PART V
CALCIUM AND BONE DISEASE
Chairman: Professor J B Rosenfeld
Calcium Loss in Renal Osteodystrophy as Measured by Neutron Activation Analysis

G R D CATTO, J A R McINTOSH, M MacLEOD
University of Aberdeen, Scotland, UK

The progressive metabolic bone disease associated with chronic renal failure produces symptoms in only a minority of patients and displays wide geographic variations in incidence (Stanbury, 1972). Although the condition is apparently uncommon in both the USA and Israel (Berlyne et al, 1973), it has been well recognised in Britain for many years. The incidence of osteodystrophy affecting patients on regular haemodialysis has wide regional differences; symptomatic bone disease is present in almost all the patients in Newcastle (Kerr et al, 1969) whereas it is not a clinical problem in London (Curtis et al, 1969).

Current techniques of assessing changes in skeletal calcium have important limitations. Such biochemical parameters as serum calcium, phosphate and alkaline phosphatase may remain within normal limits in the presence of continuing bone disease. Routine radiography is insufficiently sensitive as the most careful assessment may fail to detect changes of demineralisation until as much as 40% of skeletal calcium has been lost (Simon, 1965). Serial bone biopsies are unpleasant for the patient and difficult to quantitate accurately. Isotopic techniques assess pools of exchangeable calcium and the results are not readily interpreted. The photon absorption technique, which in vivo has a reproducibility of ± 3-4% measures not the calcium content but the total bone mineral content over a localised area of long bone such as the radius.

Neutron activation analysis may be used to measure specifically the calcium content of the tissues irradiated. Activation occurs when neutrons, travelling at the correct speed, collide with and are absorbed into the nuclei of atoms present in the tissues; if the isotopes thus produced are radioactive, their presence can be detected. A small but constant amount of naturally occurring calcium has an atomic weight of 48. When $^{48}$Ca is bombarded by slow moving or 'thermal' neutrons, $^{49}$Ca, a gamma emitter
with a half life of 8.8 minutes, is produced. By detecting and quantitating this radiation the amount of calcium present may be estimated (Fremlin, 1972). Although the technique has been successfully applied to the study of total body calcium (Chamberlain et al, 1968) the complex cyclotrons and generators used to supply the neutron flux require specially designed accommodation and skilled technical staff for their operation. Moreover, the interpretation of the results obtained from patients with renal osteodystrophy is frequently complicated by the presence of ectopic calcification (Cohn et al, 1972).

As the radiological features of renal osteodystrophy are frequently first observed in the phalanges (Siddiqui & Kerr, 1971), a simple technique for measuring small changes in the calcium content of a hand by neutron activation analysis has been developed (Catto et al, 1972). The results obtained on 19 haemodialysis patients observed for a period of nine months are presented and contrasted with results from radiological and photon absorption studies.

METHOD

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<th>Patient</th>
<th>Sex</th>
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<th>Height (m)</th>
<th>Weight (kg)</th>
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Nineteen patients dialysed for a total of 20–30 hours per week on Kiil dialysers were studied over a period of 38 weeks. The clinical details of the patients are shown in Table I. The concentration of calcium in the dialysate was maintained between 5.3 and 5.5 mg/100 ml.

A total of five activation analyses were performed on each patient. The neutron flux was provided by a powdered mixture of americium ($^{241}$Am) and beryllium ($^9$Be) doubly encapsulated in stainless steel containers measuring 10 cm long, 3 cm in diameter and sealed by argon arc welding. Alpha particles produced by the americium cause the beryllium to decay to carbon and release neutrons. These fast neutrons were slowed or 'moderated' by placing the source in a perspex tube positioned across a large water tank. The maximum thermal neutron flux was measured to be $(5.6 \times 10^5)$ neutrons per cm$^2$/sec at a distance of 2 cm from the surface of the source. During the irradiation procedure, the patients grasped the tube containing the source for 1000 seconds thus activating the $^{48}$Ca. The gamma radiation from the $^{49}$Ca was detected by placing the irradiated hand for 2000 seconds between two large sodium iodide crystals linked to a multi-channel analyser. The $^{49}$Ca content of the hand is represented by the difference between the $^{49}$Ca peak and the background radiation at the 3.1 MeV position (Figure 1).

The photon absorption technique was used to measure the total bone mineral content on each patient on six occasions. The distal third of a radius was scanned with a Norland-Cameron bone mineral analyser. A collimated beam of photons from the 200 mCi $^{125}$I source required for this.

![Gamma spectrum from an activated hand](image-url)
device passes through the combination of soft tissues and bone in the limb, and the resulting attenuation in the number of photons monitored by a suitable gamma ray detector is related to the mass of bone present. A permanent record of the scans was obtained by linking a chart recorder to the bone mineral analyser.

Twice a year each patient underwent radiological examination of hands, feet, lateral skull, chest, dorsal and lumbosacral spine, and pelvis. The films were checked and reported by the same radiologist to ensure that similar exposures were obtained on each occasion.

RESULTS

The results from the radiological and photon absorption studies are shown in Figure 2. Prior to the start of the present study 84% of the patients had radiological evidence of metabolic bone disease. Radiological changes indicating progressive demineralisation were detected in two (10.5%) patients over a period of 26 weeks; during the following six months changes were noted in a further two patients. Thus during one year evidence of progressive renal osteodystrophy was detected by radiography in 21% of the patients. During a period of 38 weeks, a net decrease in the total bone mineral content was detected by the photon absorption technique in 10 (53%) of the patients; the change in the mineral content expressed as the percentage change from the original value varied from +17.4% to -16.8% with a mean value of -1.5%.

![Changes in Bone Density Graph](image)

Figure 2. Results of radiological, photon absorption and neutron activation studies expressed as the percentage change from the initial value. Symbol 'R' indicates a radiological decrease in bone density.
Figure 3. Results of 5 activation analyses from 4 patients

Over the same period none of the 19 patients studied by neutron activation recorded a gain in skeletal calcium, and the mean calcium loss detected by this method was -12.3%. Several of the patients, for example Patient 1, 3 and 7 revealed a marked and continuing loss of $^{49}$Ca during the study whereas the $^{49}$Ca counts from Patient 10, a healthy man with no known osteodystrophy remained almost constant (Figure 3).

DISCUSSION

Although neutron activation analysis was first introduced into clinical practice in 1964 (Anderson et al, 1964) it has been used principally to study total body calcium (Nelp et al, 1970). The results obtained are reliable and repeated measurements on cadavers gave a coefficient of variation of 1.5%. The coefficient of variation for the partial body activation technique used in this study was assessed by repeated measurements of anatomical specimens and for clinical studies is between 4 and 5% (Catto et al, 1973).

The lack of detectable radiological change in 79% of the patients over one year indicates the relative insensitivity of radiology in following
progressive skeletal demineralisation. The rates of change in the total bone mineral contents of the radii (Figure 2) show several widely varying results. Patient 13, for example, a young woman with marked radiological evidence of metabolic bone disease recorded an increase of 17.4% in the bone mineral content of her left radius during a period when no alteration had been made to her treatment. The presence of ectopic calcification was suspected and the chart recorder linked to the mineral analyser produced the graph shown (Figure 4). The trace obtained from Patient 9 illustrates the changes produced in the count rate during a typical bone scan using the photon absorption technique; the decrease in the count rate noted at the point 'a' in the graph from Patient 13 occurs before the scan reaches the leading edge of the radius and is thought to indicate the presence of ectopic calcification. An x-ray of the forearm revealed extra-skeletal calcium. This finding may explain the anomalous results obtained in this series.

With few exceptions, the percentage losses of calcium detected by neutron activation analysis were considerably greater than the percentage decreases in total bone mineral content. The mean decrease in calcium content detected by the activation technique is similarly considerably greater than the mean decrease in the total mineral content detected by the photon absorption studies. It is suggested that these differences are primarily due to the greater specificity of the activation procedure. From radiographic studies it appears that the subperiosteal regions and terminal phalanges are particularly susceptible to resorption in renal osteodystrophy. Thus the changes detected by partial body neutron activation analysis although perhaps
not representative of changes in the skeleton as a whole may prove to be a sensitive and accurate indicator of the rate of progression or healing of a metabolic bone disease. Moreover, the results obtained by photon absorption are complicated by the presence of extra-skeletal deposits of calcium (Figure 4) as are the results from total body calcium studies (Cohn et al., 1972). Since the ratio of soft tissue to bone is less in the hand than in the body generally, the results from partial body activation analyses may be less influenced by ectopic calcification.

CONCLUSION

Neutron activation is the only non-destructive method which specifically measures calcium in vivo. The technique, which uses a small portable neutron source has proved sufficiently sensitive to monitor changes in skeletal calcium in patients on haemodialysis therapy and should in addition provide quantitative information on the effect of future therapy designed to limit the progress of the osteodystrophy.

ACKNOWLEDGMENT

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OPEN DISCUSSION

B H B ROBINSON (Birmingham): I was very interested in this technique because we have used techniques based on whole body neutron activation. Have you managed to do a number of measurements on different control subjects over a period? One might get very consistent results with phantoms, but there might be more difficulty in persuading normal subjects to hold their hands steady over a long period.

Secondly, I think that one should have some hesitation in extrapolating changes in calcium in the hand to the whole body. Some of our patients, in the face of progressive demineralisation of the hand, as shown radiologically, have appeared to be gaining a significant amount of metabolic calcium. Although this might be due to ectopic calcium, I don't think this is likely because of the sheer quantity of mineral involved, over ninety per cent of which is within the bone. Such radiological and tissue analyses that we have so far performed don't really suggest an explanation in terms of ectopic calcium. Many of the patients we see are not in negative calcium balance, they may even have been in a positive calcium balance, but they lose mineral from certain sites and deposit it in others, as in osteosclerosis.

CATTO: First, it is very difficult to get control subjects for a new technique of this type, and the controls were based on the hands of dead anatomical subjects. Before activating the renal patients, we activate the standard which contains a known amount of calcium. This allows calibration of the equipment. We are going to study normals and patients with other forms of bone disease. Secondly, I think the point has been made several times by your own unit and by units in the USA, that whole body calcium doesn't change to any great extent. But there is a redistribution of calcium from one area to another, and we know clinically that these patients develop fractures. What we are suggesting is that perhaps the hand is a particularly sensitive area of the body to measure. Radiographically, changes appear first in the hand. And thirdly the point about ectopic calcification. I find this one extremely difficult to answer. It has been suggested by people working in the USA that the presence of ectopic calcification is the reason that whole body techniques fail to show change.

P ZECH (Lyon): Have you any correlation of measurements by neutron activation with bone mass measurements from quantitative bone biopsies?

CATTO: Yes, we have. We irradiated dead hands, then ashed them and estimated the calcium by atomic absorption. The ratio of this to the calcium in the dead hands, was the same as the ratio of the calcium forty-nine counts obtained by activation analysis. We couldn't think of another way of doing it.
V PARSONS (London): I noticed one of your patient's hands had sticking plaster on it. Have you noticed any difference between the bone density of the hands that are being used for fistulae or shunts compared with the hands that are not being used?

CATTO: I don't think we have. We've just started looking at this very recently. I think that the differences that we found initially are explicable by ectopic calcification in blood vessels.

PARSONS: You will find that on the fistula side there is less calcium.

CATTO: It hasn't been our experience so far.