Hypercalcaemia in rheumatoid arthritis: investigation of its causes and implications

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SUMMARY When correction was made for hypoalbuminaemia, 23 of 50 ambulant patients with definite or classical rheumatoid arthritis were found to have hypercalcaemia. When these 23 patients were studied 6 months later, 7 had hypercalcaemia as defined by the correction factor for a low serum albumin level, and 6 of these patients had raised serum ionised calcium concentrations. Biochemical studies in the 23 patients indicated evidence of hyperparathyroidism, namely, hypophosphataemia, increased serum alkaline phosphatase, hyperchloraemia, and reduced tubular reabsorption of calcium. However, serum immunoreactive parathyroid hormone concentrations were normal. Only one patient had an abnormally low serum 25-hydroxy-vitamin D result: this patient had a high level of urinary D-glucaric acid and was receiving phenobarbitone for treatment of epilepsy. The biochemical features suggestive of parathyroid overactivity were particularly found in patients with raised serum calcium levels. The cause of hypercalcaemia in rheumatoid arthritis remains to be explained.

Osteoporosis is common in patients with rheumatoid arthritis even without corticosteroid therapy. Radiological studies have shown that osteoporosis is generalised, occurring to the same degree in all parts of the peripheral skeleton (Kennedy and Lindsay, 1977). Rarely, osteomalacia may be found in patients with rheumatoid arthritis, usually in elderly patients confined to home (Maddison and Bacon, 1974). However, most physicians would probably not consider that there was a general metabolic bone disorder underlying osteoporosis in rheumatoid arthritis. We were therefore surprised to find an unexpectedly high prevalence of hypercalcaemia in patients with the disease (Kennedy

et al., 1975), and this paper describes further studies on the pathogenesis of this hypercalcaemia.

Material and methods

PATIENTS STUDIED

Twenty-three of 50 randomly selected female patients suffering from 'definite' or 'classical' rheumatoid arthritis, as defined by the diagnostic criteria of the American Rheumatism Association (Ropes *et al.*, 1959) were found to be hypercalcaemic, that is, serum calcium concentration greater than 2.60 mmol/l when the total serum calcium concentration was corrected for the corresponding albumin concentration as outlined below. All these 23 patients were ambulant and all participated in the study with full knowledge of its content. All blood samples

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Table 1 Clinical and laboratory data of the patients with rheumatoid arthritis included in the study (n=23)

	Age (years)	Duration of arthritis (years)	Functional grade	Age at menopause (years)	Duration since menopause (years)	Rheumatoid factor titre	Antinuclear factor titre	Haemoglobin concentration g/l	Erythrocyte sedimentation rate mm/1 h
Mean $\pm$ SD	51.7 $\pm$ 15.6	6.0 $\pm$ 6.6	1.7 $\pm$ 0.6	45.5 $\pm$ 2.3	15.6 $\pm$ 7.3	18.2 $\pm$ 22.9	217 $\pm$ 513	12.2 $\pm$ 1.4	52.5 $\pm$ 33.5

were obtained without haemostasis between 8.30 and 9.30 a.m., the patients having fasted and taken no medication since 6 p.m. the evening before. Prior to the study all patients were taking medication: 1 patient was receiving digoxin and a diuretic, 1 was on phenobarbitone for epileptic seizures, 3 were receiving low-dose corticosteroid therapy, that is, less than 10 mg prednisolone per day, and the remainder were receiving a variety of non-steroidal anti-inflammatory analgesics. The clinical and laboratory data of the 23 patients are summarised in Table 1.

DESIGN OF CORRECTION OF SERUM CALCIUM CONCENTRATION FOR HYPOALBUMINAEMIA

The serum calcium levels were corrected for albumin employing a formula derived in the following way. A regression analysis of calcium on albumin was carried out on the results from 150 normal healthy persons, half of whom were female. Their age ranges were as follows: 17 to 69 years for females, and 18 to 70 years for males. A regression equation was then calculated on the total 150 subjects. It is of interest that when the serum albumin concentration was zero the regression line cut the Y axis at 1.43 mmol/l, which represents the ultrafiltrable calcium concentration, and corresponds to that found by Robertson (1969) (1.40 mmol/l); Pedersen (1970) (1.40 mmol/l); Rose (1972) (1.45 mmol/l); and Payne *et al.*, (1973) (1.42 mmol/l). Orrell (1971) found a somewhat higher value for ultrafiltrable calcium (1.70 mmol/l), probably in part because the subjects studied included patients with nephrotic syndrome, a condition in which there is evidence of a tendency to hypercalcaemia (Jones *et al.*, 1967).

The regression equation derived from the analysis was:

$$Y = (0.0194 \times X) + C$$

where X = serum albumin in g/l
 Y = serum calcium in mmol/l
 and C is a constant equal to 1.434
 (value of Y when $X=0$).

The mean albumin for the population was 47.0 g/l, and the following correction factor for serum calcium concentration was constructed: Ca (corrected) = $(47 - alb.) \times 0.0194 + Ca$ where Ca is the measured serum calcium in mmol/l, $alb.$ is the serum albumin in g/l, and Ca (corrected) is the serum calcium in mmol/l corrected for albumin.

This indicates that calcium concentration changes by 0.0194 mmol/l for every 1 g/l change in albumin and that therefore for every 1 g/l by which the plasma albumin exceeds the normal mean of 47.0 g/l 0.0194 is subtracted from the total plasma calcium

and a corresponding addition is made when plasma albumin is less than 47.0 g/l.

In addition, a biochemical mineral metabolism screen was carried out on each subject. This involved utilising data from blood and urine to calculate certain derived values: (1) fasting urinary calcium/creatinine ratio; (2) urinary calcium excretion (Ca_E) per litre glomerular filtrate (Nordin *et al.*, 1967); (3) theoretical maximum tubular reabsorption capacity (Tm) for calcium, $TmCa$ (Nordin *et al.*, 1976); (4) the most reliable indices of renal handling of phosphate (Peterson, 1973), i.e., $TmPO_4$ GFR (theoretical tubular maximum reabsorption of phosphate corrected for glomerular function (Bijvoet, 1969; Bijvoet *et al.*, 1969) and IPE (index of phosphate excretion) (Nordin and Bulusu, 1968).

The derivation and rationale of employing the Ca_E per litre GF and the $TmCa$ are well described by Marshall (1976) and by Nordin, Horsman and Aaron (1976). The calculation of these indices was carried out in the MRC Mineral Metabolism Unit at Leeds Royal Infirmary, and we are grateful for the co-operation extended by Professor B. E. C. Nordin and colleagues.

MEASUREMENT OF SERUM IONISED CALCIUM

A 10 ml clotted sample of blood, from which air was excluded immediately after withdrawal, was used for the estimations of ionised calcium. Serum ionised calcium concentration was measured with a flow-through electrode system (Orion 99-20) in which the electrodes were maintained at 37°C in a thermostatically controlled cabinet. At each measurement run the electrode was calibrated for direct sodium interference as well as for its response to standard calcium solutions, since the effect of sodium on the electrode has been found to vary (Watson, 1975). Immediately after each measurement of ionised calcium the pH of the same serum sample was measured at 37°C with a glass capillary electrode system (EIL Model SHH 33).

Corrections were applied to the observed ionised calcium results to take account of direct sodium interference, and for this purpose a linear relationship was assumed between $\log(Ca^{++})$ and sodium concentration (Watson, 1975). The ionised calcium at the measured pH was then adjusted to correspond to a standard pH value of 7.40. For any serum specimen the relationship between the logarithm of the ionised calcium concentration and pH is linear, but the slope of the regression line varies with albumin concentration and, to a lesser degree, with the actual ionised calcium level. Accordingly, in order to make pH corrections this slope was calculated

for each sample by a formula derived from theoretical studies of calcium binding to albumin (Pedersen, 1972) and from the results of observations of the influence of pH on serum ionised calcium with varying albumin and calcium concentrations (Watson, 1975). The reproducibility of this method has been shown to be highly satisfactory, with a coefficient of variation of 0.76% (Watson, 1975). The normal range of serum ionised calcium concentration was established in an age- and sex-matched group of healthy persons who were either members of staff or related to members of staff of the Centre for Rheumatic Diseases and in whom there was no evidence of disease associated with disturbance of calcium metabolism. These blood samples were also obtained in the fasting state and under the same stringent conditions described for the patient group.

OTHER ASSAYS

Serum immunoreactive parathormone was measured by Dr Angela Fairney of St Mary's Hospital (Fairney *et al.*, 1973) and by Mrs Catherine Hillyard of the Royal Postgraduate Medical School in London (Berson *et al.*, 1963). Calcitonin assays were performed by the latter (Coombes *et al.*, 1974). Estimation of serum 25-hydroxy-vitamin D was performed by the method of Haddad and Chyu (1971) using vitamin-D-replete rat kidney cytosol protein (McLaughlin *et al.*, 1974). Other assays included serum immunoreactive gastrin (Buchanan and McCarroll, 1971), serum immunoreactive pancreatic glucagon (Buchanan, 1973; Flanagan *et al.*, 1974), urinary D-glucuronic acid excretion (Simmons *et al.*, 1974), and urinary hydroxyproline (Goverde and Veenkamp, 1972).

In all assays reference data were obtained from estimations performed on fasting subjects matched for age and sex.

RADIOLOGICAL ASSESSMENT

Radiological films of the hands and wrists were assessed for joint space narrowing and erosions by the method described by Sharp *et al.* (1971). The metacarpal index of Barnett and Nordin (1960) was used as a measurement of osteoporosis.

STATISTICAL METHODS

The following tests were used: Student's *t* test for comparison of group means, Fisher's *F* test for comparison of group variances, and linear regression calculations for association between variables.

Results

The results of the mineral metabolic screen are summarised in Table 2. Both the corrected serum

calcium and the serum ionised calcium concentrations were significantly raised ($P < 0.001$). There was a significant correlation between the serum ionised calcium and the uncorrected serum calcium concentration ($r = 0.55$, $P < 0.05$), and this became even more significant ($r = 0.71$, $P < 0.005$) when the former was correlated with the corrected serum calcium level (Figs. 1a and b). The serum phosphate concentration was significantly reduced ($P < 0.01$), and 10 patients had raised levels of serum alkaline phosphatase. Plasma chloride concentrations were significantly raised and 5 patients had increased calcium creatinine ratios. Five patients had reduced renal tubular reabsorption of phosphate (TmPO_4/GFR) and 7 had increased phosphate excretion (IPE). When Ca_E is plotted against total serum calcium concentration (Fig. 2) the results are to the right of the mean and 7 are outside the limits of normality, indicating increased renal tubular reabsorption of calcium (Marshall, 1976).

When the serum ionised calcium, serum phosphate and alkaline phosphatase are grouped according to

Fig. 1 Correlation between serum ionised calcium and (1a) total serum calcium not corrected for albumin concentration ($r = 0.55$; $P < 0.05$); (1b) total serum calcium corrected for albumin concentration ($r = 0.71$; $P < 0.005$).

Table 2 Results (mean $\pm$ SD) of mineral metabolism screen in the patients with rheumatoid arthritis compared to

	Serum ionised calcium mmol/l	Serum calcium (uncorrected) mmol/l	Serum calcium (corrected) mmol/l	Serum phosphate mmol/l	Serum alkaline phosphatase U/l	Serum albumin g/l	Plasma bicarb. mmol/l	Plasma sodium mmol/l
Patient group	1.13 $\pm$ 0.06	2.41 $\pm$ 0.13	2.58 $\pm$ 0.12	0.98 $\pm$ 0.2	318 $\pm$ (130–1490)	38.2 $\pm$ 3.9	24.3 $\pm$ 2.2	139.3 $\pm$ 2.2
Normal reference group ranges	1.07 $\pm$ 0.05 4.23	2.39 $\pm$ 0.1	2.39 $\pm$ 0.1 8.4	1.1 $\pm$ 0.15 2.81	80–280	46.9 $\pm$ 3.3 9.7	24.5 $\pm$ 1.8	140 $\pm$ 2.0
P	<0.001	NS	<0.001	<0.01	*	<0.001	NS	NS
Number above reference range	8	1	7	0	10	0	0	1
Number below reference range	0	1	0	5	0	4	1	0

Statistical analysis invalid because of the non-normally distributed data.

Fig. 2 Plot of calcium excretion (Ca_E) per litre of glomerular filtrate, against total serum calcium concentration. The shaded area represents the limits of normality.

their 'normality' (Table 3) the following facts emerge. Of the 23 patients only 7 had all 3 results in the 'normal' range. Of the 8 patients with raised serum ionised calcium 6 had biochemical patterns which might be encountered in a hypercalcaemic hyperparathyroid state. It can be seen in Table 4 that the mean serum alkaline phosphatase lies above the upper limit of the reference range. Indeed, 10 of the 23 patients had raised serum alkaline phosphatase levels, whereas there was no patient who showed increases of serum bilirubin, aspartate

Table 3 Grouping of combinations of 'normal' and 'abnormal' serum ionised calcium, phosphate and alkaline phosphatase results

Serum ionised calcium	Serum phosphate	Serum alkaline phosphatase	Number of patients in each group
N	N	N	7 (30.9%)
↓	↑ or N or ↓	↑ or N or ↓	—
N	N	N	6 (26.0%)
↓	N	N	2 (8.6%)
↑	N	N	2 (8.6%)
↑	N	↑	3 (13.0%)
↑	↓	N	2 (8.6%)
↑	↓	↑	1 (4.3%)

Table 4 The results (mean $\pm$ SD) of serum alkaline phosphatase, bilirubin, SGOT and SGPT in the patients with rheumatoid arthritis, compared to the reference group.

	Serum alkaline phosphatase U/l	Serum bilirubin μ mol/l	Serum aspartate transaminase (SGOT) U/l	Serum alanine transaminase (SGPT) U/l
Patient group				
Mean	318	4.7	23.4	16.7
Range	130–1490	2–14	14–31	11–28
Reference range	80–280	3–14	12–38	8–41
Number of patients with results above upper limit of reference range	10	0	0	0

the normal reference group. Ranges or mean $\pm$ ISD when appropriate

Plasma potassium mmol/l	Plasma chloride mmol/l	Plasma urea mmol/l	Serum creatinine mmol/l	Maximum tubular reabsorption of phosphate (TmPO ₄ GFR) mmol/l	Index of phosphate excretion (IPE)	Calcium/ creatinine ratio (mmol/mmol)	TmCa/GFR mmol/l	Hydroxy- proline/ creatinine ratio mmol/mmol
4.1 $\pm$ 0.5	106 $\pm$ 3.3	5.4 $\pm$ 1.9	83 $\pm$ 30	0.96 $\pm$ 0.36	+0.25 $\pm$ 0.17	0.38 $\pm$ 0.28	2.04 $\pm$ 0.35	0.018 $\pm$ 0.016
4.2 $\pm$ 0.4	100 $\pm$ 2.5	2.7–7.0	35–115	0.70–135	0.70–1.35	0.06–0.46	1.68–2.08	0.02
NS	8.53 <0.001	NS	NS	*	*	*	*	*
1	9	3	1	2	7	5	12	6
3	0	0	0	5	0	1	0	0

transaminase (AST), or alanine transaminase (ALT). One patient had extremely high serum alkaline phosphatase, and this was associated with the highest serum ionised calcium in our patients. The results of the urinary hydroxyproline showed that 9 of the patients studied had levels in excess of normal limits (0.05–0.17 mmol/24h). However, when these results were subsequently calculated for accuracy of interpretation in terms of the hydroxyproline/creatinine ratio only 6 patients had abnormally high results. Since the urinary hydroxyproline and the fasting calcium/creatinine ratio suggest evidence of bone destructions (Nordin *et al.*, 1976) the results for urinary calcium and urinary hydroxyproline levels were correlated in the patients studied (Fig. 3). It can be seen that there is a statistically significant positive correlation ($r=0.54$, $P<0.005$) between these indices.

The results of the immunoreactive parathormone assay (Fairney *et al.*, 1973) and other hormone assays are shown in Table 5. It can be seen that no patient had an elevated parathormone level, and indeed in 16 the level was below the lower limit of the normal range. In the other assay (Berson *et al.*, 1963) all levels lay within the normal range. Only one patient (patient 11) had an raised serum immunoreactive gastrin level: this patient also had raised serum calcitonin and ionised calcium levels, a low parathyroid hormone (PTH) level, and the highest serum alkaline phosphatase. The patient with the low 25-OH vitamin D level (patient 23) had a markedly raised urinary D-glucuronic acid concentration. This patient had been receiving phenobarbitone for epilepsy. Serum magnesium concentrations (0.80 ± 0.06 mmol/l) were within normal limits.

Fig. 3 Correlation between the calcium ratio and the hydroxyproline ratio derived from fasting urine samples ($r=0.54$; $P<0.005$).

Patients with an elevated serum ionised calcium level tended to have lower serum phosphate levels and TmP/GFR and higher TmCa/GFR (Table 6). No significant differences were noted in the clinical and other laboratory features between the patients with elevated and normal serum ionised calcium levels.

Analysis of the bone damage indices revealed a significant correlation between the erosion score and the metacarpal index ($r=0.6$, $P<0.01$) and the erosion score and joint space narrowing score

Table 5 The individual results of the serum calcium concentrations, parathyroid hormone, calcitonin, 25-hydroxy-vitamin D, gastrin, and urinary calcium and hydroxyproline creatinine ratio together with the indices of joint destruction and osteoporosis and corresponding normal reference ranges

Patient's number	Age (Years)	Serum albumin g/l	Serum calcium mmol/l	Serum calcium (corr.) mmol/l	Serum ionised calcium mmol/l	Para-thyroid hormone (PTH) pg/ml	Calcitonin pg/ml	25-OH-vitamin-D ng/ml	Serum gastrin pg/ml	Urinary calcium/creatinine ratio	Urinary hydroxyproline/creatinine ratio	Meta-carpal index	Joint space narrowing score	Erosion score
1	38	38	2.40	2.55	1.04	132	<100	32.6	45	0.38	0.017	56	0.032	0.045
2	53	35	2.41	2.65	1.16	78	180	23	60	0.44	0.012	42	0.104	0.227
3	66	40	2.40	2.55	1.13	300	<100	21.4	50	0.36	0.010	56	0.74	0.024
4	24	38	2.50	2.65	1.12	380	<100	35.8	125	0.06	0.010	68	0.477	0.267
5	43	39	2.64	2.80	1.19	78	<100	19.6	145	0.22	0.035	55	0.213	0.141
6	51	43	2.54	2.60	1.12	140	<100	31.9	70	0.23	0.007	40	0.458	0.468
7	53	37	2.44	2.65	1.19	210	<100	24	75	0.34	0.010	57	0.264	0.462
8	59	35	2.35	2.60	1.05	78	<100	30.7	95	0.31	0.025	23	0.672	0.497
9	22	44	2.40	2.45	1.08	250	<100	34.7	30	0.39	0.020	72	0	0.007
10	58	47	2.60	2.60	1.11	156	<100	—	105	0.38	0.010	74	0.299	0.008
11	69	34	2.60	2.85	1.27	220	130	28.9	1250	0.41	0.028	48	0.037	0.042
12	63	39	2.49	2.65	1.16	250	<100	—	325	0.23	0.010	20	0.394	0.541
13	69	31	2.21	2.50	1.11	200	<100	19.9	55	0.28	0.008	22	0.245	0.266
14	72	36	2.34	2.55	1.17	260	—	26.7	75	0.17	0.005	45	0.071	0.155
15	53	39	2.33	2.50	1.19	78	<100	32.5	40	0.21	0.020	48	0	0.276
16	33	40	2.60	2.75	1.25	460	<100	—	125	0.89	0.026	54	0	0.007
17	66	33	2.15	2.40	1.09	78	<100	19.5	110	0.98	0.10	44	0.75	0.795
18	23	37	2.38	2.60	1.09	320	<100	29.9	30	1.07	0.030	70	0.06	0.035
19	44	36	2.34	2.55	1.09	360	<100	26.5	95	0.55	0.020	51	0.139	0.189
20	70	32	2.32	2.60	1.13	520	<100	25.9	120	0.10	0.008	—	—	—
21	56	43	2.36	2.45	1.10	240	<100	30	105	0.59	0.015	38	0.037	0.448
22	52	40	2.30	2.45	1.05	360	—	39.2	50	0.16	0.005	64	0	0.007
23	51	42	2.30	2.40	1.09	78	100	16.3	—	0.02	0.006	50	0.422	0.514
Mean	51.6	38.6	2.40	2.58	1.13	227	—	27.5	144.5	0.38	0.016	51	0.246	0.247
SD	15.1	4.0	0.10	0.12	0.06	129	—	6.2	254.5	0.28	0.010	15.3	0.249	0.227
Normal reference range		40-53	2.20-2.60	2.20-2.60	1.0-1.13	275-1208	>100	18.6-44.5	>600	0.04-0.49	0.02	0	>0	>0

Table 6 Comparison of the biochemical data between the normocalcaemic and hypercalcaemic patients as determined by their ionised calcium concentration

	Number	Serum ionised calcium mmol/l	Total serum calcium (corrected) mmol/l	Serum phosphate mmol/l	TmP/GFR mmol/l	TmCa/GFR mmol/l	Calcium/ creatinine ratio	Hydroxy- proline/ creatinine ratio
Hypercalcaemic patients >1.13 mmol/l	8	1.20±0.04	2.66±0.12	0.90±0.24	81.63±48.16	2.2±0.14	0.36±0.3	0.018±0.010
Normocalcaemic patients <1.13 mmol/l	15	1.09±0.03	2.53±0.08	1.06±0.16	103.5±25.6	1.9±0.5	0.39±0.3	0.019±0.23
t		2.15	3.45	1.97	1.43	1.42	0.22	0.12
P		<0.05	<0.005	<0.1 (NS)	NS	NS	NS	NS

($r=0.77$, $P<0.001$). No correlation existed between serum ionised calcium concentration and any of these bone damage indices.

Discussion

Bone loss is an important clinical facet of rheumatoid arthritis. When severe osteoporosis is present, compression fractures of the vertebrae may occur, and in peripheral bones thinning of cortical bone may complicate joint surgery. So far there has been little evidence that osteoporosis in rheumatoid arthritis has a 'metabolic' basis, and it was therefore of interest when we observed that a high proportion of patients had raised serum calcium concentrations when the serum calcium was corrected for the low albumin concentration (Kennedy *et al.*, 1975). It seemed relevant, therefore, to study in greater depth the pathogenesis of hypercalcaemia in rheumatoid arthritis. To this end 50 female patients with rheumatoid arthritis were randomly selected and screened for possible hypercalcaemia after correction of serum calcium for hypoalbuminaemia. Twenty-three of these patients with 'definite' or 'classical' rheumatoid arthritis were shown to have an elevated corrected serum calcium concentration and were chosen for detailed investigation of biochemical and hormonal aspects of calcium metabolism.

The first and most crucial question is whether or not the serum ionised calcium estimations validly support the contention that hypercalcaemia in rheumatoid arthritis is a real entity. The study was carefully controlled both in terms of matching patients for age and sex with healthy persons and in the methods employed to determine serum ionised calcium concentrations. The patients and controls were studied in the fasting state, and particular care was taken in withdrawal of venous blood to ensure that no air entered the syringe and that the arm was not constricted. The patients and controls were studied contemporaneously and the laboratory estimations were performed under identical con-

ditions. The temperature at which the estimations were performed was constant (37°C) for all samples, and the results were corrected for pH and sodium interference. Hence there are strong grounds for accepting that 8 of the 23 patients with rheumatoid arthritis did have raised serum ionised calcium concentrations.

It might be argued that the mean control value of serum ionised calcium concentration was somewhat lower (1.07 mmol/l) than that reported by other workers. However, it is extremely difficult to make comparisons of normal values obtained in different laboratories, in particular when there are so many variables which can affect such results. Moreover, a literature survey revealed that only 3 other groups have reported serum ionised calcium determinations using the ion electrode method employed in the present study while also studying patients in the fasting state. Robertson and Peacock (1968) and Ladenson and Bowers (1973) both obtained higher mean values, 1.23 and 1.28 mmol/l respectively, but Ryden *et al.*, (1976) obtained a mean value of 1.03 mmol/l, which was lower than that obtained in the control subjects in the present study. It should be noted that Ladenson and Bowers (1973) carried out their estimations at a temperature of 25°C, which would tend to increase the serum ionised calcium result. Ryden and her colleagues (1976) do not, unfortunately, state the temperature at which they carried out their estimations. Varghese (1973), using spectrophotometry, obtained a mean value slightly lower (1.04 mmol/l) than that obtained in the present study. His subjects were also fasting and the working temperature was 37°C. The reproducibility of the method employed in the present study was high, with a coefficient of variation of 0.76% in 20 samples from the same bulk source (Watson, 1975). Thus it seems reasonable to conclude that the 8 patients with rheumatoid arthritis had definite, though in some patients slight, elevations in serum ionised calcium levels.

Further confirmation of the finding of raised serum

ionised calcium levels in rheumatoid arthritis has been obtained in a study of 52 patients with the disease studied in South Africa. Approximately one-third (17) of the patients had raised serum ionised calcium levels when compared to a healthy control group matched for age and sex (Meyers *et al.*, 1976). This method employed an Orion calcium ion exchange electrode model SS-20 and the temperature at which estimations were performed was 37°C. The correction factor employed in the present study is also central to the investigation, and its validation is therefore crucial. As previously indicated, the regression line of serum calcium concentration on serum albumin level when extended to the Y axis when the albumin concentration is zero intersects at 1.43 mmol/l, which corresponds to the serum ultrafilterable calcium concentration found by others (Robertson, 1969; Pedersen, 1970; Rose, 1972; Payne *et al.*, 1973). Of great importance is whether there is concordance between hypercalcaemia estimated from the correction formula and hypercalcaemia confirmed by a high value of serum ionised calcium. Of the 23 patients with rheumatoid arthritis in the present study, 7 had hypercalcaemia as estimated by the correction formula for a low serum albumin concentration. Six of these 7 patients had elevated serum ionised calcium levels. Patient 4 (Table 5), who had a normal value for serum ionised calcium (1.12 mmol/l) had a corrected total serum calcium concentration just over the upper limit of normal (2.65 mmol/l). Of the 16 patients who had normal values for corrected serum calcium concentrations 2 had increases of serum ionised calcium (patients 14 and 15; Table 5). There is thus reasonable concordance between the corrected total serum ionised calcium concentration and the serum ionised calcium level, and this tends to support the validity of the correction formula. Further support is provided by the fact that the correlation between serum ionised calcium concentration and the corrected total serum calcium level ($r=0.71$, $P<0.005$) is higher than with the uncorrected total serum calcium ($r=0.55$, $P<0.05$).

The serum ionised calcium is the physiologically active fraction of total serum calcium, and consequently, when raised, it is of clinical significance. The associated biochemical results of the mineral metabolism screen are therefore of interest. The finding of hypophosphataemia, increased serum alkaline phosphatase, hyperchloraemia, reduced tubular reabsorption of phosphate, and increased tubular reabsorption of calcium suggest possible parathyroid overactivity. The elevation of serum alkaline phosphatase in patients with rheumatoid arthritis may be of either bone (Maddison and Bacon, 1974) or liver origin (Webb *et al.*, 1975). Unfortun-

nately, isoenzyme studies were not available to determine the source of the increased serum alkaline phosphatase levels in the present study. However, none of the patients had clinical evidence of liver disease, and all had normal serum levels of bilirubin and the enzymes AST and ALT. It is of interest that of 6 patients with elevated hydroxyproline/creatinine ratios 4 had elevated serum alkaline phosphatase levels. However, 6 patients with elevated serum alkaline phosphatase levels had normal hydroxyproline/creatinine ratios. Increased hydroxyproline excretion in the urine reflects an increased turnover of collagen, and has been reported by some (Hartmann *et al.*, 1969) but not all workers (Ziff *et al.*, 1956) in patients with rheumatoid arthritis. The significant correlation between the increased urinary calcium and hydroxyproline results (Fig. 3) suggests that the underlying cause for this is likely to be increased bone turnover.

The question arises whether this evidence suggesting increased bone turnover and the other biochemical abnormalities noted might be interpreted as indicating hyperparathyroid activity. It is therefore of interest that patients who had raised serum ionised calcium level tended to have lower serum phosphate levels and TmP/GFR and higher TmCa/GFR. Thus, one can only tentatively conclude that there is some evidence of increased bone turnover and possible parathyroid overactivity. In this context the results of the hormone assays were of particular interest.

The most accurate test for parathyroid overactivity is regarded as the direct measurement of serum PTH. By both methods of immunoassay employed in this study the serum levels of PTH were normal or reduced. Serum calcitonin levels were elevated in two patients only, and it is of interest that 1 of those had the highest serum ionised calcium concentration (patient 11, Table 5). The cause of the elevated calcitonin level may be a response to the elevation of serum ionised calcium as has been shown in some mammalian species (Gray and Munson, 1969), but not yet in man (Schneider and Sherwood, 1974).

Hypercalcaemia and hyperparathyroidism may be associated with hypergastrinaemia (Turbey and Passaro, 1972; Barreras, 1973), and rheumatoid arthritis may also be associated with high serum immunoreactive gastrin levels (Rooney *et al.*, 1976). This suggests a possible link between enteric hormones and anomalies in calcium metabolism occurring in rheumatoid arthritis. However, only 1 of the patients in the present study (patient 11, Table 5) had a raised serum immunoreactive gastrin level, though it is of interest that this patient had the highest serum ionised calcium concentration. In

addition this patient had a raised serum calcitonin level, and it might be due to the fact that hypergastrinaemia can stimulate calcitonin secretion in man (Hennessy *et al.*, 1973). Serum glucagon levels were normal in all patients studied.

The role of vitamin D in calcium metabolism in patients with rheumatoid arthritis has not been thoroughly investigated. In a recent study of 5 patients with rheumatoid arthritis bone biopsy showed osteomalacia and secondary hyperparathyroidism, and these findings were considered to be secondary to a dietary deficiency of vitamin D, assessed retrospectively by dietary histories (Maddison and Bacon, 1974). However, the analysis of serum 25-hydroxy-vitamin D levels in this study revealed only 1 abnormally low result, in a patient with associated high level of urinary D-glucuronic acid who was receiving phenobarbitone for treatment of epilepsy. It seems reasonable to postulate that the low level of vitamin D was due to liver microsomal enzyme induction secondary to anticonvulsant therapy (Hahn *et al.*, 1972; Rowe and Stamp, 1974). A number of antirheumatic drugs are also known to be associated with liver microsomal enzyme induction in experimental animals (Conney, 1967), but there was no evidence of this occurring in patients in the present study as judged by urinary D-glucuronic acid levels.

The patients in Maddison and Bacon's study were selected because they had bone fractures and they tended to be older and more severely affected by the arthritis than the patients in this study. In addition it seems germane to note that in the radiological studies of skeletal status in very large numbers of patients with rheumatoid arthritis previously reported no circumstantial evidence of osteomalacia was found (Kennedy and Lindsay, 1977). In addition, the absence of supportive biochemical evidence in a prior study (Kennedy *et al.*, 1975) would seem to suggest that the incidence of hypovitaminosis D is probably confined to a very small select group of the rheumatoid population and indeed perhaps not to any greater extent than that in the general population.

Examination of the indices of articular bone damage showed significant correlation between the erosion score and the metacarpal index, tending to suggest that whatever is occurring at the joint surface is also being reflected at the midpoint of the shaft of the metacarpal. Thus, the metacarpal index would seem to provide a reasonably simple guide to the extent of bone damage occurring in the hand of a rheumatoid patient. There was also a good correlation between the erosion score (Dc) and the joint space narrowing score (JSNc), suggesting that the development of bony defects parallels the development of cartilage destruction.

None of the patients in this study had any evidence of any alternative disease which could have explained the hypercalcaemia such as the milk-alkali syndrome, hypervitaminosis D, sarcoidosis, or malignancy. It is possible that the increased serum calcium concentrations might be due simply to erosion of bone, in which case it might be anticipated that they would correlate with activity of joint disease as judged by clinical, laboratory, and radiological parameters. There was no such correlation between hypercalcaemia and disease activity based on the patient's pain index, articular index of joint tenderness, functional grade, haemoglobin concentration, erythrocyte sedimentation rate, and serum protein concentration, nor with the radiological indices of bone damage. However, this does not totally exclude the possibility of any influence of disease activity on the production of hypercalcaemia, since the number of patients studied was small and the disease relatively long standing. Nevertheless, the fact that the hypercalcaemia is apparently not a constant feature for any one patient could be consistent with variation in the rate of bone destruction, which is not an unlikely possibility in a disease with such clinical variation.

Another variable which might explain the elevated serum ionised calcium levels is administration of antirheumatic drugs. None of the patients had taken any medication within 12 hours of the study, but persistence of drug effect cannot be discounted. Also it is possible that non-steroidal anti-inflammatory drugs or corticosteroids may interfere with plasma binding of calcium, thus producing abnormalities in serum ionised calcium measurements. Against, this however, is the fact that all the patients in this study had had no drug therapy for a minimum of 12 hours before its start, and additionally there is no reported evidence that these drugs do produce interference in this way.

It might be argued that cessation of therapy in itself could cause a release of calcium from its binding sites on albumin. However, it is of interest that the patients with rheumatoid arthritis studied in South Africa (Meyers *et al.*, 1976) had approximately the same incidence (30%) of increased serum ionised calcium levels and all were currently receiving non-steroidal anti-inflammatory drugs. Northover (1973), using amounts of indomethacin in excess of normal therapeutic doses, has shown in vitro that the drug will reduce calcium binding to the membranes of endothelial cells, but the relevance of this single observation to the present clinical studies is not apparent. Careful search of the literature has failed to find any other publication on the effects of non-steroidal anti-inflammatory drugs on serum ionised calcium determinations.

If hyperparathyroidism is not the cause of the increased serum ionised calcium concentrations and the other biochemical abnormalities observed, the question arises of what they may be due to. The situation in rheumatoid arthritis has some resemblance to that pertaining to increased serum ionised calcium concentrations in neoplasm, where in the presence of biochemical evidence strongly suggestive of hyperparathyroidism immunoassay of PTH may be normal (Heath, 1976; Woodhead and Walker, 1976). Conceivably in rheumatoid arthritis and in certain neoplasms polypeptides may be produced which have PTH-like activity but cannot yet be measured by the current immunoassay procedures. An alternative explanation is that the hypercalcaemia may be the result of effects of prostaglandin E_2 . Prostaglandin levels are increased in the synovial fluid in rheumatoid arthritis (Higgs *et al.*, 1974), and Robinson *et al.* (1975) have shown in bone culture studies in vitro that all the bone-resorption-stimulating activity in rheumatoid synovial fluid can be accounted for by prostaglandin E_2 .

Krane (1974) also demonstrated bone resorbing factor from synovial cell culture media but did not consider this to be due to prostaglandin E_2 . To date no measurement has been made of prostaglandin E_2 in the sera of patients with rheumatoid arthritis, but conceivably this, if it were raised, could cause bone resorption and hypercalcaemia. It is not known whether prostaglandin E_2 could account for the other biochemical abnormalities of mineral metabolism observed in the present study, and clearly this is worthy of further study. It is interesting to note that sera from patients with rheumatoid arthritis have been shown to cause resorption of bone in tissue culture and that this effect was most marked in serum with high calcium concentrations (Kennedy *et al.*, 1976).

Another substance with PTH-like action is osteoclast-activating factor, OAF (Horton *et al.*, 1972), a substance which has been isolated from lymphocytes. The effects of OAF on bone in vitro resemble those of PTH (Raisz *et al.*, 1975), but effects of injection of equipotent in vitro doses of the 2 substances in vivo in rats differ in that PTH affects serum calcium and phosphate while OAF does not, suggesting that OAF is a local rather than a systemic bone resorbing factor. This would also seem an obvious area for further investigation. Biochemical effects particularly regarding renal handling of calcium and phosphate have, like PGE_2 , not been studied with OAF, and clearly require further investigation.

In conclusion, the results of the present study indicate that hypercalcaemia is a not uncommon feature of rheumatoid arthritis. The hypercalcaemia

is associated with biochemical abnormalities suggestive of hyperparathyroidism, but serum concentrations of immunoreactive parathyroid hormone are normal. Since sera of patients with rheumatoid arthritis, especially those with hypercalcaemia, cause bone resorption in vitro (Kennedy *et al.*, 1976) there seems little doubt that bone loss in rheumatoid arthritis is an active process. Finally, the fact that calcitonin and hydrocortisone block the resorptive effect of rheumatoid sera on bone in vitro (Kennedy and Lindsay, 1977) may have therapeutic possibilities.

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