

# Some aspects of calcium metabolism in malignant disease of bone

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**SYNOPSIS** The rate of calcification of new bone (accretion rate) was measured by a radioisotope technique in 20 patients with carcinoma of the breast, 14 patients with multiple myelomatosis, five patients with Paget's disease of bone, and in six patients with solitary, non-osseous tumours. The rate of bone destruction was assessed in these patients by the measurement of the rate of urinary calcium excretion.

In the patients with carcinoma of the breast and bone metastases there was a marked increase both in the accretion rate and in the urinary calcium excretion suggesting an osteoblastic response to bone destruction. An osteoblastic response was also present in the patients with multiple myelomatosis but was not correlated with the urinary calcium excretion. In patients with Paget's disease of bone, high values of accretion rate were found indicating very active new bone formation.

Episodes of hypercalcaemia and hypercalciuria may be associated with malignant tumours of widely differing types and sites of origin. These disturbances of calcium metabolism are common in patients with carcinoma of the breast, particularly in those with bone metastases. Similar disturbances associated with bone destruction are observed in many patients with multiple myelomatosis (Gutman, Tyson, and Gutman, 1936; Woodard, 1953).

It has been shown that during these episodes patients are in negative calcium balance and that the hypercalcaemia is not due to excessive absorption of calcium from the gut (Laszlo, Schulman, Bellin, Gottesman, and Schilling, 1952). Studies of the osteoblastic response to the destruction of bone in these patients have depended on the indirect evidence of the levels of alkaline phosphatase in the plasma, and it has been assumed that the increased levels of this enzyme observed in patients with metastatic tumours of bone have reflected, in the absence of obvious liver damage or metastases, increased new bone formation. Conversely, the normal or low levels of plasma alkaline phosphatase observed in patients with multiple myelomatosis have been interpreted as evidence of a failure of compensatory growth of new bone in the face of destruction by myeloma tissue (Woodard, 1953; Gutman *et al.*, 1936).

The availability in recent years of the isotopes <sup>45</sup>Ca, <sup>47</sup>Ca, and <sup>85</sup>Ca has enabled a more detailed study to be made of the turnover of calcium in the body. Short-term studies of the retention of calcium in the body are of some clinical value but they do not give information concerning the way in which the body utilizes the retained calcium (Spencer, Laszlo, and Brothers, 1957). Methods based on the measurement of isotope clearance rates have indicated that a proportion of the calcium that leaves the extracellular fluid compartment does so by routes other than those of excretion. The rate of loss of this fraction of the extracellular fluid calcium has been assumed to be a measure of the rate of calcification of newly formed bone and has been termed the 'bone accretion rate' (Bauer, Carlsson, and Lindquist, 1955, 1957; Heaney and Whedon, 1958; Fraser, Harrison, and Ibbertson, 1960; Glass and Nordin, 1963). Despite the limitation of such an assumption (Heaney and Whedon, 1958) these methods provide useful tools for the assessment of bone activity in a variety of clinical conditions (Bauer *et al.*, 1957; Fraser *et al.*, 1960).

In the investigation presented here, the accretion rate was determined as a measure of new bone formation, and the rate of urinary calcium excretion was used to assess the degree of bone destruction in patients with carcinoma of the breast, multiple myelomatosis, and Paget's disease of bone. The patients were maintained on a normal hospital diet.

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## METHODS

**SELECTION OF PATIENTS** Six patients with primary malignant tumours who were otherwise in good health and without evidence of bone disease were selected for the control group. Twenty patients with carcinoma of the breast were investigated of whom 14 had radiographic evidence of bone metastases. The latter patients had previously received a variety of treatments including steroids, radiotherapy or adrenalectomy, but at the time of investigation were in relapse. Five patients with Paget's disease of bone and 14 patients with multiple myelomatosis who had skeletal deposits as well as characteristic bone-marrow and plasma protein changes were also investigated.

**TECHNIQUE USING  $^{45}\text{Ca}$  AS  $\text{CaCl}_2$**  25 to 50  $\mu\text{c.}$ , was diluted with 10 ml. of 0.9% saline, sterilized, and injected intravenously. The volume of the injected dose was determined by weighing the container before and after charging the syringe, and by radioassay of syringe residues.

Excreta were collected for 10 to 14 days after the administration of the dose. Urine was passed into 2.5 litre Winchester bottles containing 10 ml. concentrated HCl; faeces were wrapped in polythene sheeting and placed in waxed containers. The volume of each 24-hr. urine sample was measured and an aliquot (usually 50 ml.) was concentrated by wet-ashing with 10 ml. fuming nitric acid. A few drops of hydrogen peroxide were added when the ashing was almost complete to prevent charring of the sample. The white precipitate was dissolved in 2 ml. of 2N HCl and made up to 10 ml. with distilled water. Faecal samples were ashed for 12 hours in a muffle furnace at 600°C. The ash was weighed and a known aliquot (about 0.1 g.) dissolved in 2N HCl and made up to 100 ml. Of these solutions, 1 ml. aliquots were adjusted to pH 5 with 25% ammonia, the calcium precipitated with saturated ammonium oxalate and estimated by titrating with potassium permanganate (Clark and Collip, 1925). Further aliquots of the solutions containing 3 to 4 mg. calcium were then pipetted on to 1 cm.<sup>3</sup> plastic planchettes fitted into specially constructed perspex centrifuge tubes. Four ml. of a solution containing 1 mg. of Ca/ml. was added to a suitable aliquot of syringe washings and to standard solutions of  $^{45}\text{Ca}$ . These were treated in the same way as the test samples. After standing overnight the tubes were centrifuged at 2,000 r.p.m. for 20 minutes. The supernatant fluid was decanted and the plastic planchettes were removed and dried at room temperature.

All samples were assayed for radioactivity with a thin end-window GM tube and conventional scaler.

**TECHNIQUE USING 35 TO 40  $\mu\text{c.}$   $^{47}\text{Ca}$**  The dose was prepared and administered, and the excreta collected as for  $^{45}\text{Ca}$ . In some cases 20 ml. samples of blood were collected at intervals during the first 24 hours.

The excreta were counted directly after dilution to constant volume by placing the Winchester bottle within a ring of 16 GM tubes. Two standard solutions were prepared each containing 5% of the dose, one in 2.5

litres of water in a Winchester bottle and the second in a wax carton containing cotton wool. A lead filter 2 mm. thick was placed between the specimen and the GM tubes to avoid recording the radiations from  $^{47}\text{Scandium}$  which is a daughter isotope of  $^{47}\text{Ca}$  and a weak emitter. Fifty ml. aliquots of urine were wet-ashed and the calcium estimated as above. Blood samples were centrifuged and the calcium was precipitated from 10 ml. of plasma with an equal volume of saturated ammonium oxalate. After washing with 2% ammonia the precipitate was dissolved in 2N HCl for radioassay in a well-type scintillation counter. A standard sample was prepared from a suitable aliquot of the injected solution. The  $^{47}\text{Ca}$  was assayed on an E.H.T. voltage and discriminator bias such as to avoid recording activity from  $^{47}\text{Scandium}$ . The calcium content of each plasma sample was determined by permanganate titration after the radioassay.

**TECHNIQUE USING 1 TO 5  $\mu\text{c.}$   $^{47}\text{Ca}$**  Excreta were collected as above. The volume of each urine specimen was measured and 20 ml. wet-ashed and made up to 10 ml. for calcium estimation as described for  $^{45}\text{Ca}$ . The remainder of the specimen was evaporated to a small volume and made up to 300 ml. Faecal samples were ashed in a muffle furnace, dissolved in hot dilute HCl, and made up to 300 ml. A standard solution of  $^{47}\text{Ca}$  containing a known fraction of the dose was prepared in a similar volume. Samples were assayed by placing each bottle between two scintillation probes constructed with 7 × 5 cm. NaI (Tl) crystals mounted in a low background room (Trott, Parnell, Hodt, and Entwistle, 1963) and the activity of energy above 300 Kev. recorded.

**CALCULATION OF THE RATE OF CALCIUM ACCRETION IN BONE** Specific activities of urine were expressed as percentage per milligram dose of calcium. Preliminary studies of both blood and urine specimens showed that the mean specific activity of a sample of urine was similar to that of a sample of plasma obtained after an interval of time equal to one third of the period in which the urine was collected. Thus the mean specific activity of a 24-hour urine approximated that of a sample of plasma obtained eight hours after the start of the urine collection. Owing to the small doses of  $^{47}\text{Ca}$  used and its short half-life (4.7 days) low activities were present in the plasma; thus the measurement of the mean urinary specific activity obviated the use of large volumes of plasma. The specific activity of plasma calcium, whether measured directly or deduced from the urine data, was adjusted for losses in the excreta by dividing the observed specific activity by the fraction of the dose retained in the body at that time (Fig. 1). The adjusted specific activity values were plotted on semi-logarithmic paper. A curve was obtained for each investigation which could be analysed into a number of exponential functions. The early mixing components were ignored for these purposes and the last linear part of the curve was assumed to represent the rate at which calcium was lost from the extracellular fluid into semi-permanent or permanent bone. The percentage dose retained in the body plotted semi-logarithmically showed a deviation from linearity in the early part of the curve resulting from a disproportionate loss of activity in

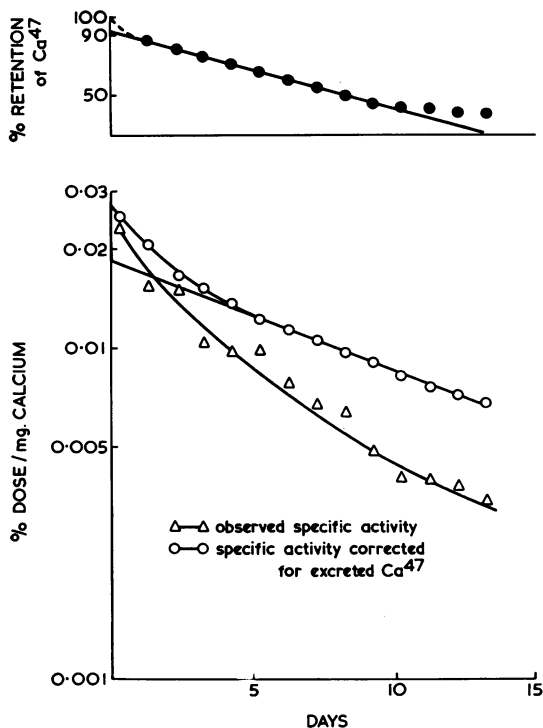


FIG. 1. Example of retention of dose of radioactive calcium and alterations in specific activity of calcium corrected for excreted losses.

urine during the early mixing phases (Fig. 1). The percentage dose retained at zero time obtained by extrapolating the linear part of this curve was used in the following calculations.

$$1 \text{ Exchangeable calcium pool (mg.)} = \frac{\text{Extrapolated percentage retained at zero time}}{\text{Extrapolated specific activity at zero time}}$$

$$2 \text{ Rate constant (fraction/day)} = 0.693$$

$$\text{Half-time of linear part of specific activity curve (days)}$$

$$3 \text{ Accretion rate (mg. Ca/hour)} = \frac{\text{Exchangeable pool size} \times \text{rate constant}}{24}$$

RESULTS

The values for the exchangeable calcium pool, the calcium accretion rate, and the mean 24-hourly urinary calcium excretion are given in Table I. The six control patients had a mean accretion rate of  $24.8 \pm 7.7$  (S.D.) mg./hr. Although the mean value of  $16.7 \pm 10.2$  mg./hr. for patients with carcinoma of the breast without metastases was lower, this

TABLE I  
INDIVIDUAL VALUES FOR EXCHANGEABLE CALCIUM, ACCRETION RATE, AND URINARY CALCIUM

Group	Rate and Urinary Calcium		
	Mean Urinary Calcium (mg./24 hr.)	Accretion Rate (mg.Ca/hr.)	Exchangeable Calcium (g.)
<i>Control</i>			
1	245	25.2	4.38
2	175	14.7	3.23
3	219	30.7	4.63
4	257	33.3	3.70
5	170	28.7	8.00
6	226	16.4	5.68
<i>Carcinoma of breast (no bone metastases)</i>			
1	318	18.6	4.50
2	315	33.0	3.90
3	281	4.8	2.62
4	150	7.0	6.60
5	264	20.7	3.23
6	228	16.2	4.62
<i>Carcinoma of breast (with bone metastases)</i>			
1	79	32.5	4.00
2	109	112.0	12.70
3	116	28.4	5.60
4	334	31.5	4.85
5	478	45.8	4.60
6	277	29.2	5.30
7	523	97.5	11.40
8	69	35.2	7.88
9	302	28.2	4.87
10	296	25.5	4.60
11	69	18.5	4.68
12	362	68.0	5.67
13	400	42.1	4.23
14	470	39.0	5.93
<i>Multiple myelomatosis</i>			
1	374	25.7	4.82
2	132	36.8	6.70
3	328	24.6	4.10
4	215	31.1	6.07
5	431	35.2	4.15
6	169	31.4	4.27
7	223	45.2	7.30
8	530	58.6	6.70
9	85	18.6	5.65
10	383	41.0	5.75
11	203	41.8	3.83
12	300	36.9	3.08
13	193	51.5	8.00
14	459	21.2	6.90
<i>Paget's disease of bone</i>			
1	186	214.0	13.30
2	100	40.8	8.30
3	130	99.0	11.00
4	76	79.5	9.35
5	69	157.0	11.10

difference was not statistically significant ( $0.1 < p < 0.2$ ). Patients with bone metastases showed an increase in the accretion rate with a mean value of  $45.2 \pm 27.9$  mg./hr. but only the group of patients who excreted more than 300 mg. of calcium in 24 hours showed an increase in accretion rate, i.e.,  $50.3 \pm 24.6$  mg./hr. which was significantly higher than the control value ( $0.02 < p < 0.05$ ).

The mean accretion rate for the patients with

TABLE II  
RATES OF BONE ACCRETION AND OF URINARY EXCRETION OF CALCIUM IN PATIENTS  
WITH CARCINOMA OF BREAST, MULTIPLE MYELOMATOSIS, AND PAGET'S DISEASE

Group	No. of Patients	Mean Bone Accretion Rate (mg./hr. $\pm$ S.D.)	Significance of Difference from Control	Mean Urinary Excretion (mg./24 hr. $\pm$ S.D.)
Control	6	24.8 $\pm$ 7.7		207.0 $\pm$ 51.0
Carcinoma of breast without bone metastases	6	16.7 $\pm$ 10.2	0.1 < P < .2	259.3 $\pm$ 63.2
Carcinoma of breast with bone metastases				
(a) All cases	14	45.2 $\pm$ 27.9	P = 0.1	277.4 $\pm$ 162.8
(b) Cases with urinary excretion > 300 mg./day	7	50.3 $\pm$ 24.6	0.02 < P < 0.05	409.9 $\pm$ 82.3
Multiple myelomatosis	14	35.7 $\pm$ 11.4	P = 0.05	287.5 $\pm$ 133.5
Paget's disease	5	118.1 $\pm$ 68.0	P < 0.01	112.2 $\pm$ 47.6

multiple myelomatosis, 35.7  $\pm$  11.4 mg./hr., was somewhat higher than that for the controls ( $p = 0.05$ ). However, the group of patients with Paget's disease of bone showed a very great increase in accretion rate, the mean being 118.1  $\pm$  68.0 mg./hr. ( $p < 0.01$ ) (Table II).

The mean urinary calcium excretion rate of control patients on an unrestricted diet was found to be 207.0  $\pm$  51.0 mg./24 hr. (Table II). Patients with carcinoma of the breast showed an increased urinary calcium excretion rate, particularly those with bone metastases whose mean rate was 277.4  $\pm$  162.8 mg./24 hr. The patients with multiple myelomatosis showed the greatest rate of urinary calcium excretion, the mean value being 287.5  $\pm$  133.5 mg./24 hr. The

excretion rate in the group of patients with Paget's disease was 112.2  $\pm$  47.6 mg./24 hr.

#### DISCUSSION

Among the patients with carcinoma of the breast studied those who had bone metastases and excreted more than 300 mg. of calcium in 24 hours showed the highest accretion rates (Table II). In contrast, of the six patients without evidence of bone metastases, four had normal and two had unusually low accretion rates. Only two of the patients in this group excreted more than 300 mg. calcium in 24 hours. Similar low accretion rates have been reported in patients with idiopathic or post-menopausal osteoporosis (Nordin, 1961; Glass and Nordin, 1963). Although the number of patients in this group is small our findings suggest that in a proportion of patients with carcinoma of the breast without bone metastases there may be an increased breakdown of bone without a compensatory increase in bone growth.

The accretion rate obtained for one of the patients with carcinoma of the breast and bone metastases was 112 mg./hr., or 2.4 standard deviations higher than the mean for the whole of this group of patients (Table II). If this patient is excluded from the group a significant correlation is seen between the rates of accretion and of urinary calcium excretion (correlation coefficient = 0.623;  $P = 0.02$ ). This suggests that increased new bone formation may be associated with osteolytic secondary deposits.

In contrast, in patients with multiple myeloma, there was no correlation between excretion and accretion rates, suggesting that the process of new bone formation in this condition is not dependent on that of bone destruction. The isotope investigations were repeated after radiotherapy in three of the patients in this group (Table III). Reductions in accretion rates were noted in all three patients. This may have been due to damage to osteoblasts in the irradiated bone, but in view of the local nature of the treatment it is more likely that it reflects the natural history of the disease.

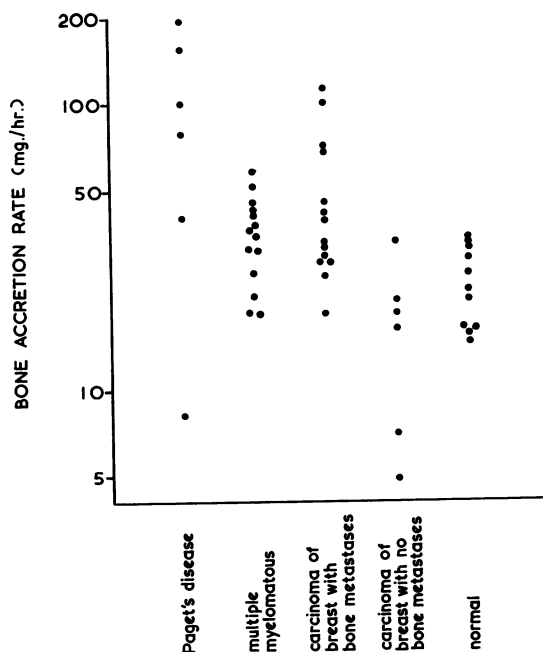


FIG. 2. Distribution of values of accretion rate in carcinoma of breast, multiple myelomatosis, and Paget's disease of bone.

TABLE III

REPEATED OBSERVATIONS ON THREE PATIENTS  
WITH MULTIPLE MYELOMATOSIS

Patient	Observation	Accretion Rate (mg./hr.)	Mean Urinary Calcium (mg./24 hr.)
1	1	25.7	374
	2	23.8	161
	3	19.9	314
5	1	35.2	431
	2	32.0	386
10	1	41.0	383
	2	15.8	409

The high values of accretion rate observed in the patients with Paget's disease of bone are associated with relatively low excretion of calcium in the urine (Table II). Previous observations of accretion rate in patients with Paget's disease have shown similar high values and they probably reflect the great activity in calcium turnover in the affected bone (Heaney and Whedon, 1958; Bauer *et al.*, 1961).

Our findings provide some confirmation for the conclusions derived from the earlier observations of the changes in the levels of serum alkaline phosphatase. Woodard (1953) observed raised levels of alkaline phosphatase in about half of her cases of carcinoma of the breast with metastatic bone disease. She also observed that a rise in serum alkaline phosphatase was less common in multiple myelomatosis, and indeed the level of this enzyme was often reduced. Thus by measuring the urinary

calcium excretion and the bone accretion rate of calcium by isotope techniques a useful assessment can be made of the rate of calcification of newly formed bone relative to the degree of bone destruction.

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