements involving more than 125 individuals.

To test reliability, sequential measurements were made on a skeletal phantom and on 15 normal volunteers. Sequential measurements also were made on five patients with osteoporosis, studied before treatment and at intervals up to 1 year on treatment. The results are compared with data obtained from a similar study on one patient suffering from osteomalacia.

METHOD

In IVNAA, ^{48}Ca in the body is converted by neutron capture to ^{49}Ca. This isotope is radioactive and decays with a half-life of 8.8 min, emitting a 3.1-MeV gamma ray that may be detected by NaI(Tl) crystals in a whole-body counter.

In our procedure, the subject is irradiated for 20 min in a shielded neutron chamber, the walls of which are 18-in. concrete. The neutrons are produced by 12 Pu-Be sources*, each of 5 Ci and with neutron output of approximately 1.2×10^7 neutrons/sec. The sources are placed symmetrically above and below the trunk; the source-to-skin distance is fixed for any one individual and is about 12 cm in all cases. Figure 1 illustrates the irradiation geometry. The subject lies on a wooden board of a stretcher. The subject is positioned definitively with respect to the stretcher and the sources using the sternal notch as a reference point. The wooden board has a thickness of 4 cm and acts as a premoderator for the fast neutrons from below. There is a similar wood premoderator above the subject but with an air space of 8 cm between the skin and the bottom of the premoderator. Above the upper sources (and correspondingly below the lower ones) are thick (>30 cm) hydrogenous reflectors that increase the neutron flux in the irradiation volume and also provide additional shielding. Thermal flux measurements show that the area irradiated (to a line where the thermal flux at the surface drops off to 50% of its maximum) is 60 cm × 30 cm. This area is constant for all subjects.

Correspondingly, the counting geometry is such that (to the 50% counting efficiency line) a similar area of the subject is measured by the four fixed NaI(Tl) crystals of the whole-body counter. Once

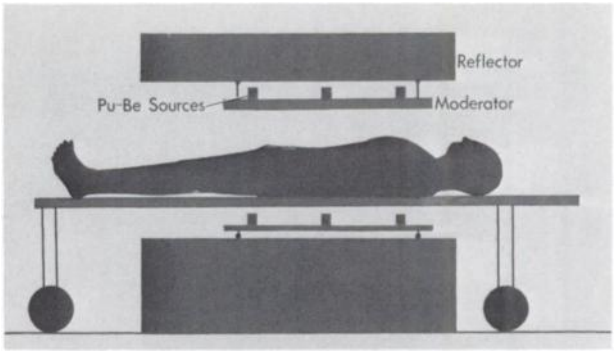

FIG. 1. Diagram of subject in neutron chamber.

again the sternal notch is used for positioning. Thus, a constant area is irradiated and counted. For different subjects this area will accommodate different proportions of the total bone in the body. With a six-foot man, approximately ⅓ of the total bone is viewed whereas with a four-foot person the figure is about 60%. These estimates are based on measurements of the bones of human skeletons.

The counting procedure consists of a 20-min count starting 3 min after the end of the irradiation. The calcium count is obtained by measuring the area under the ^{49}Ca photopeak at 3.1 MeV and subtracting from this a standard background and an amount determined by the magnitudes of the ^{24}Na and ^{38}Cl peaks at 2.75 and 2.17 MeV, respectively. These corrections are determined from phantom studies. They are made necessary by incomplete resolution of the photopeaks of ^{24}Na and ^{49}Ca at 2.75 and 3.1 MeV, respectively, and the accidental summing of the two gamma rays of ^{24}Na and because of the similar summing that is possible from ^{38}Cl. In a typical normal man, the gross count is about 1,800 in the ^{49}Ca peak region, and from this is subtracted 590 counts for background, 80 counts due to the ^{24}Na contribution, and 40 counts due to the ^{38}Cl contribution. Of this last contribution, 20 is due to summing and 20 to the production of ^{37}S.

The stability of the counting apparatus with respect to both overall efficiency and resolution is checked by counting a thorium standard before and after the main counting procedure. A continuing check on reproducibility of the method is made by measuring the calcium in a skeletal phantom†.

The dose received by a patient during the irradiation procedure is about 0.4 rem (*10*). Fission track detectors are used as dosimeters (*12*). A quality factor of 10 for fast neutrons was used. The dose is almost entirely (>90%) due to the neutrons, with smaller contributions coming from gamma rays from

* Monsanto Chemical Company.

† "Remad phantom with standard skeleton," Alderson Research Labs, Inc.

the α-Be sources (from ^{12}C) and from induced radioactivity (*11*).

SUBJECTS

The subjects consist of 15 normal volunteers, one osteomalacic patient and five osteoporotic patients, who were all studied sequentially.

The diagnosis of osteoporosis was based on radiological evidence of a generalized decrease in bone density and multiple vertebral compression fractures together with normal values for serum calcium, phosphorus, alkaline phosphatase, total protein, and protein electrophoresis. The patients with osteoporosis showed normal endocrine, liver, kidney, and gastrointestinal function with the exception of Case 14 who had hypogonadism. Testosterone replacement therapy had been given to Case 14 from 36 to 50 years of age, but the therapy had been discontinued 2 years before the present study. He showed elevated urinary calcium (370 mg/day) and urinary hydroxyproline (60 mg/day); normal values were found in the other four cases. After the initial study, the five patients were started on treatments (Table 1) including various combinations of calcium and phosphate supplements, large doses of vitamin D_2, hormones, and hydrochlorothiazide (*13*)*.

The patient was osteomalacia (Case 24) was a 19-year-old man who since infancy had suffered from vitamin D dependent rickets—an unusual form of refractory rickets that heals completely with massive doses of vitamin D_2 (*14*). He had never received adequate vitamin D therapy, and for the 3 years before the present study, no vitamin D therapy had been given. Pertinent data are shown in Table 2. Skeletal x-rays showed severe generalized loss in bone density, coarse trabecular pattern, pseudofractures, deficient mineral deposition at the sites of epiphyseal plates, dwarfism, and ricketic deformities. After the initial study, the patient was started on vitamin D_2 200,000 IU per day for 2 weeks and subsequently maintained on vitamin D_2 100,000 IU per day. His dietary calcium including supplements was 1.8 gm per day. He responded dramatically to this treatment. At 6 months, when the bone calcium IVNAA was repeated, the biochemical values had returned to normal (Table 2) and skeletal roentgenograms showed newly deposited bone mineral throughout the skeleton, particularly subperiosteally.

* Hydrochlorothiazide has been shown to improve calcium balances and to decrease rates of bone calcium turnover in some patients with osteoporosis.

RESULTS

In a series of 31 measurements over a period of 12 months, the skeletal phantom gave a mean of 801 counts for calcium; the variations from the mean are consistent with a Gaussian distribution with a standard deviation of $\pm 4.8\%$, and this is almost entirely due to the statistical accuracy of the individual counts ($\pm 4.6\%$). Grouping the results shows that there is no variation with time. The results for the sequential counts made on 15 volunteers over a period of 1 year are shown in Table 3. The variations of subsequent counts from the initial counts expressed as percent of initial count showed a standard deviation of 6.4%. This indicates that the use of human subjects introduces some variability additional to the statistical error.

Sequential counts for the one osteomalacic and the five osteoporotic patients are shown in Fig. 2. The osteomalacic showed increased bone calcium of 77% from the pretreatment value, a change which is sup-

TABLE 1. TREATMENTS FOR FIVE PATIENTS WITH OSTEOPOROSIS

Case no.	43	15	14	16	48
Sex	F	F	M	M	M
Age	71	62	52	55	39
Ca supplement	0.9 gm/day*	0.9 gm/day*	1.0 gm/day†	0.9 gm/day*	—
Vitamin D_2	50,000 IU 2 × weekly	50,000 IU 1 × weekly	—	—	—
Androgen	—	—	Testosterone cypionate 200 mg/month	Methyl testosterone 20 mg/day	—
Estrogen	Estrogen 1.25 gm/day	—	—	Ethinyl estrodiol 0.5 mg/2 days	—
Hydrochlorothiazide	—	100 mg/day	—	—	100 mg/day

* Ca supplement was 4 gm dicalcium phosphate, containing 0.9 gm Ca + 0.72 gm P.
† Ca supplement was Ca gluconate.

TABLE 2. CASE 24—VITAMIN D DEPENDENT RICKETS

	Pretreatment study	Repeat study after 6 months on vitamin D_2	Normal range
Serum calcium mg/100 ml	6.4	9.7	8.9–10.2
Serum inorganic phosphorus mg/100 ml	3.3	4.4	3.0–5.2
Alkaline phosphatase KA units	87	24	<25
Generalized renal amino aciduria	++	0	0
Oral ^{47}Ca absorption % dose (by whole-body retention (18))	18.5	75	15–45

TABLE 3. PERCENTAGE CHANGE IN CALCIUM COUNTS FROM INITIAL MEASUREMENTS

	Total no. of repeat measurements	Mean % change	s.d. (%)	P
15 volunteers	20	+2.37	6.4	
4 osteoporotics with Ca supplement	10	+8.73	7.8	<0.002
1 osteoporotic without Ca supplement	2	−18.0	—	<0.002
5 osteoporotics	12	+4.3	13.0	>0.1

ported by biochemical and radiological evidence that the osteomalacia had healed (Table 2). Changes in the osteoporotic patients were far less dramatic. Four patients showed either no significant change or only transiently increased calcium counts. The fifth (Case 48) had a value at 4 months that was 10% less than the initial count and at 8 months 26% less. Case 48 was given hydrochlorothiazide alone, and his dietary calcium was estimated to be 500 mg per day. Changes in his calcium counts are significantly ($p < 0.002$) different from those of the other four given calcium supplements together with various other treatments. For all five patients, no skeletal changes were observed by radiological assessment during the period of study. Table 3 shows the distribution of the changes from initial values for the five osteoporotic patients and for the normals. The standard deviation in each case is the standard deviation (approximately) of the Gaussian curve obtained for these distributions. For the four patients with calcium supplements taken together, the mean increase of 8.7% in calcium counts is significant compared with the normal data ($p < 0.002$), and for the one case without calcium supplements the mean decrease is significant ($p < 0.002$). If the five are taken as one group, the decrease in the one patient cancels much of the increase in the other four, and the mean value of change is not significantly different from that of the normal volunteers.

DISCUSSION

The variability found in the reproducibility studies with the skeletal phantom is 4.8% (1 s.d.). Other groups quote values of less than 2.5% (*3,11*). Our higher value is due to the smaller number of counts obtained with the present setup (i.e., to the greater statistical error), itself due to a lower neutron flux than that obtained from a cyclotron (as at Birmingham) and to a smaller volume of NaI(Tl) than that used at Brookhaven. Against these disadvantages may of course be set the absolute reliability of the Pu-Be sources for their production of neutrons and the much lower cost of the NaI(Tl) assembly at Toronto. No figures are available as yet for reproducibility studies with normal human volunteers from other laboratories. Our results indicate that with living subjects reproducibility will be poorer than with phantoms.

A dramatic increase in bone calcium as a result of successful treatment for osteomalacia is to be expected. Based on our normal data, the observed 77% change in calcium in this patient may well represent a change in total calcium of about 200 gm over a 6-month period, assuming all bones to be

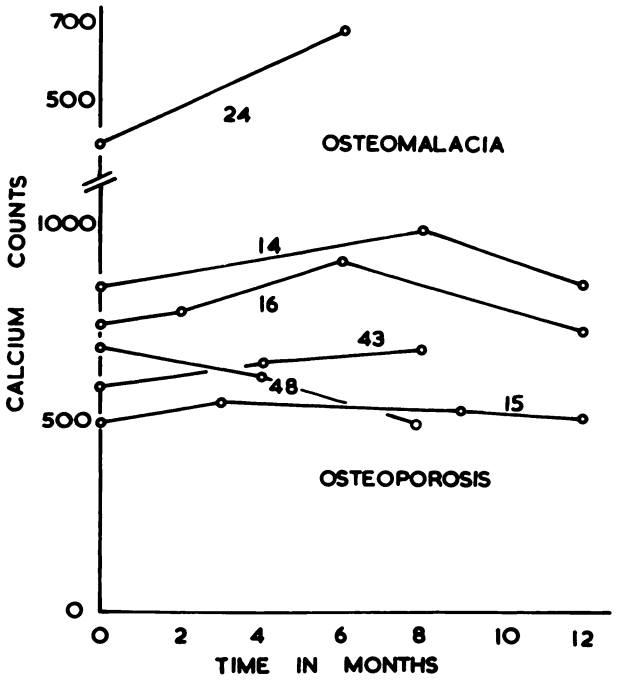

FIG. 2. Sequential calcium counts for osteoporotic (below) and osteomalacic (above) patients. Statistical errors are 5% in all cases.

equally affected. This patient was known (by a subsidiary experiment) to have a calcium absorption of 75% at the time of repeat measurement, and his dietary intake was 1.8 gm per day. Over 180 days therefore a gain of 200 gm is easily possible.

In the patients with osteoporosis, significant changes in total-bone calcium were not expected although positive calcium balances of perhaps 200 mg per day are not uncommon during the first months on treatment (*13,15,16*). Over 6 months this would represent a total of 36 gm which, in a person with 700 gm of calcium in the whole body, would mean a change of perhaps 5%. The present technique, however, restricts its measurements to about one-third of the bone, the part which includes much cancellus bone; on the latest model for alkaline earth metabolism in man (*17*), cancellus bone is assigned a turnover rate four times that of compact bone, and therefore the portion of the bone looked at by the present technique may be regarded as being the most active metabolically. A 5% increase in the total-body calcium could well therefore represent a 15% increase in the third of the skeleton studied, assuming no change in skeletal calcium of the head and extremities.

In Case 48, whose calcium counts showed a decrease of 26% over 8 months, balance data (before treatment) showed that the subject was losing 180 mg of calcium per day. Over 8 months, this represents 43 gm of calcium and a 6% decrease in total calcium, assuming an initial total calcium of 700 gm. Using the factor of 3 as discussed above, implies a possible 18% change in his calcium count. The subject had periodic roentgenography but, as previously stated, did not show evidence of bone mineral loss by that technique. The imprecision of standard x-ray is such that this is not surprising. The subject has now been placed on calcium supplements.

It may well be that investigating the the most metabolically active areas of the skeleton, that is, using partial body IVNAA, will show small changes in bone calcium not readily detectable by total-body IVNAA.

The partial-body IVNAA technique is shown to be reliable, and the results of sequential measurements on patients show that changes in calcium status can be observed. This procedure of measuring the trunk rather than the whole body detects small changes in bone calcium that may be less readily detected by total-body IVNAA.

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