bimodal distribution which might account for the clinical finding that some depressed patients respond and some do not respond to these drugs. In fact there was no evidence of a bimodal distribution. On the other hand, there was a wide scatter of values, some patients showing very little increase in amine concentration compared with controls. (Alexanderson *et al.* (1969) showed a pronounced variability in the steady-state plasma levels of patients on tricyclic antidepressants and this may be correlated with the therapeutic response (Åsberg *et al.*, 1971). We found that tissue response with M.A.O.I.s may show similar variability. Some patients evidently need a larger dose of M.A.O.I.s and Akindele *et al.* (1970) showed a correlation between clinical improvement and disappearance of rapid eye movement (R.E.M.) sleep which in certain patients occurs only with large doses of an M.A.O.I.

By doubling the dose of isocarboxazid or giving an equivalent (oral) dose of nialamide intramuscularly previously unresponsive patients may improve (Pare and Jenner, 1966). This is borne out biochemically when the findings of the present investigation are combined with those of Maclean *et al.* (1965). Ten out of 44 patients receiving a "standard" M.A.O.I. (isocarboxazid 30 mg/day, nialamide 300 mg/day, clorgyline 20 mg/day) for a minimum period of 10 days had brain 5HT concentrations of less than 0.6 µg/g. Of those patients receiving clinically more effective therapy (tranylcypromine 30 mg/day, isocarboxazid 60 mg/day, or nialamide 500 mg intramuscularly four times a week) only one out of 24 had a brain stem concentration of less than 0.6 µg/g. Nevertheless some patients do appear clinically quite unresponsive to M.A.O.I.s yet respond dramatically to tricyclic antidepressants, and, of course, the reverse is also true. The familial association of these cases has led to the suggestion that

they may represent genetically different types of depression (Angst, 1961, 1964; Pare *et al.*, 1962; Pare and Mack, 1971).

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Changes in Total Body Calcium and Sodium in Osteomalacia

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Summary

Serial measurements were made of total body calcium and sodium, using whole body neutron activation analysis, in six patients being treated for osteomalacia. All showed a rise in total body calcium ranging from 3 to 30%; this continued for up to 24 months after the start of treatment, though complete healing had apparently occurred after 6 months on the basis of histological examination of biopsy specimens of bone. As the total body calcium rose there was a fall in the non-exchangeable sodium pool in bone.

Introduction

Quantitation of the changes in bone mineralization which occur in osteomalacia is difficult. Assessment by conventional

radiography is relatively insensitive (Simon, 1965). Measurement of cortical thickness or area (Barnett and Nordin, 1960) was a useful advance but is more suited to investigation of osteoporosis than osteomalacia. In this latter condition the cortex may be ill-defined and difficult to measure precisely. Cortical width may often be relatively well preserved and less affected than cortical bone density. Techniques using γ-ray absorption (Cameron and Sorenson, 1963) or x-ray densitometry (Anderson *et al.*, 1966) offer a considerable improvement in sensitivity, detecting changes in bone mineralization with an error of around 5% in practice. These measurements made on the peripheral skeleton suffer from the disadvantage that in some metabolic bone diseases cortical and trabecular bone may not be affected to the same degree. This may be a feature of osteoporosis or uraemic osteodystrophy, but the use of spinal densitometry (Nordin *et al.*, 1968) in combination with measurements of the peripheral skeleton might be expected to avoid this difficulty.

Measurement of total body calcium avoids problems of asymmetrical skeletal involvement in metabolic bone disease and provides information about the natural history or response to treatment.

Previous studies (Anderson *et al.*, 1964; Chamberlain *et al.*, 1968a, 1968b) showed that in-vivo neutron activation analysis in man was technically feasible and able to provide an assessment of total body calcium and sodium while administering a radiation dose (Bush, 1971) which was ethically acceptable.

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With this technique the changes in total body calcium of $\pm 5\%$ should be detectable, and since more than 99% of body calcium is in bone the changes may be taken as representative of skeletal calcium.

This paper reports the results of 23 sequential measurements of total body calcium and sodium in a group of patients with osteomalacia as their bone lesions healed under the influence of treatment.

Patients and Methods

The six patients studied had clinical, biochemical, radiological, or histological evidence of osteomalacia and were studied for periods of up to three years after starting definitive treatment. The four with adult coeliac disease were treated with a gluten-free diet and only one (Case 2) had vitamin D₃ supplements, while the patient with postgastrectomy malabsorption (Case 3) had 100,000 units of calciferol daily for 22 months and the adult immigrant with nutritional osteomalacia (Case 4) had 50,000 units daily for 12 months (Fig. 1). One (Case 2) was given effervescent calcium, 2 g daily, throughout the period of this study. None of the other subjects received calcium supplements, though a normal dietary intake of calcium was maintained.

FIG. 1—Changes in total body calcium in healing osteomalacia. The confidence limit for each point is $\pm 5\%$,—that is, 2 standard deviations.

Each patient was studied at approximately six-monthly intervals by means of the neutron activation facility attached to the Nuffield cyclotron in the University of Birmingham. The theoretical and practical considerations of this method have been described (Chamberlain *et al.*, 1970), in which the calcium-49 activity induced is related to the neutron dose

received by the patient and used as an indication of total body calcium, subsequent measurements being expressed as a percentage change of the original value. Though total body calcium by this method is not expressed in absolute terms, the direction, magnitude, and rate of change of total body calcium for each patient can be determined. The relatively uniform distribution of sodium throughout the body, as compared with calcium, permits the calibration of appropriate phantoms and the expression of total body sodium in absolute terms (Chamberlain *et al.*, 1968b).

Uncalcified and decalcified sections of iliac crest bone were examined from each case at the onset of the study and again after six months' treatment in Cases 1 and 2. All biopsy specimens were assessed by Dr. E. L. Jones in the department of pathology, University of Birmingham. The width of the osteoid seams was measured on undecalcified sections and at least 10 separate fields were examined. The mean figures given in Table I are derived from all trabeculae examined, excluding those obviously cut tangentially. The biopsy specimens were cut after rapid freezing, and because of possible distortion artefact accurate measurement of the relative areas of calcified and uncalcified osteoid was not undertaken. Serum sodium concentrations were measured by flame photometer. Serum calcium and alkaline phosphatase were determined by autoanalyser, the former by the cresolphthalein complexone method and the latter by a modification of the King-Kind technique. Exchangeable sodium was measured by a conventional isotope dilution technique (Veall and Vetter, 1958) with 20 μ Ci of ²⁴Na.

Results

Each of the six cases showed a rise in total body calcium, which varied from 3 to 30% and continued for up to 24 months after beginning treatment (Fig. 1). In general, the increase correlated with the severity of the osteomalacia, as judged by the thickness of the osteoid seams on the initial diagnostic bone biopsy (Table I). In the two patients in whom bone biopsy was repeated after six months' therapy complete healing of osteomalacia was found histologically, though the total body calcium continued to rise for the next two years. Total body measurements, changes in serum biochemical values, and bone histology are shown for Case 2 in Figs. 2 and 3. One patient (Case 4) with only slight osteomalacia nevertheless showed a 10% rise under the influence of vitamin D₃, though this was not maintained after therapy was discontinued. After the 10% rise in the first six months the subsequent changes in total body calcium of 3%, 4%, and 2% respectively are within the errors of the method. A fall in total body calcium of 7% from the sixth to the eighteenth month is likely to be significant, but it is not certain whether this was due to a uniform fall in total body calcium over the 12-month period or whether, as seems more likely, the fall occurred after withdrawal of vitamin D₃.

The serum calcium was below normal in only two patients (Cases 1 and 2) but rose to within normal limits after six months' treatment. In general, there was no correlation between changes in serum calcium and changes in total body calcium (Fig. 2,

TABLE I—Summary of Clinical Features

Case No.	Age	Sex	Diagnosis	Ca (mg/100 ml)	Alk. Phos. (K.A. units/100 ml)	Bone Biopsy	Radiology
1	50	F.	Adult coeliac disease	7.5	22.3	Mean osteoid width 30 μ . Normal six months later	Skeletal survey normal
2	64	M.	Adult coeliac disease	8.1	59.5	Florid osteomalacia. Osteoid 50 μ wide. Returned to normal after six months' treatment	Looser's zones in ribs and pelvis. Loss of cortex in hands. Skeletal survey normal one year later
3	68	F.	Postgastrectomy	9.6	33	Osteoid seams of 50 μ mean width	Looser's zones in left tibia. Healed six months later
4	31	F.	Nutritional osteomalacia	9.4	28	Osteoid seams of 20 μ mean width	Looser's zones in femur. Healed six months later
5	52	F.	Adult coeliac disease	9.7	17	Osteoid seams of 18 μ mean width	Skeletal survey normal
6	38	M.	Adult coeliac disease	9.6	41	Osteoid seams of 30 μ mean width	Looser's zones in ribs and pubic rami. Healed six months later

Table II). Radiological changes proved a very insensitive reflection of changes in total body calcium. Looser's zones were found initially in four cases (Table I); in Cases 2 and 3 the total body calcium continued to rise for two years after Looser's zones had healed, while in Case 4 the total calcium fell, though radiological healing was maintained. One case has been followed for only six months and a rise in total body calcium has been associated with healing of the radiological features of osteomalacia. Changes in the level of the serum alkaline phosphatase were a more sensitive indicator of a rise in total body calcium and presumably therefore of healing of osteomalacia than either radiology or bone biopsy.

The inverse relationship between rising whole body calcium and falling serum alkaline phosphatase is illustrated by the behaviour of Case 2 (Fig. 2). Case 5 showed a small rise in alkaline phosphatase from a previously normal value with therapy, but this is a not uncommon finding in mild osteomalacia. Case 3 had a persistently raised level of alkaline phosphatase but there was also a rise in the serum 5-nucleo-

FIG. 2—Case 2. Relationship between total body measurements, serum calcium, and alkaline phosphatase.

TABLE II—Biochemical and Total Body Measurements

Case No.	Ca (mg/100 ml)	Alk. Phos. (K.A. units/100 ml)	Total Na (mEq)	Exch. Na (mEq)	Non-Exch. Na (mEq)	Total Na (% Change)	Total Ca (% Change)
1	7.5	22.3	2,988	2,283	705	0	0
	8.5	10.5	2,509	2,192	317	-17	+4
	9.1	7.3	2,335	2,045	290	-22	+9
	9.1	6	2,563	2,015	548	-15	+10
	8.1	59.5	3,259	2,517	742	0	0
2	10.1	34.6	2,727	2,691	36	-16	+12
	10.8	18	2,749	2,480	269	-16	+24
	9.5	17	2,684	2,450	234	-18	+20
	9.7	10.5	2,855	2,371	484	-13	+31
	9.6	14	2,696	2,394	302	-17	+27
3	8.6	24.7	2,506	2,010	496	0	0
	10.0	46.3	2,190	1,831	359	-13	+16
	10.0	54	1,965	1,857	108	-22	+19
	9.8	51.5	1,888	1,695	193	-25	+20
	9.5	29	2,166	1,900	266	0	0
4	9.2	11	2,175	1,858	317	0	+10
	9.8	10	2,210	2,003	117	+2	+7
	9.7	9.5	2,273	1,935	338	+5	+3
	9.1	7.0	2,443	2,131	312	+13	+5
	9.9	13	2,724	2,160	564	0	0
5	9.4	15	2,565	1,934	631	-6	+3
	9.6	40	2,516	2,197	319	0	0
6	9.3	26	2,851	2,760	91	+13	+6

FIG. 3—Case 2. Bone biopsy (von Kossa, $\times 48$). Above: 14 June 1968. Osteoid seams 50 μ wide (arrowed). Below: 15 November 1968. Normal biopsy; thin osteoid seam 10 μ wide (arrowed).

FIG. 4—Changes in total body sodium in healing osteomalacia. The confidence limit for each point is $\pm 5\%$ —that is, 2 standard deviations.

tidase, which suggested that the raised serum alkaline phosphatase was partly due to enzyme of hepatic rather than bony origin.

The changes in total body sodium were less consistent, four cases showing a fall and two a rise (Fig. 4). In general, there was an inverse relationship between total body calcium and sodium. In the three most severe cases of osteomalacia (Cases 1, 2, and 3) the fall in total sodium was due partly to a fall in the non-exchangeable fraction (the difference between total and exchangeable sodium—see Table II). In two (Cases 4 and 5) there was no change in non-exchangeable sodium, and it may be relevant that their osteomalacia, as judged by the thickness of their osteoid seams, was less severe than in the other cases. The rise in total sodium in Case 4 over the last six months of study was probably related to the oral contraceptive which had been taken during this time. In the only case not showing an inverse relationship between calcium and sodium with healing of osteomalacia (Case 6) there was a rise in exchangeable sodium associated with clinical oedema but a 35% fall in the non-exchangeable fraction.

Discussion

Knowledge of the reproducibility of the techniques of measuring whole body calcium and sodium is obviously critical in the evaluation of these results. We were advised by the Medical Research Council against repeated studies on normal subjects whose body composition might be expected to remain constant. Therefore, an estimate of the reproducibility was obtained from cadaver studies. There is reason to believe that the figure of $\pm 3.7\%$ for the standard deviation of the mean value for total body calcium in repeated measurements derived from the cadaver studies (Chamberlain *et al.*, 1968c) is larger than that which is appropriate to the present clinical studies. This is because the uniformity of the activating neutron flux has been improved by technical modifications, so that accurate repositioning of the patient in the irradiation field is much less critical (Chamberlain *et al.*, 1970). A more recent series of cadaver studies has shown a deviation about the mean of $\pm 2.5\%$ for total body calcium measurements.

Sequential studies in other patients of ours whose whole body calcium might be expected to change very little—for example, young adults on low doses of corticosteroids for non-bony disease and quiescent osteoporotics—have shown values for whole body calcium varying by only 2% or so over periods of up to 18 months. We are therefore encouraged to suggest a figure of approximately $\pm 2.5\%$ as a reasonable estimate of the reproducibility of the method, so that measured changes in total body calcium and sodium greater than $\pm 5\%$ have a 95% chance of being significant.

The inability in this study to express calcium measurements in absolute terms does not prevent the demonstration of direction, rate, and magnitude of change of total body calcium, nor their comparison in a particular group of patients. It is not possible to say that when the total body calcium has risen to a constant value this represents normality for that particular patient. Nor is this likely to be the case in view of the common association of osteoporotic change in the bone disease of malabsorption (Garner and Ball, 1966). Bone lost from the porotic phase may never be replaced (Dent and Watson, 1966), despite healing of the malacic component, so that body calcium might be expected to be below normal.

It was not an unexpected finding that total body calcium rose as osteoid seams were replaced by mineralized bone once treatment for the osteomalacia was begun. The results emphasize the general insensitivity of histological and radiological criteria in the assessment of the degree of osteomalacia. Osteoid seams on biopsy and Looser's zones on radiographs had usually disappeared by the end of the first year of treatment, though total body calcium subsequently continued to rise for periods of up to 24 months.

The two most severe cases, as might be expected, showed the greatest rise in total body calcium. Vitamin D₂ was given in both. In Case 2 it is difficult to assess the relative importance of the gluten-free diet and the vitamin D₂ therapy. The latter is likely to be of importance since in Case 4, in which vitamin D₂ was also given, there was a greater rise in total body calcium than in the patients with equivalent degrees of osteomalacia who were treated with gluten-free diet but no vitamin D supplements.

In the osteomalacia of adult coeliac disease vitamin D treatment might result in the level of total body calcium at complete healing being reached more rapidly, rather than exceeding that achieved by gluten-free diet alone.

Fourman and Royer (1968) quoted from the German literature, where Gassman compared values for the composition of bone in a normal infant and one with rickets. These results showed proportionately less calcium and phosphate but more sodium and magnesium in the rachitic bone. These findings, based on chemical analyses, are supported by the present study using neutron activation, where all but one of the cases showed an inverse relationship between total body calcium and sodium. In the most severely affected cases this fall is partly due to a reduction in the non-exchangeable fraction, though any conclusions drawn from such changes suffer from the disadvantage that they are derived from the small difference between two large measured quantities. The studies of Edelman *et al.* (1954) showed that bone is the only source of a non-exchangeable sodium pool, and the present findings suggest that bone sodium falls as osteomalacia heals.

In theory, sodium may be displaced from bone crystal or matrix. There is some evidence (Neuman *et al.*, 1962) that sodium may become substituted for calcium in the hydroxyapatite crystal. Though the major part of the increase in total body calcium is no doubt taken up in recalcifying osteoid, there may be a smaller qualitative change in the hydroxyapatite crystals, whereby sodium is replaced by calcium. Alternatively, sodium may be displaced from osteoid as mineralization occurs. There may either be a change in the sodium content of the matrix itself or a loss of extracellular-fluid sodium. This latter alternative seems less likely, because then we would have expected a more significant change in the exchangeable, rather than non-exchangeable, sodium fraction.

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