BONE LOSS IN RHEUMATOID ARTHRITIS: A CLINICAL, RADIOLOGICAL AND BIOCHEMICAL STUDY

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PREFACE

The work on which this thesis is based was carried out during the tenure of a Medical Research Council Clinical Research Followship and as a Senior Registrar in Rheumatology at the Centre for Rheumatic Diseases and the University Department of Medicine, Royal Infirmary, Glasgow. The work was begun in May 1972 and took five years to complete.

Some of the work in this thesis has already been published.

Kennedy, A. C. et al (1974)
Osteoporosis in rheumatoid arthritis.
Rheumatology, 4, 25-35.

Kennedy, A. C. et al (1974)
Osteoporosis in rheumatoid arthritis: its natural history and statistical prediction.
Rheumatology, 4, 441-445.

Kennedy, A. C. et al (1975)
Bone loss in patients with rheumatoid arthritis.
Scandinavian Journal of Rheumatology, 4, 73-79.

Kennedy, A. C. et al (1975)
Abnormalities in mineral metabolism suggestive of parathyroid overactivity in rheumatoid arthritis.
Current Medical Research and Opinion, 3, 345-358.

Kennedy, A. C. et al (1975)
Generalized localized bone loss in patients with rheumatoid arthritis.
Scandinavian Journal of Rheumatology, 4, 209-216.

Kennedy, A. C. et al (1976)
Bone-resorbing activity in the sera of patients with rheumatoid arthritis.
Clinical Science and Molecular Medicine, 51, 205-207.

Kennedy, A. C. and Lindsay, R. (1977)

Bone involvement in rheumatoid arthritis.

in Clinics in Rheumatic Diseases, 3, 403-420.

Eds. Bluestone, R. and Bacon, P. A.

W. B. Saunders Co. Ltd., London, Philadelphia and Toronto.

Lindsay, R. and Kennedy, A. C. (1978)

Aspects of bone disease in rheumatoid arthritis.

in Bone Disease and Calcitonin Symposium Proceedings, 171-181.

Ed. Kanis, J. A.

Armour Pharmaceutical Co. Ltd., Eastbourne, England.

Kennedy, A. C. et al (1978)

Investigation of the aetiology of hypercalcaemia of rheumatoid arthritis.

Annals Rheumatic Diseases (submitted)

I have also communicated the results of several of the chapters at the following scientific meetings:

The Conference on Metabolic Bone Disease to mark the 21st birthday of the National Fund for Research into Crippling Diseases, Glasgow. September, 1973.

The Royal Medico-Chirurgical Society, Glasgow, November, 1974.

The Ulster Medical Society, Belfast, February, 1975.

The Heberden Society, Norwich, March, 1975.

The XI European Symposium on Calcified Tissues Elsinore, Denmark, May, 1975.

The VIII European Rheumatology Congress, Helsinki, June, 1975.

The Scottish Society of Physicians, Glasgow, October, 1975.

The Medical Research Society, London, December, 1975.

The Fifth South African Conference on Rheumatism, Arthritis and Allied Disorders. Cape Town, South Africa, May, 1976.

The South African Orthopaedic Association. Johannesburg, South Africa, September, 1976.

The Association of Physicians of South Africa. Bloemfontein, South Africa, October, 1976.

The Heberden Society. London, November, 1977.

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Mrs. D. Harvey, Mrs. A. Cox and Miss C. Johnson typed the manuscript and Miss Brenda Burns made the figures and illustrations. I am grateful to them for their forbearance.

SUMMARY

The first patient I saw when I took up my clinical duties at the Centre for Rheumatic Diseases in Glasgow was a 59 year old spinster who had rheumatoid arthritis for 39 years. She had been bedridden for eight years and had received oral corticosteroid therapy for 15 years. The patient was in severe agony from multiple vertebral body collapse and from rib and long bone fractures. On x-ray the bones were almost transparent, and extensive biochemical tests revealed no evidence of osteomalacia.

The patient's problem of osteoporosis intrigued me and on reading the literature I was surprised to find that very little had been done to study the problem of bone loss in patients with rheumatoid arthritis. For example, it was not clear from reading the literature how much oral corticosteroid therapy was responsible for bone loss compared to the disease itself.

Chapter I of this thesis describes an extensive radiological investigation of osteoporosis in patients with rheumatoid arthritis in order to assess its extent and severity. The results show that osteoporosis

in rheumatoid arthritis is a generalised phenomenon, which is particularly evident in patients of both sexes over the age of 45 years. In addition to the duration of the arthritis, corticosteroid therapy was shown to be the other important variable in producing this osteoporosis.

Chapter II describes an extensive clinical study of calcium metabolism in patients with rheumatoid arthritis in an attempt to explore further possible factors in the bone loss occuring in this disease. One of the surprising results of this investigation indicated that hypercalcaemia is a not uncommon feature in rheumatoid arthritis. Circumstantial evidence pertaining to serum and urine biochemistry together with tentative conclusions from a calcium absorption study suggested that the hypercalcaemia might be part of a "hyperparathyroid-like" state. However, immunoassay of serum parathyroid hormone levels clearly indicated that this hormone was not elevated and was thus not responsible for these biochemical abnormalities. Similarly serum 25-hydroxyvitamin D levels were normal but an interesting trend towards elevation of serum calcitonin levels was noted.

The study was then extended to examine the effects of rheumatoid sera on a bone culture in vitro. Bone

resorption activity was demonstrated in the sera of those patients who were hypercalcaemic. It is tentatively concluded that this bone resorption is due to a substance, as yet unidentified, which has some, but not all, of the properties associated with osteoclast activating factor.

These observations may subsequently be shown to have therapeutic implications in the treatment of rheumatoid arthritis.

CHAPTER I

RADIOLOGICAL INVESTIGATION OF

OSTEOPOROSIS IN RHEUMATOID ARTHRITIS

OSTEOPOROSIS IN RHEUMATOID ARTHRITIS

One of the major difficulties when researching the historical literature on rheumatoid arthritis is the lack of any definite diagnostic criteria for the various arthritides.

Certainly one can, from the many excellent clinical descriptions in older texts, establish with reasonable certainty those cases which would be regarded as rheumatoid arthritis by more modern criteria. Nevertheless, nomenclature at least up to the middle of this century has bedevilled the interpretation of clinical disease of joints.

The term rheumatoid arthritis was first applied by Alfred Baring Garrod in 1859. Although in many older writings one can read descriptions suggestive of rheumatoid arthritis, the disease was not actually described per se until 1800 when Landré Beauvais published his thesis on "Goutte Asthenique Primitif".

Juxta-articular osteoporosis is a well recognised radiological feature of early rheumatoid arthritis (Bywaters, 1960), and in patients with severe long-standing disease generalised osteoporosis may also be found (Boyle and Buchanan 1971). Despite the fact that generalised osteoporosis may lead to pathological fractures of bones very little work has been published on osteoporosis in rheumatoid arthritis. This is perhaps surprising in view of the recognition of the widespread manifestations of rheumatoid arthritis, and the suggestion by some workers that the term 'rheumatoid disease' would be more appropriate (Ellman and Ball 1948). Before reviewing what

is known regarding osteoporosis in rheumatoid arthritis a brief review of the general problem of osteoporosis would seem germane.

Essentially bone consists of collagen fibres in a groundwork of calcific inorganic matter (calcium phosphate and carbonate, fluorine, sodium, magnesium, zinc and many other trace elements). Since bone is largely an inorganic substance lending rigidity and hardness to the skeleton there is an understandable tendency to consider bone mineral as if it were metabolically inert. It is therefore salutory to be reminded that bone has greater reparative power than any other tissue in the body save blood (Last 1963). It should also be noted that 30 per cent of body sodium resides in the skeleton (Collins 1966) and that at any point in time extensive exchange of elements is occurring between bone and other tissues via blood.

In 1885 Pommer distinguished between osteoporosis and osteomalacia at the histological level, but for many years osteoporosis remained enigmatic. The clinical entity of osteoporosis was first clearly described in 1941 by Albright, Burnett, Cope and Parsons. Currently osteoporosis is defined as a condition in which the volume of bone tissue per volume of anatomical bone is reduced. In practice this is usually diagnosed radiologically.

Howell (1966) has stated that osteoporosis is the commonest metabolic bone disorder encountered in medical practice. Certainly if the definition of osteoporosis as porosity or thinning of bone is accepted the prevalence is extremely high in both sexes in the

older age groups (Urist 1960, Gitman and Kamholtz 1965, Epker, Kelin and Frost 1965, Smith and Rizek 1966, Nordin, McGregor and Smith 1966, Morgan, Spiers, Pulvertaft and Fourman 1967, Smith, Anderson, Shimmins, Spiers and Barnett 1969). A relationship between osteoporosis and fractures is well established (Newton-John and Morgan 1968) and it is of interest that the incidence of bone fractures rises with age (Knowleden, Buhr and Dunbar, 1964).

It has long been recognised that bone ceases to grow longitudinally with the closure of the epiphyses. However, some workers have provided evidence that growth continues after closure of the epiphyses by bone being laid down on both the external and internal surfaces of the shafts. (Epker and Frost 1965, 1966, Smith and Rizek, 1966, Garn, Rohmann, Wagner and Ascoli 1967). Trotter, Broman and Peterson (1960) provided a fuller picture of osteoporosis as it applied to the whole of the adult life span in an attempt to establish criteria of early as well as advanced osteoporosis. They established that the weight/volume ratio (apparent density) of whole dry bones obtained at autopsy fell with age similarly in both sexes and in whites and negroes from twenty years of age onwards. Subsequently Arnold, Bartley, Tont and Jenkins (1966) showed that ash weight per unit volume in lumbar vertebrae decreased from about thirty years of age onwards. Other workers results on post mortem material have tended to confirm this impression of bone density peaking at the third to fourth decade and subsequently declining (Atkinson 1965, Saville 1965, Sissons, Holley and Heighway 1967).

In vivo studies employing X-ray densitometry techniques have also tended to confirm this post mortem evidence (Nordin, Young, Bentley, Ormondroyd and Sykes 1968, Smith 1970).

Rose (1967) concluded on reviewing the literature, that in most cases, osteoporosis is not a disorder which comes on suddenly in late adult life, rather it is a condition to which all people gradually progress when they pass 20 years of age. Dent and Lyall-Watson (1966) concur with this and regard the term "senile osteoporosis" as a misnomer if it is meant to imply that something new and fresh is happening in old age.

The study of osteoporosis has been bedevilled by methodological difficulties in assessment. A variety of methods have been used: simple radiological measurements (Barnett and Nordin 1960) quantitative microradiographic techniques (Jowsey 1966), tetracycline labelling (Frost 1963), densitometeric techniques (Anderson, Shimmins and Smith (1966) and calcium balance studies (Fraser, Harrison and Jones 1960, Dymling 1964, Nordin, Smith and Glass (1964), Lafferty, Spencer and Pearson 1964). None of these methods, however, has proven entirely adequate in terms of accuracy.

The simplest methods of assessment are radiological where the cortical thickness of bone is measured. However, these are naturally on selected parts of the skeleton and do not necessarily apply to the skeleton as a whole. Modifications on the basic formula of the metacarpal index of Barnett and Nordin (1960) have been introduced (Morgan 1973a) but there is little evidence to suggest that any individual method is better than any other. From radiological assessments, percentile charts of bone density have been compiled (Exton-Smith, Millard, Payne and Wheeler 1969, Smith 1970). These charts indicate that the density of bone reached a maximum at age 35

to 40 years of age in both sexes, and then falls especially in females, confirming the post-mortem data of Trotter, Broman and Peterson (1960).

X-ray densitometry is a more complex method of measuring the amount of mineral in a given segment of bone. It relies on the calculation of the absorption of X-rays by the mineral bulk. However, the variation in the amount of soft tissue overlying the bone and technical radiographic factors tends to complicate these procedures (Baker, Shraer and Yalman 1959). This technique was found to be more sensitive than the metacarpal index, but the correlation between the two methods was reasonable (Anderson, Shimmins and Smith 1966) suggesting that in epidemiological surveys, the simpler metacarpal index was an adequate measurement of osteoporosis.

The aetiology of osteoporosis remains in dispute. Albright and Reifenstein (1948) produced experimental evidence to suggest that osteoporosis was due to a deficiency of matrix with calcium loss occurring secondarily. On the other hand, Nordin challenged this concept and produced evidence that calcium deficiency per se was the primary cause of osteoporosis (Nordin 1961). The skeleton possesses approximately 1000 g. of calcium in the young adult, and Dent (1969) pointed out that only 50 mg of calcium loss per day was required to produce an average degree of osteoporosis in an older subject.

In any patient therefore who is losing bone mass, it is reasonable to assume that he or she is in negative calcium balance either by cause or effect. Calcium balance status at any point in time is dependent on dietry intake, absorption capacity, urinary excretion, faecal excretion and sweat loss. Studies of calcium balance are therefore difficult, time consuming and tend to have a fairly high degree of imprecision (error of 5 to 10 per cent) (Smith 1970).

Investigations of patients who have been immobilised have shown negative calcium balances of as much as 600 mg. per day with markedly raised urinary calcium values (Rose 1966). However, in many studies of calcium balance in patients with primary osteoporosis, the calcium balances are not markedly negative and urinary calcium levels do not tend to be elevated (Whedon 1959, Nordin 1961, Spencer, Menczel, Lewin and Samachson 1964, Rose 1965). This suggests that the osteoporotic patient has a negative calcium balance, little more than normal individuals, confirming Dents' rather pessimistic view of the difficulties of investigating osteoporosis from the calcium balance point of view (Dent 1969).

Other aspects of the metabolic status in osteoporosis have similarly been rather unhelpful in defining its pathogenesis. Serum calcium levels are essentially within normal limits and in post-menopausal women may lie in the upper limit of the normal range, and urinary calcium tends to follow a similar pattern. In the majority of patients with primary osteoporosis plasma phosphate and urinary phosphate lie within normal limits (Nordin 1973). Investigation of calcium absorption have led to some apparently conflicting conclusions. Dent and Friedman (1965) and Rose (1965), produced data which suggested that calcium absorption was low in cases of spinal osteoporosis. However, Avioli, McDonald and Lee (1965) suggested that calcium absorption was no lower in such patients than in normal control subjects of the same age.

By now it will have been appreciated that the term osteoporosis is used for a condition about which relatively little is known. It has been convenient to apply this term to almost any condition or disease in which there is demineralisation of bone which does not fit into a definite pattern of known bone disease, e.g. hyperparathyrodism or osteomalacia. Thus, "osteoporosis" was claimed as a secondary

phenomenon to thyrotoxicosis (Albright and Reifenstein 1948). Although the histological appearance of bone in hyperthyroidism is not different to that in primary osteoporosis, Follis (1953) demonstrated osteitis fibrosa in bone biopsies from patients with hyperthyroidism, suggesting that the bone disorder in thyrotoxicosis was not solely explained by osteoporosis. However, at this point in time, it is still generally accepted that osteoporosis may occur secondary to corticosteroid therapy (Murray 1960) Cushings Syndrome (Sprague, Randall, Salassa, Scholz, Priestley, Walters and Bulbulian 1956), immobilisation of a limb (Sissons 1952, Stevenson 1952), alcoholism (Saville 1965), primary biliary cirrhosis (Atkinson, Nordin and Sherlock 1956) and other clinical conditions (Nordin 1973).

The question of treatment of osteoporosis is probably one of the most controversial topics in what is already a highly controversial condition. To date there is no general agreement that osteoporosis can be reversed (Rose 1967), but some workers advocate therapy with calcium and small doses of Vitamin D (Nordin 1973), or albumin infusions (Dent and Lyal Watson 1966) in an attempt to improve calcium balance and improve symptoms arising from the osteoporotic state. The use of sex hormone therapy and thyrocalcitonin has not been shown to reverse osteoporosis, but possible may retard its development. Suffice to say that evidence of a satisfactory response to treatment of this condition is lacking and the verdict on all therapies must remain the Scottish Legal one of "not proven".

As the "Sage of Norwich" in his treatise on Urn Burial, noted "Teeth, bones and hair give the most lasting defiance to corruption" and were it not for the fact that "Time which antiquates antiquities and hath an art to make dust of all things hath yet spared these minor monuments" we might not have appreciated that rheumatoid arthritis was present in the world even in ancient times.

The oldest human bones showing evidence of "arthritis" were those found in Lower Egypt by Petrie (1890) with a suggested date of circa 1300 BC. Next came the "arthritis" discovered by Eve (1890) in ancient human remains in Egypt dated to the Ptolemaic period (second century BC), however, in retrospect these changes of "arthritis" were probably due to osteoarthritis. The bones of a Norse Viking, found in the Christianed fjord; skeletal remains in Pompeii doscovered by Chiaje (1853); those in the Convent of Marienthon in Pomerania by Virchow (1869); those in the Roman Sarcophagus at Smithfield by Dr. Norman Moore (1883) and those in the Catacombs of Paris found by Lebert (1859) all testified to the existence of arthritis in ancient peoples. However, it is not certain whether or not these were rheumatoid arthritis. In the latter part of the last century during the course of excavations undertaken by the survey department of the Egyptian Government in the tract of Nubia lying immediately south of the First cataract of the Nile over 6,000 skeletons were discovered ranging from early pre-dynastic times to the fifth century A.D. Professor Elliott Smith and Dr. Wood Jones who examined these specimens stated in the Nubian Survey Bulletin that "the disease which shows itself with by far the greatest frequency in the bodies of all periods is rheumatoid arthritis" (Elliott-Smith and Wood Jones 1910). An excellent photograph of bony destruction typical of rheumatoid arthritis (erosions, thinning of bones and bony ankylosis) of the hand and wrist occurring in a specimen from Ancient Nubia is evidenced by Lawford Knaggs in his book, to support the antiquity of rheumatoid arthritis (Lawford Knaggs 1926). These archaeological finds testify to the existence of rheumatoid arthritis in ancient times and also indicates its presence in various geographical areas. Significantly it was the bones which provided information and yet to a large extent the involvement of these same structures by the rheumatoid process has been relatively

ignored by medical scientists down the centuries. Certainly an excuse can be made for these workers prior to the twentieth century being unable to investigate the bony complications of rheumatoid arthritis because of the absence of X-ray facilities. William Balfour in 1816 in his description of the disease mentions that rheumatism (rheumatoid arthritis) affects muscle, nerves, blood vessels and all manner of organs, but does not mention any effect on bone. However, Barwell (1865) in his book is in no doubt that in chronic rheumatic arthritis, (from his description of this disease it probably embraces both rheumatoid and osteoarthritis) "the morbid action does not begin in the synovial membrane, but in the bones".

Although many of his premises and conclusions are seen in hindsight to be wrong he does give a very good pathological description of the effect of rheumatoid arthritis on bone. "At a certain period of the inflammation the thickened and condensed bone becomes gradually lighter. The bone does not become soft, but rarified. The external shell of the bone and the walls of the cancelli have become thinned. The gradual rarefaction of the bone and the thinning of both internal and external lamellae are due, I believe, to the slow ossification of the bone cells which starves the intercellular osseous material and allows its gradual absorption. Hence the peculiar action termed osteoporosis, (enlargement of the Haversian canals) is produced by absorption of the bony linings of those channels". Similarly, Bannatyne in 1896 writes, "On examining sections of bone (from rheumatoid patients) and also of medullary tissues, I was struck by the appearance of some of the latter. They were large granular cells with many nuclei and in a few instances I found places where they occurred in depressions of the bone. From these circumstances there could be no doubt as to their nature. They were osteoclasts and it is evident that the nature of the process is a rarefying osteitis".

In 1905 Odery Symes noted that when bones of patients with rheumatoid arthritis were examined by X-rays, they appeared more translucent than normal bones. He also contrasted this finding with the fact that in osteoarthritis the bones were more opaque and considered these features might be a useful diagnostic point in the differential diagnosis of the two diseases. The claim that demineralisation of bone occurring in rheumatoid arthritis is due to osteoporosis, is now a commonly held concept. (Soila 1958, Bywaters 1960, Castillo, El Sallab and Scott 1965, McConkey, Frazer and Bligh 1965, Saville and Kharmosh 1967, Martel 1968 and Bjelle and Nilson 1970), and the occurrence of localised "osteoporisis" in relation to the affected joints in patients with rheumatoid arthritis is well recognised (Soila 1963, Bywaters 1960, Bjelle and Nilsson 1971, Duncan, Frost, Villanueva and Sigler, 1965, Martel 1968. Generalised osteoporosis is also said to be present in patients with this disease (Soila, 1958, Berens, Locki, Lin and Norcross, 1964, McConkey, Frazer and Bligh 1965, Saville and Kharmosh 1967, Virtama, Helela and Kalliomaki 1968, Mueller and Jurist 1973). The aetiology of the loss of bone occurring in rheumatoid arthritis is still not understood. Local inflammation, immobilisation, generalised catabolic processes and poor nutrition in rheumatoid patients have all been implicated as causative factors, but to date no concrete incriminatory evidence has been discovered. In addition, osteoporosis is a well known complication of corticosteroid therapy; hence in patients with rheumatoid arthritis receiving such therapy, it is quite likely that the osteoporosis associated with rheumatoid arthritis might be further aggravated. However, there is a marked paucity of quantitative information in relation to the effects of age, sex, the severity of the clinical symptoms, the duration of the disease, and the effects of treatment on

bone mass in patients with rheumatoid arthritis. This is all the more surprising since quantitative measurements have been available for over a decade. The techniques range from the morphological measurements such as cortical widths of the metacarpal, femur and radius (Barnett and Nordin, 1960, Meema 1963) to the use of X-ray and γ-ray densitometric techniques (Nordin, Barnett, MacGregor and Nisbet, 1962; Keane, Speigler and Davis 1959; Doyle 1961; Anderson, Shimmins and Smith 1966; Cameron and Sorensen 1963; Shimmins, Anderson, Smith and Aitken 1972; Shimmins, Smith, Aitken, Anderson and Gillespie 1972). Various studies have also shown that age and sex differences affect normal populations (Smith, Anderson, Shimmins, Speirs and Barnett, 1969) and may influence the effects of disease and therapy (Smith 1970). Thus, most of the research into "osteoporosis" in rheumatoid arthritis, has been channelled towards an assessment of the degree and extent of demineralisation present and also the role of corticosteroid therapy in its genesis. The vexed question of whether osteoporosis occurring in rheumatoid arthritis is simply a localised effect secondary to inflammation and immobilisation or a more non-specific generalised condition has yet to be answered.

PERSONAL OBSERVATIONS

The object of the investigations in this chapter is to attempt to answer some of the problems related to osteoporosis in rheumatoid arthritis. First, I will describe studies using the metacarpal index (Barnett and Nordin, 1960) to define the relationship between osteoporosis and the clinical features of the disease and corticosteroid therapy. Secondly, by studying the relationship between the metacarpal index and the clavicular cortical width (Anton, 1969) and femoral index (Barnett and Nordin, 1960). In these patients I will attempt to ascertain the relative importance of local and systemic factors in the pathogenesis of osteoporosis in rheumatoid arthritis.

MATERIALS AND METHODS

The methods used for measuring changes in bone mass of the femur and metacarpal was that described by Barnett and Nordin, (1960). X-rays of the left femur and right second metacarpal were taken, the thigh and hand being placed in contact with the film, at a film focal distance of 100cm. The internal and external diameters of the bone at the midpoint were measured with a Vernier calliper. The femoral and metacarpal indices were obtained by dividing the sum of the cortical widths by the external diameter so as to allow for differences in size, in order that direct comparisons could be made, for example, between male and female patients. The thickness of the upper cortex of the right clavicle, hereafter termed Clavicular Cortical Thickness (CCT) was measured at the midpoint of the clavicle from postero-anterior chest radiographs using a Vernier calliper. (Anton 1969)

Subjects studied

Four hundred and fifty-three patients with rheumatoid arthritis were studied. The definition of rheumatoid arthritis used was the diagnostic criteria proposed by the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox and Jessar, 1959). The patient group was composed of 158 males and 295 females.

A metacarpal index result was obtained in each of these patients. In three hundred and sixty-one of these patients it was possible to obtain a femoral index score and in three hundred and seven patients it was possible to obtain in addition to the metacarpal and femoral indices a measurement of clavicular cortical thickness.

The results of the metacarpal and femoral indices were compared with control values obtained in normal subjects, these being the relatives and friends of patients admitted to the general medical and surgical wards of the Western Infirmary, Glasgow. The object of the study was fully explained and the subject asked if he/she would volunteer to have an X-ray of the hand and femur carried out. In this way, metacarpal indices of 312 male and 317 female normal subjects were obtained and also the femoral index values of 119 female and 76 male normal controls.

A standard questionnaire was completed at the time to ensure that anyone suffering from disease liable to affect bone mass was excluded from the study. These normal subjects have been studied separately and reported on in detail elsewhere (Smith, Anderson, Shimmins, Speirs and Barnett, 1968; Smith, Anderson, Shimmins, Speirs and Barnett, 1969; Smith, 1970). Normal values for the clavicular index were obtained from 221 relatives of patients who attended the Medical and Surgical wards of Stobhill General Hospital, Glasgow, care being taken to exclude people who had disease likely to affect bone mass.

Of these 221 volunteers, 114 were males and 107 were females. The mean age of the male subjects was 47.4 $\pm$ 15.2 years (range 21 to 78 years), and female subjects, 48.1 $\pm$ 16.6 years (21 to 79 years).

RESULTS

Table I:1 gives an analysis of the clinical and diagnostic features of the patients in the first part of this study. This includes the number of patients studied in each of four groups, comprising male and female patients both treated and not treated with corticosteroids. The statistics show the mean (± 1 standard deviation) of age, the duration of the arthritis, titre of rheumatoid factor (Ball, 1952), age of onset of menopause, serum albumin and globulin, haemoglobin concentration and erythrocyte sedimentation rate. Only minor differences emerged between the groups. The patients receiving corticosteroid therapy were on average slightly younger, and had had the condition for longer. However, these differences were not statistically significant. The titre of rheumatoid factor was higher in those patients who had been given steroid therapy but this was not statistically significant. The mean haemoglobin was significantly higher in the male compared with female subject, but the steroid-treated groups did not differ significantly from the non-steroid treated subjects of the same sex. Serum albumin and globulin levels, and erythrocyte sedimentation rates did not differ significantly between the groups. The mean age of onset of the menopause was nearly identical in the two female patient groups at 45.2 and 45.8 years of age. Of the 158 male patients studied, 24 were receiving treatment with corticosteroids, and 134 were not. In Fig.I:1 (a and b) the metacarpal index was related to age in the non-treated group (Fig.I:1a) and steroid treated group (Fig.I:1b).

	Males			Females		
	Non-Steroid Treated Subjects A	Steroid Treated Subjects B	A + B	Non-Steroid Treated Subjects A	Steroid Treated Subjects B	A + B
Numbers	134	24	158	186	109	295
Age in Years*	56.4 ± 11.8	59.9 ± 13	57.3 ± 11.2	53 ± 33.6	50.5 ± 12.8	51 ± 13.8
Duration of Arthritis In Years*	12.2 ± 13.9	15 ± 10.5	12 ± 8.1	9 ± 10.8	12.3 ± 10.4	10.5 ± 14.3
Rheumatoid Factor Titre*	141.1 ± 670	264.7 ± 283.4	226.8 ± 530.7	205.9 ± 458	336.2 ± 645.4	251.6 ± 533
Age of Menopause In Years*	-	-	-	45.2 ± 6.7	45.8 ± 4.4	45.4 ± 7
Serum Albumin G/L* ...	35.5 ± 4.3	34.4 ± 5.9	35.1 ± 4.1	33.4 ± 4.6	32.4 ± 7.4	33.0 ± 4.2
Serum Globulin GL/* ..	36.8 ± 8.0	36.7 ± 7.4	36.8 ± 8.2	35.5 ± 7.6	35.2 ± 6.7	35.4 ± 7.3
Erythrocyte Sedimentation Rate* .	47.9 ± 30.2	50.8 ± 28.2	48.7 ± 29.8	44.9 ± 28.7	50.1 ± 31	50 ± 29.9
Haemoglobin Concentration in G/100 ml	13.7 ± 1.7	13.6 ± 1.9	13.7 ± 1.6	12.1 ± 1.7	11.9 ± 2.8	12 ± 1.5

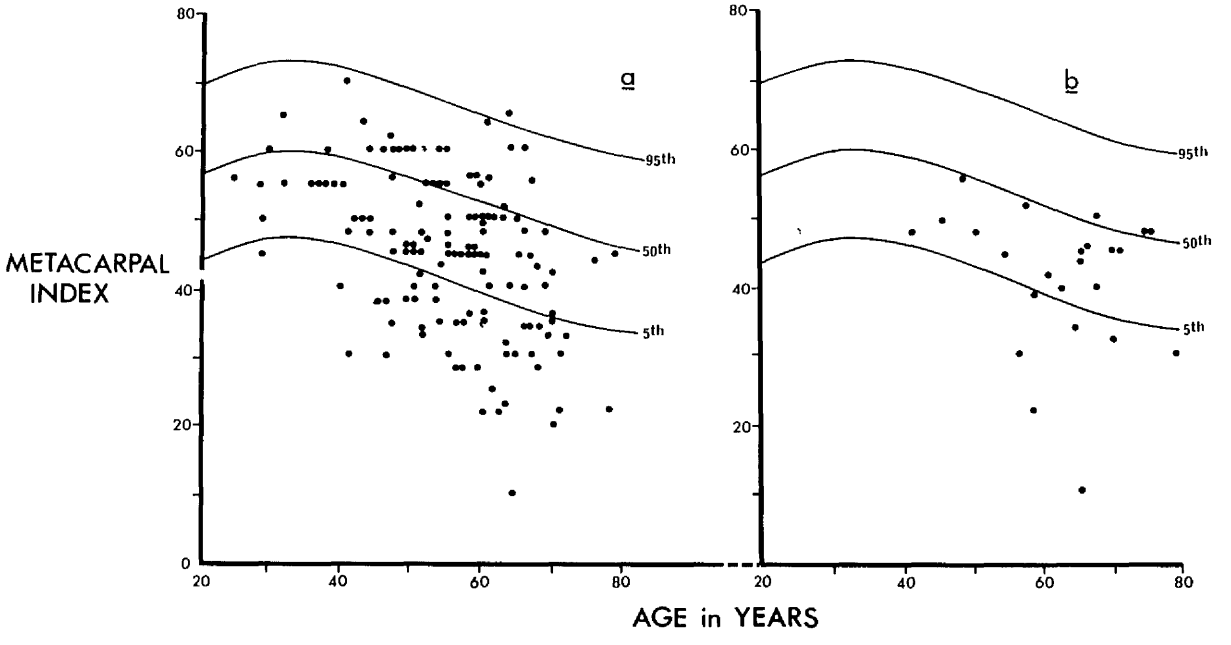
TABLE I:1

Clinical and diagnostic features in the male and female subjects treated or not treated with corticosteroids

(Mean * Mean ± 1 Standard Deviation)

Figure I:1

The Metacarpal Indices in male patients with rheumatoid arthritis plotted against age. Those patients not receiving corticosteroid therapy are shown in (a) and those who were receiving corticosteroid therapy are shown in (b).
The normal 5, 50 and 95 percentile values are shown by continuous lines.


METACARPAL INDEX
80
60
40
20
0
a
b
95th
50th
5th
20
40
60
80
AGE in YEARS

Superimposed on these results were the 5, 50 and 95 percentile lines obtained from the 312 normal male subjects.

Table I:2 shows the percentage of patients below the 5th percentile in each group after sub-dividing the patients into groups 45 years of age and above, and those below 45 years of age. This age division, is of course, arbitrary and was chosen because the mean age of the menopause occurred at this age in the female subject, and it therefore seemed logical to divide them into these groups for comparison.

Of the 114 male patients not on steroid therapy and aged 45 years and over, 42 (36.8%) were below the 5th percentile (Table I:2). Only 3 of the patients (15%) under 45 years of age were below the 5th percentile. Only one male patient who was treated with corticosteroids was below the age of 45, and his metacarpal index lay on the 10th percentile. Of the 23 patients aged 45 and over, 7 (30%) were below the 5th percentile. Of the 295 female patients studied, 109 were receiving treatment with corticosteroids and the remaining 186 were not. In Fig.I:2 (a and b), the metacarpal index was plotted in relation to age in the non-steroid treated group (Fig.I:2a) and the steroid-treated group (Fig.I:2b). Superimposed on these results were the 5, 50 and 95 percentile values obtained from the 317 normal female subjects. Table I:2 shows the percentage of patients below the 5th percentile in these two groups after dividing them into groups showing those below 45 years and those above 45 years.

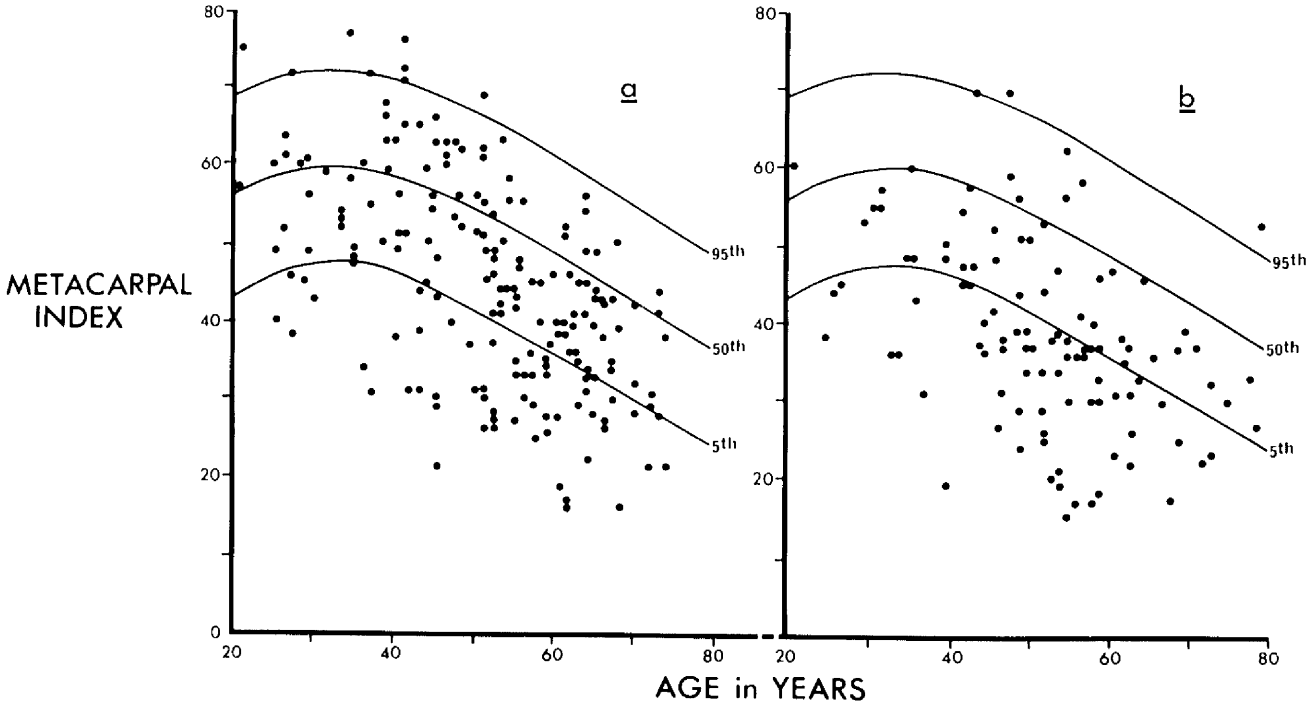
	Male		Female	
	Non-Corticosteroid treated	Corticosteroid treated	Non-Corticosteroid treated	Corticosteroid treated
Under 45 Years of age	15%	-	22.4%	40.7%
45 Years of age and Over	36.8%	30.4%	36.7%	62.2%

TABLE I:2

The Percentage of Patients below the 5th Percentile treated or not treated with corticosteroids

Fig I: 2

The Metacarpal Indices in female patients with rheumatoid arthritis plotted against age. Those patients not receiving corticosteroid therapy are shown in (a) and those who were receiving corticosteroid therapy are shown in (b).
The normal 5, 50 and 95 percentile values are shown by continuous lines.


METACARPAL INDEX
80
60
40
20
0
a
b
95th
50th
5th
20
40
60
80
AGE in YEARS

Of the 186 non-steroid treated female patients, 58 were below 45 years of age and the remaining 128 were 45 years or over. As in the male group, those patients 45 years and over appeared more severely affected than the patients under 45 years, 36.7% of those 45 years and over lying below the 5th percentile compared with 22.4% of those under 45 years of age (Table I:2).

Of the 109 steroid-treated patients, 26 were under 45 years and 40.7% of them were below the 5th percentile. Of the remaining 82 female patients 45 years or over, 62.2% were below the 5th percentile.

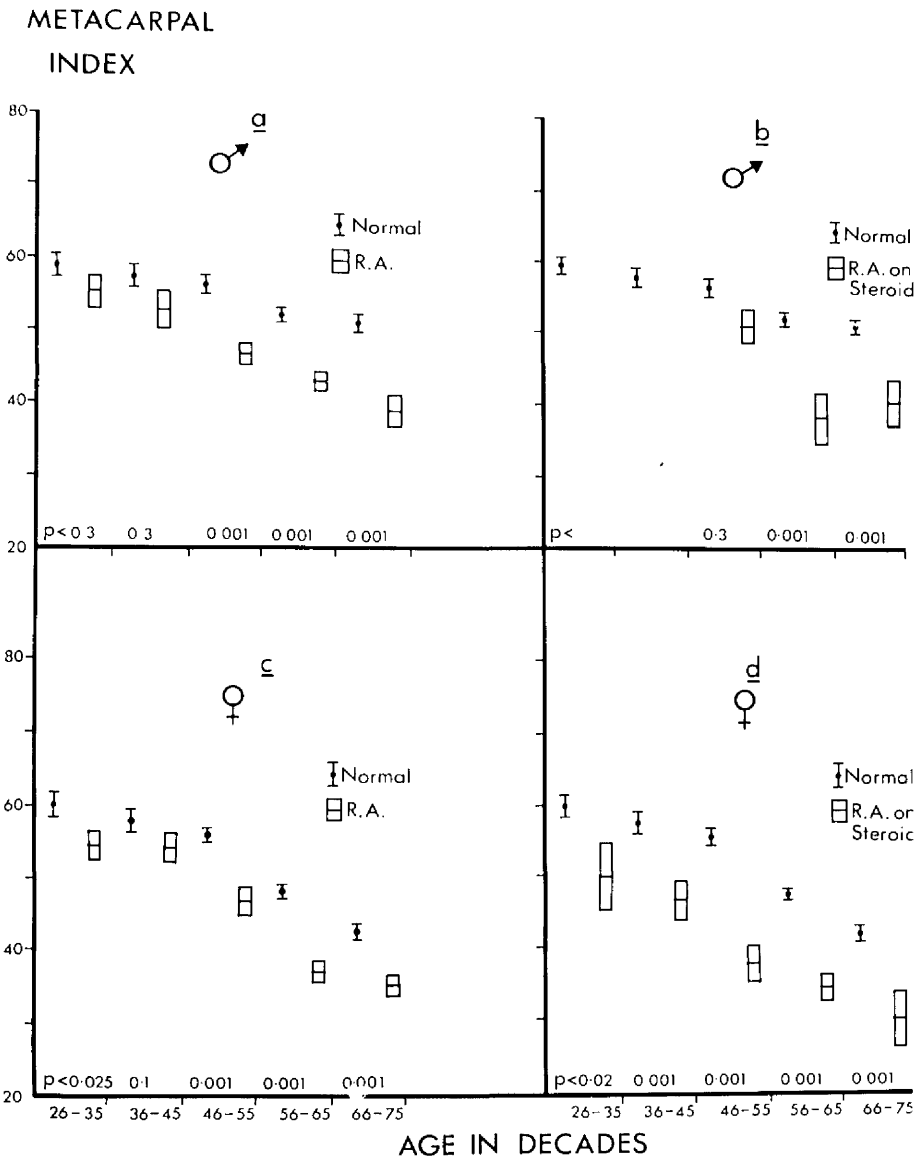
In order to test for statistical differences between the normal subjects and the patients with rheumatoid arthritis, it is necessary to look for differences by decades. This is because of the fall in the metacarpal index which occurs with increasing age in normal male and female subjects. Fig.I:3a shows the mean and standard error of the metacarpal index for the normal male subjects and the male subjects with non-steroid treated rheumatoid arthritis. No significant difference between the normal subjects and the patients was found under the age of 45 years.

In the patients aged 45 years and over, however, a highly significant difference was seen ($P < 0.001$) at the three decades from 46 to 74 years of age, the patients all having lower metacarpal indices. In the small group of steroid-treated male patients (Fig.I:3b) statistical differences were apparent ($P < 0.001$) in the two decades between 56 and 74 years of age, again the patients having lower metacarpal indices.

Fig I: 3

The mean and standard error range in the Metacarpal Index for normal male and female patients, and patient groups are shown plotted against age.

(a) shows the values for male patients with rheumatoid arthritis and normal male subjects.
(b) Male patients with rheumatoid arthritis treated with corticosteroids and normal subjects.
(c) Female patients with rheumatoid arthritis and normal subjects.
(d) Female patients with rheumatoid arthritis treated with corticosteroids and normal subjects.


METACARPAL
INDEX
a
b
c
d
Normal
R.A.
R.A. on Steroid
R.A. or Steroic
80
60
40
20
p<0·3
0·3
0·001
0·001
0·001
p<
0·3
0·001
0·001
p<0·025
0·1
0·001
0·001
0·001
p<0·02
0·001
0·001
0·001
0·001
26–35
36–45
46–55
56–65
66–75
AGE IN DECADES

A similar picture emerged in the non-steroid treated female patients (Fig.I:3c). Significant differences were observed between 26 and 34 years of age, but none between the ages of 36 and 45 years. In the three decades between 45 and 74 years of age, the patients metacarpal indices were significantly lower than those of the normal subjects ($P < 0.001$). In the steroid-treated group (Fig.I:3d) the female patients in the decades between 25 and 74 had statistically significantly lower metacarpal indices than the normal subjects.

The Effects of Duration of Rheumatoid Arthritis, and the Effects of Duration of Steroid Therapy on Bone Mass

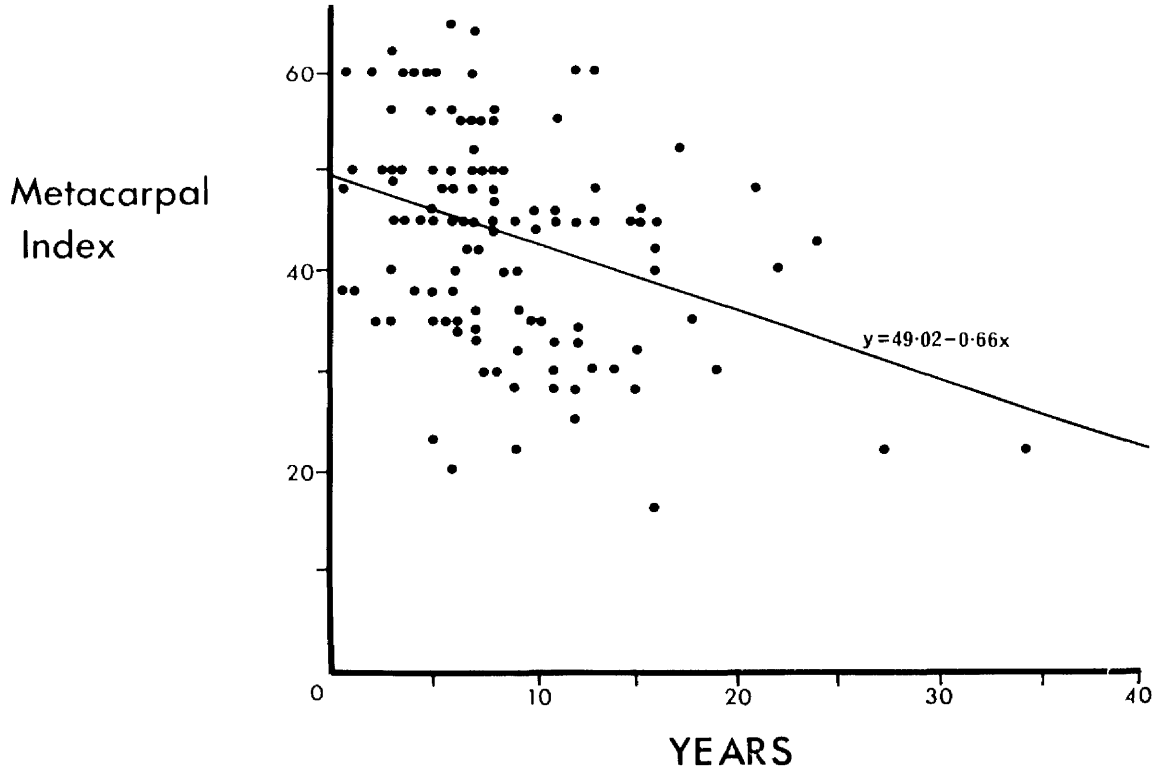
The effects of duration of this disease and the effect of treatment with corticosteroids in the patients has been tested in two ways: firstly, as a direct measurement of the metacarpal index to the duration of the rheumatoid arthritis, or rheumatoid patients treated with steroid therapy; secondly, as a measurement of the percentile value in relation to the duration of the disease or steroid therapy. The latter is necessary to exclude any influence of age on the results. When this was considered for the four groups as a whole, no significant relation to duration of the disease or duration of steroid therapy was found. However, scrutiny of Fig. I:3 suggests that older patients are more severely affected and, therefore, male and female patients over the age of 45 years were examined.

The effects of the duration of rheumatoid arthritis in male patients over the age of 45 years are illustrated in Fig.I:4 and detailed in Table I:3. In the male patients, the metacarpal index

Fig I: 4

The Metacarpal Index for male patients with rheumatoid arthritis is shown plotted against the duration of the disease.
There is a wide scatter but the fall in Metacarpal Index is significantly related to the duration of the disease ($P<0.001$).

see Table I: 3


Metacarpal Index
60
40
20
0
10
20
30
40
y=49·02−0·66x
YEARS

x	Group	Sex	No. of Sub-jects	a	b	r	t	p
Metacarpal Index	Non-steroid treated	M	114	49.02	-0.66	-0.35	3.92	< 0.001
Metacarpal Index	Steroid treated	M	23	48.66	-2.00	-0.56	3.13	< 0.01
Percentile (M.I.)	Non-steroid treated	M	114	31.31	-.82	-0.17	1.86	> 0.05 (N.S.)
Percentile (M.I.)	Steroid treated	M	23	38.25	-3.19	-0.41	2.18	< 0.05
Metacarpal Index	Non-steroid treated	F	128	49.19	-1.17	-0.59	8.25	< 0.001
Metacarpal Index	Steroid treated	F	82	40.78	-1.63	-0.34	3.26	< 0.001
Percentile (M.I.)	Non-steroid treated	F	128	39.12	-1.89	-0.41	5.13	< 0.001
Percentile (M.I.)	Steroid treated	F	82	23.48	-2.98	-0.28	2.54	< 0.02

TABLE I:3

The relation between the Metacarpal Index, the duration of rheumatoid arthritis and corticosteroid therapy (years) in male and female patients over the age of 45 - measured as the change in the M.I. and change in percentile values of the M.I. ($y = a + bx$).

is inversely related to the duration of the disease. However, when considered as percentile values it fails to reach significance. In the steroid-treated group of male patients over 45 years of age, the fall in metacarpal index was inversely related to the duration of the disease ($P < 0.01$) and when considered as a fall in the percentile value ($P < 0.05$). In the female subjects (Table I:3) a highly significant fall was found in both the metacarpal index and the percentile values in both the steroid and non-steroid treated groups over the age of 45 years of age.

The possible effects of age on bone loss in the non-steroid and steroid-treated patients are examined in Table I:4. Since the bone mass, measured as the metacarpal index falls with age in both males and females, the bone loss over and under 45 years of age is expressed as the percentile value so that the relative changes from normality can be determined. As can be seen, the patients in all eight groups at 45 years and over have lower percentile values than those under 45 years of age. However, it is only in the non-steroid treated female patients that statistical significance is achieved. There was only one male patient under 45 years of age who received steroid therapy.

The possible effects of the patient's sex on bone loss in the non-corticosteroid treated groups, and those patients treated with corticosteroids, is examined in the same way in Table I:5 using the percentile values of the metacarpal index. No significant differences emerged in any of the groups. Again there was only one male patient under the age of 45 receiving steroid therapy so no comparison could be made in this group.

	Sex	Age	No. of Sub-jects	Mean M.I. Percen-tile	S.E.	t	P
Non-steroid Treated Patients ..	M	< 45	20	32.15	6.13	1.5	> 0.1 (n.s.)
	M	> 45	114	24.42	2.54		
	F	< 45	56	37.05	4.42	2.44	< 0.02
	F	> 45	128	25.41	2.50		
Steroid Treated Patients	M	< 45		Numbers Insufficient			
	M	> 45					
	F	< 45	26	19.92	5.37	0.95	> 0.5 (n.s.)
	F	> 45	82	14.61	2.65		

TABLE I:4

The effects of rheumatoid arthritis and steroid therapy on the percentile values of the Metacarpal Index in male and female patients over and under the age of 45 years

	Sex	Age	No. of Sub-jects	Mean M.I. Percen-tile	S.E.	t	P
Non-steroid treated Patients ...	M	< 45	20	32.15	5.80	0.60	N.S.
	F	< 45	58	37.05	4.42		
Steroid-treated Patients	M	< 45	Insufficient Numbers				
	F	< 45					
Non-steroid treated Patients ...	M	> 45	114	24.42	2.53	0.6	N.S.
	F	> 45	128	25.41	2.50		
Steroid-treated Patients	M	> 45	23	20.78	3.95	1.14	N.S.
	F	> 45	82	14.61	2.65		

TABLE I:5

Comparison of the percentile Metacarpal Index between male and female subjects under 45 years, and 45 years and over, and the effects of rheumatoid arthritis and steroid therapy

The effects of corticosteroid therapy were tested (Table I:6) by comparing the percentile differences in metacarpal index in the non-steroid and steroid treated patients who were 45 years of age and over and those under 45 years of age. Again there was only one male patient under 45 years, so no comparison could be made in this group. In the male patients who were 45 years and over, no significant differences was seen. However, in the female patients who were treated with steroid therapy the bone mass was found to be significantly lower than that found in the patients with rheumatoid arthritis who had not been treated with steroid therapy.

However, difference in bone loss could arise between the patients above and below 45 years of age because of the duration of the arthritis or the duration of corticosteroid therapy. From Table I:7 it can be seen that the males above and below the age of 45 years of age had rheumatoid arthritis for a longer mean time than the female subjects, but these differences were not statistically significant. Male and female patients aged 45 years and above had had rheumatoid arthritis for significantly longer than the patients under 45 years of age.

Male patients aged 45 years and over had been treated with steroids for significantly longer than the female patients of 45 years and above. There was, however, no significant difference in the length of time that female patients under the age of 45 years had been treated compared with those over the age of 45 years.

	Sex	Age	No. of Sub-jects	Mean M.I. Percen-tile	S.E.	t	P
Non-steroid treated Patients ...	F	< 45	58	37.05	4.4	2.3	< 0.02
Steroid-treated Patients	F	< 45	27	19.92	5.37		
Non-steroid treated Patients ...	F	> 45	128	25.41	2.50	2.86	< 0.001
Steroid-treated Patients	F	> 45	82	14.61	2.65		
Non-steroid treated Patients ...	M	< 45		Insufficient Numbers			
Steroid-treated Patients	M	< 45					
Non-steroid treated Patients ...	M	> 45	114	24.42	2.54	0.5	N.S.
Steroid-treated Patients	M	> 45	23	20.78	3.95		

TABLE I:6

Comparison between the percentile Metacarpal Index in male and female patients, non-steroid treated and steroid treated, and under and over the age of 45 years.

Sex	Age Group (Years)	Mean Duration of Rheumatoid Arthritis	No. of Sub-jects	S.E.	t	P
M	< 45	9.69	20	1.09	1.95	> 0.05 N.S.
F	< 45	6.57	58	0.8		
M	> 45	12.36	114	0.63	1.06	> 0.2 N.S.
F	> 45	11.26	128	0.73		
F	< 45	6.57	58	0.81	3.75	< 0.001
F	> 45	11.26	128	0.73		
M	< 45	9.69	20	1.13	5.80	< 0.001
M	> 45	12.36	114	0.63		
		Mean Duration of Steroid Therapy (Years)				
F	> 45	3.24	82	0.26	2.28	< 0.025
M	> 45	4.58	23	0.63		
F	< 45	2.85	26	0.41	0.76	> 0.2 N.S.
F	> 45	3.24	82	0.26		

TABLE I:7

Comparison of the duration of Rheumatoid Arthritis and Corticosteroid Therapy in male and female patients below 45 years of age and aged 45 years and above

In three hundred and fifty-one of the patients investigated in the first part of this study it was possible to obtain a femoral index value. The results of the analysis of their clinical and diagnostic criteria are shown in Table I:8. These are shown as the mean and 1 S.D. in each patient group. The mean age of the male patients was significantly greater than that of the female patients. This difference was still apparent after subdividing them into corticosteroid treated and non-corticosteroid treated groups, the male patients being significantly older than the female patients. There was no significant difference in age between the steroid and non-steroid treated male patients or between the steroid and non-steroid treated female patients. A similar pattern emerged when considering the duration of the arthritis which was, on average, present for longer in the male than in the female subjects. The mean titre of rheumatoid factor was higher in both the male and female corticosteroid treated groups than in the non-steroid treated patients, though these differences did not reach statistical significance. In the female patients, the age of onset of the menopause was almost identical in the corticosteroid treated and non-corticosteroid treated groups. The mean serum albumin and globulin levels were slightly, but not significantly higher in the male patients. The mean erythrocyte sedimentation rate was not significantly different in any of the groups. The male patients had a significantly higher haemoglobin level than the female patients in all three groups. However, there was no significant difference between the steroid and non-steroid treated male patients and this was also true for the female patients.

	A + B	Male Patients		A + B	Female Patients	
		Non-Steroid Treated A	Steroid Treated B		Non-Steroid Treated A	Steroid Treated B
umbers	150	126	24	201	130	71
ge (Years)	57.1 ± 11.0	56.1 ± 11.7	59.9 ± 13.0	50.3 ± 12.8	52.1 ± 28.4	48.9 ± 11.9
uration of Arthritis (Years)	12.6 ± 9.0	12.7 ± 13.4	15.0 ± 10.5	7.8 ± 10.7	6.8 ± 9.2	8.9 ± 6.2
heumatoid Factor Titre	228.0 ± 506.7	144.6 ± 590.0	264.7 ± 283.4	226.7 ± 530.0	196.4 ± 395.0	312.5 ± 634.6
ge at Menopause (Years)				45.3 ± 6.2	45.4 ± 6.2	45.6 ± 4.1
erum Albumin g/l	3 54± 51	3 62± 5.	3 44± 59	3 28± .44	3 24± 42	3 31± 8.
erum Globulin g/l	3 68± 88	3 69± 85	3 67± .74	3 54± 72	3 54± 75	3 54± 69
rythrocyte Sedimentation Rate	47.9 ± 29.1	46.2 ± 28.0	50.8 ± 28.2	48.4 ± 30.4	46.0 ± 30.1	49.2 ± 30.7
aemoglobin Concentration g/100 ml	13.8 ± 1.8	13.9 ± 1.8	13.6 ± 1.9	12.1 ± 1.7	12.4 ± 1.8	12.0 ± 2.4

TABLE: I:8

Clinical and Diagnostic features of the male and female patients with rheumatoid arthritis included in the second part of the study

Bone loss is well known to occur in both male and female subjects with increasing age, as measured by the femoral index Nordin, McGregor and Smith (1966); Smith (1970). The femoral indices in the patient groups have therefore been compared by decades with the control subjects. In Fig.I:5 the femoral indices of the noncorticosteroid treated female patient group is seen to be significantly lower than those of the control subjects in each decade between 25 and 74 years of age. In the female corticosteroid treated group, the femoral indices were significantly lower than in the normal subjects in all age groups, except in the decade 25 to 34 years of age (Fig.I:6). When the femoral indices in the steroid and non steroid treated groups were compared (Fig.I:7) the steroid treated groups at each decade had marginally lower mean values, but in no decade did this reach statistical significance.

In the male patients who had not been treated with corticosteroids, the mean femoral indices were all lower than those of the control subjects in each decade between 25 and 74 years of age (Fig.I:8), but only reached statistical significance between 45 and 64 years. The number of male patients who were treated with corticosteroids was small (twenty-four), one patient was below 45 years of age, and the rest ranged between 45 and 74 years of age. The mean values were lower in each of the three decades, compared with the control subjects, but only reached statistical significance in the 55 to 64 year age group (Fig.I:9). When the steroid treated male patients with rheumatoid arthritis were compared with the non-steroid treated group in the three decades 45 to 74 years of age, no significant difference between these groups was found (Fig.I:10).

Fig I: 5

The mean and standard error of the femoral index shown by decades in normal female subjects and in the female patients with rheumatoid arthritis not receiving corticosteroid therapy.

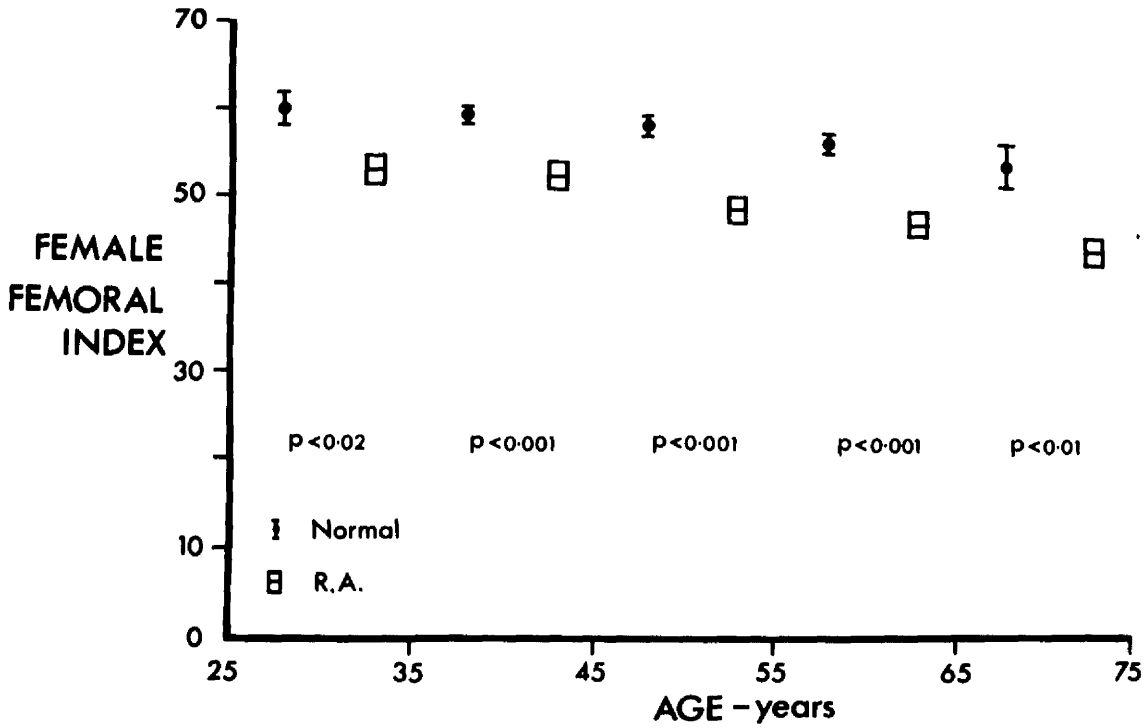

70
50
30
10
0
FEMALE
FEMORAL
INDEX
p<0·02
p<0·001
p<0·001
p<0·001
p<0·01
Normal
R.A.
25
35
45
55
65
75
AGE – years

Fig I: 6

The mean and standard error of the femoral index shown by decades in normal female subjects and in the female patients with rheumatoid arthritis who were receiving corticosteroid therapy.

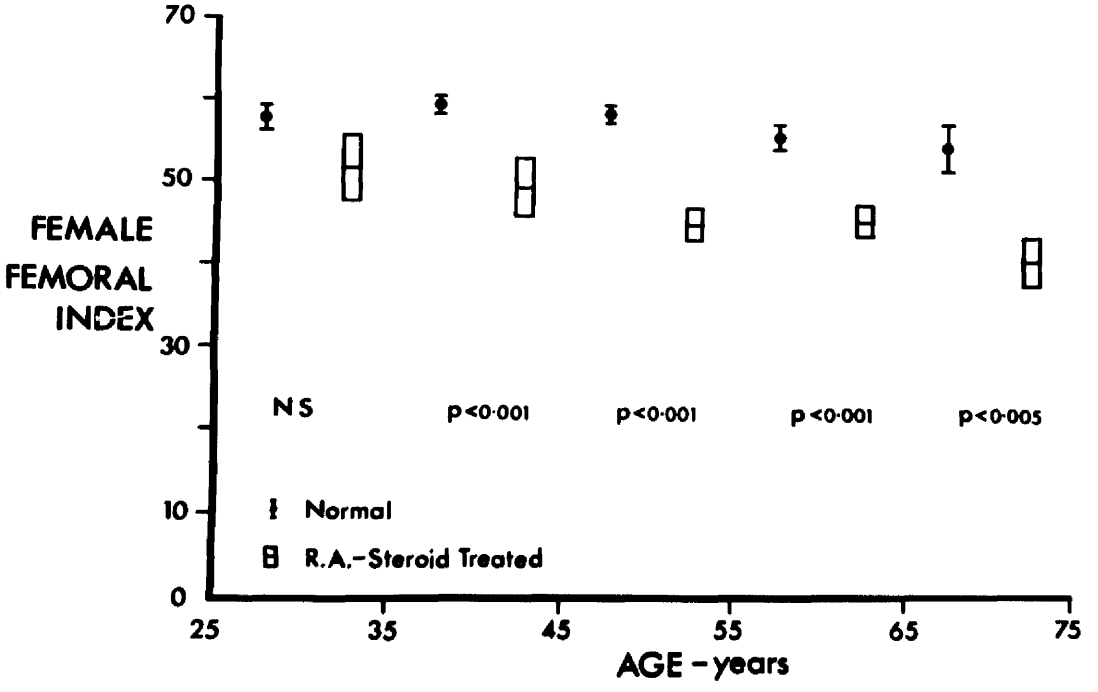

70
50
30
10
0
FEMALE
FEMORAL
INDEX
NS
p<0·001
p<0·001
p<0·001
p<0·005
Normal
R.A.–Steroid Treated
25
35
45
55
65
75
AGE – years

Fig I: 7

The mean and standard error of the femoral index shown by decades, in female patients with rheumatoid arthritis not receiving corticosteroid therapy and those female patients who were receiving corticosteroid therapy.

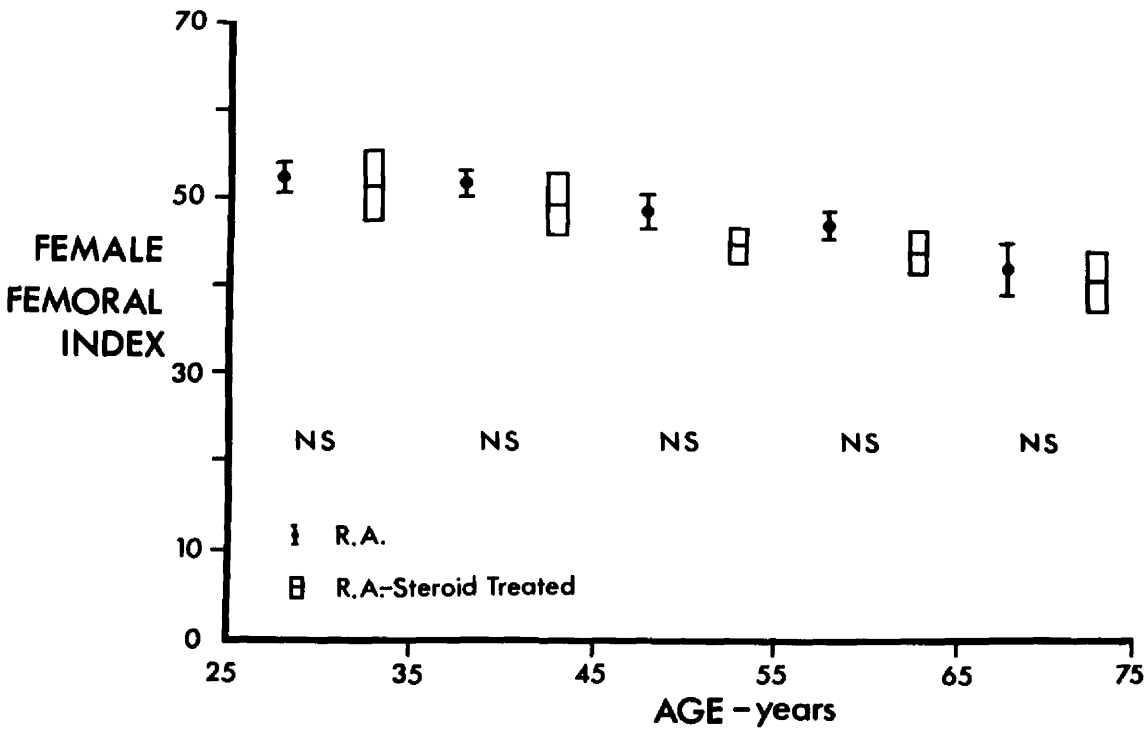

70
50
30
10
0
FEMALE
FEMORAL
INDEX
NS
NS
NS
NS
NS
R.A.
R.A.-Steroid Treated
25
35
45
55
65
75
AGE – years

Fig I: 8

The mean and standard error of the femoral index shown by decades in normal male subjects and in the male patients with rheumatoid arthritis not receiving corticosteroid therapy.

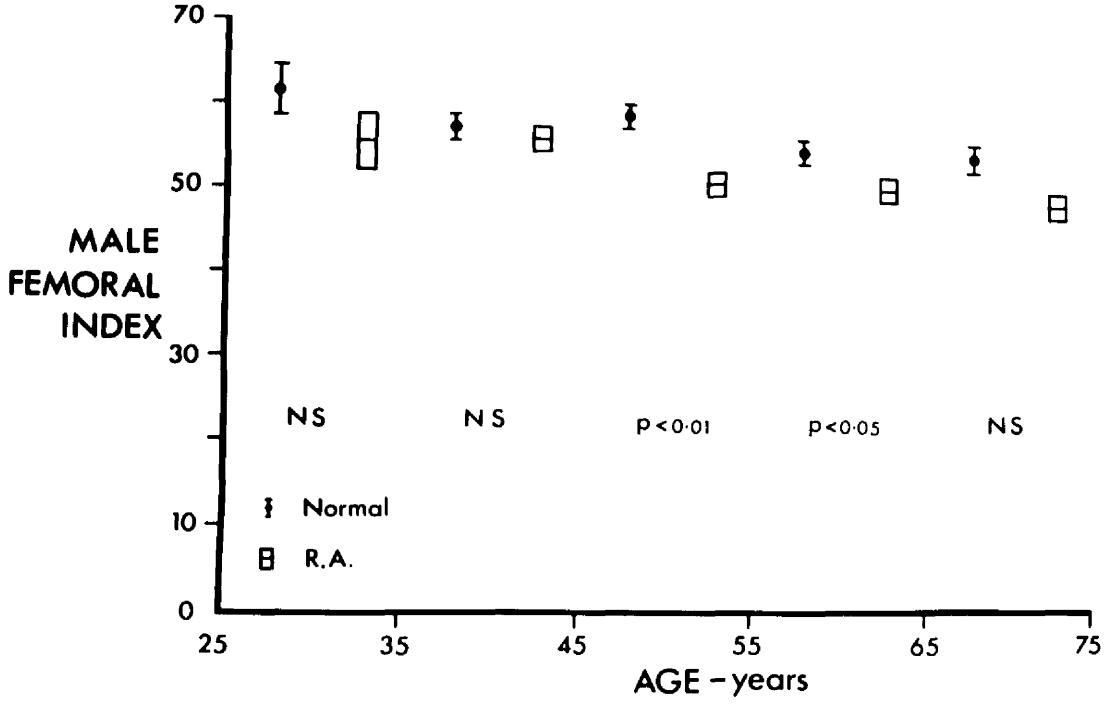

70
50
30
10
0
MALE
FEMORAL
INDEX
NS
NS
P<0·01
P<0·05
NS
Normal
R.A.
25
35
45
55
65
75
AGE – years

Fig I: 9

The mean and standard error of the femoral index shown by decades in normal male subjects and in the male patients with rheumatoid arthritis who were receiving corticosteroid therapy.

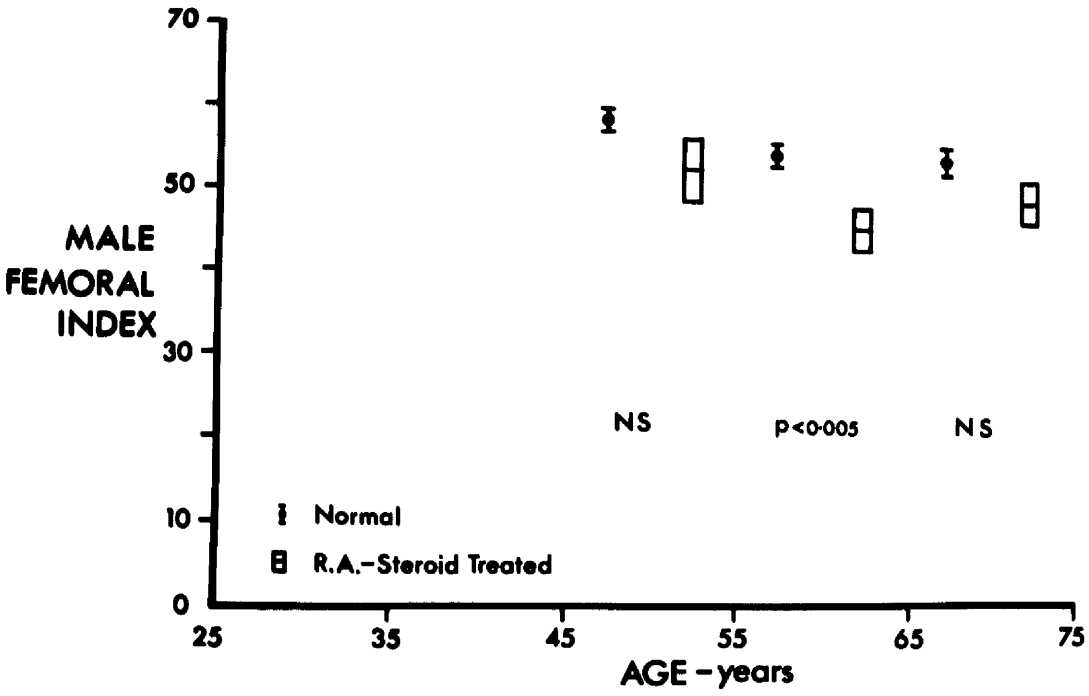
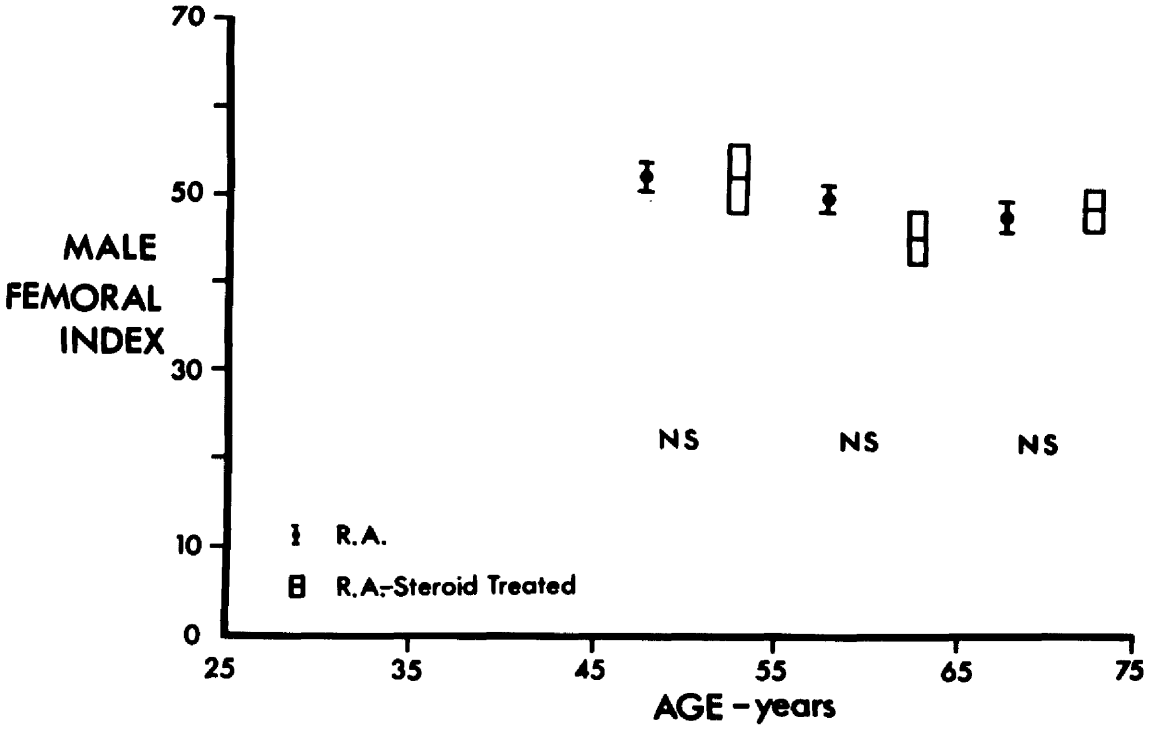

70
50
30
10
0
MALE
FEMORAL
INDEX
NS
p<0·005
NS
Normal
R.A.–Steroid Treated
25
35
45
55
65
75
AGE – years

Fig I: 10

The mean and standard error of the femoral index shown by decades in male patients with rheumatoid arthritis not receiving corticosteroid therapy and those male patients who were receiving corticosteroid therapy.


70
50
30
10
0
MALE
FEMORAL
INDEX
NS
NS
NS
R.A.
R.A.-Steroid Treated
25
35
45
55
65
75
AGE - years

The relation between the femoral and metacarpal indices in the various patient groups is shown in Table I:9. The patients have been divided into two groups - 45 years of age and above, and those below 45 years - since this is the mean age of the menopause after which the rate of bone loss accelerates. The males also have been divided into those 45 years and above, and those below 45 years, so that the different male and female age groups correspond. The relation between the femoral indices and metacarpal indices in the entire male and entire female groups is also shown. Only one male patient under 45 years of age was treated with corticosteroids. The femoral and metacarpal indices are clearly related except in the corticosteroid treated female patients under the age of 45 years. However, the highest correlation coefficient, 0.736 for the corticosteroid treated male patients, gives a coefficient of determinancy of only 0.542.

In order to test this further, the femoral index and metacarpal index have been examined for concordance and discordance compared with the normal values. The results are shown in Table I:10. The patients have been divided into male and female groups and each of these in turn into steroid treated and non-steroid treated. Finally, the groups have been sub-divided into those 45 years of age and above, and those below 45 years of age. In the male patients, concordance ranges from 62 to 100% when values for both the femoral index and metacarpal index lie within the 2 S.D. range of the normal values, and 16 to 21.7% when both values lie without the normal 2 S.D. range. In the female patients, the corresponding concordance ranges between 45.2% and 62.7% and 4.6% and 12.6% for values within and without the 2 S.D. range for normal subjects.

Clinical Group	Sex	Age Group (Years	No. of Sub-jects	a	b	r	t	P
Non-corticosteroid-treated	M	< 45	18	22.419	0.614	0.639	3.322	<0.002
Non-corticosteroid-treated	M	> 45	108	24.881	0.688	0.583	9.727	<0.001
Corticosteroid-treated	M	< 45	1	-	-	-	-	-
Corticosteroid-treated	M	> 45	23	-6.804	0.962	0.736	4.987	<0.001
Total	M	All Ages	150	25.2412	0.5735	0.7124	-2.5562	<0.01
Non-corticosteroid-treated	F	< 45	43	35.647	0.277	0.339	2.305	<0.05
Non-corticosteroid-treated	F	> 45	87	34.344	0.306	0.385	3.844	<0.001
Corticosteroid-treated	F	< 45	18	34.022	0.305	0.405	1.771	n.s.
Corticosteroid-treated	F	> 45	53	33.058	0.309	0.336	2.547	<0.02
Total	F	All Ages	201	34.2153	0.3037	0.4471	7.0153	<0.001

TABLE I:9

Correlation between Femoral Index (F.I.) and Metacarpal Index (M.I.) in male and female patients with rheumatoid arthritis

Clinical Group	No. of Sub-jects	Sex	Age Group (Years)	F.I. N	M.I. N (%)	F.I. S	M.I. S (%)	F.I. N	M.I. S (%)	F.I. S	M.I. N (%)	Total Con-cora-ance	Total Dis-cord-ance (%)
N-CT	18	M	<45	18	100			-	-		-		100
N-CT	108	M	>45	67	62	20	18.5	16	14.8	5	4.6	80.6	19.4
CT	1	M	<45		-		-		-		-	100	-
CT	23	M	>45	15	65	5	21.7	3	13	1	4.3	82.6	17.4
TOTAL	150	M	All Ages	100	66.5	25	16.0	19	12.6	6	4.0	83.3	16.7
N-CT	43	F	<45	27	62.7	2	4.6	1	2.3	13	30.2	67.4	32.6
N-CT	87	F	>45	50	57.4	11	12.6	20	22.9	6	6.8	70.1	29.9
CT	18	F	<45	10	55.5		-	2	11.1	6	33.3	55.6	44.4
CT	53	F	>45	24	45.2	6	11.3	20	37.7	3	5.6	56.6	43.4
TOTAL	201	F	All Ages	111	55.2	19	9.4	43	21.3	28	13.9	64.7	35.3

TABLE I:10

Concordance and discordance of Femoral (F.I.) and Metacarpal (M.I.) Indices in patients with rheumatoid arthritis

CT = Corticosteroid treated: N-CT = Non-corticosteroid treated; N = Normal (i.e. within $\pm$ 2 S.D.); S = Subnormal (i.e., out-with - 2 S.D.)

Discordance ranged from 13% to 14.8% in the male subjects when the femoral indices were normal and the metacarpal indices abnormal, and 4 to 4.6% when the metacarpal index values were normal and the femoral index values were abnormal. In the female patients, 2.3% to 37.7% and 5.6% to 33.3% were discordant when normal femoral indices and normal metacarpal indices were considered. Total concordance and discordance percentages are shown in the last two columns Table I:10. Numbers of patients are small in some groups due to the division of the patients into eight categories. However, if the total value for each of the male and female subjects is considered, the metacarpal index is more frequently abnormal than the femoral index - 12.6% compared with 4% in the male patients, and 21.3% and 13.9% in the female patients.

The duration of arthritis in the steroid and non-steroid treated groups of patients is shown in Table I:11. Only one male patient below the age of 45 years was treated with corticosteroids. In the remaining groups, the steroid treated patients had had rheumatoid arthritis for a longer period than had the non-steroid treated patients. These differences reached statistical significance in the male patients of 45 years of age and above, and in the female patients under 45 years of age. There is no significant correlation between the duration of the arthritis and the femoral index in either the steroid or non-steroid treated male patients, as is shown in Table I:12. In the female patients, the femoral index is significantly correlated to the duration of the disease in both the steroid and non-steroid treated patients over the age of 45 years, but not under the age of 45 years. In Table I:13 correlation between the duration of steroid therapy and the femoral index is shown. There is a statistically significant correlation in both male and female patients over the age of 45 years.

Clinical Group	Sex	Age	Duration of Arthritis	t	p
Non-corticosteroid-treated	M	< 45	9.0 ± 1.2	-	-
Corticosteroid-treated	M	< 45	-	-	-
Non-corticosteroid-treated	M	> 45	8.6 ± 0.5	2.60	< 0.02
Corticosteroid-treated	M	> 45	12.0 ± 1.6		
Non-corticosteroid-treated	F	< 45	4.8 ± 0.8	2.2	< 0.05
Corticosteroid-treated	F	< 45	8.2 ± 1.4		
Non-corticosteroid-treated	F	> 45	6.9 ± 0.6	1.1	n.s.
Corticosteroid-treated	F	> 45	8.1 ± 0.86		

TABLE I:11

The duration of Rheumatoid Arthritis in male and female patients treated with corticosteroid therapy and not so treated

Clinical Groups	No. of Sub-jects	Sex	Age	a	b	r	t	P
Non-corticosteroid-treated	18	M	< 45	59.30	-0.38	0.296	1.28	n.s.
Non-corticosteroid-treated	108	M	> 45	52.25	-0.25	0.15	1.58	n.s.
Corticosteroid-treated	1	M	< 45	-	-	-	-	-
Corticosteroid-treated	23	M	> 45	52.33	-0.24	0.19	0.83	n.s.
Non-corticosteroid-treated	43	F	< 45	52.81	-0.03	0.03	0.16	n.s.
Non-corticosteroid-treated	87	F	> 45	50.88	-0.68	0.41	4.12	< 0.001
Corticosteroid-treated	18	F	< 45	49.75	-0.11	0.08	0.35	n.s.
Corticosteroid-treated	53	F	> 45	49.24	-0.55	0.43	3.06	< 0.005

TABLE I:12

Correlation between the duration of rheumatoid arthritis and Femoral Index (F.I.) in male and female patients ($y = a + bx$)

No. of Subjects	Sex	Age Group (Years)	a	b	r	t	p
1	M	< 45	-	-	-	-	-
23	M	> 45	53.6	1.07	0.40	2.08	< 0.05
18	F	< 45	52.16	0.83	0.17	0.67	n.s.
53	F	> 45	49,94	1.84	0.42	3.21	< 0.005

TABLE I:13

Correlation between the duration of steroid therapy
and the Femoral Index in male and female patients with rheumatoid arthritis
($y = a + bx$)

The third part of this study looks at the metacarpal and femoral indices together with the clavicular cortical thickness in three hundred and seven of the patients included in the initial part of the study.

Table I:14 summarises the clinical and laboratory data of these patients and shows the mean (± 1.S.D.) of the following: age; duration of arthritis; titre of rheumatoid factor; age at onset of the menopause; serum albumin and globulin concentrations; haemoglobin concentration and erythrocyte sedimentation rate. Only minor differences existed between the groups and none of these reached statistical significance. Fig I:11 shows the changes in the clavicular cortical thickness (CCT) in normal and non-corticosteroid-treated rheumatoid male subjects in 10-year age groups. Both groups show a fall with increasing age, however, and in each 10-year age group the patients had statistically significantly lower mean values than the normals.

The number of male subjects receiving corticosteroids was relatively small, and there was only one patient below 45 years of age(Fig. I:12). Only those patients between 55 and 65 showed significantly lower values than the normals. The CCT in the corticosteroid- and non-corticosteroid-treated male subjects is shown in Fig. I:13. No significant differences were found between the two groups.

The data from the female patients was examined in the same way. In Fig. I:14 it can be seen that the clavicular cortex decreases with age in both the normal subjects and the non-corticosteroid-treated female patients. There is a significantly lower mean CCT in each of

	Male Patients			Female Patients		
	A + B	Non-Steroid Treated A	Steroid Treated B	A + B	Non-Steroid Treated A	Steroid Treated B
Numbers	150	126	24	157	99	58
Age (Years)	57.1 ± 11.0	56.1 ± 11.7	59.9 ± 13.0	51.4 ± 13.1	53.0 ± 29.1	49.2 ± 11.6
Duration of Arthritis (Years)	12.6 ± 9.0	12.7 ± 13.4	15.0 ± 10.5	7.9 ± 10.2	7.0 ± 9.5	8.3 ± 5.9
Rheumatoid Factor Titre	228.0 ± 506.7	144.6 ± 590.0	264.7 ± 283.4	220.9 ± 516.0	190.7 ± 406.0	305.4 ± 607.5
Age at Menopause (Years)	-	-	-	45.5 ± 7.0	45.3 ± 6.1	45.8 ± 4.0
Serum Albumin g/l	3 54± 51	3 62± 5.	3 44± 59	3 26± 42	3 26± 42	3 39± .59
Serum Globulin g/l	3 68± 88	3 69± 85	3 67± 74	3 55± 73	3 53± 72.	3 54± .65
Erythrocyte Sedimentation Rate	47.9 ± 29.1	46.2 ± 28.0	50.8 ± 28.2	45.5 ± 28.2	43.2 ± 29.1	47.1 ± 30.1
Haemoglobin Concentration in G/100 ml	13.8 ± 1.8	13.9 ± 1.8	13.6 ± 1.9	12.2 ± 1.5	12.2 ± 1.7	12.1 ± 2.6

TABLE I:14

Clinical and diagnostic features in male and female subjects with rheumatoid arthritis included in the third part of the study

Fig I: 11

The mean and standard error of the clavicular score (C.C.T.) in ten year age groups in normal male subjects and in male patients with rheumatoid arthritis not receiving corticosteroid therapy. Significant differences between the normal subjects and patients studied are indicated.

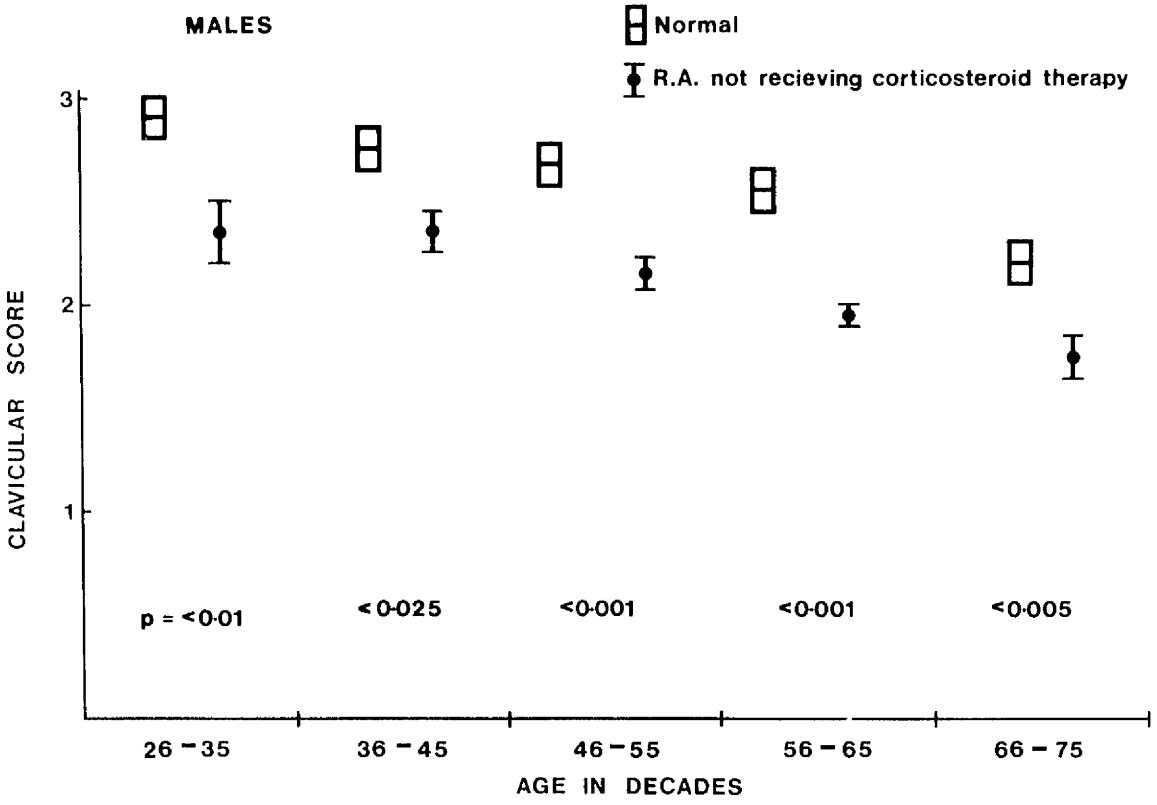

MALES
Normal
R.A. not recieving corticosteroid therapy
CLAVICULAR SCORE
3
2
1
p = <0·01
<0·025
<0·001
<0·001
<0·005
26 – 35
36 – 45
46 – 55
56 – 65
66 – 75
AGE IN DECADES

Fig I: 12

The mean and standard error of the clavicular score (C.C.T.) in ten year age groups in the normal male subjects and in the male patients with rheumatoid arthritis who were receiving corticosteroid therapy. Significant differences between the normal subjects and the patients studied are indicated.

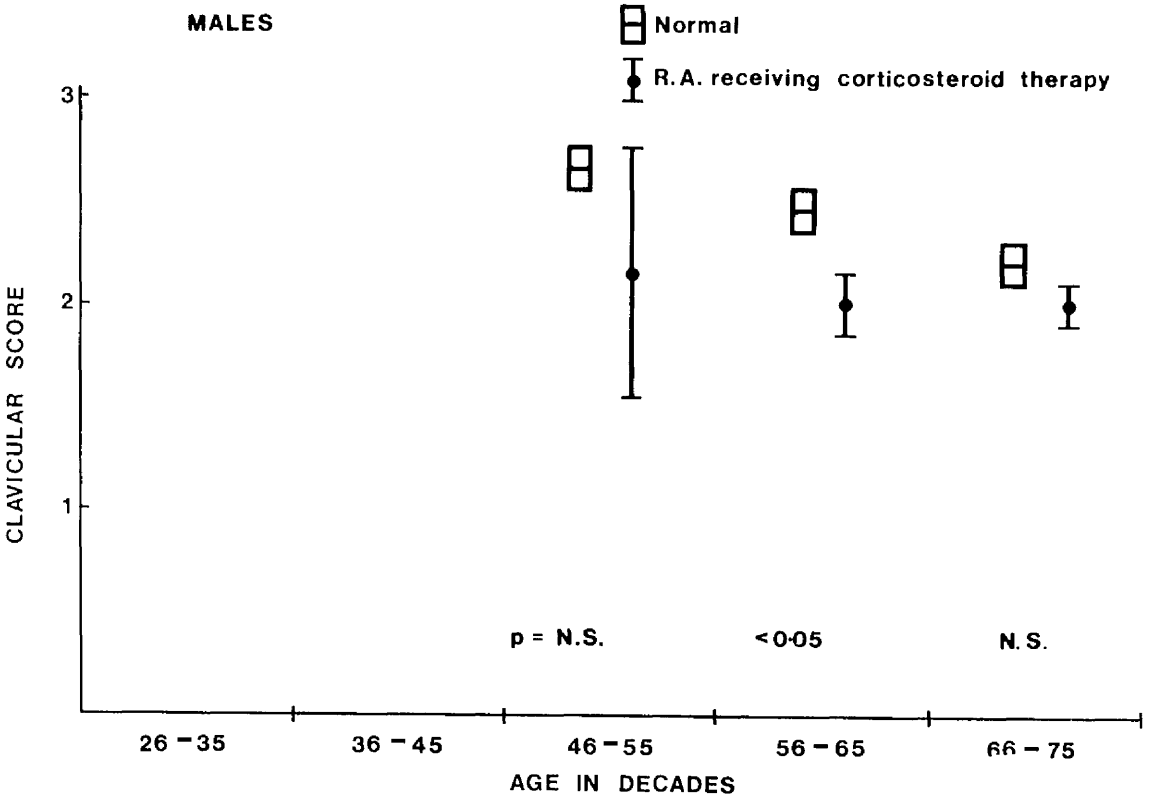

MALES
Normal
R.A. receiving corticosteroid therapy
CLAVICULAR SCORE
3
2
1
p = N.S.
<0·05
N.S.
26 – 35
36 – 45
46 – 55
56 – 65
66 – 75
AGE IN DECADES

Fig I: 13

The mean and standard error of the clavicular score (C.C.T.) in ten year age groups in male patients with rheumatoid arthritis and male patients with rheumatoid arthritis who were receiving corticosteroid therapy. Significant differences between the two groups are indicated.

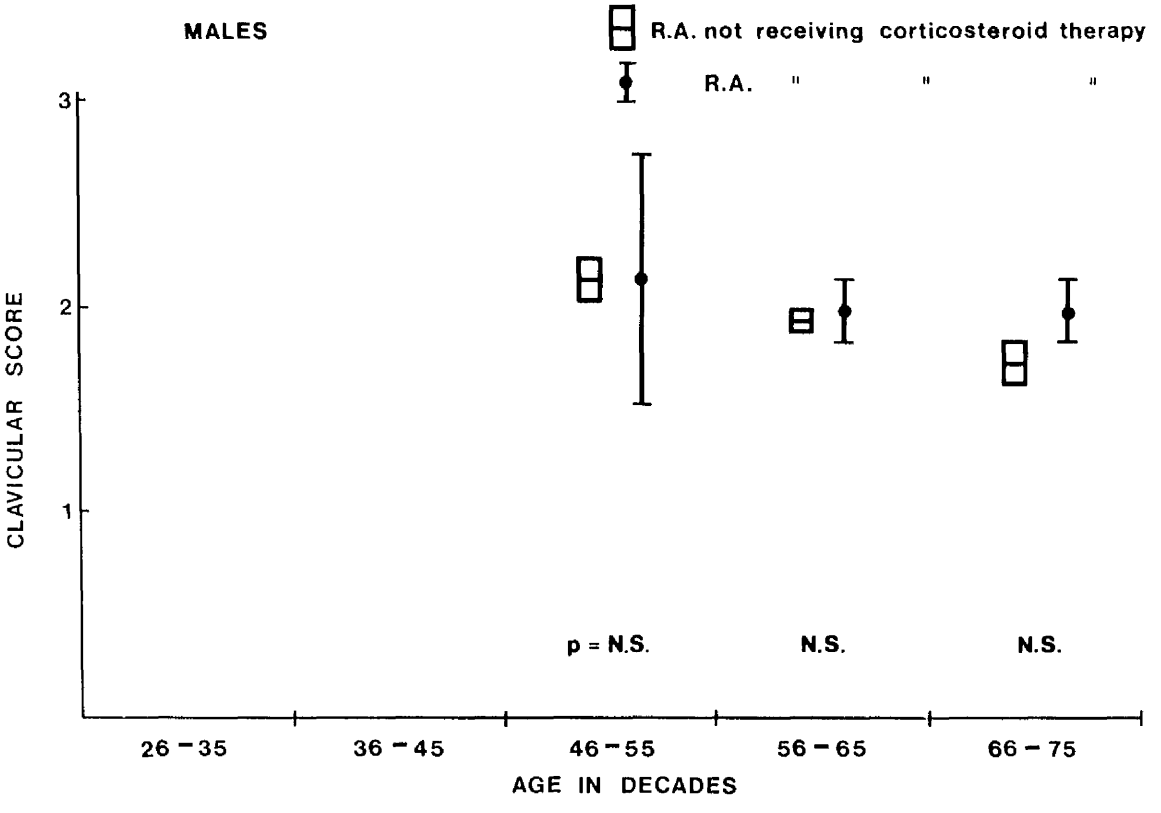
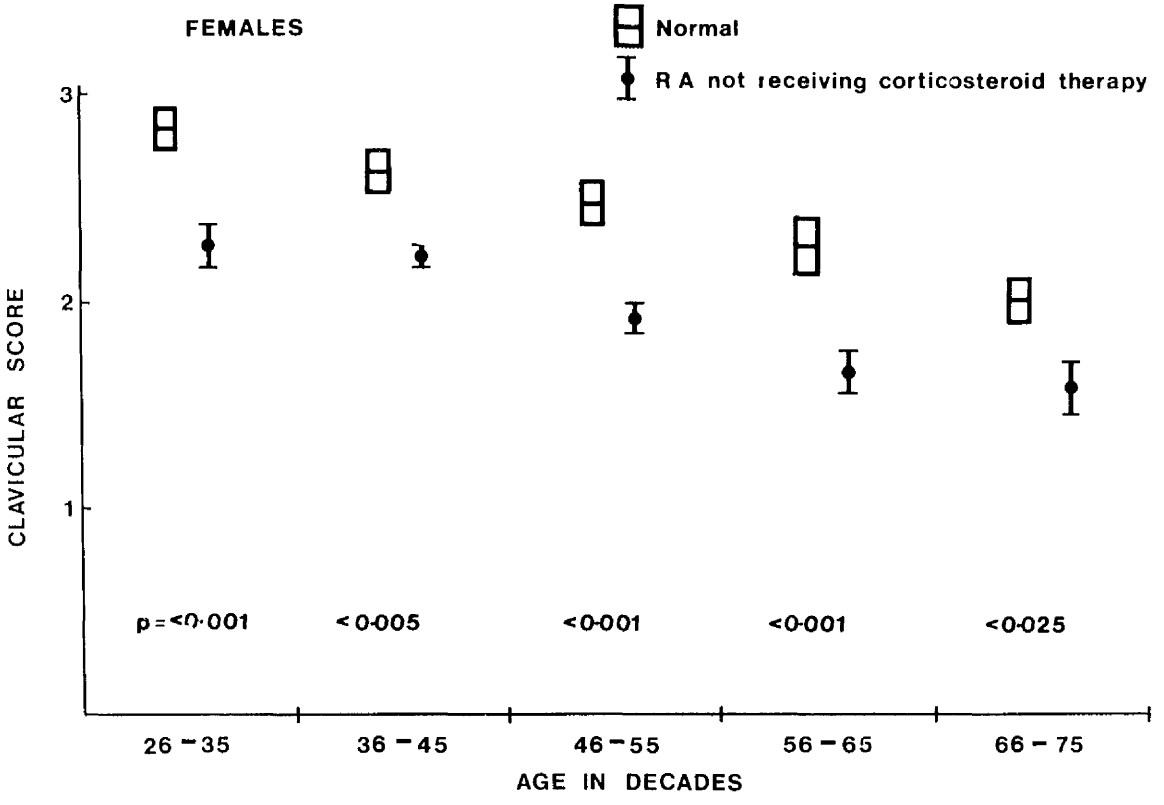

MALES
R.A. not receiving corticosteroid therapy
R.A. " " "
CLAVICULAR SCORE
3
2
1
p = N.S.
N.S.
N.S.
26 - 35
36 - 45
46 - 55
56 - 65
66 - 75
AGE IN DECADES

Fig I: 14

The mean and standard error of the clavicular score (C.C.T.) in ten year age groups in normal female subjects and in female patients with rheumatoid arthritis not receiving corticosteroid therapy.
Significant differences between the normal subjects and patients are indicated.


FEMALES
Normal
R A not receiving corticosteroid therapy
CLAVICULAR SCORE
3
2
1
p = <0·001
< 0·005
<0·001
<0·001
<0·025
26 – 35
36 – 45
46 – 55
56 – 65
66 – 75
AGE IN DECADES

the 10-year age groups when the patients are compared with the normal subjects.

Fig. I:15 shows a comparison between the normal subjects and the corticosteroid-treated rheumatoid patients. Again, in each 10-year age group the patients show significantly lower values than the normal controls. There was, however, no significant difference between the corticosteroid- and non-corticosteroid-treated female patients (Fig. I:16).

The relation between the duration of the arthritis in the patient groups is shown in Table I:15. The female patients were divided into those above and below the age of 45 years (the average age at menopause), and the male patients were divided into the same age groups, for comparison. Significant results were found only in the male subjects. Both the corticosteroid and non-corticosteroid groups over the age of 45 years showed a significant fall in the CCT with increasing age.

The CCT has been correlated with the metacarpal and femoral indices and the results are shown in Tables I:16 and 1:17. The patients were divided into corticosteroid- and non-corticosteroid-treated groups. There was only one male patient below 45 years of age who had been treated with corticosteroids. Significant correlations were found between the CCT and the metacarpal index (Table I:16) in all the groups except for the corticosteroid-treated female patients under the age of 45 years. There was a significant correlation between the CCT and the femoral index in all patient groups (Table I:17) except again for the female patient group below 45 years of age receiving corticosteroid therapy.

Fig I: 15

The mean and standard error of the clavicular score (C.C.T.) in ten year age groups in normal female subjects and in the female patients with rheumatoid arthritis who were receiving corticosteroid therapy. Significant differences between the normal subjects and the patients studied are indicated.

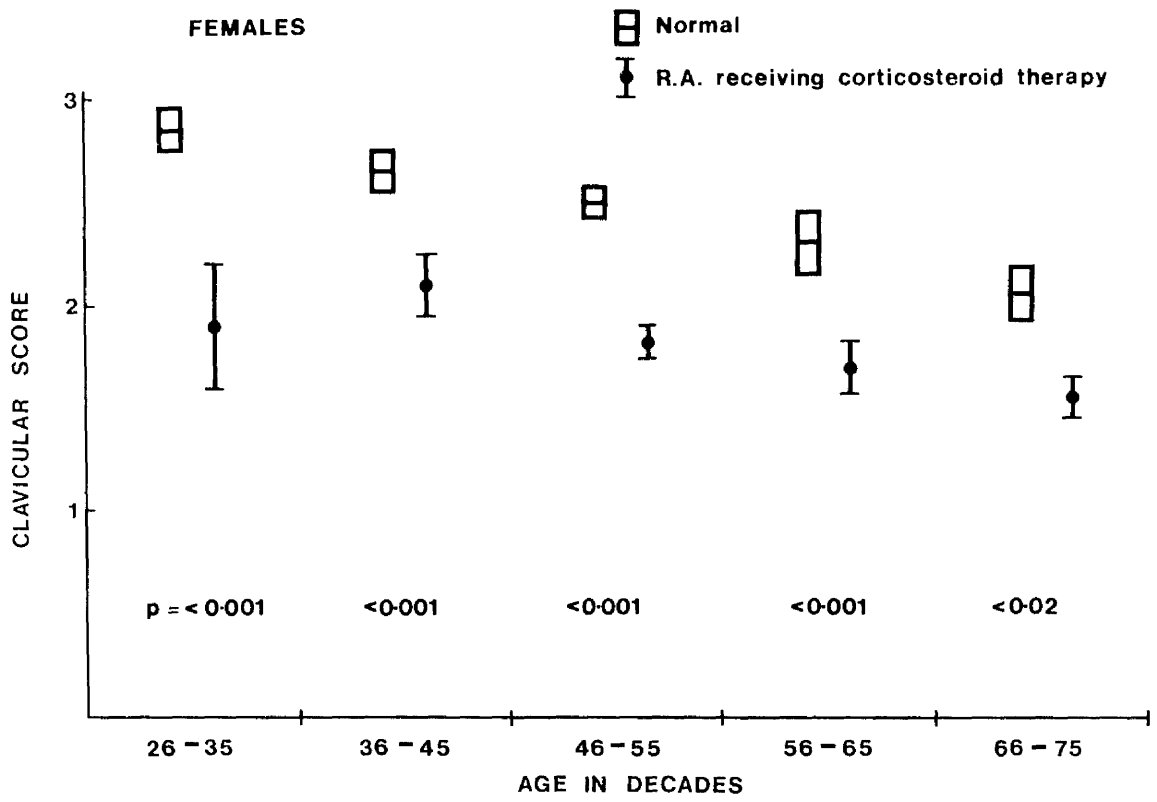
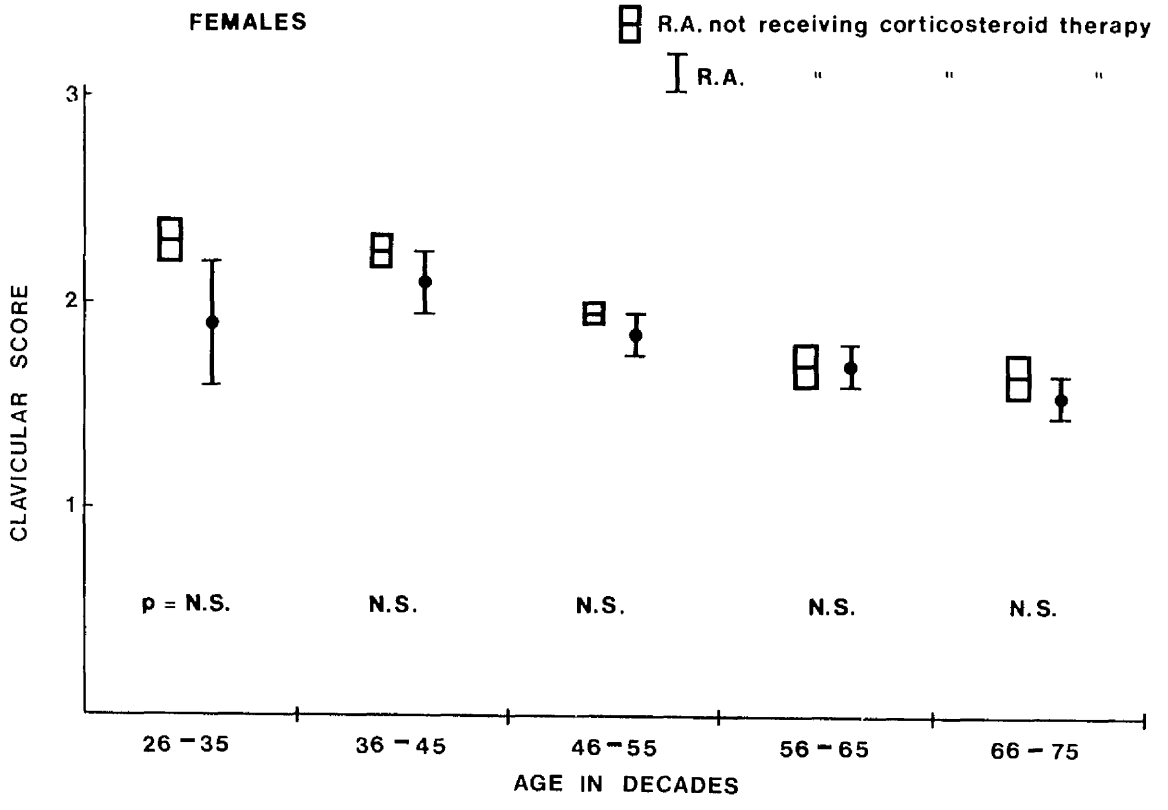

FEMALES
Normal
R.A. receiving corticosteroid therapy
CLAVICULAR SCORE
3
2
1
p = < 0·001
<0·001
<0·001
<0·001
<0·02
26 – 35
36 – 45
46 – 55
56 – 65
66 – 75
AGE IN DECADES

Fig I: 16

The mean and standard error of the clavicular score (C.C.T.) in ten year age groups in female patients with rheumatoid arthritis and in female patients with rheumatoid arthritis who were receiving corticosteroid therapy.
Significant differences between the two groups are indicated.


FEMALES
R.A. not receiving corticosteroid therapy
R.A. " " "
CLAVICULAR SCORE
3
2
1
p = N.S.
N.S.
N.S.
N.S.
N.S.
26 – 35
36 – 45
46 – 55
56 – 65
66 – 75
AGE IN DECADES

Clinical Group	Sex	Age	No. of Sub-jects	a	b	r	t	p
Non-corticosteroid-treated	F	<45	37	0.01	2.24	0.001	0.014	n.s.
Corticosteroid-treated	F	<45	17	0.43	2.32	0.12	0.43	n.s.
Non-corticosteroid-treated	F	>45	65	0.41	1.99	0.03	0.41	n.s.
Corticosteroid-treated	F	>45	38	0.52	2.04	0.12	0.52	n.s.
Non-corticosteroid-treated	M	<45	18	2.55	0.02	0.34	1.49	n.s.
Corticosteroid-treated	M	<45	1	-	-	-	-	
Non-corticosteroid-treated	M	>45	108	2.17	0.02	0.25	2.73	<0.01
Corticosteroid-treated	M	>45	23	2.46	0.10	0.64	3.89	<0.001

TABLE I:15

The relation between clavicular cortex width (y) and the duration of the disease (x) in male and female patients with rheumatoid arthritis (y = a + bx)

n.s. = not significant

Clinical Group	Sex	Age	No. of Sub-jects	a	b	r	t	p
Non-corticosteroid-treated	F	< 45	37	31.8	11.2	0.42	2.73	< 0.01
Corticosteroid-treated	F	< 45	17	43.2	4.5	0.21	0.85	n.s.
Non-corticosteroid-treated	F	> 45	65	11.64	16.79	0.66	7.15	< 0.001
Corticosteroid-treated	F	> 45	38	8.5	16.8	0.64	5.06	< 0.001
Non-corticosteroid-treated	M	< 45	18	22.64	13.68	0.72	4.38	< 0.001
Corticosteroid-treated	M	< 45	1	-	-	-	-	
Non-corticosteroid-treated	M	> 45	108	7.54	18.2	0.87	18.52	< 0.001
Corticosteroid-treated	M	> 45	23	3.9	18.1	0.78	5.76	< 0.001

TABLE I:16

Correlation coefficient between clavicular index and metacarpal index in patients with rheumatoid arthritis

n.s. = not significant

Clinical Group	Sex	Age	No. of Sub-jects	a	b	r	t	p
Non-corticosteroid-treated	F	< 45	37	31.9	8.75	0.43	2.9	< 0.005
Corticosteroid-treated	F	< 45	17	35.5	7.0	0.44	1.89	> 0.05
Non-corticosteroid-treated	F	> 45	65	39.3	3.97	0.24	2.06	< 0.05
Corticosteroid-treated	F	> 45	38	22.06	12.43	0.5	3.56	< 0.001
Non-corticosteroid-treated	M	< 45	18	24.39	13.57	0.77	4.87	< 0.001
Corticosteroid-treated	M	< 45	1	-	-	-	-	
Non-corticosteroid-treated	M	> 45	108	23.59	13.49	0.76	12.27	< 0.001
Corticosteroid-treated	M	> 45	23	24.33	12.37	0.69	4.44	< 0.001

TABLE I:17

Correlation coefficient between clavicular index and femoral index in patients with rheumatoid arthritis

Table I:18 summarises the results of comparison of the clavicular cortical thickness with the metacarpal and femoral indices in terms of concordance and discordance of results. It can be seen that there is an overall high concordance of results, but that the metacarpal and femoral indices were more frequently subnormal in both male and female patients than the measurement of clavicular cortical width. The definition of a subnormal value in any of the measurements was a value lying below the value of 2 S.D. in the normal subjects, taken in decades.

	Sex	No. of Sub-jects	Total Con-cordance	Total Dis-cordance	Discordance	
					When CCT Normal	When CCT Low
CCT and Metacarpal Index	M	150	121 (81%)	29 (19%)	28	1
CCT and Femoral Index	M	150	128 (85%)	22 (15%)	18	4
CCT and Metacarpal Index ...	F	157	119 (76%)	38 (24%)	26	12
CCT and Femoral Index	F	157	120 (76%)	37 (24%)	25	12

TABLE I:18

Concordance and discordance of the clavicular cortical thickness (CCT) with the metacarpal index (MI) and femoral index (FI) in patients with rheumatoid arthritis

DISCUSSION

It is widely accepted that bone loss occurs in patients with rheumatoid arthritis, and that excess of circulating corticosteroids exacerbate the bone loss which occurs naturally with increasing age (Sissons, 1956; Villanueva, Klein and Frost, 1964; Saville, 1970; Robbins and Saville, 1971). However, there are a number of questions related to the magnitude of these losses which remain unanswered. In the first part of this study employing the metacarpal index alone, an attempt was made to answer the following questions:-

1. Is the magnitude of the bone loss in rheumatoid arthritis significantly greater than that which occurs in normal men and women with increasing age?

2. Is the bone loss related to the length of time the patients have had the disease?

3. Is increasing age an important factor in determining the amount of the bone loss?

4. Is the bone loss in rheumatoid arthritis more severe in the male or female patients?

5. Does corticosteroid therapy significantly affect the degree of bone loss?

6. Is the younger patient (below 45 years of age) or the older patient more severely affected in the degree of their bone loss by the use of corticosteroids?

7. Do corticosteroids cause more bone loss in male or female patients?

In the initial part of the study I measured only the bone loss in the second metacarpal of the right hand measured by the metacarpal index of Barnett and Nordin (1960). Rheumatoid arthritis is known to affect the joints of the hand early and often more severely than other joints. Clearly local factors could therefore be important in the bone loss and I have not initially attempted to suggest that the changes seen in the metacarpal represent changes elsewhere in the skeleton.

When the patient groups were broken down into the corticosteroid- and non-corticosteroid-treated groups, the titre of rheumatoid factor was higher in the steroid treated groups but these differences were not statistically significant. The erythrocyte sedimentation rate, haemoglobin levels, plasma proteins and other clinical and laboratory indices in each group were very similar. Comparisons were therefore, possible between the groups, and each group was in turn compared with normal subjects.

From the evidence accumulated in the initial part of this study, the answers to the seven questions posed seemed as follows:-

1. The bone mass was significantly decreased in both male and female patients compared with normal subjects. In the female patients the mean values of the metacarpal index are significantly lower than in the normal female subjects from 26 years of age upwards. In the males below 45, the mean values are lower than in the normals, but significant differences do not emerge except in those above the age of 45 years.

2. The bone loss, measured as the metacarpal index, falls significantly with the duration of the disease. Since the changes are also evident when the percentile metacarpal index is used this relation cannot be an artefact caused by older patients having had the disease for a longer time than the younger patients.

3. Since increasing age itself is accompanied by bone loss in normal subjects and would be expected to occur in the patients, it was necessary to compare the bone loss in the patients above and below the age of 45 years by comparing the mean percentile changes. The male patients aged 45 years and above had a lower mean percentile metacarpal index than those below the age of 45 years (24.42 compared with 34.40) but this difference was not statistically significant. The female patients aged 45 years and over did show a significantly lower mean percentile value than those below the age of 45 ($P < 0.02$). However, it can be

seen from Table I:7 that the older patients have, not surprisingly, had their disease for significantly longer. The bone loss was also significantly directly related to the duration of the disease. These differences could, therefore, arise simply due to this and do not necessarily indicate that the older patient is more susceptible to bone loss secondary to hormonal or other changes.

4. Normal female subjects showed a greater loss of bone than normal males, with the accelerated bone loss occurring after the menopause. Disease may, therefore, have exacerbated bone loss to a greater extent in the female subjects. However, examination of the percentile values of the metacarpal index in male and female patients under 45 years of age, and 45 years of age and above showed no significant differences between the male and female patients. That is, sex was not a significant determining factor in the magnitude of the bone loss in patients with rheumatoid arthritis.

5. Female patients with rheumatoid arthritis who had been treated with corticosteroids had significantly lower mean metacarpal indices than normal subjects between 26 and 75 years of age. In the male patients on corticosteroids, significant lower values only emerged between the ages of 55 and 75 years. When the steroid treated female patients were compared with the non-steroid treated, the former group was found to have significantly lower percentile metacarpal values in both the under and over 45 year age groups. This suggests that corticosteroids had caused a greater degree of bone loss than

that which would have occurred as a result of the disease alone. However, the possibility that the steroid treated group may have had more bone loss due to the slightly (but not significantly) longer duration of the disease, cannot be completely ruled out.

6. There was no evidence to suggest that the steroid treatment has more severely affected the female patients of 45 years of age and over compared with the female patients below 45 years in spite of the longer duration of the steroid therapy and of the disease in the older steroid treated group. This comparison was not possible in the male patients because of insufficient numbers.

7. When the bone loss in steroid treated male patients of 45 years of age and upwards was compared with that in females in this age group, there was a greater mean loss of bone in the female patients, but this was not statistically significant.

The conclusion reached in the first part of this study was that rheumatoid arthritis led to a loss of bone in both male and female patients as measured in the second metacarpal. This loss was directly related to the duration of the disease. However, the bone loss was not significantly different when male and female patients were compared. The bone loss was greater in patients who had been receiving steroids than those who had not been so treated. There was some evidence to suggest that the older patient was more severely affected by the disease, but since the older patient had generally had the disease for longer this cannot be accepted as proven.

There was no evidence to suggest that female patients were significantly more severely affected by steroid treatment than the males.

Having shown that significant bone loss occurs in the metacarpal of patients with rheumatoid arthritis and taking into consideration the fact that rheumatoid arthritis is a generalised disease affecting many tissues remote from the joints such as the heart and lungs, the question of the extent of bone loss in rheumatoid arthritis is raised. Any such generalised loss of bone could be the result of immobility resulting from joint pain and stiffness, the generalised inflammatory nature of the disease or from the use of corticosteroids. It is fair to note that the joints of the hands and wrists are especially subject to rheumatoid changes and thus the local effects of the disease might be more liable to affect bone mass in the metacarpal, especially as it is a relatively small bone, rather more than the larger more central bones of the body.

The femoral index and the clavicular cortical thickness were considered less likely to be subject to local disease activity, thus these indices were measured and compared with the metacarpal indices in the same patients in order to assess the extent of bone loss occurring in rheumatoid arthritis. The changes occurring were noted and compared as individual indices with age and sex matched normal subjects. In addition information relating to the effect on bone of corticosteroid therapy in rheumatoid arthritis was established.

The administration of corticosteroid hormones is known to produce osteoporosis in other conditions (De Martini Grokoest and Ragan, 1952., Teicher and Nelson, 1952., Reifenstein, 1958.) and this condition of bone is also known to occur in Cushings Syndrome (Knowlton, 1953., Wang and Robbins, 1956). However, the effects of corticosteroids on patients with rheumatoid arthritis are less predictable.

Corticosteroids reduce the local and generalised inflammatory changes, and could therefore improve mobility. In this way, they could reduce bone loss, and the end result would be a balance between the two opposing effects of the steroid therapy.

The investigations showed that there is a significant loss of bone in the femur, compared with normal, age-matched subjects, measured as the femoral indes in both male and female subjects with rheumatoid arthritis. Moreover, the bone loss was much more significant in the female subjects. In the male subjects, significant bone loss was seen only in patients 45 years of age and over. There was a very similar pattern of bone loss in the patients with rheumatoid arthritis treated with corticosteroids, compared with normal, age-matched controls. When the bone loss in patients with rheumatoid arthritis was compared with the bone loss in the patients treated with corticosteroids, no significant differences emerged, either in the male or in the female groups, suggesting that the steroid therapy had not contributed to bone loss in these patients.

The study also showed that the clavicular cortical thickness was significantly diminished in both male and female patients with rheumatoid arthritis at all ages and in those patients who were treated with corticosteroids. Like the results of the femoral indices, but unlike those of the metacarpal indices, the clavicular cortical thickness was not shown to be significantly more reduced in patients treated with corticosteroids than those not so treated.

When the metacarpal index was compared with the femoral index, highly significant correlations were obtained between the two measurements, except in the corticosteroid treated female patients under 45 years of age. These results suggested that similar changes are occurring in both these bones as the result of the rheumatoid arthritis. When tested for concordance, the results are in keeping with the occurrence of greater bone loss in the metacarpal, except in the female patients under 45 years of age, whether or not they were treated with corticosteroids. However, the numbers of subjects in the various categories are small, and the results should be interpreted with caution.

The male patients were, on average, older than the female subjects and had had rheumatoid arthritis longer, yet it is interesting to note that the female patients appeared to be more severely affected than the male patients, in terms of bone loss; this appears to be so, whether the patients were treated with corticosteroids or not. There was a much poorer correlation between the duration of rheumatoid arthritis and the fall in the femoral index than for the correlation with the metacarpal index observed earlier in the study. Only the female patients over the age of

45 years in both the steroid and non-steroid treated groups showed a statistically significant correlation. There was also a statistically significant correlation between the femoral index and duration of steroid therapy in the male and female patients over the age of 45 years.

The results of the study also show that the clavicular cortical thickness is related to the metacarpal and femoral indices. The results of the study of concordance and discordance confirm this but also show that the metacarpal and femoral indices were more frequently subnormal than the clavicular cortical thickness.This suggests that while the C.C.T. is a useful measure of bone loss, it is less sensitive than the metacarpal and femoral indices.

These results also suggest that osteoporosis is a generalised, and not merely a localised condition, in patients with rheumatoid arthritis. This is more in keeping with the possibility that the bone loss is either primarily or secondarily due to the generalised inflammatory nature of the condition and not only to the immobilisation due to joint pain and stiffness.

Moreover, though the relationships of the various indices of osteoporosis may be causally related, it must be remembered that other factors such as age, sex, and the severity of the disease, as well as the duration of corticosteroid therapy, and of the rheumatoid arthritis, could be contributory to the significance of these results. On the present data, it would seem more likely that the duration of the rheumatoid arthritis, rather than the duration of the corticosteroid therapy, was the important contributory factor in the bone loss.

This is an interesting finding, as the development of osteoporosis is regarded as a hazard in patients treated with corticosteroids.

The results of the study on the femoral indices and clavicular cortical thickness did not indicate any potential aggravation of the bone loss occurring in rheumatoid arthritis by corticosteroid therapy, although the study on the metacarpal indices suggested a slight tendency for this to happen. This potential hazard therefore would appear to be minimal in patients with rheumatoid arthritis.

Having collated a large mass of data pertaining to osteoporosis in patients with rheumatoid arthritis, I thought it would be of interest to have further more sophisticated statistical analysis carried out using a digital computer.

This section deals with a detailed statistical computer analysis carried out in collaboration with the Department of Biomathematics, Oxford University (Dr. John A. Anderson). In addition, I thought it might prove of practical value if a statistical prediction of the degree of osteoporosis present in a patient could be ascertained, and the results of this study are also included in this section of the chapter.

Study A

STATISTICAL ANALYSIS

604 consecutive patients entering the Centre for Rheumatic Diseases were selected for inclusion in this study, many of these were previously included in the clinical study.

The patients were divided into two groups by sex. The females were further classified as rheumatoid factor positive and rheumatoid factor negative. All the males were rheumatoid factor positive. The groups consisted of 405 females (of whom 88 were sero negative) and 199 male patients.

The rheumatoid factor negative patients had a milder form of the disease than the rheumatoid factor positive patients.

There were fifteen measurements to be recorded for each patient but there were a number of missing values.

The variables which could have been recorded for each patient were:

(1) Sex F = 0 M = 1

(2) Diagnosis

(3) Age

(4) Age at onset of disease

(5) Duration of disease in years

(6) Functional Grade (An assessment of physical capability measured on a four point scale)

(7) X-ray classification (Measured on a three point scale)

(8) Rheumatoid Factor Titre

(9) Erythrocyte Sedimentation Rate

(10) Metacarpal Index

(11) Femoral Index

(12) Clavicular Cortical Thickness

(13) Dose of Prednislone

(14) Duration of steroid therapy

(15) Standard aluminium equivalent ('s.a.e.')

The data were transferred into a file in the computer and verified.

Variables (10), (11) and (12) were chosen to be the parameters of osteoporosis. The s.a.e. was only recorded for the females.

The groups of primary interest were the rheumatoid factor positive males and females. The other group (rheumatoid factor negative females) was included in the initial examinations of the data mainly for comparison.

Thus, measures of osteoporosis in each patient was based on some or all, of observations (10), (11), (12) and (15) (for the females). The other observations were included as 'explanatory' variables.

Initially the metacarpal index, femoral index, clavicular cortical thickness and the standard aluminium equivalent were assessed as measurements of osteoporosis in our patients.

For each of the groups separately the vectors of means and covariance matrices of these four variables were found. Missing values were taken account of in the following way. The mean of a particular score in a group was found by summing that score over all patients in the group and dividing by the number of patients who had the score recorded. The variances were calculated by subtracting the score mean from each score and using the formula

$$\text{Var}\ (X_1) = \sum_i \frac{(X_{i1} - \bar{X}_1)^2}{n - 1}$$

where $\bar{X}$ is the score mean

and i summed is over all patients (n) with the score recorded.

The covariance between any two scores was calculated using

$$\text{Cov}\ (X_1, X_2) = \sum_i \frac{(X_{i1} - \bar{X}_1)\ (X_{i2} - \bar{X}_2)}{m - 1}$$

where i summed over all patients (m) with both scores recorded.

This estimate of the covariance was biased because $\bar{X}_1$ and $\bar{X}_2$ were not the means of their respective scores in this summation group but they were the means over all the patients. The correlations between two scores which were also calculated were also biased because the variances were not the true variances over the summation groups.

These biases should, however, have been small enough to neglect since the numbers of patients were quite large and it was hoped that the increased precision resulting from better estimates of the means and variances more than offset the small bias.

These means, variances and covariances and the correlation matrices are given in tables I:19 - I:22. The means of the bone scores and standard aluminium equivalent for the rheumatoid factor negative females were larger than those for the corresponding rheumatoid factor positive females perhaps suggesting that the osteoporosis was correlated with the severity of the disease.

The primary interest was in the correlations of the different bone scores and the standard aluminium equivalent throughout the groups. There did not, however, seem to be a consistent pattern over these groups.

All the correlations were positive. The rheumatoid positive females had smaller correlations, on average, than the other three groups, the femoral index and clavicular cortical thickness having a correlation of only 0.15. In these other three groups the correlations between the bone scores were all greater than 0.52, that of the metacarpal and clavicle always being the largest.

The correlations between the standard aluminium equivalent and the bone scores did not add further information. They were high (greater than 0.77) for the rheumatoid factor negative patients but except for the metacarpal in the rheumatoid factor positive, they were about 0.3.

	FEMALE R.F. Positive	MALE R.F. Positive	FEMALE R.F. Negative
Metacarpal Index	43.09	44.09	54.4
Femoral Index	47.41	50.62	53.0
Clavicular Cortical Thickness	1.89	2.0	2.20
Standard Aluminium equivalent (s.a.e.)	23.73	-	29.50

Table I:19

Means of osteoporotic scores in patients with rheumatoid arthritis.

	Metacarpal Index	Femoral Index	Clavicular Cortical Thickness CCT	S.A.E.
Rheumatoid Factor Positive Females .. Table 1:20	177.69 (305) .41 (198) .32 (210) .78 (142)	51.98 91.26 (227) .15 (163) .33 (90)	3.51 1.17 .68 (214) .39 (88)	88.65 26.16 2.67 67.41 (141)
Rheumatoid Factor Positive Males .... Table 1:21	135.77 (155) .69 (151) .82 (152)	72.91 81.72 (150) .77 (150)	4.89 3.56 .26 (152)	-
Rheumatoid Factor Negative Females .. Table 1:22	181.81 (75) .52 (51) .74 (51) .99 (23)	55.10 61.49 (51) .56 (39) .77 (19)	4.38 1.99 .19 (53) .81 (15)	119.83 53.64 3.18 79.77 (23)

TABLE I:20-22

The Variance-Co-variance and Correlation Matrix in patients with rheumatoid arthritis. The correlation matrices are given in the lower triangle and the variance-co-variances in the upper triangle and diagonal.

The brackets denote the number of observations on which the comparison is based.

A principle component analysis was carried out on the correlation matrices. This was achieved using a standard N.A.G. Library routine to calculate the eigenvalues and eigenvectors of a real symmetric matrix. The first principal component and corresponding principal value are presented in table I:23. It can be seen that the first principal component has approximately equal components in all three groups, suggesting that it is a 'size' component. So, if a single measure of the patient's osteoporotic condition were required an average of the standardised scores in the groups is suggested.

The standard aluminium equivalent did not appear to add much extra information and since none of the males had this measure recorded it was decided not to include the standard aluminium equivalent as a parameter in the assessment of the patients' osteoporotic condition.

The effect of corticosteroid therapy

To investigate the effect of corticosteroid therapy on the patients in the three clinical groups, the groups were further sub-divided into corticosteroid and non-steroid treated patients. If steroid therapy were having an effect on the osteoporotic condition of the patient, the mean of the scores for the steroids should be significantly lower than those of the non-steroids.

Female R.F.Positive	Male R.F.Positive	Female R.F.Negative
2.26	2.5	3.22
- .59	- .57	- .51
- .39	- .56	- .43
- .38	- .59	- .49
- .59		- .56

Table I:23

Principal Value and First Principal Component

The means, corresponding correlation and covariance matrices were calculated for both the steroid and non-steroid group as before. Tables I:24 and I:25 give details of these. 'Students t' was tabulated for the differences in corticosteroid and non-corticosteroid treated groups. The rheumatoid factor positive females were the only patients who showed a significant effect due to corticosteroid therapy. In this group the mean ages of those on therapy and those not were approximately the same. The changes in the mean metacarpal and clavicular scores give a significant t value at the two per cent level while for the femur the difference between corticosteroid and non-corticosteroid groups giving significance at the ten per cent level. Metacarpal and femoral scores for the corticosteroid group became less correlated while the femoral and clavicular scores both had increased correlation with the metacarpal index.

There were only eleven rheumatoid negative females on corticosteroid therapy. For the rheumatoid factor negative female patients the metacarpal index score for those on corticosteroid therapy was appreciably lower than those not on therapy but the corticosteroid treated group had a large standard error. The decrease was not significant. Here again a general increase in correlation of bone score due to corticosteroid therapy was evident. The metacarpal and clavicular scores showed a correlation of .99 which is probably slightly inflated by the fact that there were only six comparisons made. High correlations for the bone scores were a feature of the corticosteroid group.

		Corticosteroid treated			Non corticosteroid treated			Significance
		No.of patients	Mean Score	Standard error of mean	No.of patients	Mean Score	Standard error of mean	t
Metacarpal Index	Female R.F.Positive	90	39.64	1.28	215	44.53	.93	3.10 **
	Male R.F. Positive	22	41.45	2.3	133	44.53	1.04	1.22
	Female R.F.Negative	9	45.44	5.94	66	55.62	1.59	1.66
Femoral Index	Female R.F.Positive	90	45.79	1.28	137	48.16	.80	1.63 *
	Male R.F. Positive	21	49.71	2.3	130	50.76	.67	.56
	Female R.F.Negative	6	52.5	4.42	45	53.06	1.13	.12
Clavicular Cortical Thickness	Female R.F.Positive	62	1.73	.0038	138	2.5	.86	3.10 **
	Male R.F.Positive	23	2.07	.0082	130	2.01	.0021	.5731
	Female R.F.Negative	7	2.0	.048	46	2.04	.0038	.997

Table I:24

Means and standard errors of the osteoporotic scores in corticosteroid and non corticosteroid treated patients with rheumatoid arthritis.

** denotes significance at 2%

* denotes significance at 10%

	Cortico-steroid treated			Non-cortico-steroid treated		
	Metacarpal Index	Femoral Index	Clav-corticol thickness	Metacarpal Index	Femoral Index	Clav-Corticol Thickness
Rheumatoid Factor positive females	144.7	40.35	3.02	184.43	53.52	3.36
	.35	91.85	1.79	.41	89.20	.63
	.52	.39	.23	.27	.07	.85
Rheumatoid Factor positive males	111.52	54.22	3.46	138.44	75.41	5.18
	.71	52.39	1.69	.76	86.30	3.87
	.77	.55	.18	.83	.58	.28
Rheumatoid Factor Negative females	282.69	138.50	8.92	155.63	43.42	3.28
	.83	97.91	4.00	.46	56.60	1.53
	.99	.76	0.29	.64	.49	0.17

Table I:25

Variable Analysis in patients with rheumatoid arthritis. The covariances lie in the upper triangle and diagonal and correlations in the lower triangle.

The rheumatoid factor positive male patients on corticosteroid therapy were on average 4.3 years older than those not on corticosteroid therapy. The osteoporotic score correlations were high, those for the corticosteroid treated patients being slightly lower than those of the non-corticosteroids. Corticosteroid therapy did not appear to be having as much effect on the correlations in the males as it did in the females. The males did not show an overall significant decrease in osteoporotic scores due to corticosteroid therapy.

A principal component analysis was carried out (table I:26) on the correlation matrices and again there was approximately equal weighting in the first principal component.

Thus, corticosteroid therapy did seem to be affecting the rheumatoid positive females but none of the other groups. The rheumatoid factor positive female patients had also much lower bone score correlations.

Females R.F. Positive		Males R.F. Positive		Females R.F. Negative	
Steroid	Non Steroid	Steroid	Non Steroid	Steroid	Non Steroid
1.84	1.52	2.35	2.4	2.72	2.07
- .59	.68	- .60	- .61	- .60	- .59
- .53	.59	- .55	- .55	- .55	- .54
- .61	.43	- .57	- .57	- .58	- .60

Table I:26

The Principal Components and Principal Component of the Correlation Matrix of the male and female corticosteroid and non-corticosteroid treated patients with rheumatoid arthritis.

Dependence of Osteoporosis on other factors

Other possible factors which might contribute to the development of osteoporosis were now studied. This was achieved by using a regression analysis on the three clinical groups.

It was decided that an average of the osteoporotic scores would be a good indication of the patients general osteoporotic condition. The scores were standardised by dividing by their standard deviation which was calculated over all patients in the group. This measure of osteoporosis enabled the maximum number of patients to be included in the regression analysis.

A library regression analysis package was used (FAKAD) with the dependent variable the measure of osteroporosis and the independent variables expected to be of importance:

(2) age,

(3) duration of arthritis,

(4) functional grade

(5) X-ray classification

(6) Rose Waaler Titre

(7) Erythrocyte sedimentation rate

(8) Receiving corticosteroid therapy or not coded by 1 or 0.

In each group a multiple regression model was set up to minimise the residual sum of squares i.e. to explain as much of the variation as possible.

The first regression was carried out in each group using the linear model

$Yi = \alpha + \beta Xi + ei$

X is the independent variable

α, β are constant

Y is the dependent variable

e is the error term

A linear regression was carried out with each of the seven variables as the independent variable and that one chosen which minimised the residual sum of squares. Now using this variable as one independent variable a multiple regression model was posed with two independent variables, the second being chosen from the remaining six so as to minimise the residual. At each stage a test of significance was carried out to ascertain if inclusion of the new dependent variable in the regression model made a significant reduction in the residual sum of squares. This process was continued until the inclusion of a further variable gave no significant reduction in the residual sum of squares. The results are presented in Tables I:27 - I:29.

All the patients, except for the rheumatoid factor negative patients, showed a highly significant regression on age with the two groups of rheumatoid factor positive patients showing a significant regression on the duration of the disease having taken account of age.

Age, therefore, seemed to be the most important factor emerging from these results with perhaps the duration of the disease being important when age has been allowed for. The relationship of age to

Source Independent variables in the model	d.f.	Sums of Squares	F.
X_2	1	44.8	71.4 **
Residual	302	190.0	
X_4 , X_2	2	52.49	445
Residual	301	181.31	
X_4 /X_2	1	8.69	14.43 **
X_4, X_2, X_3	3	56.2	31.5
Residual	300	178.6	
X_3,/X_4, X_2	1	2.71	4.53 **
X_3, X_4, X_2, X_8	4	58.2	24.6
Residual	299	176.6	
X_8/X_4, X_2, X_3	1	2.0	3.39

Table I:27

The results of the regression analysis carried out on three hundred and four rheumatoid factor positive female patients for whom the complete list of independent variables was available.

** denotes significance at 5%

Source Independent variables in the model	d.f.	Sums of Squares	F.
X_2	1	20.9	25.6 **
Residual	154	125.6	
X_6, X_2	2	24.91	16.2
Residual	153	121.0	
X_6/X_2	1	4.6	5.82 **
X_6, X_2, X_3	3	28.91	12.5
Residual	152	117.6	
X_3/X_6, X_2	1	3.4	4.39 **
X_2, X_6, X_3, X_7	4	31.41	10.3
Residual	151	115.1	
X_7/X_6, X_3, X_2	1	2.5	3.26

Table I:28

The results of the regression analysis carried out on one hundred and fifty six rheumatoid factor positive male patients for whom the complete list of independent variables was available.

** denotes significance at 5%.

Independent Variables in the Model	d.f.	Sums of Squares	F
X_5	1	7.9	9.95**
Residual	46	36.2	
X_5, X_3	2	10.1	6.71
Residual	45	33.98	
X_5/X_3	1	2.22	2.51

TABLE I:29

The Results of the regression analysis carried out on 48 rheumatoid factor negative female patients for whom the appropriate list of independent variables was present

** Denotes significance at 5 per cent.

the development of osteoporosis was considered in the rheumatoid factor positive males and females tables I:30 and I:31. The two groups of patients were classified as those receiving and those not receiving corticosteroid therapy. Figs. I:17 to I:22 give graphical descriptions of the tables. Only non-steroid treated males are shown because there were too few males receiving steroids in the age groups. One standard error above and below the mean is indicated on each graph. All the graphs show, for the non-steroid group, a fairly constant mean cortical width of the particular bone until the patient reaches his middle forties, about forty-five and then a uniform deterioration. All the graphs show the same effect. This suggests that there is some change in the body about this age after which the patient suffering from rheumatoid arthritis becomes more osteoporotic as he gets older even if he is not on corticosteroid therapy.

In figs I:17 and I:19 the graphs for the hand and clavicular scores on the non-steroid treated groups were similar to those of the steroid treated females, except that the graph for the steroid group lies below that of the non-steroid group.

Corticosteroid therapy did not have such a marked effect on the femur, it happens on two occasions that those on therapy have a larger osteoporotic score mean that those not on therapy. It is interesting to note that the femur was not as highly correlated to the other two bone scores as they were to each other.

Osteoporotic Index	Age	Corticosteroid treated			Non-corticosteroid treated			Significance **=2%
		No.of patients	Mean Score	Standard error of mean	No.of patients	Mean Score	Standard error of mean	t
Metacarpal Index	0-34	12	50.33	3.95	28	55.71	2.15	1.19
	35-39	2	45.5	2.5	12	56.91	3.05	2.89 **
	40-44	9	48.89	3.2	23	53.30	2.54	1.08
	45-49	14	43.92	3.44	21	49.85	2.83	1.33
	50-54	18	36.11	2.53	33	45.12	2.12	2.73 **
	55-59	16	34.31	2.05	32	37.12	1.56	1.09
	60-64	9	33.33	2.41	28	37.5	2.13	1.30
	65-69	5	35.0	3.56	23	34.78	2.03	.05
	70	3	28.8	3.2	15	33.8	2.25	1.28
Femoral Index	0-34	10	49.6	3.23	18	53.94	1.64	1.19
	35-39	2	36.5	3.5	8	52.25	3.07	3.38 **
	40-44	8	44.5	5.81	17	52.76	1.51	1.38
	45-49	12	45.17	2.65	9	49.22	2.34	1.06
	50-54	8	47.75	1.75	19	48.26	1.63	.21
	55-59	12	49.0	2.61	19	47.63	2.23	.40
	60-64	4	38.0	1.15	20	45.45	1.73	3.58 **
	65-69	4	46.75	1.55	17	41.71	2.47	1.73
	70	4	38.75	2.06	10	43.6	4.88	.92
Clavicular Cortical Thickness	0-34	8	1.85	.25	21	2.19	.13	1.20
	35-39	2	2.25	.25	12	2.18	.067	.26.
	40-44	8	1.93	.15	18	2.22	.085	1.64
	45-49	13	1.85	.13	16	2.00	.11	.89
	50-54	10	1.61	.09	22	1.97	.07	3.06 **
	55-59	11	1.67	.16	19	1.66	.096	.05
	60-64	4	1.45	.17	19	1.63	.12	.87
	65-69	4	1.38	.13	15	1.55	.04	.05
	70	2	1.25	.25	11	1.5	.15	.85

Table I:30

The effect of age on the indices of osteoporosis in corticosteroid and non-corticosteroid treated female patients with rheumatoid factor positive rheumatoid arthritis

Osteoporotic Index	Age	Corticosteroid treated			Non corticosteroid treated			Significance **=2%
		No.of patients	Mean Score	Standard error of mean	No.of patients	Mean Score	Standard error of mean	t
Metacarpal Index	0-34	-	-	-	7	55.1	2.44	-
	35-39	(1)	(48)	-	12	54.75	2.23	-
	40-44	2	52	3	14	49.29	2.89	.89
	45-49	(1)	(48)	-	20	46.1	1.92	-
	50-54	4	36.25	6.69	20	43.05	2.3	.96
	55-59	2	41	1.0	28	42.5	2.2	.62
	60-64	6	31.7	5.9	19	40.34	3.2	.17
	65	6	41.33	3.32	13	34.61	2.56	1.6
Femoral Index	0-34	-	-	-	7	55.28	2.4	-
	35-39	(1)	(52)	-	12	56.17	1.87	-
	40-44	2	54	4	14	52.93	2.8	.22
	45-49	(1)	(58)	-	20	49.7	1.85	-
	50-54	4	43.5	44	19	50.47	2.16	1.41
	55-59	2	44.5	.5	28	49.54	1.69	2.84 **
	60-64	6	51.67	3.1	18	49.3	2.36	.684
	65	5	50.25	2.73	12	47.5	3.18	.64
Clavicular Cortical Thickness	0-34	-	-	-	7	2.29	.15	-
	35-39	(1)	(2.5)	-	12	2.37	.10	-
	40-44	2	2.75	.25	14	2.18	.19	1.81
	45-49	(1)	(2.3)	-	20	2.05	.09	-
	50-54	4	1.87	.24	19	1.97	.10	.378
	55-59	2	2	-	28	1.91	.09	.93
	60-64	6	1.91	.2	18	1.83	.13	.345
	65	7	2.07	.13	12	1.74	.14	1.72

Table I:31

The effect of age on the indices of osteoporosis in corticosteroid and non-corticosteroid treated male patients with rheumatoid factor positive rheumatoid arthritis. Brackets are employed where only one score is available

Fig I: 17

The mean and standard error of the metacarpal index, plotted against age, in non corticosteroid and steroid treated female patients with rheumatoid arthritis.

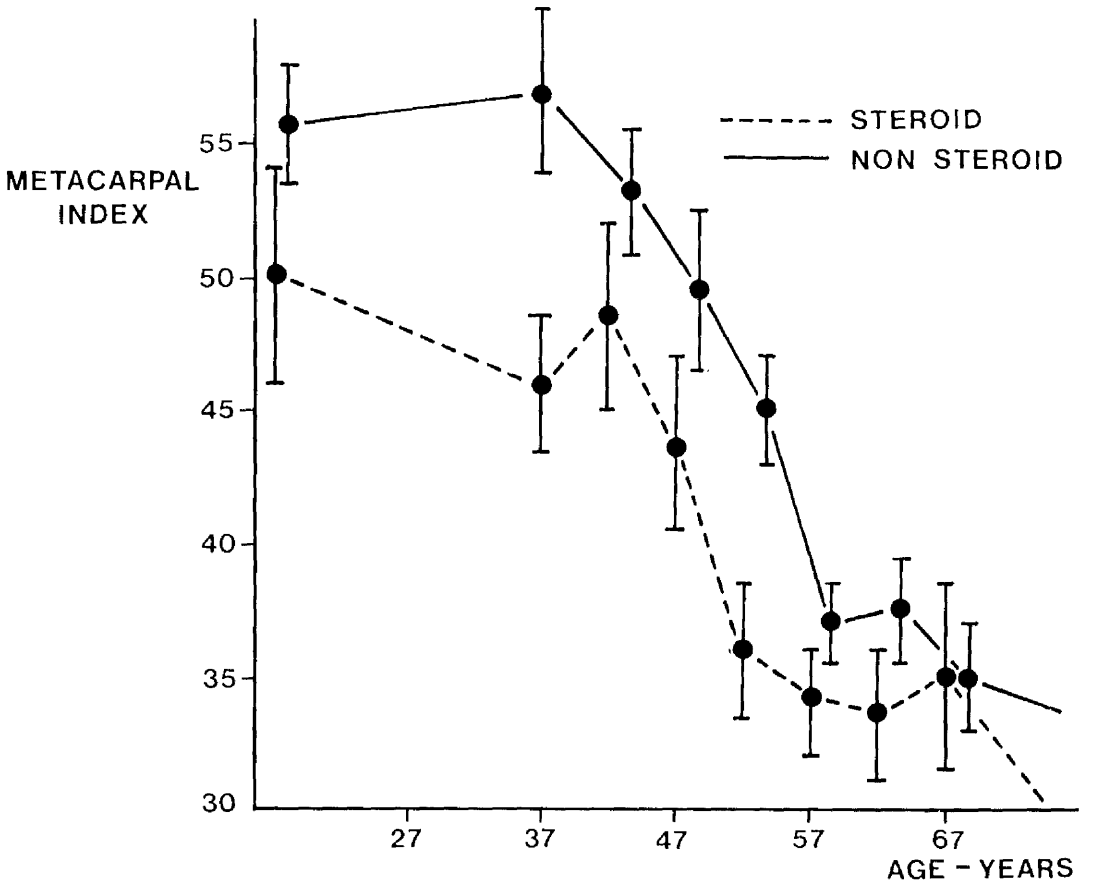

METACARPAL INDEX
55
50
45
40
35
30
27
37
47
57
67
AGE - YEARS
STEROID
NON STEROID

Fig I: 18

The mean and standard error of the femoral index plotted against age in non corticosteroid and steroid treated female patients with rheumatoid arthritis.

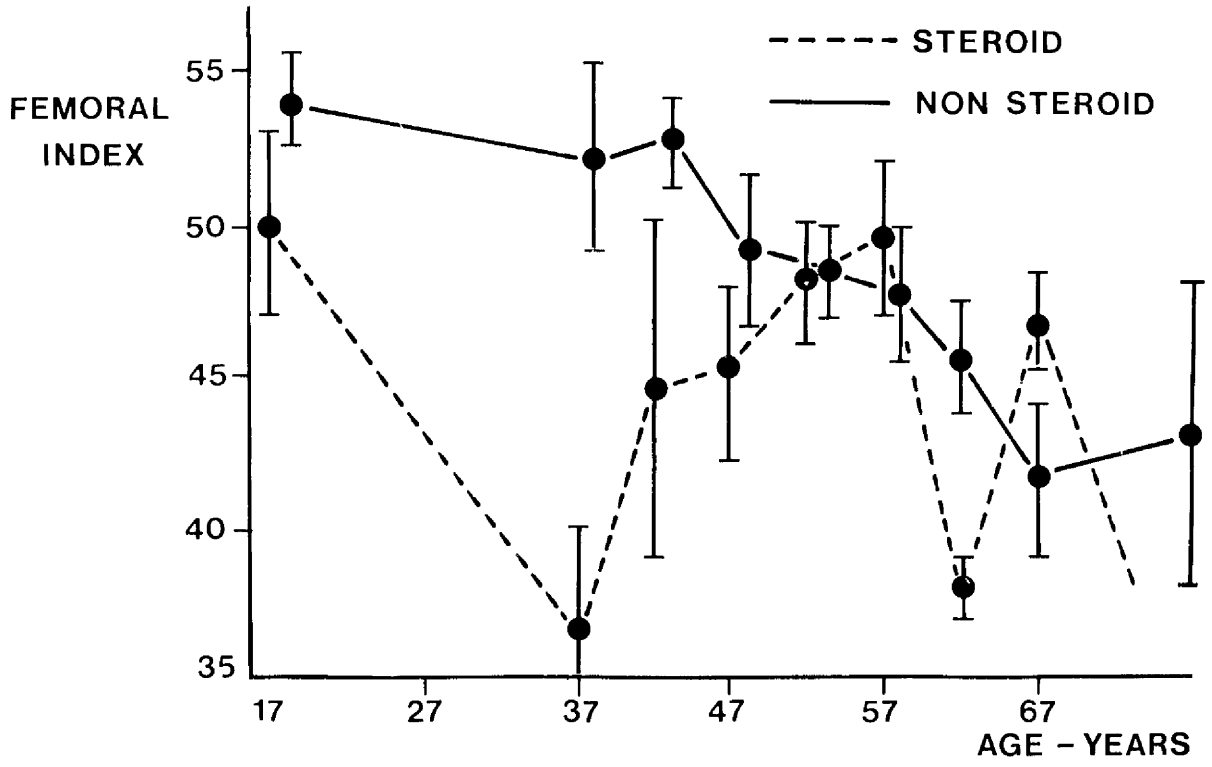

STEROID
NON STEROID
FEMORAL INDEX
55
50
45
40
35
17
27
37
47
57
67
AGE – YEARS

Fig I: 19

The mean and standard error of the clavicular cortical thickness in non corticosteroid and steroid treated female patients with rheumatoid arthritis.

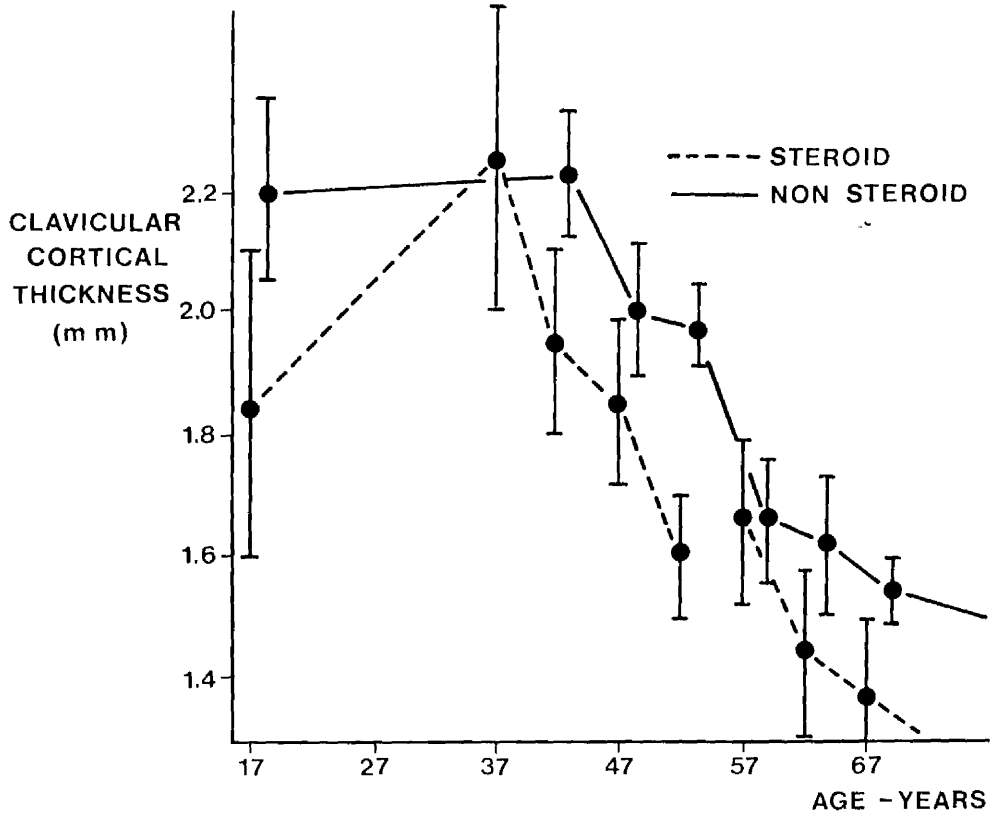

STEROID
NON STEROID
CLAVICULAR CORTICAL THICKNESS (m m)
2.2
2.0
1.8
1.6
1.4
17
27
37
47
57
67
AGE - YEARS

Fig I: 20

The mean and standard error of the metacarpal index in non corticosteroid treated male patients with rheumatoid arthritis.

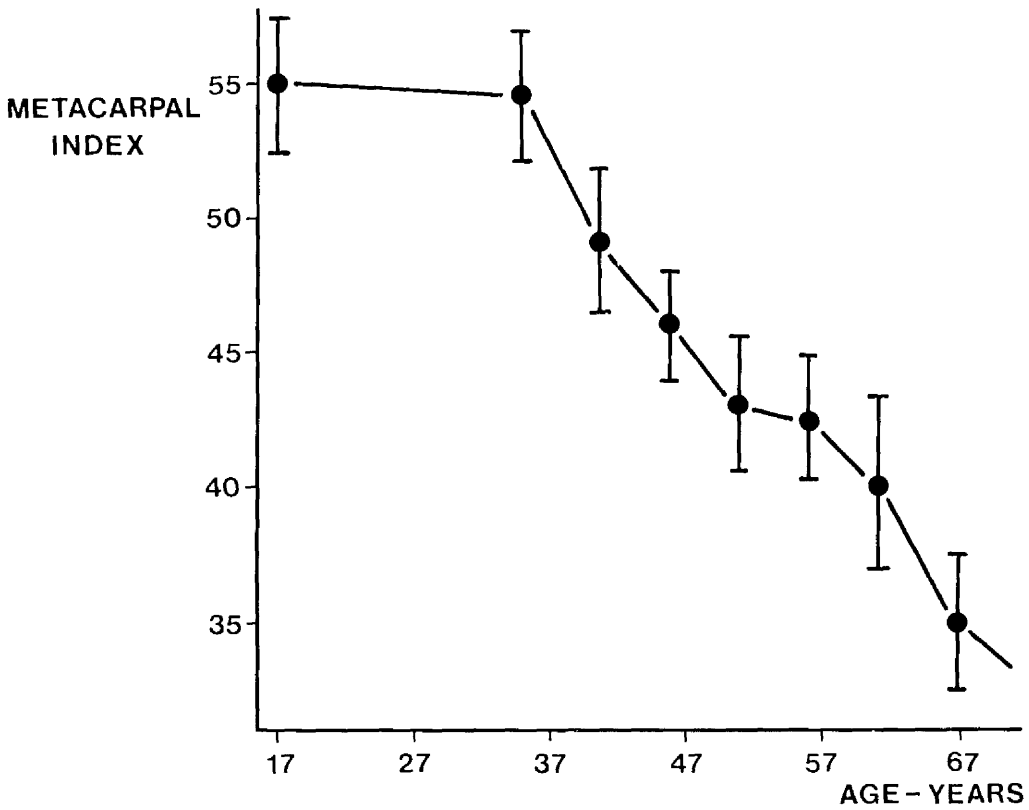

METACARPAL
INDEX
55
50
45
40
35
17
27
37
47
57
67
AGE - YEARS

Fig I: 21

The mean and standard error of the femoral index in non corticosteroid treated male patients with rheumatoid arthritis.

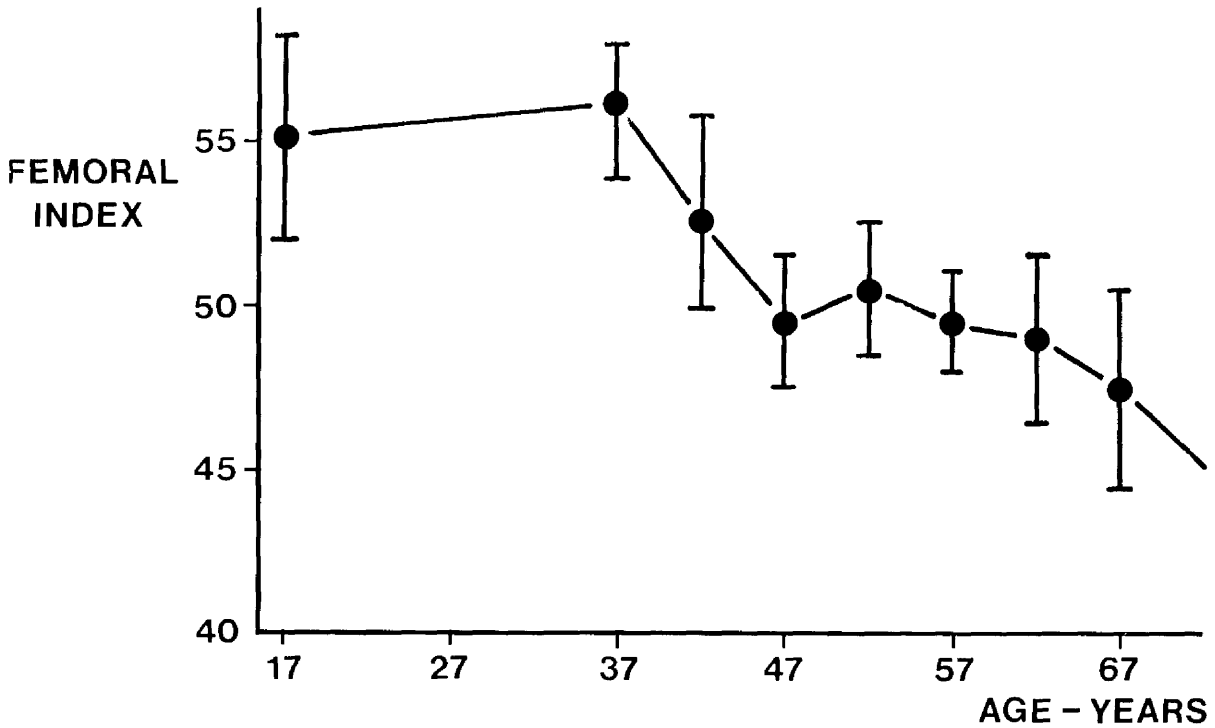
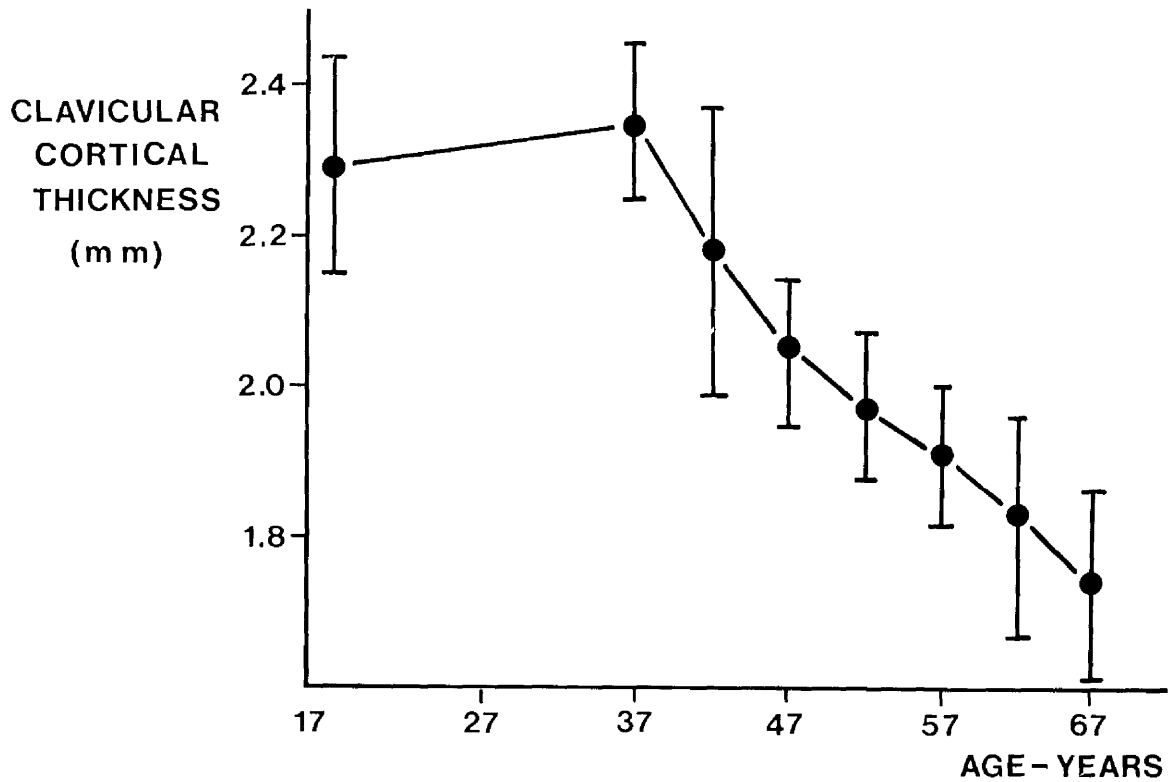

FEMORAL
INDEX
55
50
45
40
17
27
37
47
57
67
AGE - YEARS

Fig I: 22

The mean and standard error of the clavicular cortical thickness in non corticosteroid treated male patients with rheumatoid arthritis.


CLAVICULAR
CORTICAL
THICKNESS
(mm)
2.4
2.2
2.0
1.8
17
27
37
47
57
67
AGE - YEARS

Since the change with respect to age took place after age forty five, some of the regressions on just those patients who had attained that age were repeated.

The observations which were included in the regression model as independent variables were

(2) age

(3) duration of the disease

(4) steroid therapy coded by 1 or 0

The duration of the disease for patients getting rheumatoid arthritis before they were forty five was modified by assuming, as suggested from fig.I:17 to fig.I:22, that the disease only markedly affects osteoporosis after the patient reaches forty five. The metacarpal index was chosen to be the dependent variable because it was now thought to be a more sensitive measure of the osteoporotic condition than the previous average of the standardised osteoporotic scores.

A linear model was posed for the regression of the metacarpal index on variables (2), (3) and (4). As expected, the regressions on age proved to be highly significant for both males and females. Now having taken account of age, the regression of the metacarpal index on duration of the disease and whether or not a patient had been treated with steroids were calculated. The females showed significant regression on steroid therapy but not on the duration (Table I:32 (a)). The regression on the duration was not significant if both age and steroid therapy were taken account of. In the

Source	d.f.	Sums of Squares	F	p
X_2	1	.41 $X10^4$	34.43	< 0.02
Residual	217	2.55 $X10^4$		
X_3/X_2	1	.02 $X10^4$	2.9	N.S.
X_4/X_2	1	.12 $X10^4$	10.6	< 0.02
Residual (X_3)	216	2.51 $X10^4$		
Residual (X_4)	216	2.43 $X10^4$		
X_3/X_4, X_2	1	.01 $X10^4$	.94	.NS
Residual	215	2.43 $X10^4$		

Table I:32 (a)

The regression analysis of the metacarpal index on age (X_2), duration of disease (X_3) and presence of steroid therapy (X_4) in two hundred and nineteen rheumatoid factor positive female patients with rheumatoid arthritis.

previous regression analysis the regression on duration was significant (after age 45) but this sample included those patients under forty-five. The males showed significance on neither steroid therapy nor duration of disease (table I:32(b), this may have been biased by the fact that there were only 21 males on such therapy and 114 not on therapy.

Since the regression of the metacarpal indices for the females, showed as significant for steroid therapy the regression on duration of the dose was calculated allowing for both age and the patients being on corticosteroid therapy: The regression was not significant (table I:33) which is surprising.

Table I:34 shows values of the duration of the disease tabulated in the different age groups for the rheumatoid factor positive female metacarpal indices only. For all osteoporotic indices within a specified duration of the disease there was a definite trend of decrease but within any age group the duration of the disease did not have any trend. This table may not be a completely fair representation as there were more patients available for comparison in cells along the diagonal.

Age, therefore, seemed to be the most important single factor determining the osteoporotic status of patients of both sexes. The results showed a definite increase in the severity of osteoporosis in patients over forty-five. Allowing for age, steroid therapy appears to contribute to the development of osteoporosis in the rheumatoid positive females over forty-five.

Source	d.f.	Sums of Squares	F	p
X_2	1	.18 $X10^4$	15.2	<0.02
Residual	133	1.58 $X10^4$		
X_4/X_2	1	.003 $X10^4$	.24	N.S.
X_3/X_2	1	.004 $X10^4$	.24	N.S.
Residual (X_4)	132	1.577 $X10^4$		
Residual (X_3)	132	1.576 $X10^4$		

Table I:32 (b)

The regression analysis of the metacarpal index on age (X_2), duration of disease (X_3) and presence of steroid therapy (X_4) in one hundred and thirty five rheumatoid factor positive male patients with rheumatoid arthritis.

Source	d.f.	Sums of Squares	F	p
X_5/X_2, X_4	1	.03 X10	2.7	N.S.
Residual	215	2.40 X10		

Table I:33

The regression analysis of the metacarpal index on the duration of the dose of corticosteroid therapy (X_5), allowing for age (X_2) and presence of steroid therapy (X_4) in the female patients with rheumatoid arthritis.

	Age Group (Years)	Duration of Disease in Years						
		0-2	3-5	6-8	9-11	12-14	15-17	18-20
Corticosteroid treated:								
Metacarpal Index	45-49	40.7 (7)	46.4 (8)					
	50-54	27 (1)	39.6 (5)	33.1 (9)	42.3 (3)	-	-	-
	55-64	28.7 (3)	34.8 (4)	39.0 (5)	26.0 (5)	36.5 (5)	31 (1)	33.6 (3)
	65+	-	37 (1)	-	38 (1)	-	-	-
Non-corticosteroid treated:								
Metacarpal Index	45-49	50.1 (18)	48.3 (3)	-	-	-	-	-
	50-54	34.8 (5)	49.5 (15)	43.2 (11)	47.0 (3)	-	-	-
	55-64	33.5 (4)	38.8 (10)	33.7 (7)	39.9 (16)	35.4 (12)	38.9 (12)	36.6 (5)
	65+	37 (1)	-	31.5 (2)	34.5 (4)	32.4 (5)	-	-

TABLE I:34

Mean Metacarpal Index tabulated in terms of age group and duration of arthritis in corticosteroid and non-corticosteroid treated female patients with rheumatoid arthritis.
The numbers in brackets denote the number of indices on which each group mean was based.

Discussion

The evidence available from this study of rheumatoid arthritic patients suggested that osteoporosis in rheumatoid arthritis was directly linked to the age of the patient. The two groups of major concern, the rheumatoid positive males and females, showed a change in the development of osteoporosis in patients after they were forty five. The osteoporotic condition of the patients, as measured by the means of cortical thickness of three bones, the metacarpal, the femur and the clavicle, was found to be fairly constant for those patients under forty five and then to decrease linearily with age.

Those rheumatoid factor positive females over forty five who had received corticosteroids were significantly more osteoporotic than those who had not (taking into account the development with age). In fig I:17 - Fig I:19 this development of osteoporosis can be seen for steroid and non-steroid groups, those receiving steroids showed basically the same trend as those not receiving therapy except that the mean osteoporotic scores (for metacarpal and clavicle) were all slightly smaller. The femur did not give such a marked difference as the other two bones but the femur was shown not to be as highly correlated with the other osteoporotic scores in the steroid treated females as the scores were with each other. The question which must be asked on consideration of these graphs is whether those patients given steroids were more osteoporotic, in the first instance, than a patient of an equivalent age not given this therapy. In the first set of regressions for this group, however, the severity of the disease as measured by functional grade and X-ray classification

were not significant. The duration of disease was not significant when age was taken into account and duration of steroid therapy not significant when age and a patients being on corticosteroid therapy were eliminated.

The rheumatoid factor positive males given steroid therapy were not found to be more osteoporotic than those not on therapy. The crude difference between the three osteoporotic scores for these groups was more than offset by the natural development of osteoporosis with age. This might explain the very low F ratio for the significance of steroids eliminating age (table I:32).

Study B

PREDICTION OF OSTEOPOROSIS

As far as I am aware no-one has attempted to predict the degree of osteoporosis which would be expected in a patient with rheumatoid arthritis. I therefore decided to study patients who had been documented some years before and whom I was able to reassess at a later date. Could I evolve a statistical procedure whereby I could determine whether a patient had more extensive osteoporosis than expected from the clinical and laboratory features of the arthritis? That is the question which this section of the chapter attempts to answer.

METHOD AND MATERIALS

Patients were selected at random from patients seen at the Centre for Rheumatic Diseases between the years 1965 to 1968. Since the greatest changes in osteoporotic indices had been noted to occur around the age of 45 years, the patients studied were selected from the age group 35 to 60 years. In this way it was hoped to maximise change in the osteoporotic indices.

All patients studied satisfied the diagnostic criteria of the American Rheumatism Association for "definite" or "classical" rheumatoid arthritis (Ropes et al 1959). There were 26 males with a mean age of 47.7 years ($\pm$ S.D. 4.5, range 38 to 55) of whom six were receiving oral corticosteroid therapy, and 29 female patients with a mean age of 46.8 years ($\pm$ S.D. 5.0, range 35 to 70), of whom thirteen were receiving oral corticosteroid therapy. Relevant clinical and laboratory details of these patients at the beginning and end of the study are shown in Table I:35.

Each patient had three osteoporotic indices measured: metacarpal, and femoral indices (Barnett and Nordin 1960), and clavicular cortical thickness (Anton 1969). These measurements were made twice. The mean time interval between the two measurements for male patients being 6.5 $\pm$ S.D. 1.3 years and for females 8.7 $\pm$ 1.5 years.

	Male				Female			
	First Measurement	Second Measurement	t	p	First Measurement	Second Measurement	t	p
Functional grade	2.4 ± 0.13	1.6 ± 0.1	4.26	<0.001	2.3 ± 0.12	2.0 ± 0.12	-	N.S.
Sheep Cell agglutination test (reciprocal of titre)	340 ± 8.7	345.5 ± 92.3	-	N.S.	216.2 ± 40.9	112 ± 32.2	2.0	<0.05
Antinuclear factor (reciprocal of titre)	86.5 ± 51.5	22.5 ± 13.1	-	N.S.	26.2 ± 11.5	20.7 ± 9.8	-	N.S.
Serum Albumin g/l	36.8 ± 0.7	44.3 ± 0.9	6.6	<0.001	34.6 ± 0.6	43.5 ± 0.6	10.2	<0.001
Serum Globulin g/l	36.0 ± 1.2	37.5 ± 1.4	-	N.S.	33.9 ± 0.7	33.7 ± 0.9	-	N.S.
Haemoglobin g/100 ml	13.8 ± 0.3	13.8 ± 0.4	-	N.S.	12.5 ± 0.2	13.1 ± 0.3	-	N.S.
Erythrocyte sedimentation rate (Westergren) mm.1st hour.	44.8 ± 6.	39 ± 5.8	-	N.S.	40.3 ± 4.0	29.5 ± 4.4	-	N.S.
Metacarpal Index	48.4 ± 2.0	40.6 ± 2.1	2.63	<0.02	48.7 ± 2.2	36.4 ± 2.1	3.97	<0.001
Femoral Index	53. ± 1.7	50.9 ± 2.0	-	N.S.	50.6 ± 1.2	49.3 ± 1.6	-	N.S.
Clavicular cortical thickness (mm).	2.1 ± 0.1	2.0 ± 0.1	-	N.S.	2.1 ± 0.01	1.7 ± 0.1	2.87	<0.01

Table I:35

Clinical and Laboratory Data of Male and Female Patients at the

RESULTS

When the clinical and laboratory data obtained at the outset and at the termination of the study are compared (Table I:35) it can be seen that for male patients there was a significant improvement in the functional grade (Steinbrocker, Traeger and Batterman 1949) and the serum albumin levels, but a significant deterioration in the metacarpal index. In the female patients there was a significant improvement in serum albumin, and a reduction in titre of rheumatoid factor which was barely significant. Both the metacarpal index and clavicular cortical thickness measurements showed significant deterioration in the female patients.

Initially regressions were carried out of change in osteoporosis measurements against the time elapsed between the two measurements. None of these were statistically significant, but this is not surprising when it is noted that there is little variation in the time interval between observations. To avoid this problem it was assumed, not unreasonably, that the regression line of change in measure against change in time passed through the origin. With this model, the regressions were recalculated. As before, only patients reaching ages over 40 years by the time of the second observation were considered and the duration was taken to be the actual interval of time less any time spent below aged 40 years.

The patients were divided into four groups determined by sex and whether or not the patient was receiving corticosteroid therapy. The regression model $y = \beta x$ was fitted on each of these four groups separately, where y is the change in osteoporotic score and x is change

in time. The coefficient β was estimated by least squares to be $\beta = \Sigma xy/\Sigma x^2$ with standard error $\sigma / \sqrt{\Sigma x^2}$ where σ^2 is the residual mean square about the regression line, $\sigma^2 = \Sigma x^2 - (\Sigma xy)^2/\Sigma x^2$ The estimates of β, together with their standard errors are shown in Table I:36. In all cases it can be seen that the gradients are negative, that is, on average the osteoporotic scores are decreasing in time. However, it is only for the metacarpal index that these gradients are significantly different from zero and even here, the gradient for the male steroid group (which had a small sample size) was not significant. The gradients of the metacarpal index in the four groups are fairly similar, except for the female steroid group but none of the between group differences are statistically significant.

The question then arises of trying to predict the change on the metacarpal index of a patient over a period of say x_o years. (The other indices were excluded from further consideration as their gradients were not significantly different from zero). A point estimate of the change in index is given by $y_o = \hat{\beta} x_o$ but of course there is an associated standard error $\sigma \sqrt{1 + x_0^2/\Sigma x^2}$ For small values of x_o this will be approximately σ. Table I:37 gives values of the predicted change on the index and its standard error over various periods of time. It can be seen that the predicted changes have high standard errors, indeed it is only over the 15 and 20 year periods that the predicted change exceeds twice its standard error. Thus there is a high variability about the trend line.

Clinical Group	Metacarpal Index			Femoral Index			Clavicular cortical thickness		
	n	mean	S.E.M.	n	mean	S.E.M.	n	mean	S.E.M.
Female non-cortico steroid	13	-1.5	0.4	11	-0.76	0.72	11	-.027	0.02
Female cortico-steroid	16	-2.0	0.5	16	-0.2	0.19	15	-.008	0.019
Male non-cortico steroid	20	-1.2	0.4	20	-0.16	0.68	20	-.41	0.47
Male cortico-steroid	6	-1.2	0.9	6	-0.65	0.5	6	-.016	0.55

Table I:36

The mean gradients ($\pm$ S.E.M.) of the Metacarpal Index, Femoral Index and Clavicular Cortical Thickness in Male and Female patients with rheumatoid arthritis.

	FEMALE				MALE			
	Non-corticosteroid treated		corticosteroid treated		Non-corticosteroid treated		Corticosteroid treated	
Time in years	Predicted change	S.E.	Predicted change	S.E.	Predicted change	S.E.	Predicted change	S.E.
1	1.5	10.8	2.1	14.2	1.2	8.7	1.2	12.7
5	7.5	11.0	10.5	14.4	6.0	8.8	6.0	13.5
10	15.0	11.6	21.0	14.9	12.0	9.4	12.0	15.7
15	22.5	12.5	31.5	15.7	18.0	10.2	18.0	18.8
20	30.0	13.6	42.0	16.8	24.0	11.2	24.0	22.4

Table I:37

The predicted Change in the Metacarpal Index ($\pm$ SEM) over various time periods in male and female patients with rheumatoid arthritis.

As indicated earlier these conclusions are based on patients aged between 40 and 50 years of age who were followed for periods of approximately 8 years. Thus, the predictions over the 15 and 20 years periods are extrapolations.

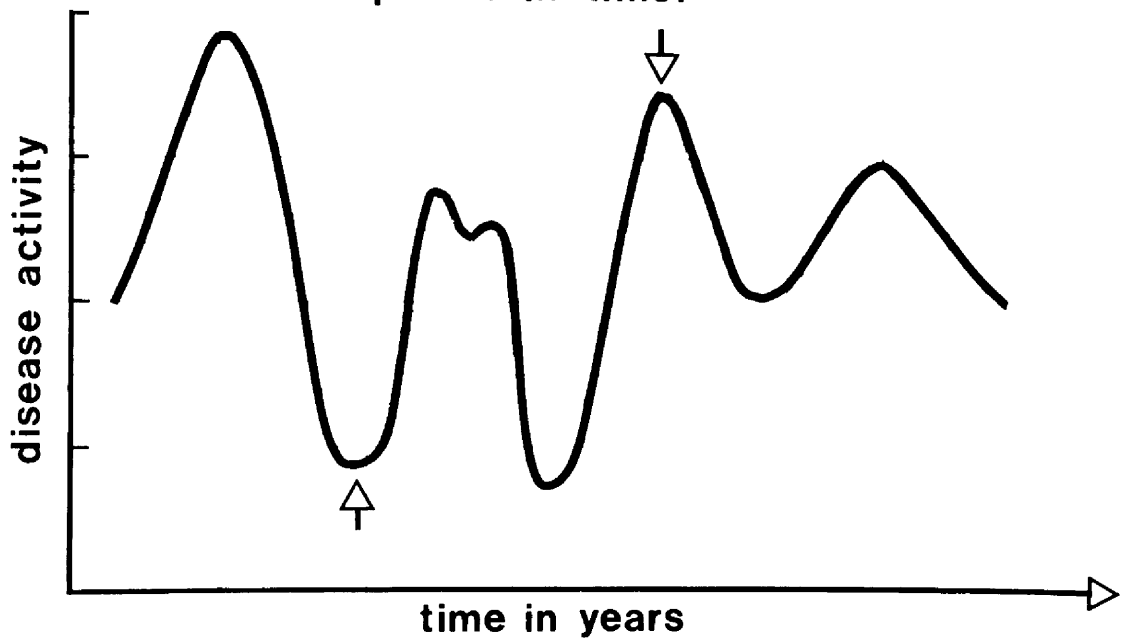
DISCUSSION

In the final part of this study I have attempted to answer the question: can one, knowing the clinical features of the arthritis and whether or not the patient has had corticosteroid therapy, predict what the degree of osteoporosis will be over a period of time? Certainly the study has shown that osteoporosis develops over a period of time, but the statistically predicted degree of osteoporosis has a very high standard error and consequently any prediction must have a high degree of error. This is probably due to the marked variation in clinical activity of arthritis which can occur during two points in time, as illustrated schematically in Fig. I:23. In this respect it is interesting that although improvement occurred in several clinical and laboratory parameters in both male (functional grade and serum albumin concentration) and female patients (functional grade, haemoglobin and serum albumin concentrations, erythrocyte sedimentation rate, and titre of rheumatoid factor), osteoporosis continued to develop, especially in females. It would be necessary to measure 'total' disease activity over a period of time if more accurate prediction of osteoporosis was to be obtained. This conceivably could be done by making assessments at regular short periods of time e.g. three monthly, although it is doubtful whether the results would justify the effort and time involved.

Fig I: 23

Schematic diagram of possible variations in the activity of rheumatoid arthritis occurring between two points in time.

Possible variations in disease activity occurring between two points in time.



I deliberately chose patients around the age of 45 years where change in osteoporosis was likely to be most marked. Presumably, therefore, if much younger or older patients had been studied the error in prediction would probably have been even greater. Only a very gross statistical outlier could be identified and it is quite probable that the variable responsible e.g. osteomalacia would be clinically apparent otherwise.

In summary, it must be concluded that although the degree of osteoporosis developing in a patient with rheumatoid arthritis can be predicted statistically, the margin of error was too great for it to be clinically meaningful.

SUMMARY

The results of the studies in this chapter on radiological osteoporosis in rheumatoid arthritis show that:

1. Bone mass as measured by the metacarpal index is significantly decreased in both male and female patients compared to age and sex-matched normal subjects.
2. Bone mass falls significantly with duration of disease.
3. Female patients over 45 years of age have significantly more osteoporosis than those under 45 years, but this trend is not present in male patients.
4. The sex of the patient in itself did not appear to be a significant determining factor in bone loss occurring in patients with rheumatoid arthritis.
5. Female patients over 45 years of age treated with corticosteroids had significantly more osteoporosis than those females over 45 years not so treated.
6. Corticosteroid therapy did not more severely affect female patients over 45 years of age than those under that age.
7. Both femoral indices and clavicular cortical thickness measurements show significant reduction in patients with rheumatoid arthritis of both sexes compared to normal values.
8. Correlation coefficients and concordance/discordance analysis of the three indices of osteoporosis suggests that osteoporosis occurring in rheumatoid arthritis is a generalized phenomenon.
9. A predictive index of the development of osteoporosis in rheumatoid arthritis was determined, but the margin of error was too great to give clinically meaningful prediction.

CHAPTER II

STUDIES OF CALCIUM METABOLISM IN RHEUMATOID ARTHRITIS

INTRODUCTION

Having established that radiological evidence of osteoporosis was widespread in rheumatoid arthritis, the question arose as to why this bone loss occurred. Osteoporosis results when bone resorption exceeds bone formation, irrespective of the rate of each. Bone formation is a function of osteoblast activity and bone resorption usually of osteoclast activity. There is, however, evidence that bone resorption may occur in the absence of osteoclasts, and be mediated by endothelial cells (Cameron, 1961), mast cells (Goldhaber, 1965; Frame and Nixon, 1968) and osteocytes (Meunier, Bernard and Vignon, 1971), and such factors may be important in pathological states such as rheumatoid arthritis.

The study of bone formation and resorption (osteoblast and osteoclast function) at molecular level is extremely complex, but it is possible to make certain indirect measurements in the clinical situation. The introduction to this chapter provides a background to some of the investigative procedures which were employed to study the biochemistry and endocrinology of bone metabolism in patients with rheumatoid arthritis. In this introduction it will be apparent that considerable time is devoted to outlining and discussing some of these procedures, particularly those relating to the serum calcium fractions and their derived values. This selectivity is felt necessary in view of the heavy dependence of the data in the chapter on these same criteria.

CALCIUM METABOLISM

Calcium is the fifth most common element in the human body, and accounts for approximately 1300 g. i.e. 1.9 per cent, of an average 70 Kg. man (Nordin, 1976). Nearly all the calcium present in the body is present in bone, and constitutes just over 20 per cent of the wet weight of bones in the adult (Woodard, 1964).

The National Food Survey (1973) considered that the mean calcium intake per day was of the order of 1000 mg. per person, and Nordin (1976) considered that 95 per cent of the U.K. population consumed 500 to 1500 mg. of calcium per day. The principal source of dietary calcium is milk, which accounts for approximately 50 per cent of the total daily calcium (Nordin, 1976). The net calcium absorption from the gastro-intestinal tract is approximately 25 to 30 per cent of the dietary intake (Nordin, 1976). The measurement of true calcium absorption from the gastro-intestinal tract is complicated by the fact that faecal loss of calcium includes not only unabsorbed dietary calcium but also loss of calcium in the digestive juices. The magnitude of endogenous faecal calcium loss has been estimated to be between 76 and 260 mg. per day in normal subjects (Melvin, Hepner, Bordier, Neale and Joplin, 1970; Saville, 1973). Calcium absorption is measured in clinical practice by a single or double isotope method. In the former a dose of radioactive calcium is administered in a calcium carrier, and blood levels of radioactivity are monitored over a period of time. In the latter procedure two

different calcium isotopes are administered, one orally and the other intravenously. The rate of absorption from the gastro-intestinal tract is measured by the difference between the specific activities of the two isotopes in plasma. There is a progressive reduction in calcium absorption in normal persons with advancing age, and this is especially so in the female, perhaps due to reduced oestrogen levels (Nordin, 1973). Increased calcium absorption is often present in primary hyperparathyroidism (Hodgkinson, 1963; Stanbury, 1968; Sjoberg, 1970), idiopathic hypercalciuria (Henneman, Benedict, Forbes and Dudy, 1958; Libermann, Sperling, Atsmon, Frank, Modan and De Vries, 1968; Caniggia, Gennari and Cesari, 1965; Nordin, Peacock and Wilkinson, 1972), hypervitaminosis D and may also be present in sarcoidosis (Anderson, Dent, Harrer and Philpot, 1954; Henneman, Dempsey, Carrol and Albright, 1956; Reiner, Sigurdsson, Nunziata, Malik, Poole and Joplin, 1976).

The normal excretion rate of calcium in the urine is less than 8 mmols per day, 20 per cent of the calcium being ionised and the remainder complexed (Fourman and Royer, 1968). The urinary excretion of calcium is depressed with chronically low dietary calcium (Nordin, Hodgkinson and Peacock, 1967), advancing age (Davis, Morgan and Rivlin, 1970; Bulusu, Hodgkinson, Nordin and Peacock, 1970; Nordin, 1973) and renal failure (Cochran and Nordin, 1971; Morgan, 1973). Urinary calcium excretion is greater

in males due to their greater body weight (Nordin, et al, 1967), but the sex difference disappears when calcium is related to creatinine (Bulusu, et al, 1970). During the summer months in some climates there is increased calcium excretion in the urine, probably due to ultraviolet synthesis of vitamin D resulting in an increased absorption of calcium from the gastro-intestinal tract. There is a diminution in urinary calcium excretion with exercise (Nordin, et al, 1967). Prolonged bed rest or immobilisation causes a marked increase in the urinary excretion of calcium (Whedon and Shorr, 1957), which does not appear to be due to increased intestinal absorption of calcium (Nordin, et al, 1967) and restricting dietary calcium does not influence this phenomenon (Howard, Parson and Bingham, 1945). Whedon and Shorr (1957) considered that the fundamental cause of hypercalciuria occurring with immobilisation was due to the absence of weight bearing on bones.

Fourman and Royer (1968) estimated that the filtered calcium load at the renal glomerulus was approximately 225 mmols per day, and that approximately 215 mmols of this was reabsorbed by both the proximal and distal tubules. Intravenous infusion of calcium, increases calcium concentration in the glomerular filtrate, and it is believed that approximately half of this increase is excreted in the urine (Peacock, and Nordin, 1968; Peacock, Robertson and Nordin, 1969). It is not possible to determine the calcium "threshold", or tubular maximum reabsorption of calcium (TmCa) because there is an upper limit to which the plasma calcium

level can be raised which is compatable with life. Nevertheless, Marshall, Peacock and Nordin (1973) have calculated a theoretical TmCa by extrapolating from data obtained from calcium infusions in normal subjects and patients with osteomalacia. The TmCa is best expressed in m.mol/l of glomerular filtrate as this excludes variation due to differences in renal mass and as TmCa/GFR is more appropriate than the absolute TmCa (Nordin, 1976). Tubular reabsorption of calcium is a highly complex process and can be calculated from the difference between filtered and excreted calcium if both are expressed in the same units. Thus,

$$C_F = Ca_E + Ca_R$$

where Ca_F = Filtered load of calcium

Ca_E = Excreted calcium

Ca_R = Reabsorbed calcium

Tubular reabsorption can be obtained from plotting the plasma calcium against Ca_E, and normal ranges have been derived (Nordin, 1976).

In clinical practice the urinary calcium/creatinine (Ca/Cr) ratio obtained while the subject is fasting has proved a convenient method for estimating increase in net bone resorption. The ratio may be elevated in normal post-menopausal females, hyperparathyroidism and conditions associated with hypercalcaemia provided renal failure is not present (Nordin, Horsman and Aaron, 1976). If calcium absorption and/or intake is excessive an

increased Ca/Cr ratio may derive from the previous day's absorbed calcium even after a 12 hour fast, especially so, if the GFR is reduced since this would delay excretion of absorbed calcium (Nordin, et al, 1976).

SERUM CALCIUM

Rona and Takahashi (1911) showed by passing plasma through a protein-impermeable membrane that plasma calcium consisted of a protein-bound non-diffusible fraction and a free diffusible fraction. This confirmed the hypothesis of a free and protein-bound fraction of serum calcium proposed some forty years earlier by Pribam (1871). Following the observations of Rona and Takahashi there was uncertainty as to the nature of the protein-binding and composition of the diffusible fraction (Neuhausen and Marshall, 1922; Greenberg and Greenberg, 1932; Thomson and Collip, 1932; Tendeloo, 1936) until the classical experiments of McLean and Hastings (1934, 1935a, 1935b). These workers established the first acceptable direct measurement of serum ionised calcium employing an isolated frog's heart preparation. They also showed that the frog's heart responded only to the ionised calcium, but not to protein-bound or complexed calcium (McLean and Hastings, 1935a, b). In addition, McLean and Hastings showed that there was a linear relationship between serum calcium concentration and serum protein levels, and from this they constructed a nomogram from which serum ionised calcium could be derived given the values for total serum calcium and protein concentrations.

It is now recognised that serum calcium exists in three fractions: approximately 50 per cent is ionised, 40 per cent is protein bound, and 10 per cent is ligand bound. The measurement, therefore, of the total serum calcium can at best be only useful as a diagnostic screening test, since although the concentration of ionised calcium correlates reasonably well with the total serum calcium (Ladenson and Bowers, 1973) elevation or diminution of ionised calcium may occur in the presence of a normal total serum calcium concentration (McLean and Hastings, 1935b; Walser, 1962; Pittinger, Chang and Faulkner, 1971; Ladenson and Bowers, 1973) (Table II:I). The serum ionised calcium is, as McLean and Hastings demonstrated the physiologically important fraction of serum calcium and its measurement is therefore of great importance.

The calcium in blood which is bound to protein is mostly in the form of calcium albuminate (Pfordte and Ponsold, 1971; Pedersen, 1972e; Van Slyke, Hastings, Hiller and Sendroy, 1928). Albumin accounts for 90 per cent of protein-binding at pH 7.4 (Pedersen, 1971b, 1972 a-c, e). The effect of changes in pH on calcium protein-binding has been long established namely that increases in pH cause increase in protein binding both in vivo and in vitro, and conversely decreases in pH reduces protein binding (Moore, 1970; Seamonds, Towfighi and Arvan, 1972). Prasad and Flink (1957) suggested that pCO_2 and bicarbonate have greater effects on calcium protein-binding than changes in pH, but Pedersen (1971a) clearly showed that there was up to a 20 per cent

Condition	Effect on ionised calcium	Effect on total calcium
Acute acidosis	Elevated	Normal
Chronic acidosis	Normal	Decreased
Acute alkalosis	Decreased	Normal
Chronic alkalosis	Normal	Elevated
Hyperparathyroidism	Elevated	Elevated
Hypercalcaemia of malignancy	Elevated	Elevated
Vitamin D 'poisoning'	Elevated	Elevated
Hypoparathyroidism/tetany	Decreased	Decreased
Renal failure	Variable	Decreased

Table II: I

Effect of various conditions on serum ionised and total calcium concentrations.

change in calcium protein-binding with a change in pH from 7 to 8 and this was independent of the mechanism of the pH change. Decrease in protein-binding of calcium has also been observed with decrease in temperature (Toribara, Terepka and Dewey, 1957; Munday and Mahy, 1964; Robertson and Peacock, 1968; Pedersen, 1969). The albumin sites for binding of calcium may be competed for by other ions, such as magnesium (Pedersen, 1969; Pedersen, 1972b). However, only changes in pH have been shown, as yet, to have any potential clinical relevance. It is of interest, however, that Gosling, Robinson and Sammons (1975) have shown that during haemodialysis ionised calcium fell and bound calcium increased. Although there was a rise in blood pH they could not explain these changes in serum fractions as being solely due to the pH change.

It is still uncertain what the relative strengths of calcium binding are to different globulin fractions. Early work (Gutman and Gutman, 1937; Drinker, Green and Hastings, 1939; Rawson and Sunderman, 1948; Prasad and Flink, 1958) showed considerable variation in calcium binding affinity within the various globulin fractions, and there was little evidence of binding of calcium to gamma globulin. Held and Freeman (1964) found no significant differences between binding of calcium to albumin, alpha and beta globulin, and confirmed very little binding to gamma-globulin. Pfordte and Ponsold (1971) studied calcium binding to ultracentrifugated preparations of different proteins and showed that beta globulin bound more than the other globulin fractions and between 3.7 to 6.5 times more than albumin. Unfortunately neither Held

and Freeman (1964) nor Pfordte and Ponsold (1971) mentioned the ionised calcium concentration at which their studies were carried out, and the latter authors failed to provide evidence of saturation of binding capacity of serum proteins at a level of total serum calcium of 25 m.mol/l.

The protein-bound serum calcium in normal subjects has a mean value of about 1 m.mol/l. (Dale and Kellerman, 1967; Raman, 1971, Putman, 1972). Clearly changes in total serum calcium may be expected if there are marked alterations in concentration of serum proteins, particularly albumin. However, it is not yet clear exactly what effects changes in serum protein concentration have on the level of total serum calcium and on the protein-bound and ionised fractions.

There are four case reports in the literature of patients with dysproteinaemias or multiple myeloma who had increased total serum calcium concentrations due to abnormal binding of calcium to abnormal plasma protein (Glueck, Wayne and Goldsmith, 1962; Lingarde and Zettervall, 1973; Soria, Soria, Dao, James, Bousser and Bilski-Pasquier, 1976). However, to date there is little other evidence to suggest that this type of anomaly is in any way common. In hepatic cirrhosis conflicting results have been found. Thus, Adamski and Smarz (1969) found increased calcium protein-binding and a decreased ionised calcium concentration, whereas Moore (1971) found decreased protein-binding and increased ionised

calcium in patients with the same disease. In an extensive study Pedersen (1972e), as might be expected, concluded that patients with hypergammaglobulinaemia exhibited a decrease in calcium binding per gram of serum protein, and that this decreased progressively as the gamma globulin concentration rose. This decrease of binding occurred irrespective of whether the hypergammaglobulinaemia was diffuse or monoclonal.

CORRECTION OF SERUM CALCIUM FOR HYPOALBUMINAEMIA

Changes in concentration of serum proteins, especially albumin, have a marked effect on the total serum calcium concentration. As stated, attempts have been made to correct the serum calcium concentrations for changes in serum protein levels, and, indeed, as far back as 1935 McLean and Hastings introduced their nomogram for this purpose. Payne, Little, Williams and Milner (1973) studied 200 consecutive serum specimens sent to a biochemistry laboratory for "liver function tests". These sera showed a wide range of abnormal protein concentrations. The authors found that the total serum calcium concentration correlated closely with albumin levels ($r = 0.867$), but less closely with total protein. They suggested a simple formula for adjusting calcium concentration derived from the regression equation of calcium on albumin.

Adjusted calcium = calcium - albumin + 4.0, where calcium is in mg./100 ml. and albumin mg./100 ml. Using this formula Payne et al (1973) found that 95 per cent of the serum calcium

concentrations after adjustment for regression of calcium on albumin were identical with the limits of the normal range in healthy individuals. The Gaussian distribution of corrected serum calcium values suggests that any skewing with diagnostically significant calcium values is minimal (Cook, Levell and Payne, 1970). It is of interest that the regression coefficient of calcium on total protein obtained by Payne et al (1973) (0.689) was of the same order (0.676) obtained by Dent (1962) based on serum calcium and plasma specific gravity. The regression coefficient of calcium on albumin obtained by Payne et al (1973) was considerably higher, however, (0.989) than that (0.707) obtained by Orrell (1971) on some 945 serum specimens, but of the same order as that obtained by Jones, Peters, Morgan, Coles and Mallick (1967) and Cockel, Kendall, Becker and Hawkins (1971) in sera from patients with a variety of disorders influencing serum protein concentrations, such as malabsorption, hepatic cirrhosis, malnutrition and rheumatoid arthritis. The lower regression coefficient obtained by Orrell (1971) for calcium on albumin may have been due to inclusion of sera from patients with nephrotic syndrome. In this condition there is evidence that patients may have higher calcium concentrations (Jones, Peters, Morgan, Coles and Mallick, 1967) than patients with similar levels of serum albumin possibly due to the presence of other abnormal serum proteins which more effectively bind calcium than albumin (Pedersen, 1972e). In addition, it is possible, as suggested by Payne et al (1973), that patients studied by Orrell (1971) may have included some with hypercalcaemia due to other causes. At zero albumin concentration the intercept on serum calcium found by

Payne et al (1973) was 1.43 m.mol/l, which corresponds to the ultra-filterable calcium concentration. This value is close to that reported by others (Robertson, 1969; Pedersen, 1970; Rose, 1972), but lower than that found (1.70m.mol/l) by Orrell (1971). The formula which Payne etal (1973) proposed was based on correction to the mean serum calcium concentration rather than the mean serum albumin concentration, because the between-batch coefficients of variation at normal serum calcium concentration were 1.2 per cent for calcium but 3.5 per cent for albumin.

It is of interest that in the same edition of the British Medical Journal Berry and his colleagues (Berry, Gupta, Turner and Burns, 1973) came to similar conclusions and recommended a correction factor of 0.023 m.mol/l. change in calcium for each 1 g/l change in albumin. Berry et al (1973) also validated the correction for specific gravity (Dent, 1962) but recommended a more exact adjustment of 0.23 mg. of calcium per 100 mls. for every 0.001 change in specific gravity.

The publications of these two papers sparked off a lively correspondence in the British Medical Journal and Clinical Chemistry. Sanderson (1974) could not understand why he obtained different corrected serum calcium levels using the formulae proposed by Berry et al (1973) and Payne et al (1973). These differences arose as a result of differences in the reference values for serum albumin, which were 46g/l and 40g/l respectively. However, it is important to note that the regression coefficients of total plasma calcium on

albumin derived by both these groups were very similar (Berry, Dent, Flynn, Gupta and Turner, 1974).

Parfitt (1974) criticised the formulae for correcting serum calcium for changes in serum albumin concentration on the grounds that these formulae were only accurate if the serum ionised calcium was normal. He proposed that a proportional rather than an absolute correction be employed and that such could be deduced from the McLean and Hasting's nomogram (1935a, b). The nomogram refers to concentration in water rather than plasma, but a computer programme to solve the quadratic equation on which the nomogram is based, has been written (Parfitt, 1969). Parfitt (1974) also suggested a more complicated proportional correction, which he claimed gave more accurate correction for changes in protein due to posture (Husdan, Rapoport and Locke, 1973) than Dent's original specific gravity formula (Dent, 1962). The formula proposed by Parfitt (1969) assumes that 55 per cent of total calcium is diffusible, with a reference value of total protein of 7.2 g./100 ml. The formula proposed was

Corrected calcium = measured calcium/0.55 +
Total Protein/16.

This formula Parfitt claimed agreed closely with the nomogram for calcium values lying between 1.5 and 3.5 m.mol/l.

Payne et al (1974a) agreed with Parfitt's criticism that significant errors might be introduced in correcting total serum

calcium for albumin concentration when the ionised calcium level was either abnormally high or low. However, they pointed out that the adjusted values are higher than they should be if the ionised calcium is high and lower than they should be when the ionised calcium is low, with the consequence that the clinical usefulness of their correction formula was enhanced. Marshall and Nordin (1974), however, took issue with this conclusion of Payne et al (1974a) and pointed out that with a low ionised calcium level the adjustment applied is larger than it should be, whereas with a raised calcium level it is smaller. Consequently they maintained that only where the adjustment is subtracted from the measured total will the enhancement claimed by Payne et al (1974a) be observed. This would, argued Marshall and Nordin, only occur if the serum albumin concentration were higher than normal. Moreover, they pointed out that when the serum albumin is low the adjustment must be added, so giving an adjusted total higher than it would be with a low ionised calcium, and lower than it would be with a high ionised calcium. Marshall and Nordin (1974) recommended the McLean-Hastings nomogram as the most appropriate method for allowing for changes in serum protein concentrations "because it is based on a proportionate change in total calcium with protein rather than an absolute change". An improved nomogram would be based on the serum albumin rather than the total serum protein concentration, and this these authors have now produced (Hodgkinson and Knowles, 1976). In reply to Marshall and Nordin, Payne et al (1974b) suggested that their proposed nomogram might be of more value in determining

non-protein bound calcium, and that the correction formula which they had proposed was still a valid screening procedure.

In 1975 Pain, Rowland, Phillips and Duncan challenged the concept of using a correction formula for estimating total serum calcium when the serum albumin was reduced. These authors criticised the use of an "average" regression coefficient for serum calcium on albumin obtained from a pool of sera from a large number of subjects. They found a wide individual variation in the regression coefficients and therefore recommended that the individual's own regression coefficient should be established by a tourniquet test which increases the serum protein concentration in the veins of the constricted arm (Husdan, Rapoport and Locke, 1973). Pain et al (1975) found that although the average regression coefficient for total serum calcium concentration on serum albumin concentration was 25 μu.mol. of calcium per gram of albumin, there was more than a sevenfold variation in regression coefficients between individuals, the 95 per cent fiducial limits being 7 to 53 μu.mol/g. The method which Pain and Phillips (1976) suggested for determining an individual regression coefficient was to obtain blood at 0, 5, 10 and 15 minutes with venous occlusion. Husdan, Rapoport, Locke and Oreopoulos (1976) criticised this method proposed by Pain et al (1975, 1976) on the grounds of impractability in clinical practice and on the fact that venous occlusion over a period of 15 minutes especially when the hand

is pumped might result in accumulation of lactic acid and fall in pH, with a consequent increase in ionised calcium and fall in protein-bound calcium. Other correspondents (Payne and Little, 1976; Ramsay and Shelton, 1976; Hodkinson, 1976; Payne, Little, Williams and Milner, 1976) also disagreed with the conclusions of Pain and his colleagues on similar grounds.

Calcium Binding and Globulins

Hodkinson (1974) questioned the validity of adjusting for low serum concentration when estimating total serum globulin on the grounds that hypoalbuminaemia is frequently associated with hyperglobulinaemia, and that globulins may contribute significantly to the protein-binding of calcium. That this occurs in certain paraproteinaemias is unquestionable (Glueck, et al, 1962; Lingarde and Zettervall, 1973; Soria, et al, 1976) but the cases reported are very few. However, as pointed out by Payne, et al (1974a) the correlation between calcium and total globulins is relatively weak (0.299 for 200 paired sera). Furthermore, as previously mentioned the intercept of the regression line at zero albumin concentration found by Payne et al (1973) was close to reported values for normal plasma ultrafilterable calcium. In addition, as previously mentioned, Pedersen (1972d) estimated that less than 4 per cent of normal total calcium was bound to globulin. Thus, the average regression coefficient obtained by Payne et al (1973) and other workers (Dent, 1962; Robertson, 1969; Pedersen, 1970; Orrell, 1971; Rose, 1972; Berry et al, 1973) is not likely to be seriously affected by changes in serum globulin concentrations.

The current consensus of opinion regarding the correction of calcium is in favour of employing some formula or nomogram for routine purposes (British Medical Journal, 1977; Husdan, et al, 1976). However, it is necessary for each laboratory to establish its own ranges and criteria for correction. A recent editorial in the British Medical Journal (1977) concludes that for most clinical purposes "the "corrected" plasma calcium is an adequate measure of ionised calcium on almost all occasions".

SERUM IONISED CALCIUM

There is a general agreement that ionised calcium is the metabolically active fraction of serum calcium and is under hormonal control. It therefore follows that a direct measurement is highly desirable in investigation of disturbances of calcium metabolism.

The first workers to measure the serum ionised calcium concentration were McLean and Hastings (1934). These authors used a bio-assay employing a frog's heart preparation. Others have also employed bio-assay techniques (Paupe, 1955; Yendt, Connor and Howard, 1955; Soulier and Crosnier, 1958), but these have not proved as reliable as the McLean and Hasting's method (Marshall, 1976). However, the bio-assay procedure is unsuitable for routine laboratory use, and has been superseded by spectrophotometry and selective ion electrodes. The latter method has been most widely employed and has been the procedure employed in this thesis. There has been only one comparative study of the two laboratory methods

(Raman, 1972), and this showed a significant correlation ($r = 0.72$, coefficient of determination 51.8 per cent) with a tendency for higher values to be obtained by the spectrophotometric method.

The development of ion-selective electrodes dates back to the discovery of the hydrogen ion selective glass electrode at the beginning of the century (Eisenman, Rudin and Casby, 1957). The pH electrode is now a standard piece of equipment in most clinical laboratories. Calcium electrodes, although available for several decades, were however unsuitable for use in serum because of "poisoning" by proteins and because selectivity for calcium over other cations was inadequate (Truesdell and Christ, 1967). The major advance in measurement of ionised calcium in serum came with the development of ion-exchange calcium electrodes which had a high specificity for calcium ions (Ross, 1967). This method has enabled accurate measurement of serum ionised calcium under anaerobic conditions using small amounts of serum.

Measurement of serum ionised calcium using an ion-selective electrode is fraught with technical difficulties (Moore, 1970). The pH of the serum has profound effects on ultrafiltrable calcium (Toribara, Terepka and Dewey, 1957; Prasad, 1960; Loken, Havel, Gordan and Whittington, 1960) and a comparable effect on the electrode measurement of ionised calcium (Loken, et al, 1960; Moore, 1970) (Table II:I). It is therefore important for comparative purposes that measurement of serum ionised calcium be carried out, or standardised to a pH of 7.4 (Watson, 1975). Ion

interference obviously may be important when an electrode method is used. If the serum sodium is, for example, increased it may interfere with the estimation (Watson, 1975). In clinical practice the serum sodium concentration is usually normal lying within a narrow range and interference is therefore uncommon. Although theoretically other ions may cause difficulties and should be considered when measurements are made on pathological specimens, for clinical purposes serum sodium correction usually suffices. Temperature may also affect measurement (Hansen and Theodorsen, 1971). Thus, for example, in normal sera the ionised calcium concentration is 4 per cent lower at 37^{o}C than at room temperature (Watson, 1975). It is therefore important that measurement of both sera and standard solution be performed at the same temperature, preferably at body temperature (Watson, 1975). Technological problems may also exist and include variation in pressure of the pump system, sample flow rate, drift instability and sluggish response (Rushton et al, 1973; Watson, 1975).

There is considerable variation in the mean values of serum ionised calcium as estimated by the ion selective method (Table II:II). However, the intra-individual variation is acceptable; (vide infra) Ladenson and Bowers (1973) reported a variation of 1.2 per cent. Marshall (1976) found a variation of 2.5 per cent and also found the inter-individual variation to be 3.7 per cent. Robertson (1976) concluded: "The variance of the ionized calcium within and between normal fasting individuals is very small and

Author	Material	Numbers	Fasting (F) or not (N.F.)	Technique	Temperature	Mean Serum ionised calcium concentration mmols/l (rang
McLean and Hastings (1935)	Serum	11	N.F.	Frog Heart	Room	1.18 (1.05 – 1.30)
Paupe (1955)	Serum	?	?	Toads Heart	?	1.16 (1.06 – 1.26)
Ettori and Scoggan (1961)	Serum	21	N.F.	Spectrophotometry	Room	1.25 (1.10 – 1.40)
Walser (1961)	Plasma	20	N.F.	Spectrophotometry	36°C	1.18 (0.95 – 1.40)
Arnold et al (1968)	Serum	27	N.F.	ion electrode	Room	1.28 (0.95 – 1.60)
Oreskes et al (1968)	Plasma	50	N.F.	ion electrode	Room	1.15 (0.95 – 1.40)
Robertson and Peacok (1968)	Serum	47	F	ion electrode	37°	1.23 (1.16 – 1.32)
Sachs et al (1969)	Serum	?	?	ion electrode	?	1.1 (0.90 – 1.30)
Moore (1970)	Serum	47	N.F.	ion electrode (static)	37°	1.12 (0.96 – 1.28)
	Serum	17	N.F.	ion electrode (flow thru')	25°	1.15 (1.09 – 1.23)
Raman (1970)	Serum	20	N.F.	ion electrode	26±2°	1.18 (0.90 – 1.48)
Hattner et al (1970)	Serum	23	N.F.	ion electrode	Room	0.98 (0.90 – 1.05)
Pedersen (1970)	Serum	32	N.F.	Spectrophotometry	37°	1.15 (1.10 – 1.20)
Schwartz et al (1971)	Serum	30	N.F.	ion electrode	27°	0.98 (0.90 – 1.05)
Hansen and Theodorsen (1971)	Serum	35	N.F.	ion electrode	Room	1.10 (1.03 – 1.18)
Pittinger et al (1971)	Serum	132	N.F.	ion electrode	?	1.08 (0.96 – 1.20)
Cham (1972)	Plasma	18	N.F.	Spectrophotometry	Room	1.10 (1.00 – 1.20)
Putman (1972)	Plasma	12	N.F.	Ultrafiltration	Room	1.10 (0.95 – 1.25)
Rose (1972)	Plasma	15	F	Ultrafiltration	37°	1.40 (1.25 – 1.55)
Fuchs et al (1972)	Serum	?	?	ion electrode	25°	1.23 (1.13 – 1.33)
Schwille and Ernestberger (1972)	Serum	?	?	ion electrode	?	1.13 (0.95 – 1.28)
Rushton et al (1973)	Serum	?	N.F.	ion electrode	37°	0.98 (0.90 – 1.03)
Ladenson and Bowers (1973)	Serum	7	F	ion electrode	25°	1.28 (1.18 – 1.39)
Varghese (1973)	Plasma	19	F	Spectrophotometry	37°	1.04 (0.90 – 1.18)
Leme et al (1973)	Serum	17	N.F.	ion electrode	?	1.05 (0.99 – 1.11)
Freaney et al (1974)	Serum	24	N.F.	ion electrode	23°	1.18 (1.02 – 1.33)
Ryden et al (1976)	Serum	15	F	ion electrode	?	1.03 (0.93 – 1.13)

Table II: II

Serum ionised calcium concentrations for normal populations demonstrating the variation in results and methodology.

of the same order as the variance of the analytical methods by which it is measured". The day-to-day variation is no greater than 0.025 m.mol/l, which is not surprising when the level of serum ionised calcium is under hormonal control.

Slight (0.05 m.mol/l) but not significant differences in serum ionised calcium have been noted between the sexes (Walser, 1961; Hattner, Johnson, Bernstein, Wachman and Brackman, 1970; Petersen, 1972d; Husdan, et al, 1973). There does, however, appear to be a reduction in the serum ionised calcium concentration in people over the age of 60 years (Lingarde, 1972; Marshall, 1976).

With a few exceptions (Moore, 1970; Pittinger, et al, 1971), most workers have found a good correlation between the serum ionised calcium and the total serum calcium concentration (Ladenson and Bowers, 1973). However, it has been shown that both an increased and decreased serum ionised calcium concentration may occur in the presence of a normal total serum calcium level (Table II:I) (McLean and Hastings, 1935b; Walser, 1962; Pittinger, Change and Faulkner, 1971; Ladenson and Bowers, 1973). This reinforces the importance of direct measurement of the serum ionised calcium wherever possible in any study of abnormalities in calcium metabolism.

REGULATION OF CALCIUM HOMEOSTASIS

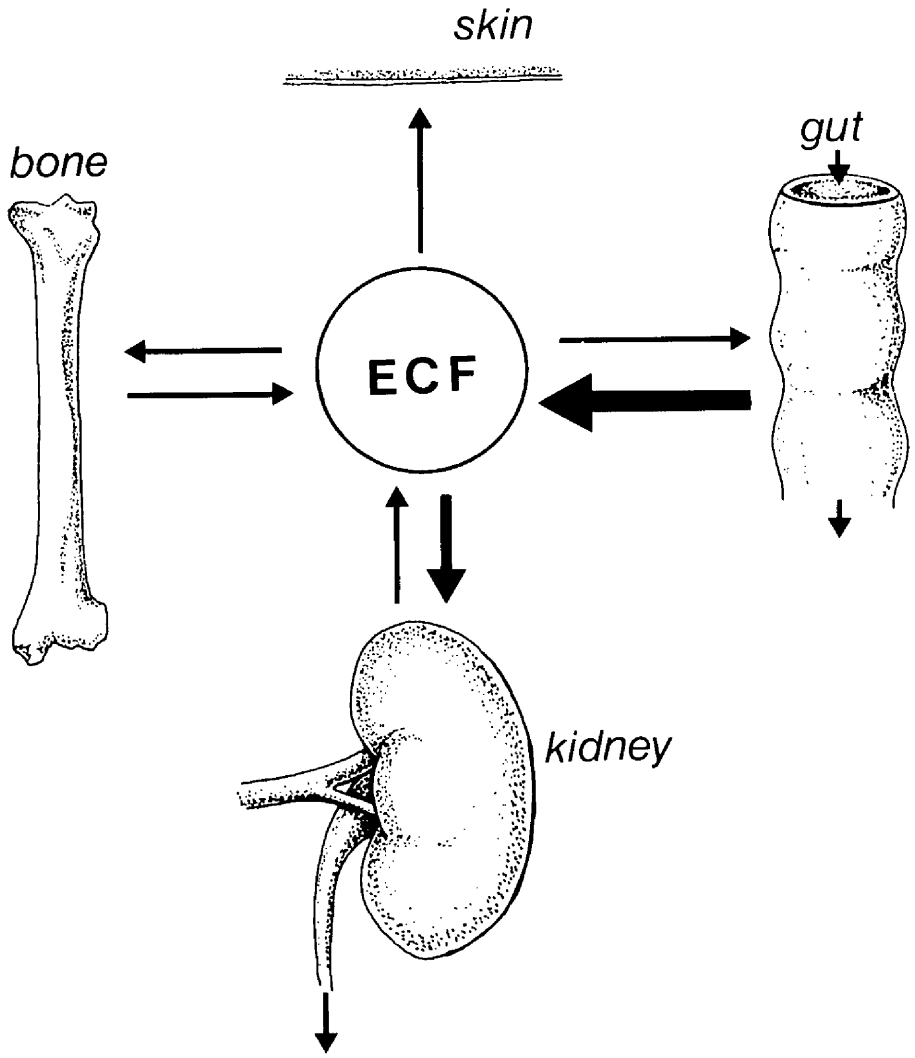
Serum calcium is maintained in health within relatively narrow limits, and is the resultant of the rate at which calcium enters the blood and the rate it leaves the blood (Figure II:1). As previously mentioned calcium enters the blood stream by absorption from the gastro-intestinal tract and in normal adults, net absorption is approximately 6 m.mol per day. The net input from bone is the difference between the rate at which bone is being resorbed and the rate at which bone mineralisation is taking place: generally in healthy subjects these two rates are equal. Net renal loss of calcium is the difference between the total filtered calcium and the amount of calcium reabsorbed by the tubules. Normally this net excretion must approximate to the net absorbed calcium from the gastro-intestinal tract (Figure II:1).

Thus, the three major tissues involved in calcium homeostasis in addition to blood are intestine, bone, and kidney. In vertebrates calcium homeostasis probably involves at least three major serum factors, parathyroid hormone (PTH), calcitonin and vitamin D.

The physiological role of PTH is to raise the plasma calcium concentration to that level necessary to maintain normal cellular metabolism, and the parathyroid glands are stimulated by hypocalcaemia to produce PTH. The hormone has an action within minutes upon the kidney to increase the tubular reabsorption of calcium, so reducing urinary calcium loss. PTH also acts very

Figure II:1

Diagrammatic representation of calcium fluxes occurring between the main body pools in the normal state.


skin
bone
gut
ECF
kidney

rapidly upon the pool of calcium, which is possibly contained in "bone fluid", to mobilise calcium into the blood. In addition, osteoclastic resorption of bone is stimulated after serveral hours, which results in bone breakdown and release of bone mineral. After a period of one to two days PTH also appears to enhance intestinal absorption of calcium from the diet via its effect on vitamin D metabolic pathway. These actions of PTH are demonstrated diagrammatically in Figure II:2. In Figure II:3 the hydroxylation steps involved in the conversion of vitamin D to the active 1,25 dihydroxycholecalciferol which like PTH is involved in maintenance of normal calcium and phosphate metabolism are outlined. The 1,25 dihydroxycholecalciferol has a direct effect upon the rate of intestinal absorption of calcium. PTH is thought to either increase the production or enhance the activation of renal 1-hydroxylase which in turn causes increased production of 1,25 dihydroxycholecalciferol, thereby exerting its indirect action on the intestinal absorption of calcium (Parsons, 1976).

Where there is excess secretion of PTH then the rates of both bone formation and resorption are accelerated, although the latter predominates giving rise to an overall bone loss. Intestinal absorption of calcium is also increased, which also contributes to hypercalcaemia. Although renal excretion of calcium is increased, the proportion of the filtered load of calcium which is reabsorbed by the renal tubules will also be increased, and so contribute to the hypercalcaemia. The increased excretion of calcium and phosphate by the kidney can lead to the formation of renal stones.

Fig II:2

Diagrammatic representation of the mode of action of parathyroid hormone (P.T.H.) on calcium fluxes occurring between the main body pools. A + sign indicates an increase in flow due to the effect of P.T.H.

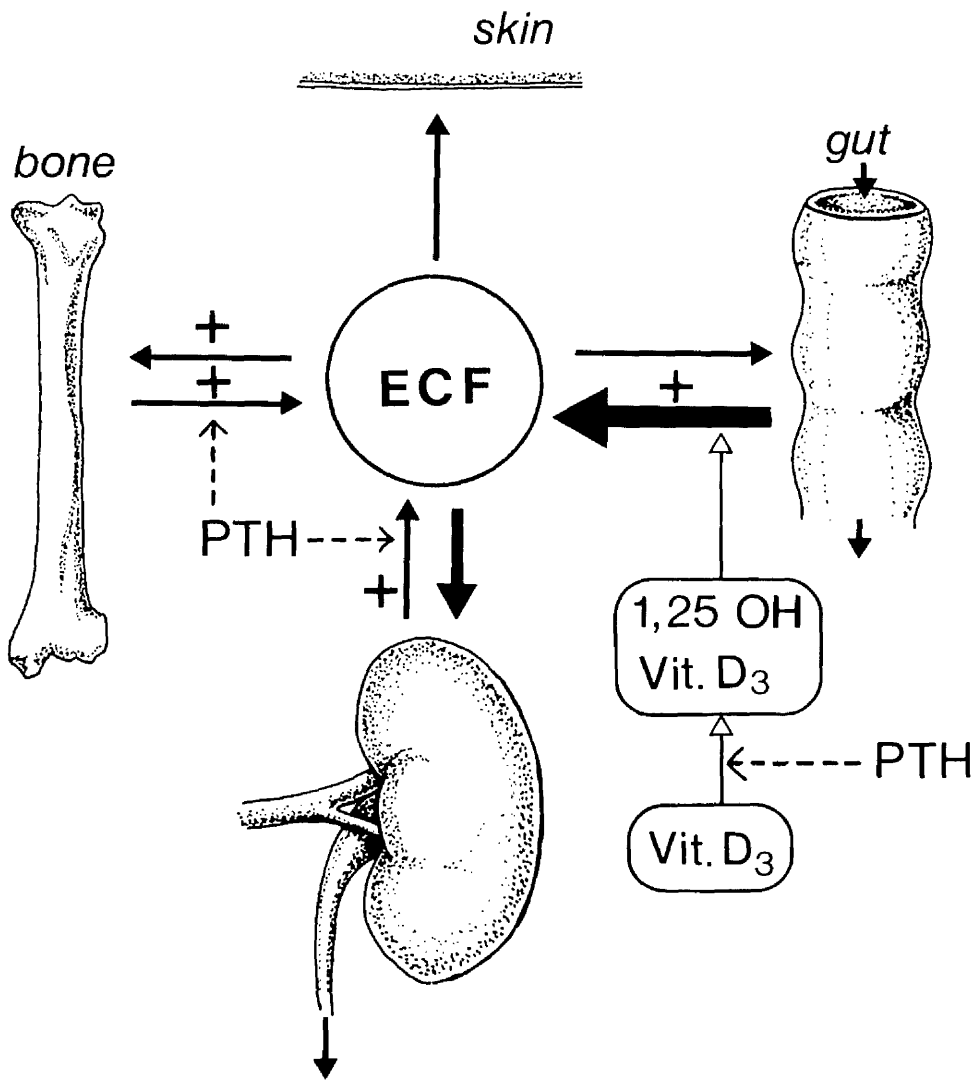
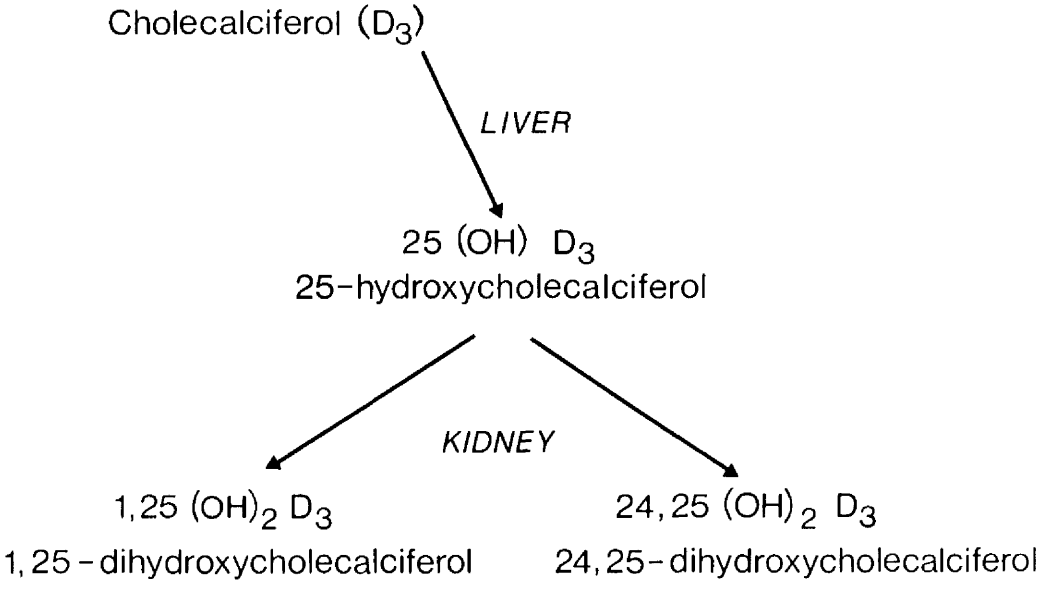

skin
bone
gut
ECF
+
+
+
PTH
+
1,25 OH
Vit. D3
PTH
Vit. D3

Fig II:3

Schematic representation of the hydroxylation steps involved in the metabolism of vitamin D.

Hydroxylation of cholecalciferol



Chemistry of PTH

Mammalian PTH is a single polypeptide chain comprising 84 amino acids (Brewer and Ronan, 1970; Niall, Keutmann, Sauer, Hogan, Dawson, Aurbach and Potts, 1970). The complete amino-acid sequence is not, as yet, known for human PTH, but is known for bovine and porcine hormones (Reiss and Canterbury, 1973; Russell, 1976). The structural requirements for biological activity has been determined, and shown to lie in the first 32 to 34 amino acids reading from the N terminal (Potts, Murray, Peacock, Niall, Tregear, Keutmann, Powell and Deftos, 1971; Keutmann, Dawson, Aurbach and Potts, 1972). There is some evidence from *in vitro* receptor assay systems that the amino acid at position 1 (serine for human and porcine, alanine for bovine hormone) is important for biological activity (Potts, et al, 1971).

Biosynthesis and Secretion of PTH

In common with other hormones, e.g. insulin (Steiner, Clark, Nolan, Rubinstein, Margoliash, Aten and Oyer, 1969) PTH is synthesised as a prohormone, which contains six extra amino acids on its N-terminal end, and a further precursor (pre-pro PTH) with a total of 115 amino acids has also been identified in *in vitro* studies (Habener, Kemper, Ernst, Rich and Potts, 1975). There is evidence that PTH is secreted from the parathyroids as the 1-84 hormone, but is cleaved within minutes of entering the circulation to yield a carboxy-terminal fragment or fragments. These fragments comprise about two thirds of the intact molecule, and when there is

normal renal function the circulating half-life is at least ten-fold longer than that of the intact hormone (the $T_{\frac{1}{2}}$ is approximately 20 to 40 minutes). In renal failure the $T_{\frac{1}{2}}$ may be several hours. The fate of the cleaved amino terminal one third is not yet known (Habener and Potts, 1976), but since this is the biologically active fraction of PTH, the relevance of assaying biologically inert fragments generated by metabolic cleavage still poses serious questions (Zanelli and Rafferty, 1976).

Measurement of PTH

Berson and Yalow (1958), Berson, Yalow, Aurbach and Potts (1963), were the first to develop a radio-immunoassay for PTH. They subsequently suggested that PTH in plasma was possibly heterogeneous (Berson and Yalow, 1968). There is evidence that PTH derived from the parathyroid glands and PTH in serum may differ as a result of fragmentation of the hormone either within the gland (Silverman and Yalow, 1973) or on its release into the blood stream (Habener, Mayer, Dee and Potts, 1976). Detailed descriptions of radio-immunoassay methods have been published by Arnaud, Tsao and Littledyke (1971) and Segre, Habener, Powell, Tregear and Potts (1972) and the methodology has been extensively reviewed by Segre, Tregear and Potts (1975) and by Habener and Potts (1976). The brief outline of radio-immunoassay of PTH which follows is largely based on the studies of the above authors. Most immunoassays of PTH have employed bovine PTH and raised antibodies to either the whole hormone or to fragments of the hormone. The isolation in quantity

of human PTH (Keutmann, Barling, Hendy, Segre, Niall, Aurbach, Potts and O'Riordan, 1974) has made it possible to measure PTH using human antigen. There are a number of antigenic sites on PTH, whether human, bovine or porcine, and antisera raised against the intact hormone are heterogeneous containing antibodies to different parts of the amino-acid chain. Antibodies have been raised to both the amino-terminal and the carboxy-terminal (Barling, Hendy, Evans and O'Riordan, 1975; Papapoulos, Hendy, Tomlinson, Lewin and O'Riordan, 1977) and it has been possible to show that the active amino-terminal fragment has a short biological half-life of only a few minutes, whereas the carboxy terminal has a half-life of several hours (Barling, et al, 1975; Papapoulos, et al, 1977). Radio-immunoassays of PTH in patients with hyperparathyroidism have given conflicting results. A significant number of hyperparathyroid patients having values of PTH within the normal range (Lequin, Hackeng and Schopman, 1970; Arnaud, Littledyke and Tsao, 1970; Buckle, 1974) in contrast to Reiss and Canterbury (1971) who found elevated levels of PTH in all cases of hyperparathyroidism. Since antibodies raised against PTH are directed against different immunological determinants, the ability to detect intact metabolic fragments must vary from laboratory to laboratory.

Segre, et al (1972) studied the fragments of PTH measured by radio-immunoassay. By using antisera directed against the amino-terminal and carboxy- terminal determinants they were able

to conclude that biological activity resided in the amino-terminal determinant and that the majority of the circulating hormone was inactive. One of the paradoxes, however, is that in clinical practice radio-immunoassay methods dependent on antibodies against the carboxyl-terminal end of the molecule apparently give more reliable results in the diagnosis of hyperparathyroidism. The reason for this paradox may be due to the fact that intact PTH and the amino-terminal fragment have very short half-lives, whereas the biologically-inactive carboxyl-terminal end of the molecule has a longer half-life (Silverman and Yalow, 1973). Further work is required to characterise the varicus fragments of PTH to determine their nature and biological activity and to assess their value as diagnostic tests. Until such time as there are standard anti-sera, radio-immunoassays of PTH will probably continue to give conflicting results between different laboratories. It is, therefore, important that each laboratory indicate its results in both normal and hyperparathyroid subjects and to record the nature of the antibody employed in the radio-immunoassay.

Calcitonin was first discovered by Copp and his colleagues (Copp, Cameron, Cheney, Davidson, and Henze, 1962). Initially it was uncertain whether calcitonin was derived from the thyroid or parathyroid glands, but in most mammals it has now been clearly established to be derived from the 'C' cells of the thyroid (Pearse and Carvalheira, 1967). The stimulus to production and release of calcitonin from 'C' cells is hypercalcaemia and the

hormone has also been shown, in a bone culture situation, to inhibit the resorption of bone (Friedman and Raisz, 1965; Gaillard and Thesingh, 1968). Administration of calcitonin induces in mammals hypocalcaemia, proportionately distributed between the protein-bound and ultrafiltrable fractions (Gitelman, Kukolj and Welt, 1965), hypophosphataemia and hypomagnesaemia (Copp, et al, 1962; Hirsch, Voelkel and Munson, 1964; Milhaud, Perault-Staub and Moukhtar, 1965; Tenenhouse, Arnaud and Rasmussen, 1965; Bell, Barret and Patterson, 1966; Robinson, Martin and McIntyre, 1966; Haymovits and Rosen, 1967; Rasmussen, Anast and Arnaud, 1967). Calcitonin also has a number of other actions including reduction in faecal calcium excretion, increased calcium and phosphate urinary excretion, and reduction in urinary hydroxyproline (Milhaud and Moukhtar, 1966; Robinson, et al, 1966; Ardaillou, Vuoguant, Milhaud and Richet, 1967; Klein and Talmage, 1968; Hioco, Bordier, Miravet, Denys and Tun-Chot, 1969; Pak, Ruskin and Casper, 1969; Aldred, Kleszynski and Bastian, 1970; Bijvoet, Veer, De Vries and Van Koppen, 1971). In addition to producing hypercalciuria and hyperphosphaturia, calcitonin also causes a diuresis with increased excretion of sodium, potassium, creatinine and bicarbonate (Aldred, Kleszynski and Bastian, 1970) and this does not seem to be secondary to a hypocalcaemic induced secretion of PTH, since there is no marked change in renal excretion of cyclic AMP (Bijvoet, et al, 1971). However, it is important to note that the physiological role of calcitonin in man, as opposed to its pharmacological action,is as yet unclear.

The radio-immunoassay of CT has proved easier than that of PTH. CT has been shown in different species to have 32 amino acids with a loop of seven amino acids formed by a disulphide bond at the amino terminal (Brewer and Ronan, 1970); Schneider and Sherwood, 1974) and the entire sequence is essential for biological activity (Potts and Deftos, 1974). However, the major problem with the immunoassay of CT in man has been the unreliability of the assay in detecting basal levels of the hormone in normal subjects (Tashjian, et al, 1972; Deftos, et al, 1972). It is, of course, possible that in the normal state there is no CT present in human blood. The hormone has been readily detected in patients with medullary carcinoma of the thyroid and forms an important diagnostic criterion in the detection of the disease and the screening of family members for pre-symptomatic forms of this disease (Russell, 1976).

PERSONAL OBSERVATIONS

Hypoalbuminaemia and hyperglobulinaemia are well recognised biochemical features of rheumatoid arthritis (Cockel, et al. 1971). The serum calcium concentration is, however, generally normal. The fact that hypercalcaemia may be concealed by hypoalbuminaemia prompted an investigation into patients with rheumatoid arthritis to ascertain this possibility.

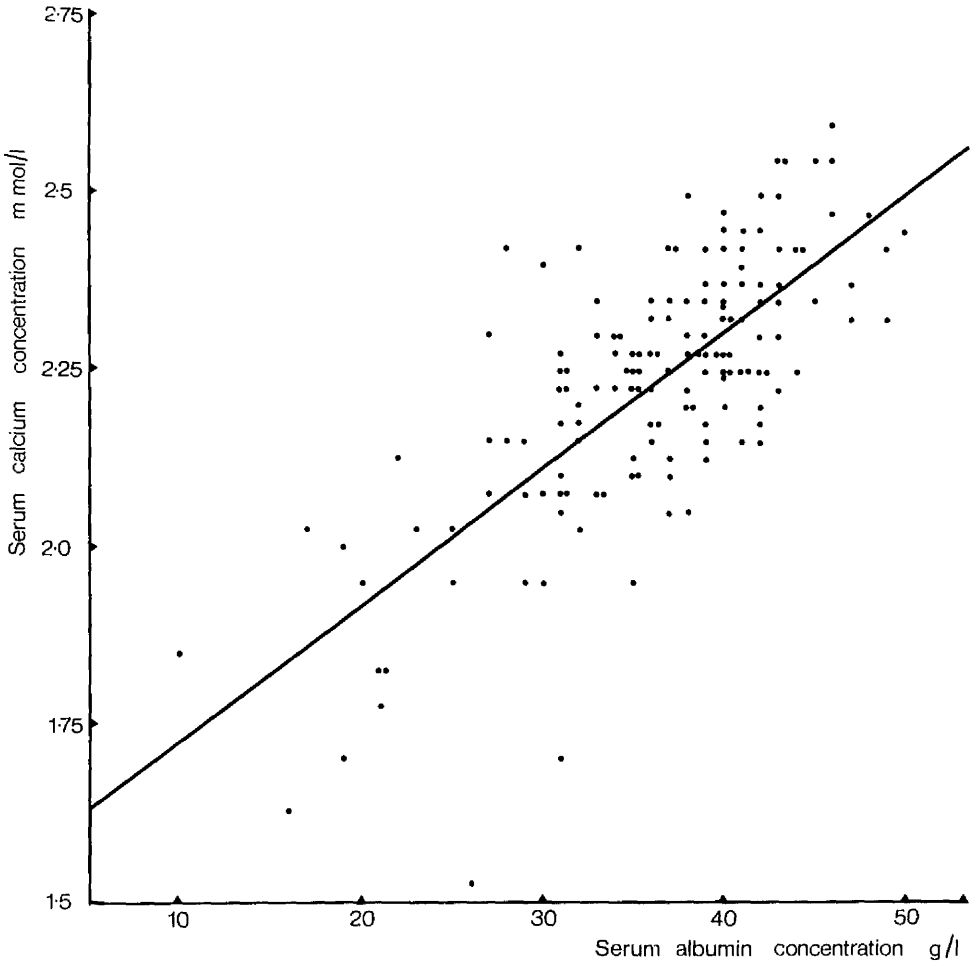
It was first necessary to design a valid correction factor to test this hypothesis.

Design of Correction of Serum Calcium Concentration for Hypoalbuminaemia

A regression analysis of calcium on albumin was carried out on the results from 100 normal healthy subjects, half of whom were female. Their age ranges were as follows: 17 to 69 years for females, and 18 to 70 years for males. Since both the serum albumin and calcium concentrations lay within very narrow normal ranges a regression analysis could not be performed. It was therefore necessary to extend the X axis by including 50 patients with hypoalbuminaemia from a variety of causes, but with no known disease which might affect calcium metabolism. Half of these patients were female, and their age range corresponded to that of the normal subjects (20 to 72 years). A regression equation was then calculated on the total 150 subjects (Fig. II:4). It is of

Fig II:4

The regression of serum calcium concentration (mmol/l) on serum albumin concentration (g/l) obtained from 150 normal controls
$Y = (0.0194 \times X) + C$ where X is serum albumin in g/l, Y is serum calcium in mmol/l and C is a constant equal to 1.434.


2·75
2·5
2·25
2·0
1·75
1·5
Serum calcium concentration m mol/l
10
20
30
40
50
Serum albumin concentration g/l

interest that when the serum albumin concentration is zero the regression line cuts the Y axis at 1.43 m.mol./l., which represents the ultrafiltrable calcium concentration, and corresponds to that found by Robertson (1969), (1.40 m.mol./l); Pedersen (1970), (1.40 m.mol./l.); Rose (1972), (1.45 m.mol./l.); and Payne et al (1973), (1.42 m.mol./l.). Orrell (1971) found a somewhat higher value for ultrafiltrable calcium (1.70 m.mol./l.) probably in part due to the fact that 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 m.mol./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 m.mol ./l.

Alb. is the serum albumin in g./l.

and Ca (corrected) is the serum calcium in m.mol ./l. corrected for albumin.

This indicates that calcium concentration changes by 0.0194 m.mol ./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.

Using the above formula for correction of serum calcium concentration for hypoalbuminaemia, 23 of 50 randomly selected patients with "definite" or "classical" rheumatoid arthritis by the diagnostic criteria of the American Rheumatism Association (Ropes, et al, 1959) were found to have hypercalcaemia (serum calcium > 2.60 m.mol./l.). These patients all were sero-positive for rheumatoid factor and all had articular erosions on joint x-ray. All patients were ambulant.

It was therefore decided to study the 23 patients who were hypercalcaemic after correction for hypoalbuminaemia in greater detail. All of the patients were studied within six months of the initial observation of their being hypercalcaemic. The studies describe the validation of the correction factor and detailed biochemical and hormonal studies of calcium metabolism.

In addition, experimental studies on an in vitro bone model are described which suggest the possibility of a bone-resorbing factor present in the serum of patients with rheumatoid arthritis.

SECTION 1

Biochemical Studies in Hypercalcaemic Patients with Rheumatoid Arthritis

MATERIALS AND METHODS

Patients

Twenty-three female patients suffering from "definite" or "classical" rheumatoid arthritis as defined by the diagnostic criteria of the American Rheumatism Association (Ropes, et al, 1959) participated in the study. Each patient was attending the Centre for Rheumatic Diseases as an out-patient and was selected on the basis of having had a serum calcium concentration greater than 2.60 m.mol./l. after correction for hypoalbuminaemia as outlined above, in the six month period prior to the study.

One patient was receiving no drugs, one was receiving digoxin and a diuretic, one was on phenobarbitone for epileptic seizures, three were receiving low dose corticosteroid therapy (i.e. < 10 mg. prednisolone per day), and the remainder were receiving a variety of non-steroidal anti-inflammatory agents. None of the patients in this study was on excessive milk intake, vitamin D or calcium supplements. The patient group had a mean age of 51.7 years with an age range of 22 - 72 years. Other clinical parameters including duration of disease, functional grade (Steinbrocker, et al, 1949), age at menopause and duration of time since onset of menopause were also recorded.

The patients were requested to fast and take no medication from 6.00 p.m. on the evening prior to commencement of the study and all blood samples were taken between 8.00 a.m. and 9.30 a.m. on the following morning.

Laboratory Procedure

Blood samples were withdrawn without haemostasis; urine collected in dilute hydrochloric acid, over a period of 4 hours. As each blood sample was collected it was divided into aliquots. Those aliquots necessary for hormone estimations were immediately immersed in ice. Thereafter the individual aliquots were handled as described below.

A 10 ml. sample of clotted blood was separated within half an hour and the serum used for estimation of calcium, magnesium, proteins, albumin, phosphate and alkaline phosphatase. A further 10 ml. of blood was collected in a lithium-heparin tube and also separated within half an hour and used for estimations of plasma electrolytes, urea and creatinine. In addition routine liver function tests, i.e. serum bilibrubin, aspartate transaminase (AST 2.6.1.1.) and alananine transaminase (ALT 2.6.1.2.) were carried out. Calcium and magnesium were measured by atomic absorption spectrophotometry using the Perkin-Elmer 403 (Fell and Peaston, 1973). Serum total proteins were estimated by the Biuret reaction as automated for Auto-Analyzer (Technicon Auto Analyzer method N14a) and serum albumin by the bromcresol green

binding method as modified for Auto-Analyzer (Northam and Widdowson, 1965). Purified dried human albumin (Boehringwerke) was used as primary standard. Phosphate was measured in serum and urine by the molybdate reaction as modified for Auto-Analyzer (Yee, 1968) and serum alkaline phosphatase activity was measured at 37°C using LKB 8600 Reaction, Rate Analyzer and Boehringer reagents. Plasma electrolytes, urea and creatinine were measured on SMA 6/60. Creatinine was also estimated by the alkaline picrate reaction (Technicon Auto-Analyzer method N11B).

The serum calcium levels were corrected for albumin employing the formula described above. A biochemical mineral metabolism screen was carried out on each subject. This involved utilising data from blood and urine to calculate certain derived values:

i) Fasting Urinary calcium/creatinine ratio

ii) Urinary calcium excretion (CaE) per litre glomerular filtrate (Nordin, Hodgkinson and Peacock, 1967).

iii) Theoretical maximum tubular reabsorption capacity for Calcium, Tm Ca (Nordin, Horsman and Aaron, 1976).

iv) The most reliable indices of renal handling of phosphate (Paterson, 1973) i.e. $TmPO_4/GFR$ (Theoretical tubular maximum reabsorption of phosphate corrected for glomerular function (Bijvoet, 1969; Bijvoet, Morgan and Fourman, 1969) and I.P.E. (Index of phosphate excretion) (Nordin and Bulusu, 1968).

Both the indices ii) and iii) were calculated by the formula employed in the MRC Mineral Metabolism Unit Leeds, and the rationale has been outlined in the introduction to this chapter.

A 10 ml. clotted sample of blood, from which air was excluded immediately after withdrawal was used for the estimations of ionised calcium.

Measurement of Serum Ionised Calcium Concentration

The Instrumentation

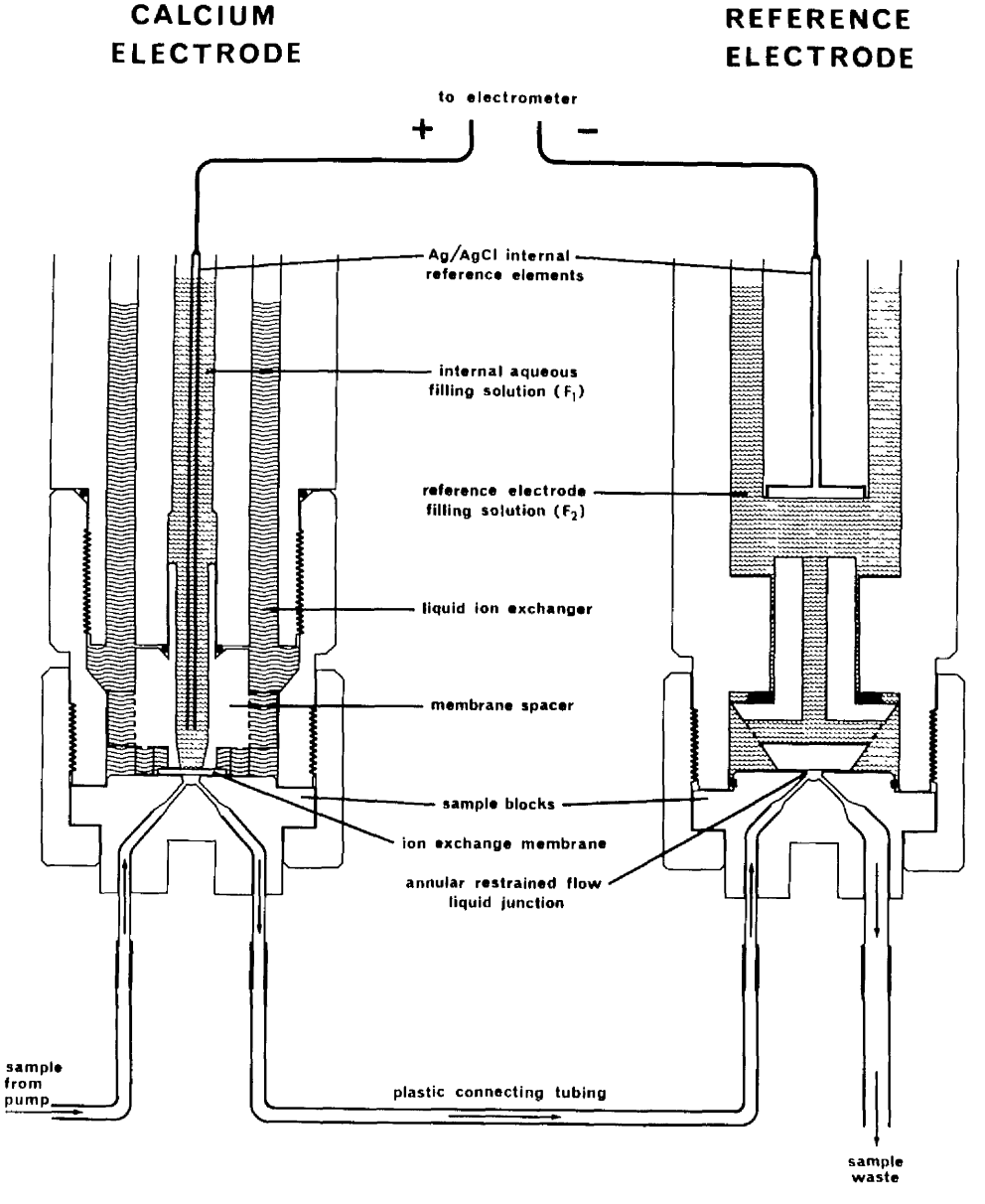
The measurement system used was a modification of a commercially available serum calcium flow-through electrode measurement system (Orion Research, model 99 - 20)*. The calcium selective electrode was of the liquid membrane type using a phosphate ester ion-exchanger and was designed to allow anaerobic measurement in small volumes of a continuously flowing sample (Orion Research, model 98 - 20). This was used in conjunction with a silver/silver chloride reference electrode which had a single restrained flow liquid junction, and was also of the flow-through type (Orion, model 90 - 03). The essential features of these electrodes are illustrated in Figure II: 5.

The electrodes were housed in a small oven which was kept at a constant temperature of 37^{o}C. The door of this oven was modified by the insertion of a perspex window with holes to accommodate the electrode leads and sample feed tubing.

* Orion Reserach Inc., 11 Blackstone St., Cambridge, Mass. 02139, U.S.A

Fig II:5

Diagrammatic representation of the calcium selective electrode and reference electrode used in the measurement of serum ionised calcium concentration.


CALCIUM ELECTRODE
REFERENCE ELECTRODE
to electrometer
+
−
Ag/AgCl internal reference elements
internal aqueous filling solution (F_1)
reference electrode filling solution (F_2)
liquid ion exchanger
membrane spacer
sample blocks
ion exchange membrane
annular restrained flow liquid junction
sample from pump
plastic connecting tubing
sample waste

Samples were fed through the electrodes from 1 ml. disposable plastic syringes* via narrow bore plastic tubing, and a continuous non-pulsatile flow of 33 µl/min was maintained by a specially designed syringe pump (Orion, model 88 - 20).

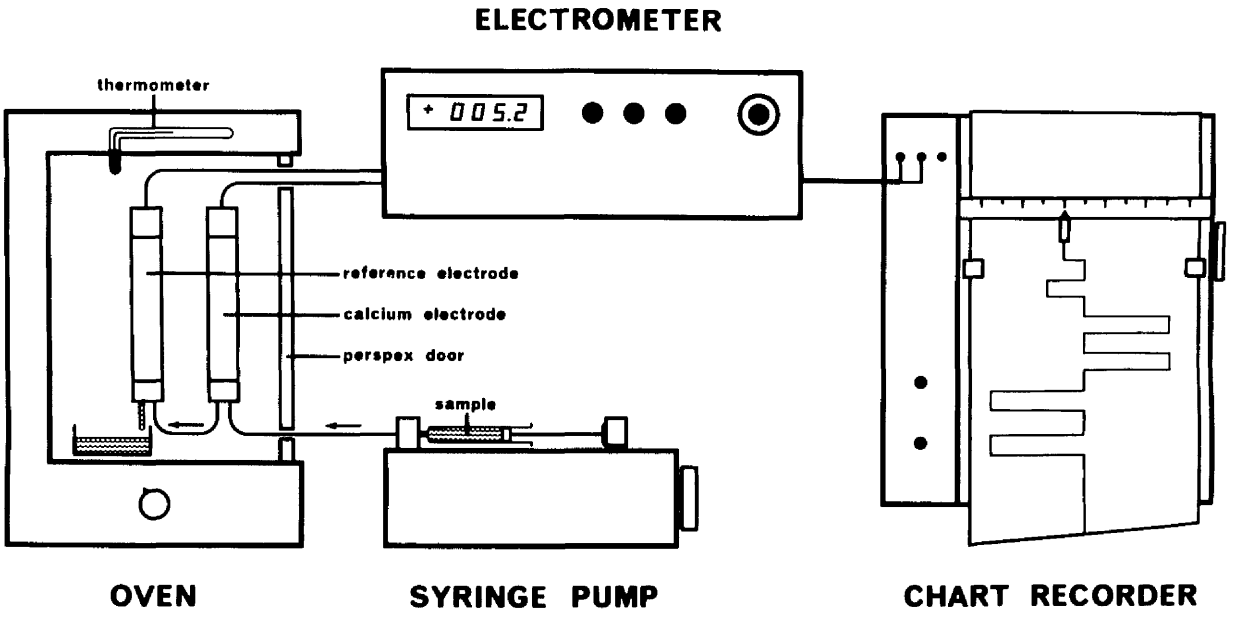
The electromotive force (EMF) of the cell was measured by a pH/mV electrometer (Orion, model 801), with a digital display accurate to ± 0.1 mV. A potentiometric strip chart-recorder (Servoscribe, RE 511) was connected to the recorder output of the electrometer to provide a continuous record of the EMF changes, the chart speed being set at 12 cm/hour. The principle advantage of the chart recorder over the digital display was in the evaluation of electrode stability and drift tendencies, but it also allowed further scale expansion of the EMF readings as required. Figure II: 6 summarises diagrammatically the instrumentation in toto.

When measurements of ionised calcium were performed on serum samples, a small amount of trypsin powder was added to each of these standard solutions (6 mg. per 10 ml.) in order to inhibit the accumulation of protein on the electrode membrane and in the connecting tubing. The trypsin was not used when measurements were restricted to aqueous standard solutions.

* Becton, Dickinson and Company Ltd., Dublin, Ireland.

Fig II:6

Diagrammatic representation of the total system of instrumentation employed in the measurement of serum ionised calcium concentration.


ELECTROMETER
thermometer
+ 005.2
reference electrode
calcium electrode
perspex door
sample
OVEN
SYRINGE PUMP
CHART RECORDER

Method of Operation

The calcium electrode was prepared according to the manufacturer's instructions (Instruction Manual; Orion Research Inc.). using the Orion serum calcium ion-exchange solution and internal reference solution. The reference electrode was filled with a solution of 1 mol/l KCl saturated with AgCl, which was prepared in the laboratory. After preparation, the electrodes were installed in the constant temperature cabinet and left for a minimum of 16 hours (i.e. overnight) to come to equilibrium at the required temperature of 37°C. Then, before use, the electrodes were shaken, as one would shake down a clinical thermometer, to dislodge any air bubbles which might have appeared in the filling solutions during warming.

At the start of a measurement run the reference electrode was examined to ensure that there was an adequate reservoir and unobstructed flow of the filling solution. 10 ml. of each standard solution was then placed in a clean plastic tube, trypsin powder was added for serum samples, and the tubes were suspended in a water bath at 37°C. Samples were drawn up in 1 ml. plastic syringes which were then sealed with closed Luer hubs and suspended in the water bath to maintain the sample at 37°C.

The base-line standard was run through the electrode first, until a stable EMF reading was achieved. This took a variable length of time from 30 to 120 minutes. A standard solution was

used to obtain a base-line standard. In a typical measurement run, once the electrcde had stabilised on this solution, another standard solution was run for about 5 minutes followed again by the original solution. This sequence was repeated with the base-line standard solution, before continuing with further aqueous standards or serum samples. The base-line standard was run before and after every other solution measured, and the difference in the electrode EMF between each serum sample and the base-line standard was noted from the digital display of the electrometer and measured from the chart recording. At the completion of a measurement run the tubing was cleared of the standard solution and left dry until the next run.

Measurement of sample pH

When the pH of the sample was measured (i.e. in all serum samples and in some aqueous standards) it was done immediately following the ionised calcium measurement in that sample.

The pH was measured using a conventional capillary electrode system (Electronic Instruments Ltd., model SHH 33 system) in conjunction with a standard expanded scale pH meter (Radiometer, pH M 22r). The glass electrode consisted of a pH sensitive glass capillary, phosphate buffer/KCl internal filling solution and Ag/AgCl internal reference element; the reference electrode was a calomel reference element in 3.8 mol/l KCl with single ceramic plug restrained flow liquid junction incorporated into a capillary

tube. Both half-cells were surrounded by water jackets through which water at 37°C was circulated from the water bath. Samples were introduced to the electrode system by means of suction by a pump.

A single phosphate buffer, pH approximately 7.4 at 37°C was used to calibrate the pH meter and the pH of this 'working' buffer together with the slope response of the electrode were periodically checked against ampoules of standard buffer solutions (Radiometer, Type S1510 pH 7.383 at 37°C, and Type S1500 pH 6.841 at 37°C). The pH measurements were performed in the following way. The working buffer, pre-heated to 37°C in the water bath, was aspirated through the electrode and the meter reading observed when stable. More buffer was aspirated and the meter reading observed again. Once a stable, repeatable reading was achieved, the meter was adjusted to read the pH of the buffer. The sample of unknown pH was then aspirated several times (usually 3 times for serum) until a stable reading was observed. This reading was noted and the pH of the buffer rechecked. Provided the second reading of buffer pH corresponded to its original setting, the pH recorded for the unknown sample was accepted, otherwise the whole procedure was repeated until satisfactory results were obtained. The sample pH was noted to the second decimal place and the precision of the measurement is estimated to be within the limits of $\pm$ 0.01 pH unit.

The electrode capillaries were left filled with working buffer solution when not in use. 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 $\left[Ca^{++}\right]$ 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 using a formula derived from theoretical studies of calcium binding to albumin (Pedersen, 1972a) and from the results of observations of the influence of pH on serum ionised calcium with varying albumin and calcium concentrations (Watson, 1975).

Reproducibility of Ionised Calcium Measurement

To assess the reproducibility of measurement during a single run, 20 ml. of fresh serum was prepared from a sample of blood from a normal adult. The serum was stored in a 20 ml. plastic syringe, free of air bubbles. Samples of 0.5 ml. were withdrawn into 1 ml. plastic syringes by means of a three-way stopcock, and, after pre-heating to 37^{o}C, the ionised calcium concentration and pH were measured. These measurements were performed on 20 samples

from this bulk source. The cell EMF was recorded by a chart recorder and the EMF readings from the digital display of the electrometer were also noted for each sample. For purposes of comparison the ionised calcium concentration for each sample was computed independently from the measured recorder deflections. The results obtained from the recorder revealed a range of 1.19 - 1.22 m.mol ./l. with a mean of 1.20 $\pm$ I.S.D. 0.009 m.mol ./l.(coefficient of variation of 0.76%). Thus the reproducibility would seem quite satisfactory in the presence of a reliable reference standard.

The normal range of serum ionised calcium concentration was established in an age and sex matched group of healthy subjects who were either members of staff or related to members of staff at the Centre for Rheumatic Diseases and in whom there was no evidence of disease associated with disorder of calcium metabolism. The samples of blood were obtained in the fasting state and under the same stringent conditions described for the patient group.

Calcium Absorption Study

Subjects

A separate group of 23 female patients with "definite" or "classical" rheumatoid arthritis and with skeletal evidence of osteoporosis had calcium absorption measurements carried out. Ten of these patients were receiving corticosteroid therapy (ranging from 5-10 mg. of prednisolone per patient per day) and the remainder were receiving standard non-steroidal anti-inflammatory

drugs. Unfortunately it was not possible to carry out these tests in the 23 patients who underwent the detailed biochemical and hormonal study.

Ten normal healthy female subjects with no known evidence of disease relating to calcium metabolism volunteered to act as controls. All were members of staff of the Centre for Rheumatic Diseases or relatives of same. It was not possible to arrange larger numbers for control purposes because of reluctance on the part of those approached to be admitted to hospital.

Due to the paucity of numbers of normal controls, an approach was made to Professor B.E.C. Nordin, M.R.C. Mineral Metabolism Unit at Leeds Royal Infirmay, who kindly supplied results from a larger number of normal controls employing exactly the same dose of calcium carrier and technique of measuring calcium absorption used in this study. From this source the results from 28 normal female subjects of similar age to local controls were used for comparative purposes. The age ranges of both normal groups and the patients with rheumatoid arthritis were comparable.

Method

The method of assessing calcium absorption was as described by Nordin (1967). Each subject was fasted for 12 hours prior to the study and then received orally 5 uCi of ^{47}Ca in a carrier of 50 mg. of calcium chloride. Following administration of the

entire dose of the radioisotope plus carrier, blood samples were taken at 30 minute intervals for a period of 3 hours. Each blood sample was centrifuged and the resultant plasma sample counted in a gamma scintillation counter (GAMMA-GUARD) for ^{47}Ca activity. In order to convert these plasma activity values into indices of calcium absorption account must be taken of the bodyweight of the subject since this determines the size of the plasma and extracellular fluid calcium pool in which the radioisotope is diluted. This stable calcium pool has been shown to correspond approximately to the plasma calcium concentration in milligrams per litre multiplied by 15 per cent of the bodyweight in kilograms. Thus the plasma activity of ^{47}Ca per litre is multiplied by 15 per cent of bodyweight (Kg) to provide a measure of the actual amount of radiocalcium present in the pool at any given time (Nordin, 1967).

HORMONE ASSAYS

Serum from aliquots immersed in ice were separated within twenty minutes and frozen at -20°C. These were used for the following analyses:

1) Parathyroid hormone: was assayed by two centres employing different immunoassay techniques:

Technique 1 Serum immunoreactive parathyroid hormone (i PTH) concentration was measured by a double antibody radioimmunoassay technique (Fairney, Jackson and Clayton, 1973) using a human

parathyroid adenoma culture media standard (Wilis, Fairney, Varghese, Tatler, Baillod and Moorehead, 1974). Professor Claude Arnaud gifted the parathyroid adenoma culture media standard and the Medical Research Council the parathyroid antiserum. The antiserum used cross reacts with both the amino and carboxyl terminal ends of the PTH molecule. The assay sensitivity and precision is such that it is capable of differentiating between low and normal levels of PTH. This assay was kindly performed by Dr. Angela Fairney at St. Mary's Hospital, London.

Technique 2 In the second assay, plasma samples were assayed in two dilutions in duplicate, using plasma from patients with hypoparathyroidism as diluent. The assay procedure was a modification of that of Berson, Yalow, Aurbach and Potts (1963), and used Burrough's Wellcome Antiserum 211/32, purified bovine PTH (kindly supplied by Dr. John Potts, Jr.) as a standard and parathyroid hormone for radio iodination (75/52) from M.R.C. National Institute for Biological Standards and Controls. This assay was kindly performed by Mrs. Catherine Hillyard of Dr. I. MacIntyre's laboratory in the Royal Postgraduate Medical School, Hammersmith Hospital, London.

Calcitonin Assay Plasma samples were thawed and serial dilutions made in calcitonin free plasma. The assay procedure has been reported previously (Coombes, Hillyard, Greenberg and McIntyre, 1974). This assay was also carried out by Mrs. Catherine Hillyard.

25-Hydroxy-vitamin D estimation - Estimation of serum 25-hydroxy-vitamin D was performed (Haddad and Chyu, 1971) using Vitamin D replete rat kidney cytosol protein (McLaughlin, Fairney, Lester, Raggatt, Brown and Wills, 1974). The results obtained for 25-hydroxy-vitamin D were compared with measurements made on normal subjects taking into account the seasonal variation and interassay variation (McLaughlin, et al, 1974). Dr. John Babcock of the Upjohn Company, U.S.A. kindly supplied the 25-hydroxy ergocalciferol.

Gastrin Assay - Serum gastrin was measured using a sensitive and specific radio-immunoassay based on antibody raised in rabbits to synthetic human gastrin. Labelled hormone was prepared using modification of the chloramine-T method (Hunter and Greenwood, 1962) and separation of the free hormone from that bound to antibody was achieved with dextran coated charcoal (Buchanan and McCarrol, 1971). Cross reaction with cholecystokinin and pancreozymin is minimal and studies indicate that the hormone not only recognises the heptadecapeptide hormone, but also "big" gastrin (Yalow and Berson, 1970).

Glucagon Assay - Two antibodies raised to pancreatic glucagon were used in the glucagon assay, namely YY89 which reacts with the C-terminal and YY57 with the N-terminal region of glucagon (Flanagan, Buchanan and Murphy, 1974). The assay procedure has been previously reported in detail (Buchanan, 1973). These two hormone assays were kindly performed by Professor Keith D. Buchanan of Belfast University.

Urinary D-glucaric acid Excretion as an indication of liver microsomal enzyme induction was assayed using the procedure of Marsh (1963) as modified by Simmons, Davis, Dordoni and Williams (1974).

Urinary hydroxyproline was measured in each patient employing the Hypronosticon Kit manufactured by Organon Limited (Goverde and Veenkamp, 1972).

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

Radiological Assessment

Radiological films of hands and wrists obtained at the time of study were examined for evidence of bone and joint damage. Each finger joint, metacarpal bone, carpal bone and the radius and ulna were examined separately for erosions. One point was assigned to each erosion and five points to an area of total destruction of the articular surface. Twenty-nine areas in each hand and wrist (14 finger joints, 5 metacarpal bones, 8 carpal bones, the radius and ulna) were read in this way giving a maximum possible defect score of 290 (Sharp, Lidsky, Collins and Moreland, 1971). A similar grading system was employed to assess joint space narrowing and the highest possible score in this method was 216.

A corrected erosion or defect score (Dc) was obtained by dividing the total number of erosion points on a single film by the maximum possible erosion score. A similar calculation was made to provide a corrected joint space narrowing score (JSNc) (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, Fischer's F test for comparison of group variances and linear regression calculations for association between variables.

The consent of each patient included in the study was obtained after a full explanation of the implications involved and approval for the study was granted by the Ethical Committee of Glasgow Royal Infirmary.

RESULTS

The clinical laboratory details of the twenty-three female patients included in this study are shown in Table II:III. The patients had had their disease for a mean period of 6.0 years (range 1 to 20 years). Thirteen of the patients had passed the menopause, and results of rheumatoid factor and anti-nuclear factor titres, haemoglobin concentration and erythrocyte sedimentation rate were consistent with varying degrees of disease activity.

Table II:IV summarises the essential findings of the mineral metabolism screen. The serum calcium concentration was not significantly different from the values of our reference group. When account is taken of the serum albumin concentration, which was significantly lower than that of the reference group ($t = 9.7$; $p<0.001$), the corrected serum calcium concentration becomes significantly elevated ($t = 8.4$; $p<0.001$). Significant elevation in serum ionised calcium concentration was noted ($t = 4.23$; $p<0.001$) in the 23 patients with rheumatoid arthritis compared to the 23 healthy age and sex-matched control subjects. Eight of the patients with rheumatoid arthritis had serum ionised calcium concentrations in the hypercalcaemic range. It is interesting to note that although a positive correlation was obtained between serum ionised calcium and the observed serum calcium ($r = 0.55$; $p<0.005$)Fig. II:7a, when the serum calcium is corrected for serum albumin concentration this correlation becomes much more significant ($r = 0.71$; $p<0.001$). (Figure II:7b).

	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 hr
Mean	51.7 ±	6.0 ±	1.7 ±	45.5 ±	15.6 ±	18.2 ±	217 ±	12.2 ±	52.5 ±
S.D.	15.6	6.6	0.6	2.3	7.3	22.9	513	1.4	33.5

Table II: III

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

	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	Plasma Potassi mmol/l
Patient Group	1.13 ± 0.06	2.41 ± 0.13	2.58 ± 0.12	0.98 ± 0.2	318 ± (130 - 1490)	38.2 ± 3.9	24.3 ± 2.2	139.3 ± 2.2	4.1 ± 0.5
Normal Reference Group Ranges	1.07 ± 0.03	2.39 ± 0.1	2.39 ± 0.1	1.1 ± 0.15	80 - 280	46.9 ± 3.3	24.5 ± 1.8	140 ± 2.0	4.2 ± 0.4
t	4.23		8.4	2.81		9.7			
p	<0.001	N.S.	<0.001	< 0.01	*	<0.001	N.S.	N.S.	N.S.
Number Above Reference Range	8	1	7	0	10	0	0	1	1
Number Below Reference Range	0	1	0	5	0	4	1	0	3

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

Table II: IV

Results (Mean ± I.S.D.) of mineral metabolism screen in the patients with rheumatoid arthritis compared to the normal referenc

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 (I.P.E.)	Calcium / Creatinine Ratio (mmol/mmol)	TmCa/GFR mmol/l	Hydroxyproline / Creatinine Ratio mmol/mmol
106	5.4	83	0.96	+0.25	0.38	2.04	0.018
±	±	±	±	±	±	±	±
3.3	1.9	30	0.36	0.17	0.28	0.35	0.016
100							
±							
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
8.53							
<0.001	N.S.	N.S.	*	*	*	*	*
9	3	1	2	7	5	12	6
0	0	0	5	0	1	0	0

Table II: IV continued

Group. Ranges or mean ± I.S.D. when appropriate.

Fig II:7a

The correlation of the serum ionised calcium concentration with the corresponding total serum calcium concentration uncorrected for serum albumin. ($r = 0.55$; $p<0.005$).

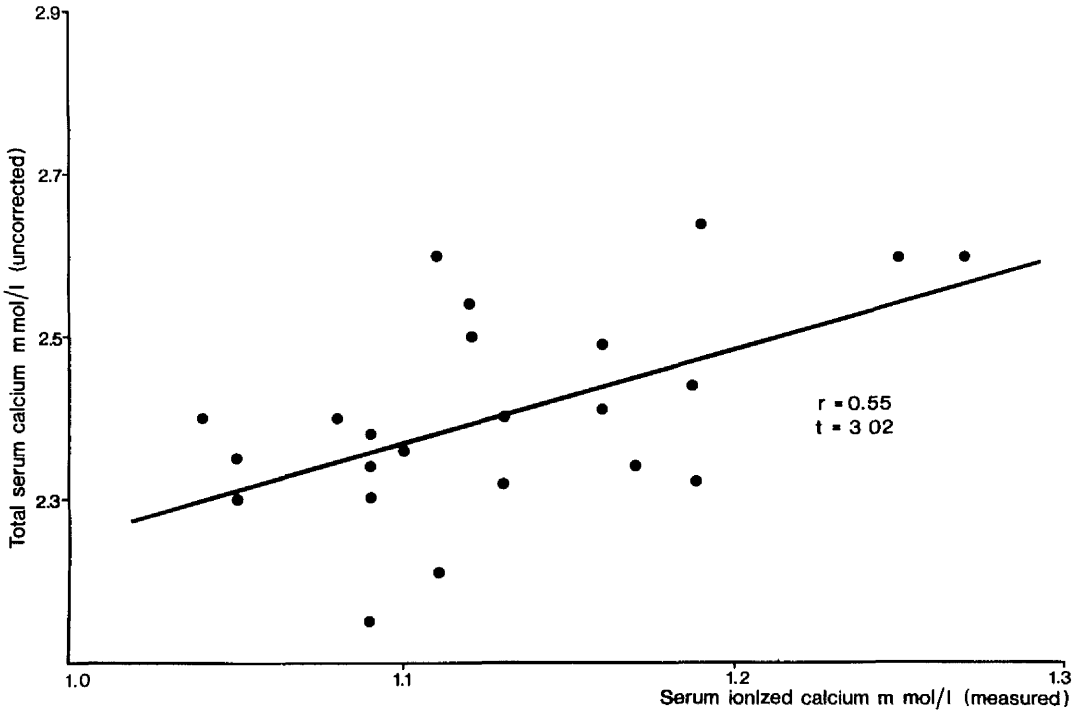
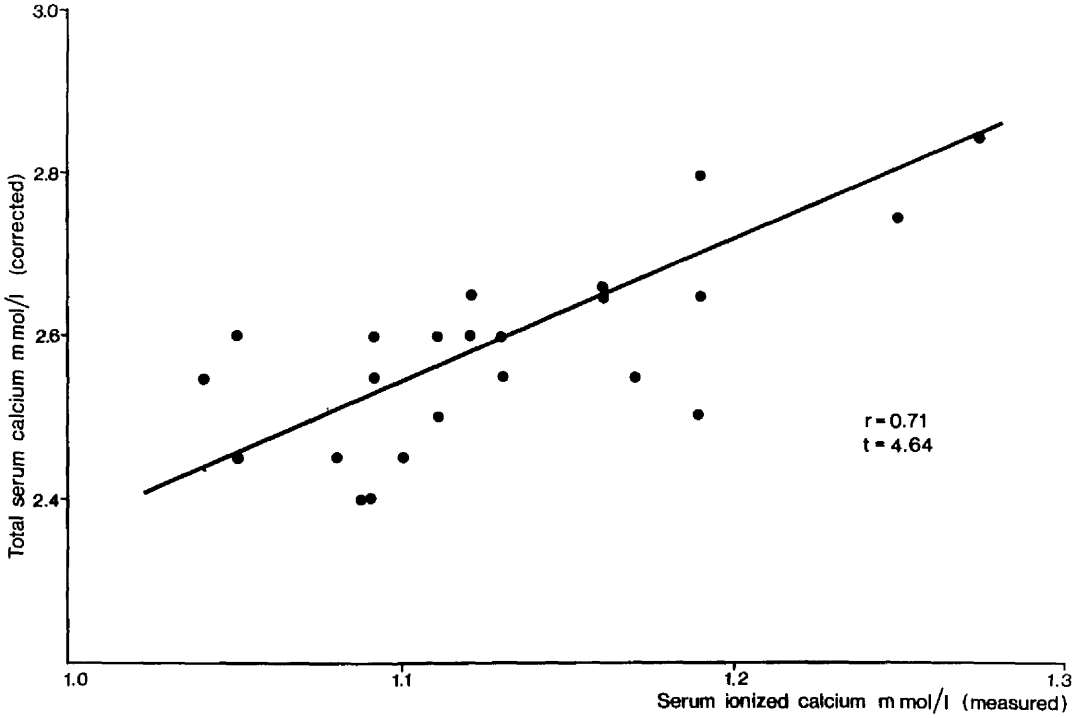

2.9
2.7
2.5
2.3
Total serum calcium m mol/l (uncorrected)
r = 0.55
t = 3 02
1.0
1.1
1.2
1.3
Serum ionized calcium m mol/l (measured)

Fig II:7b

The correlation of the serum ionised calcium concentration with the corresponding total serum calcium concentration corrected for serum albumin ($r = 0.71$; $p<0.001$).

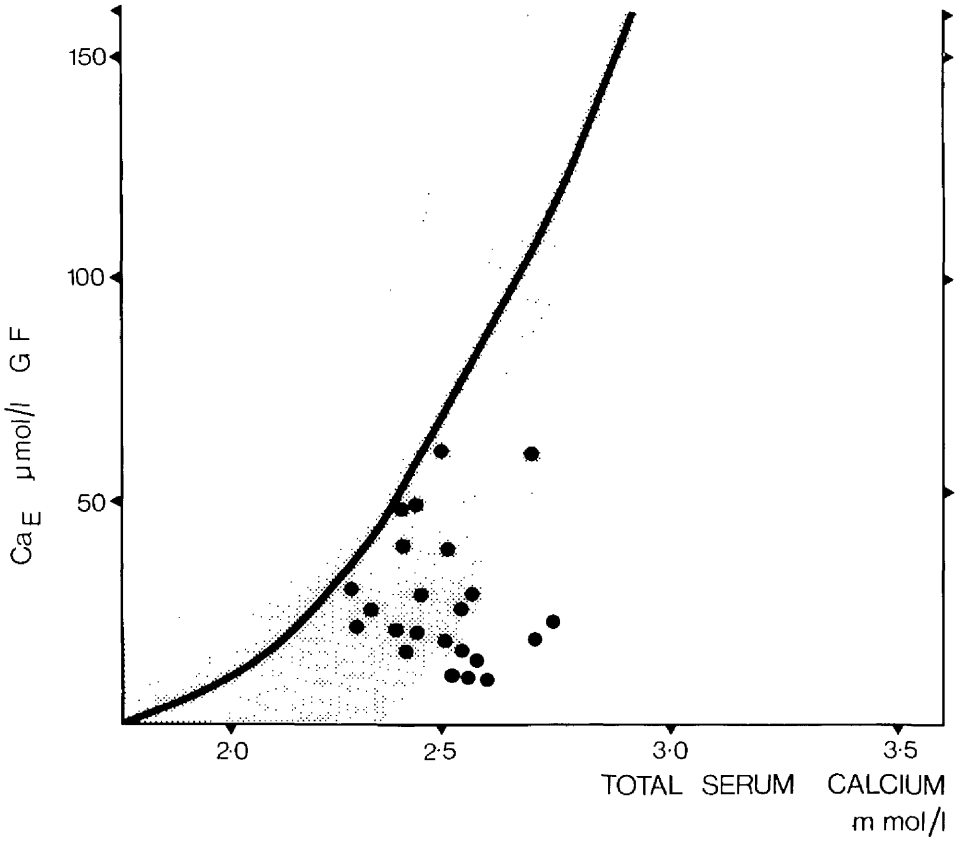

3.0
2.8
2.6
2.4
Total serum calcium m mol/l (corrected)
r = 0.71
t = 4.64
1.0
1.1
1.2
1.3
Serum ionized calcium m mol/l (measured)

Similarly, the serum phosphate concentration was significantly reduced ($t = 2.81$; $p<0.01$), and 10 of the rheumatoid patients had elevated levels of serum alkaline phosphatase. Plasma chloride concentration was significantly elevated in the group of patients with rheumatoid arthritis ($t = 8.53$; $p<0.001$) but plasma urea, potassium, sodium and total CO_2 and serum creatinine revealed no difference from the control group. Five patients had elevated calcium/creatinine ratios. Examination of the indices of renal handling of phosphate revealed that five patients had reduced renal tubular reabsorption of phosphate ($TmPO_4/GFR$) and seven had increased phosphate excretion (I.P.E.). When CaE is plotted against total serum calcium concentration (Figure II:8) it can be seen that the results are to the right of the mean value and that seven are outwith the established limits of normality, thus indicating evidence of increased renal tubular reabsorption of calcium (Marshall, 1976).

The results of theoretical maximal tubular reabsorption of calcium TmCa/GFR also show a general increase compared to normal values (Marshall, 1976). When the serum ionised calcium, serum phosphate and alkaline phosphatase are grouped according to their "normality" (Table II:V) the following facts emerge. Of the twenty-three patients only seven had all three results in the "normal" range. Of the eight patients with elevated serum ionised calcium, six had biochemical patterns which might be encountered in a hypercalcaemic hyperparathyroid state.

Fig II:8

The relation between calcium excretion (Ca_F) per 100 mls of glomerular filtrate and serum calcium concentration providing indices of tubular re-absorption of calcium, in 23 patients with rheumatoid arthritis. The thick black line represents the mean normal values and the stippled areas are the limits of normality.


150
100
50
Ca_E µmol/l G F
2·0
2·5
3·0
3·5
TOTAL SERUM CALCIUM
m mol/l

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	↑	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 II: V

Grouping of combinations of "normal" and "abnormal" serum ionized calcium, phosphate and alkaline phosphatase results.

Investigations of serum alkaline phosphatase with other tests of liver function in the form of serum bilirubin, serum AST and ALT are recorded in Table II:VI. It can be seen that the mean serum alkaline phosphatase lies above the upper limit of the normal range. Indeed 10 of the 23 patients had elevated serum alkaline phosphatase levels, whereas there was no patient who showed elevations of serum bilirubin, AST or ALT. One patient had an extremely high serum alkaline phosphatase and this was associated with the highest serum ionised calcium concentration. The results of the urinary hydroxyproline estimation showed that nine of the patients with rheumatoid arthritis had levels in excess of the normal limits (0.05 - 0.17 m.mol/24h). However, when these results were subsequently recalculated for accuracy of interpretation in terms of the hydroxyproline/creatinine ratio only six patients had abnormally high results (Table II:IV).

Urinary hydroxyproline is regarded as providing evidence of the rate of collagen destruction and also has been shown to relate to bone destruction (Turek and Goverde, 1973). Since, the fasting calcium/creatinine ratio also suggests possible evidence of bone destruction (Nordin, Horsman and Aaron, 1976) the results for urinary calcium and hydroxyproline excretion were correlated in the patients studied (Figure II:9). Their indices were not expressed in terms of the creatinine level since it was possible that a spurious correlation might be reached if each index was divided by the same denominator. It can be seen that there is a highly significant positive correlation ($r = 0.54$; $p < 0.005$) between these indices.

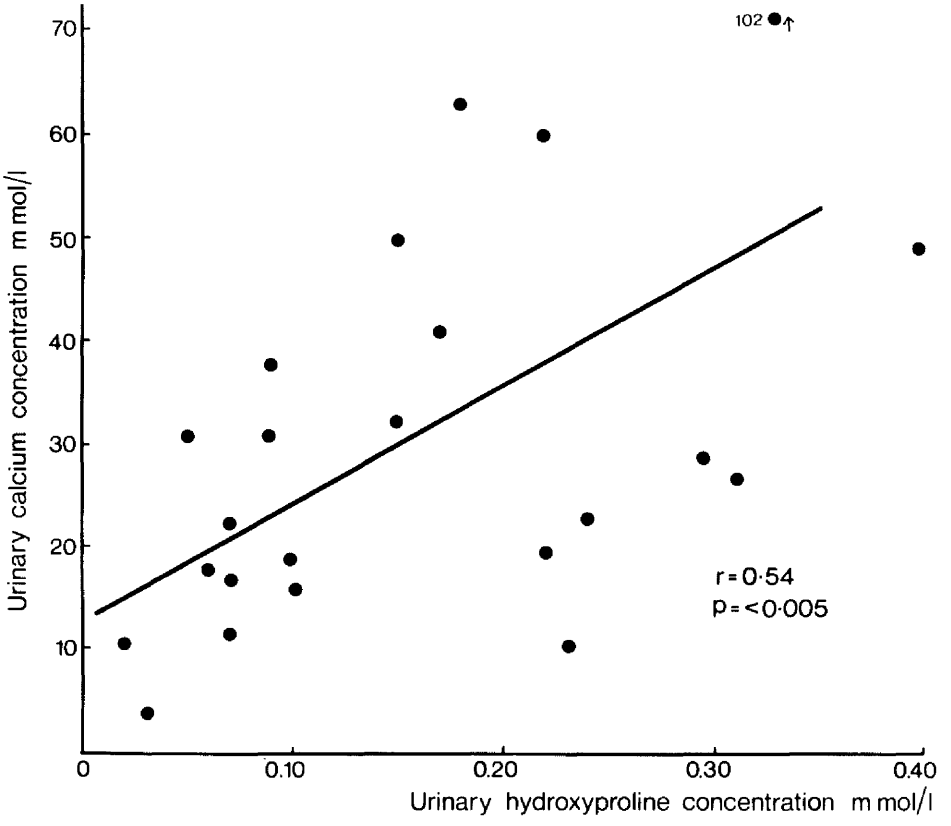
	Serum Alkaline Phosphatase u/l	Serum Bilirubin μ mol/l	Serum Aspartate Transaminase (S.G.O.T.) u/l	Serum Alamine Transaminase (S.G.P.T.) u/l
Patient Group Mean	318	4.7	23.4	16.7
Patient Group Range	130 - 1490	2 - 14	14 - 31	11 - 28
Reference Range	80 - 280	3 - 14	12 - 38	8 - 41
Number of Patients with Results Above Upper limit of reference Range.	10	0	0	0

Table II: VI

The results (mean $\pm$ I.S.D.) of serum alkaline phosphatase, bilirubin, S.G.O.T. and S.G.P.T. in the patients with rheumatoid arthritis, compared to the reference group.

Fig II:9

The correlation between the urinary hydroxyproline concentration and urinary calcium concentration obtained from 23 patients with rheumatoid arthritis in the fasting state.


102
70
60
50
40
30
20
10
0
0.10
0.20
0.30
0.40
Urinary calcium concentration m mol/l
Urinary hydroxyproline concentration m mol/l
r = 0·54
p = < 0·005

Serum magnesium concentrations in the patient groups, 0.80 $\pm$ 0.06 m.mol./l., were not significantly different from those of normal group, 0.82 $\pm$ 0.06 m.mol./l., (mean $\pm$ S.D.).

In an attempt to ascertain whether hypercalcaemia was a reflection of disease activity, those patients with elevated serum calcium concentrations were compared with those who had normal serum calcium concentrations, in terms of erythrocyte sedimentation rate, haemoglobin concentration, rheumatoid factor titre and functional grade. No significant difference between the groups emerged.

It was apparent that the patients in this study manifested many features suggestive of abnormalities in calcium metabolism, and the question arose as to whether these abnormalities were confined solely to those patients with hypercalcaemia. The patients were therefore divided into normo and hypercalcaemic groups as determined by the serum ionised calcium concentrations and their data re-analysed (Table II:VII).

It can be seen that those with serum ionised calcium concentrations exceeding 1.13 m.mol./l. had significantly higher levels ($t = 3.45$; $p<0.005$) of total serum calcium. In addition they also had lower serum phosphate concentrations and TmP/GFR and higher TmCa/GFR, but these results failed to reach significance at the 5 per cent level.

	Number	Serum Ionised Calcium mmol/l	Total Serum Calcium (corrected) mmol/l	Serum Phosphate mmol/l	TmP/ GFR mmol/l	TmCa/ GFR mmol/l	$\frac{\text{Calcium}}{\text{Creatinine}}$ Ratio	$\frac{\text{Hydroxyproline}}{\text{Creatinine}}$ Ratio
Hypercalcaemic		1.20	2.66	0.90	81.63	2.2	0.36	0.018
Patients		±	±	±	±	±	±	±
>1.13 mmol/l	8	0.04	0.12	0.24	48.16	0.14	0.3	0.010
Normocalcaemic		1.09	2.53	1.06	103.5	1.9	0.39	0.019
Patients		±	±	±	±	±	±	±
<1.13 mmol/l	15	0.03	0.08	0.16	25.6	0.5	0.3	0.23
t		2.15	3.45	1.97	1.43	1.42	0.22	0.12
p		<0.05	<0.005	<0.1 (N.S.)	N.S.	N.S.	N.S.	N.S.

Table II: VII

The comparison of the biochemical data between the normocalcaemic and hypercalcaemic patients as determined by their serum ionised calcium concentration.

Calcium Absorption Study

Table II:VIII demonstrates the number of subjects in each group and it can be seen that there is no significant difference in their respective mean ages.

Group A represent the normal values obtained from Professor Nordin, hereafter designated NORMALS (N) and Group B represent the normal values obtained in this study, hereafter designated NORMALS (K).

The plasma ^{47}Ca activity curves for each of the four groups are seen in Figure II:10. It can be seen that both the groups of patients with rheumatoid arthritis appear to have a higher rate of calcium absorption compared to the control group (K). Although this trend is also evident when the patients with rheumatoid arthritis are compared to the control group (N) the difference is not so marked.

The significance of these results are demonstrated more clearly by examining the mean two hour peak ^{47}Ca plasma activity levels of each of the four groups (Table II:VIII). It can be seen that both groups of patients with rheumatoid arthritis have significantly higher plasma ^{47}Ca levels compared to the local normal group - NORMALS (K). However, although the calcium absorption curves of both the rheumatoid groups indicate increased absorption compared to the Normal group (N) (Figure II:10) when the two hour peak plasma ^{47}Ca activities are compared, only the non-corticosteroid treated patients demonstrate evidence of increased calcium absorption which is statisically significant. Also it is important to note that there is a statistically significant difference between the Normal

Group	Number	Age in Years	2 hour peak per cent Plasma ^{47}Ca activity
A Normals (N)	28	54 ± 11.3	24.5 ± 7.2
B Normals (K)	10	56.4 ± 11.0	18.4 ± 9.3
C Rheumatoid Arthritis (non C.S.)	13	59.8 ± 8.0	28.8 ± 7.0
D Rheumatoid Arthritis (C.S.)	10	56.2 ± 9.0	32.0 ± 15.0

Comparison between groups.

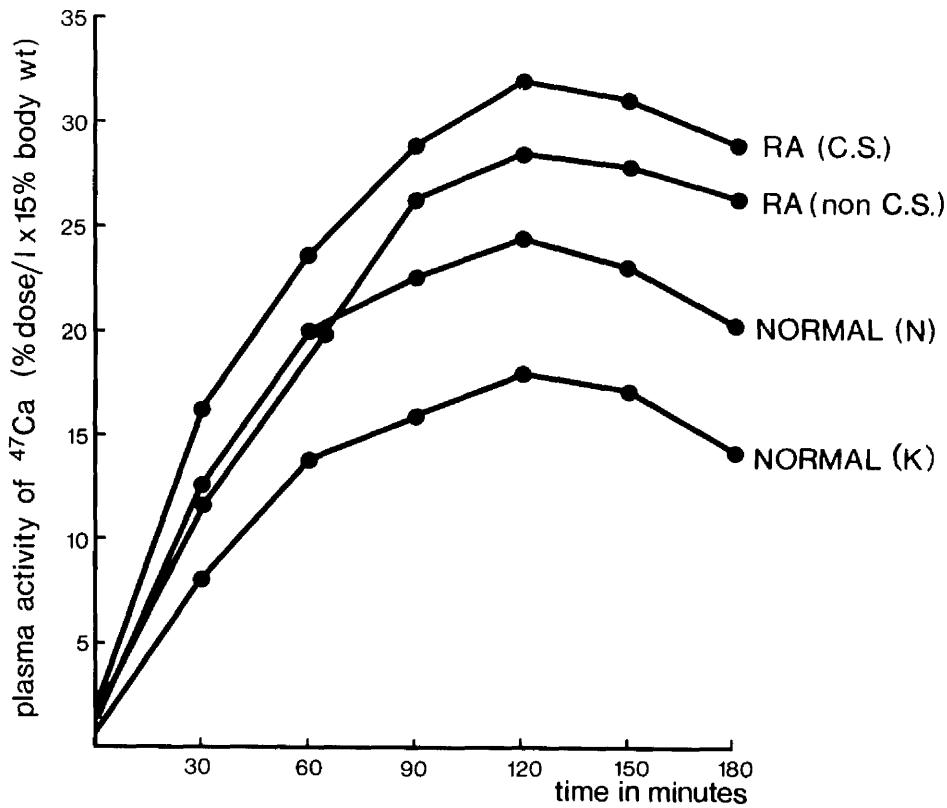
A and B	t 2.12	p	< 0.025
A and C	t 1.79	p	< 0.05
A and D	t 1.52	p	N.S.
B and C	t 3.06	p	< 0.005
B and D	t 2.43	p	< 0.025

Table II: VIII

The results of the two hour peak plasma ^{47}Ca activity (x 15 per cent body weight) in each of the four groups studied. All results are expressed as mean ± I.S.D. and statistically significant differences between the groups are noted below the table.

Fig II:10

The plasma activity of ^{47}Ca at intervals of 30 minutes for each of 4 groups, viz. patients with rheumatoid arthritis receiving corticosteroid therapy - R.A. (C.S.), those not receiving such therapy - R.A. (non C.S.) and the normal control population from Professor B.E.C. Nordin - Normal (N) together with the results for local normal controls - Normal (K).


plasma activity of ^{47}Ca (% dose/l x 15% body wt)
35
30
25
20
15
10
5
30
60
90
120
150
180
time in minutes
RA (C.S.)
RA (non C.S.)
NORMAL (N)
NORMAL (K)

Group (N) and the Normal Group (K) with the former showing a higher mean plasma ^{47}Ca level than the latter.

HORMONE ASSAYS

The results of the parathyroid hormone estimation, using both assays revealed no raised levels of this hormone. In the first technique it was possible to obtain absolute results and these have been tabulated. In the second technique all results lay within the normal range (100 - 730 pg/ml) and have not been tabulated since it was not possible to establish abnormally low results in this assay. The results of serum PTH (Technique 1), calcitonin, 25-hydroxy-vitamin D and gastrin measurements, together with serum calcium parameters, urinary calcium and hydroxy-proline measurements, and indices of bone damage, are shown in Table II:IX.

From this table it can be seen that the immunoassay result for serum PTH indicates that none of the patients had an elevated result and indeed, sixteen patients had results below the reference range. Two patients were noted to have elevated serum calcitonin levels and both of these had high serum ionised calcium concentrations together with low PTH levels. One patient had an elevated serum gastrin (patient 11) and also had elevated serum calcitonin and ionised calcium levels, together with a low PTH level and a high serum alkaline phosphatase. Analysis of the 25-OH-vitamin D levels revealed that one patient (patient 23), had a reduced concentration while all the others were within the normal range. It is interesting to note that this patient also had an elevated urinary D-glucaric

	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 Hydroxy-proline / Creatinine Ratio	Meta-carpal Index (MI)	Joint Space Narrowing Score (JSNc)	Erosion Score (Dc)
	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	1.16	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		51.7	38.2	2.40	2.58	1.13	227		27.5	144.5	0.38	0.018	49.9	0.246	0.247
		±	±	±	±	±	±		±	±	±	±	±	±	±
S.D.		15.6	4.0	0.10	0.12	0.06	129		6.2	254.5	0.28	0.016	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	70	>0

Table II: IX

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 (M.I., J.S.N.c, Dc.) and corresponding normal reference ranges.

acid concentration. The mean glucaric acid concentration for the whole group was 31.2 m.mol/l. (normal range 10-46 m.mols./l).

Immunoassay of glucagon employing both the 57 and 89 antibodies showed normal levels.

Radiological Assessment

A significant correlation was found between the erosion score, Dc, and the metacarpal index ($r = 0.6$, $p<0.01$) and the erosion score and joint space narrowing score (JSNc) ($r = 0.77$, $p<0.001$). No correlation however existed between serum ionised calcium concentrations and any of these indices of joint destruction and osteoporosis.

DISCUSSION

The first and most crucial question in this study 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 subjects and in the methodology employed in determination of 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 conditions. The temperature at which the estimations were performed was constant ($37^{o}C$) for all samples, and the results were corrected for pH and sodium interference. Hence, there are strong grounds for accepting that eight of the 23 patients with rheumatoid arthritis did have elevated serum ionised calcium concentrations.

It might be argued that the mean control value of serum ionised calcium concentration was somewhat lower (1.07 m.mol./l.) than that reported by other workers (Table II:II). 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 three other groups have reported serum ionised calcium determinations using the ion electrode method employed in the present study whilst 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 m.mol./l. respectively, but Ryden et al (1976) obtained a mean value of 1.03 m.mol./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, quote a temperature at which they carried out their estimations. Varghese (1973) using spectrophotometry obtained a mean value slightly lower (1.04 m.mol./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 per cent in 20 samples from the same bulk source (vide supra). Thus, it seems reasonable to conclude that the eight patients with rheumatoid arthritis had definite, albeit in some patients slight, elevations in serum ionised calcium levels.

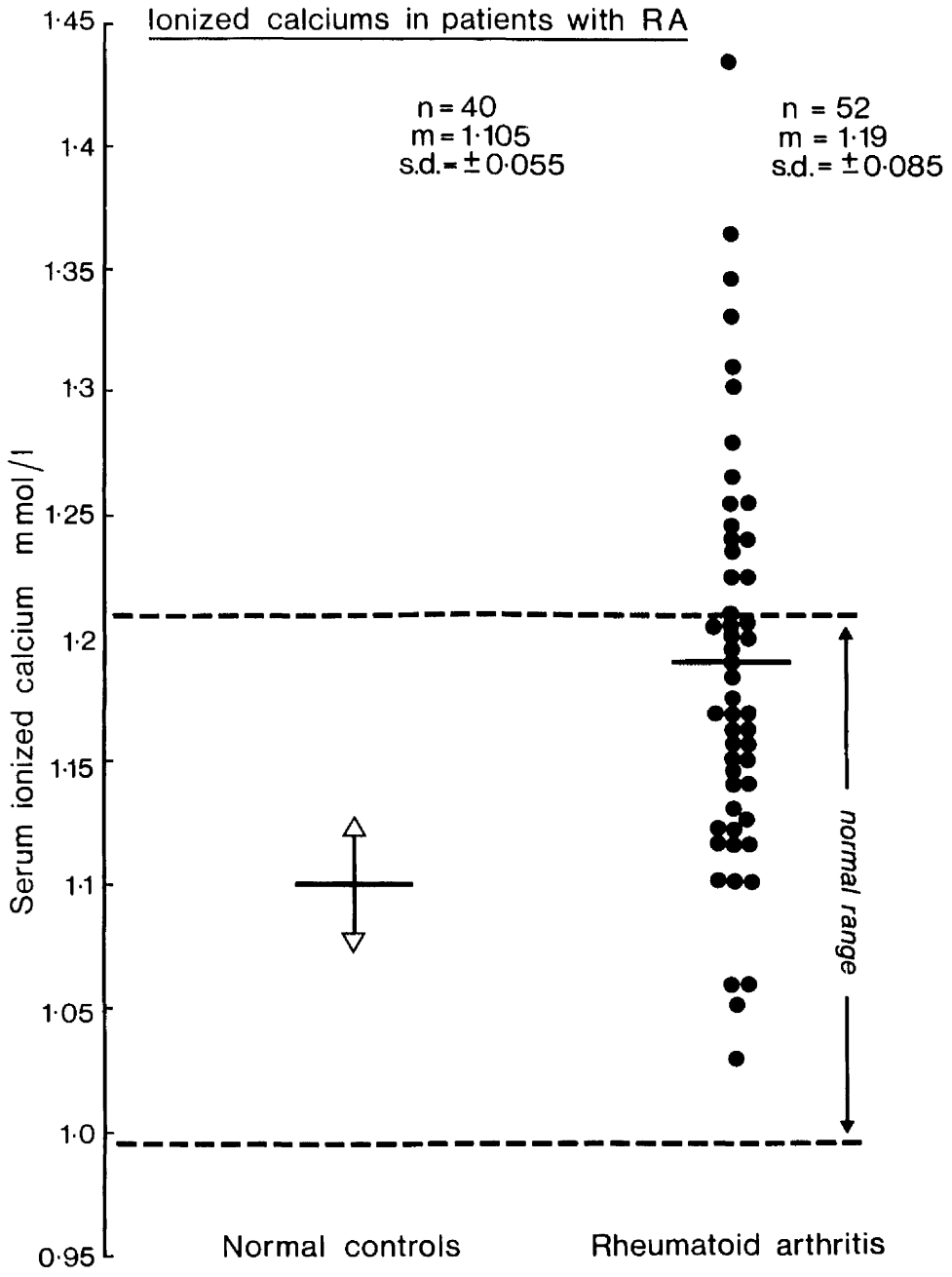
Further confirmation of the finding of raised serum ionised calcium levels in rheumatoid arthritis has been obtained in a study of 52 patients with the disease studied personally in South Africa. Approximately one third (17) of the patients had elevated serum ionised calcium levels when compared to an age and sex matched healthy control group (Meyers, Laidley, Levine, Milne, Salant and Kennedy, 1976). I am grateful to Dr. Anthony Meyers of Professor Bothwell's

Department of Medicine in Johannesburg for the estimations of serum ionised calcium concentrations (Fig. II:11). The method employed an Orion calcium ion exchange electrode model SS-20 and the temperature at which estimations were performed was 37°C.

In the introduction to this chapter the rationale and methodology of correcting serum calcium concentration for low levels of serum albumin was discussed in depth because of the relevance to this current investigation. The correction factor employed in the present study is central to the investigation, and its validation is therefore crucial. As previously discussed the regression line of serum calcium concentration on serum albumin level (Fig. II:4) when extended to the Y axis when the albumin concentration is zero intersects at 1.43 m.mol./l. which corresponds to the serum ultrafilterable calcium concentration found by others (Robertson, 1969; Pedersen, 1970; Rose, 1972; Payne, et al, 1973). Of greater 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 seven had hypercalcaemia as estimated by the correction formula for a low serum albumin concentration. Six of these seven patients had elevated serum ionised calcium levels. The patient (patient 4) (Table II:IX) who had a normal value for serum ionised calcium (1.12 m.mol./l.) had a corrected total serum calcium concentration just over the upper limit of normal (2.65 m.mol./l.). Of the 16 patients who had normal values for corrected serum calcium concentrations two had elevations of serum ionised calcium (patients

Fig II:11

The serum ionised calcium concentrations obtained from 52 female patients with rheumatoid arthritis and 40 sex-matched normal controls, in a recent South African study employing an ion selective electrode.


Ionized calciums in patients with RA
n = 40
m = 1·105
s.d. = ±0·055
n = 52
m = 1·19
s.d. = ±0·085
Serum ionized calcium mmol/l
1·45
1·4
1·35
1·3
1·25
1·2
1·15
1·1
1·05
1·0
0·95
normal range
Normal controls
Rheumatoid arthritis

14 and 15; Table II:IX). There is thus reasonable concordance between the corrected total serum ionised calcium concentration and the serum ionised calcium level, and this supports 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 ($r = 0.71$; $p<0.001$) is higher than with the uncorrected total serum calcium ($r = 0.55$; $p<0.005$) (Fig. II:7a and b).

Hypercalcaemia does not appear to be a constant phenomenon in rheumatoid arthritis. The 23 patients in the study were selected on the basis that they had been noted to have hypercalcaemia six months prior to the study as defined by correcting the total serum calcium level for hypoalbuminaemia. However, at the time of study only seven patients had an elevated corrected total serum calcium level.

The serum ionised calcium is the physiologically active fraction of total serum calcium, and consequently, when elevated, 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, Whaley, MacSween, Nuki,

Dick and Buchanan, 1975). Unfortunately 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 six patients with elevated hydroxyproline/creatinine ratios four had elevated serum alkaline phosphatase levels. However, six 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, Rohde and Schmidt, 1969) but not all workers (Ziff, Kibrick, Dresner and Giribetz, 1956) in patients with rheumatoid arthritis. The significant correlation between the increased urinary calcium and hydroxyproline results (Fig. II:9) suggests that the underlying cause for this is likely to be increased bone turnover. The question arises as to 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 an elevated serum ionised calcium level had 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 separate study of calcium absorption were of further interest.

Calcium Absorption Study

This separate study was an attempt to assess calcium absorption in patients with rheumatoid arthritis and it was interesting to observe that the results suggested that patients with this disease appeared to exhibit increased calcium absorption irrespective of treatment with oral corticosteroid therapy. This was less evident when comparing the results of the rheumatoid patients with Professor Nordin's normal group (N) but the trend was still present. The apparent non effect of corticosteroids on calcium absorption in rheumatoid arthritis is of interest since these drugs generally diminish calcium absorption (Miravet, Hioco, Debeyre, Dryll, Ryckewaert and De Seze, 1966; Gallagher, Aaron, Horsman, Wilkinson and Nordin, 1973) except in primary hyperparathyroidism where they appear to have little effect.

This study, however, suffers from several disadvantages. Firstly, it is generally acknowledged that virtually all methods of measuring calcium absorption are relatively imprecise. Even with double isotope methods, calcium pool exchange rates and processes are difficult to take account of, and therefore even in the best hands interpretation of calcium absorption studies must be treated with caution.

The interpretation of the results in this study are further complicated because the method employed was perhaps a little outdated relying on a single isotope. Also when considering possible comparisons with results of other workers, the question of calcium carrier doses is of some importance, since differences in these will produce variation in the shape of resultant calcium absorption

curves, thus negating such comparisons. There is no clear agreement on what is the ideal carrier dose, e.g., Nordin's group now favour 20 mg. calcium chloride (personal communication) whereas Dr. I.T. Boyle in Glasgow now favours 100 mg. (personal communication) in contrast to this study where 50 mg. calcium chloride was employed in all groups.

In addition, the number of controls obtained locally were unfortunately rather small, although their mean age was comparable with that of both groups of patients with rheumatoid arthritis. The relevance of comparing the results of the study with the normal results obtained from Professor Nordin may also be questioned since although the technique employed was exactly similar, the time and place of study were different.

These constraints therefore, mitigate against any definite conclusion being reached regarding calcium absorption in rheumatoid arthritis. Nevertheless, accepting these provisos, the results of the study do seem to indicate that there is sufficient evidence to suggest abnormalities in calcium absorption in patients with rheumatoid arthritis thus warranting further more accurate assessment of calcium absorption preferably carried out in a specialised calcium metabolic unit.

At this juncture in view of the biochemical evidence in one group of patients with rheumatoid arthritis and the calcium absorption evidence (although not entirely satisfactory) in another group a trend towards hyperparathyroid-like activity seemed possible.

HORMONE ASSAYS

The crucial test for parathyroid overactivity is regarded as the direct measurement of serum PTH. By both methods of immunoassay the serum levels of PTH were normal or reduced. Serum calcitonin levels were elevated in two patients only, and it is of interest that one of those had the highest serum ionised calcium concentration (Patient 11, Table II:IX). 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 immuno-reactive gastrin levels (Rooney, Kennedy, Gray, Sturrock, Buchanan and Dick, 1976). This suggests a possible link between enteric hormones and anomalies in calcium metabolism occurring in rheumatoid arthritis. However, only one of the patients in the present study (Patient 11, Table II:IX) had an elevated serum immuno-reactive gastrin level, although it is of interest that this patient had the highest serum ionised calcium concentration. In addition this patient had an elevated serum calcitonin level, and it might be due to the fact that hypergastrinaemia can stimulate calcitonin secretion in man (Hennessy, Gray, Cooper and Ontjes, 1973). Serum glucagon levels were normal in all patients studied.

The role of vitamin D in calcium metabolism in patients with rheumatoid arthritis to date has not been thoroughly investigated. In a recent study of five patients with rheumatoid arthritis, bone biopsy evidence of osteomalacia and secondary hyperparathyroidism was found 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 results in this study revealed only one abnormally low result, in a patient with associated high level of urinary D-glucaric 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, Birge, Scharp and Avioli, 1972; Rowe and Stamp, 1974). A number of anti-rheumatic 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-glucaric 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 reported in the first chapter of this thesis, no evidence of osteomalacia was found. In addition the absence of supportive biochemical evidence in this study 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 that that in the general population. Examination of the articular bone damage indices 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.

In this context it is interesting to note that the correlation coefficient between JSNc and Dc observed in this study compares very closely to the correlation coefficient between the same two indices in another study of radiological abnormalities occurring in rheumatoid arthritis (Sharp, et al, 1971) lending further support to the parallel progression of the two lesions in this disease.

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 a not unlikely possibility in a disease with such clinical variation.

Another variable which might explain the elevated serum ionised calcium levels is administration of anti-rheumatic 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 prior to the commencement of the study, and additionally there is no previous evidence in the literature 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 (32 per cent) (Fig. II:11) had approximately the same incidence of increased serum ionised calcium levels and all were non-fasting, and were currently receiving non-steroidal anti-inflammatory drugs. Northover (1973) has shown, in vitro, using amounts of indomethacin in excess of normal therapeutic doses, 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 as to 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 immuno-assay 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 immuno-assay procedures. An alternative explanation is that the hypercalcaemia may be the result of effects of prostaglandin E. Prostaglandin levels are increased in the synovial fluid in rheumatoid arthritis (Higgs, Vane, Hart and Wojulewski, 1974), and Robinson Tashjian and Devine (1975) have shown in in vitro bone culture

studies 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 elevated, 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.

Another substance with PTH-like action is osteoclast-activating factor, OAF (Horton, Raisz, Simmons, Oppenheim and Mergenhagen, 1972), a substance which has been isolated from lymphocytes. The effects of OAF on bone in vitro resembles those of PTH (Raisz, et al, 1975) but effects of injection of equipotent in vitro doses of the two 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 also require further investigation.

SECTION 2

Prevalence of Hypercalcaemia in Rheumatoid Arthritis and other Arthritides

The studies described in 23 patients with rheumatoid arthritis suggest that hypercalcaemia is not uncommon in this disease. To ascertain the prevalence of hypercalcaemia, and any effect of corticosteroid therapy upon it, a large number of patients with the disease were studied. Since the measurement of serum ionised calcium is technically very difficult and time-consuming, corrected total serum calcium levels were utilised. It has been previously shown in this chapter that there is a high correlation between the serum ionised fraction and the corrected total serum calcium level (Fig II: 7b), thus reasonably validating the use of the latter for epidemiological purposes. In addition, patients with other arthritides were studied to determine whether hypercalcaemia was peculiar only to patients with rheumatoid arthritis.

Patients Studied

The clinical and laboratory data in 364 patients with "definite" or "classical" rheumatoid arthritis (Ropes, et al, 1959) are shown in Table II: X. The age and sex distribution and duration of arthritis in patients with osteoarthritis, ankylosing spondylitis, psoriatic arthritis, and gout, are shown in Table II: XI. The diagnostic criteria for these diseases were as described in Copeman (1978). All patients included in this study were non-fasting and receiving their current anti-rheumatic therapy.

Group	Number of Patients	Age (years)	Age at menopause (years)	Duration of Arthritis (years)	Reciprocal of Rheumatoid factor titre	E.S.R. (mm/1st hour)	Hemoglobin (g/100ml)
A Male: Non-corticosteroid-treated	112	56.1 ± 10.6	-	11.1 ± 7.6	107 ± 340	44.9 ± 27.1	13.9 ± 1.5
B Male: corticosteroid-treated	23	59.9 ± 13	-	14.6 ± 9.0	252 ± 275	47.2 ± 26.0	13.7 ± 1.4
C All males	135	56.9 ± 10.2	-	11.7 ± 8.1	193 ± 164	45.2 ± 31.0	13.9 ± 1.3
D Female: Non-corticosteroid-treated	156	52.4 ± 24	45 ± 6.3	9.3 ± 10.3	217 ± 425	46.3 ± 22.5	12.2 ± 1.4
E Female: corticosteroid-treated	73	50.8 ± 14	45.9 ± 5.1	12.1 ± 10.6	352 ± 602	49.0 ± 28.0	11.5 ± 2.9
F All females	229	51.6 ± 12.4	45.1 ± 6.5	10.7 ± 12.8	264 ± 582	48.6 ± 26.2	12.1 ± 1.8
G All patients	364	53.6 ± 11.9	-	11.1 ± 11.2	237 ± 473	47.3 ± 28.1	12.7 ± 1.8

TABLE II : X Clinical and laboratory data (excluding biochemistry) in 364 patients with Rheumatoid Arthritis (mean ± 1 S.D.)

		Male Patients				Female Patients			
Disease	Total Number of Patients	Number	Mean duration of disease in yrs	Age range in years	Mean age in years	Number	Mean duration of disease in yrs	Age range in years	Mean age in years
Osteoarthritis	100	22	10.7	33-79	61.3	78	8.9	39-80	61.05
Ankylosing Spondylitis	136	120	9.5	20-71	37.6	16	13.5	21-73	43.5
Psoriatic Arthritis	60	31	3.6	18-60	35.4	29	6.2	18-69	41.3
Gout	38	37	9.4	20-61	50.1	1	28	68	68

Table II: XI

Age, sex distribution and duration of disease in the patients with osteoarthritis, ankylosing spondylitis, psoriatic arthritis and gout.

Laboratory Procedures

The methods for measuring the total serum calcium, total serum proteins, serum albumin and serum phosphate concentrations were as outlined in the Material and Methods section of the previous study. The serum alkaline phosphatase was estimated by the method of Kind and King (1954) as modified for Auto-Analyzer (Axelson, Ekman and Knutsson, 1965). The procedure for correcting serum calcium for corresponding albumin concentrations was as outlined previously.

Reference normal biochemical values were obtained from 280 healthy subjects of both sexes (age range 16-67 years).

Results

From Table IIX it can be seen that the clinical and laboratory data in the corticosteroid and non-corticosteroid treated male and female groups of patients with rheumatoid arthritis were comparable. The reciprocal of the titre of rheumatoid factor, however, was lowest in the non-corticosteroid treated male patients, and was significantly different from the other groups. No other significant differences were found. When biochemical results (serum albumin, globulins, calcium, phosphate and alkaline phosphatase) of patients were compared to those of the reference group (Table II: XII and Figure II: 12), the observed serum calcium results did not differ significantly, whereas the most noticeable abnormality was the outstanding hypoalbuminaemia in patients and, hence, the significant difference in serum albumin.

Group	Number of patients	Serum Albumin (g/l)	Serum Globulins (g/l)	Serum Calcium (m mol/l)	Serum Calcium (corrected) (m mol/l)	Serum Phosphate (m mol/l)	Serum Alkaline Phosphatase (K.A.U./100ml)
A Male:Non-Corticosteroid-treated	112	35.8 ± 4.6	37.1 ± 8.6	2.37 ± 0.15	2.59 ± 0.17	(104)* 1.078 ± 0.182	(101)* 12.6 ± 6.5
B Male:Corticosteroid-treated	23	34.7 ± 5.7	36.5 ± 7.2	2.39 ± 0.19	2.63 ± 0.24	(21)* 1.057 ± 0.197	(18)* 11.4 ± 2.5
C All males	135	35.2 ± 5.2	37.0 ± 9.1	2.38 ± 0.16	2.61 ± 0.16	(125)* 1.074 ± 0.184	(119)* 12.4 ± 6.1
D Female:Non-corticosteroid-treated	156	33.45 ± 4.9	35.8 ± 7.1	2.38 ± 0.14	2.63 ± 0.15	(146)* 1.104 ± 0.181	(153)* 12.7 ± 7.7
E Female:Corticosteroid-treated	73	32.3 ± 8.1	35.3 ± 6.2	2.38 ± 0.11	2.67 ± 0.13	(65)* 1.111 ± 0.213	(63)* 12.0 ± 7.1
F All females	229	33.0 ± 4.7	35.6 ± 8.1	2.38 ± 0.15	2.66 ± 0.16	(211)* 1.106 ± 0.190	(216)* 12.5 ± 7.5
G All patients	364	33.8 ± 5.0	36.1 ± 8.5	2.38 ± 0.15	2.64 ± 0.16	(336)* 1.094 ± 0.188	(335)* 12.46 ± 7.0
H Reference group	280	46.9 ± 3.3	30.9 ± 5.19	2.393 ± 0.101	2.393 ± 0.101	1.142 ± 0.164	8.0 ± 2.5
Comparison between group: A and H		t 23.2 p<0.001	t 7.10 p<0.001	t 1.49 NS	t 11.48 p<0.001	t 3.30 p<0.001	F 6.7 p<0.01
B and H		t 10.1 p<0.001	t 3.65 p<0.001	t 0.07 NS	t 4.70 p<0.001	t 2.26 p<0.05	t 5.59 p<0.001
C and H		t 23.9 p<0.001	t 7.20 p<0.001	t 0.86 NS	t 14.4 p<0.001	t 3.70 p<0.001	F 5.59 p<0.01
D and H		t 30.6 p<0.001	t 7.56 p<0.001	t 1.02 p NS	t 17.6 p<0.001	t 2.19 p<0 05	F 9.48 p<0.01
E and H		t 15.1 p<0.001	t 6.18 p<0.001	t 0.96 NS	t 16.9 p<0.001	t 1.1 NS	F 8.1 p<0.01
F and H		t 37.8 p<0.001	t 7.60 p<0.001	t 1.12 NS	t 21.9 p<0.001	t 2.24 p<0.05	F 9.00 p<0.01
G and H		t 39.9 p<0.001	t 9.58 p<0.001	t 1.29 NS	t 23.9 p<0.001	t 3.34 p<0.001	F 7.84 p<0.01

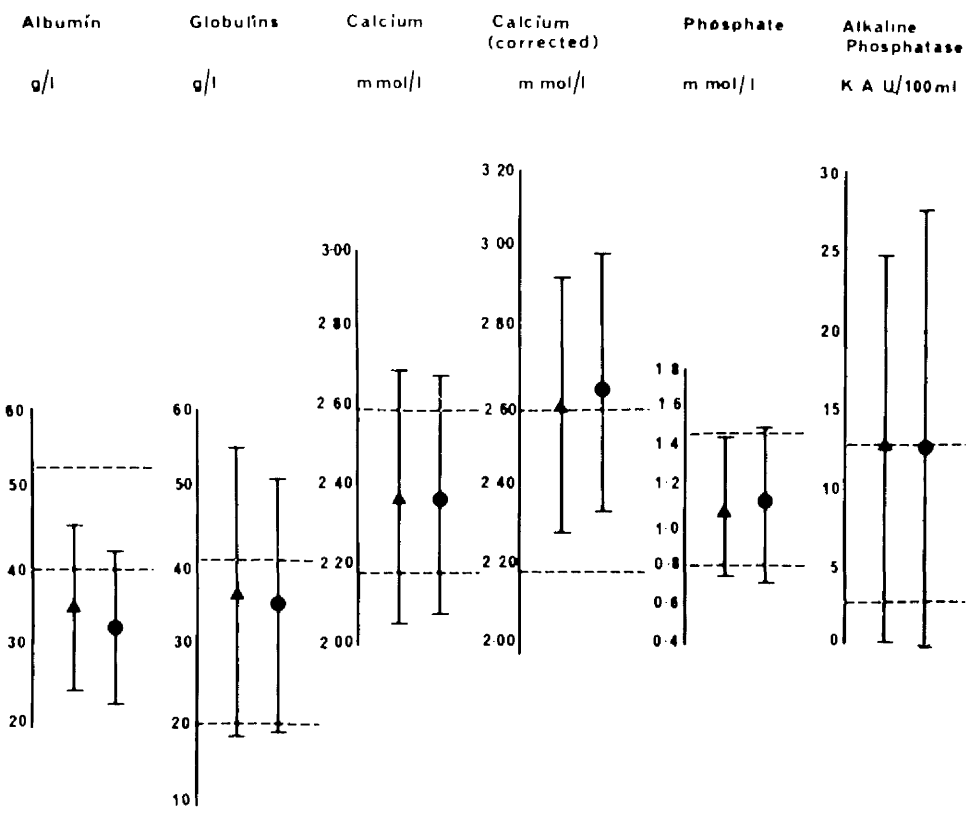
* Figures in brackets denote number of observations when different from those in first column.

TABLE II : XII Biochemical Data - 364 patients with Rheumatoid Arthritis

(mean ± 1 S.D.)

Fig II:12

The results of the biochemical analysis of sera from three hundred and sixty four patients with rheumatoid arthritis. The vertical bars represent the 2 S.D. spread on either side of the mean, the latter being represented by the symbol ▲ for males and o for females. The horizontal interrupted lines represent the mean ± 2 S.D. of results for the reference group.


Albumin
g/l
60
50
40
30
20
Globulins
g/l
60
50
40
30
20
10
Calcium
mmol/l
3·00
2 80
2 60
2 40
2 20
2 00
Calcium (corrected)
m mol/l
3 20
3 00
2 80
2 60
2 40
2 20
2·00
Phosphate
m mol/l
1 8
1 6
1 4
1·2
1·0
0·8
0·6
0·4
Alkaline Phosphatase
K A U/100ml
30
25
20
15
10
5
0

When the total serum calcium concentrations were corrected for their corresponding serum albumin concentration it can be seen (Fig.II: 12) that they now attain a statistically significant elevation compared to normal values.

Serum alkaline phosphatase activity and globulin concentration were also significantly higher. The mean values for serum phosphate concentration were lower than normal values but the difference in mean was very small (0.048 m.mol/l. Table II: XII) and although this was a statistically significant difference it was felt that this significance resulted mainly because of the comparison of two large populations.

The corrected serum calcium concentrations in both male and female patients bore no consistent relation to age (Table II: XIII), except in the male non-corticosteroid-treated subgroup where corrected serum calcium was significantly higher in those aged over 45 years than in those under 45 years old. The age of 45 years was chosen because in the studies in the first chapter 'osteoporosis' was found to be more pronounced in patients over this age.

When the number of patients with corrected serum calcium levels exceeding the two standard deviation range of the normal population (Table II: XIII) is considered, the majority are in the over 45 years of age group. Only three females had corrected serum calcium below the lower limit of normal.

	MALE PATIENTS							FEMALE PATIENTS							All Patients
				Corticosteroid Treated		Non-Corticosteroid Treated					Corticosteroid Treated		Non-Corticosteroid Treated		
	Total	<45 Years	>45 Years	<45 Years	>45 Years	<45 Years	>45 Years	Total	<45 Years	>45 Years	<45 Years	>45 Years	<45 Years	>45 Years	
Number	135	16	119	3	18	13	101	229	71	158	18	52	41	118	364
Mean Corrected Serum Calcium m mol/l ± 1 S.D.	2.61 ± 0.16	2.59 ± 0.3	2.62 ± 0.15	2.70 ± 0.3	2.60 ± 0.21	2.50 ± 0.15	2.61 ± 0.15	2.66 ± 0.16	2.63 ± 0.13	2.67 ± 0.17	2.63 ± 0.11	2.68 ± 0.13	2.64 ± 0.12	2.62 ± 0.18	2.64 ± 0.16
Number with Elevated results	65 (47%)	7 (44%)	58 (49%)	2 (66%)	8 (44%)	5 (38%)	59 (50%)	113 (49%)	33 (46%)	80 (51%)	11 (61%)	33 (63%)	22 (54%)	47 (40%)	178 (48.9%)
Number with low results	0	0	0	0	0	0	0	3 (1.5%)	0	0	0	0	0	3 (3%)	3 (0.8%)
Comparison with reference group: n = 280 Calcium 2.393 ± 0.101 m mol/l (mean ± 1 S.D.)	t 14.4 p<0.001	F 8.8 p<0.01	t 15.1 p<0.001	F 8.8 p<0.01	F 4.3 p<0.01	t 3.64 p<0.001	t 13.4 p<0.001	t 21.9 p<0.001	t 14.3 p<0.001	t 18.7 p<0.001	t 9.59 p<0.001	t 15.1 p<0.001	t 14.2 p<05001	t 12.8 p<0.001	t 23.9 p<0.001

TABLE II : XIII Serum Corrected Calcium Results in Clinical Groups Studied

When the results of serum calcium, serum phosphate, and alkaline phosphatase in individual patients (all results were available in 118 male and 212 female patients) are grouped according to their 'normality' and subsequently examined following correction of serum calcium for albumin (Table II: XIV), some interesting features become apparent. The number of patients who, prior to correction were considered to have normal serum calcium concentrations became considerably reduced in both sexes, after correction is made for their serum albumin concentrations. It is interesting to note that, even before correction for albumin concentration, four males and six females were already hypercalcaemic and, as has been shown already, these numbers are greatly increased when serum calcium was corrected for albumin. The last three patterns of abnormalities in Table II: XIV represent various combinations of abnormalities of serum calcium, phosphate and alkaline phosphatase, which may be encountered in the hypercalcaemic hyperparathyroid state.

No statistically significant correlation was found between corrected serum calcium levels and duration of arthritis or of any of the indices of osteoporosis-metacarpal index (Barnett and Nordin, 1960), femoral index (Barnett and Nordin, 1960), clavicular cortical width (Anton, 1969), and standard aluminium equivalent (Anderson, et al, 1966) (Table II: XV).

It can be seen from Table II: XVI that the corrected total serum calcium concentrations in the arthritides other than rheumatoid arthritis did not differ significantly from the control values.

Sex	Serum Calcium	Serum Phosphate	Serum Alkaline Phosphatase	Number of Patients in Each Group	
				Before Correction of Calcium for Albumin	After Correction of Calcium for Albumin
M F	N	N	N	72 (61%) 128 (60%)	42 (35%) 51 (24%)
M F	↓	N	N	6 (5%) 7 (3%)	0 2 (1%)
M F	↑	N	N	4 (3%) 6 (3%)	40 (34%) 88 (42%)
M F	↑	N	↑	1 (1%) 1 (0.5%)	14 (12%) 31 (15%)
M F	↑	↓	N	0 0	1 (1%) 2 (1%)
M F	↑	↓	↑	0 0	1 (1%) 3 (1.5%)

N = Normal Result
↑ = > Mean + 2SD of Reference Group
↓ = < Mean - 2SD of Reference Group

TABLE II: XIV Groupings of Combinations of Normal and Abnormal Serum Calcium, Phosphate and Alkaline Phosphatase Results

	Number of patients	r	p
Duration of R.A.	358	0.057	NS
Metacarpal Index	318	0.026	NS
Femoral Index	247	0.045	NS
Clavicular Cortical Thickness	256	0.015	NS
Standard Aluminium Equivalent	113	0.066	NS

TABLE II:XV

Correlation of Corrected Serum Calcium with Duration of Arthritis and Indices of Osteoporosis

Disease	Serum Calcium concentration (uncorrected) mmol/l	Serum Albumin concentration (g/l)	Serum Calcium concentration corrected mmol/l	Serum Phosphate concentration mmol/l	Serum Alkaline Phosphatase S I units
Osteoarthritis	2.37 ± 0.12	42.64 ± 4.40	2.45 ± 0.14	1.11 ± 0.15	209.0 ± 68.0
Ankylosing Spondylitis	2.36 ± 0.11	44.4 ± 3.90	2.42 ± 0.12	1.07 ± 0.13	285.7 ± 123.5
Psoriatic Arthritis	2.31 ± 0.10	42.45 ± 5.12	2.40 ± 0.12	1.08 ± 0.16	233.0 ± 93.5
Gout	2.38 ± 0.12	46.0 ± 2.86	2.40 ± 0.12	1.01 ± 0.16	223.0 ± 62.4
Normal	2.39 ± 0.10	46.9 ± 3.3	2.39 ± 0.10	1.10 ± 0.15	80-280 (range)

Table II: XVI

The mean ± I.S.D. of serum calcium, albumin, phosphate concentrations and serum alkaline phosphatase activities in patients with osteoarthritis, ankylosing spondylitis, psoriatic arthritis and gout compared to normal values.

Similarly, no significant differences emerged when serum phosphate concentration and alkaline phosphatase values from the disease groups are compared with the controls except for the group of patients with ankylosing spondylitis where the mean level of alkaline phosphatase activity lay near the upper limit of normal and 54 of these patients had elevated levels of this enzyme.

In this study of a large group of patients with rheumatoid arthritis the prevalence of hypercalcaemia was approximately 50 per cent. This contrasts to 34 per cent found in the 23 patients with rheumatoid arthritis who were studied in greater depth. In the study of 52 patients in South Africa mentioned previously (Fig. II: 11) the prevalence of hypercalcaemia was found to be 32 per cent. These differences in prevalence may be explained by differences in the size of the populations studied. Further in the group of 23 patients, the duration of disease was shorter (6 years compared to 12 years in the large group), and the patients were in the fasting state and not receiving their usual anti-rheumatic drugs in contrast to the patients in the other studies. In addition, it must be recognised that it is extremely difficult to match precisely patients with rheumatoid arthritis in terms of the numerous clinical and laboratory indices of disease activity. The three studies, although differing in the percentage presence of hypercalcaemia, do all indicate that this is a not unusual laboratory abnormality in the disease.

It is of interest that hypercalcaemia appears to have clinical specificity for rheumatoid arthritis. It should be noted, however, that hypoalbuminaemia also tends to occur more frequently in patients with rheumatoid arthritis. However, none of the other arthritides with the exception of the group with ankylosing spondylitis had biochemical abnormalities in serum phosphate and alkaline phosphatase in contrast to the patients with rheumatoid arthritis. This suggests the possibility that there may be something peculiar to rheumatoid arthritis which is causing hypercalcaemia. Inflammation, per se, cannot be the cause since this is common, although not so severe, in osteoarthritis (Boyle and Buchanan, 1971) and in psoriatic arthritis the histological changes in synovium and the radiological appearances in the joints may be identical to those in rheumatoid arthritis. The relevance of the increased serum alkaline phosphatase activity in patients with ankylosing spondylitis is uncertain, since it was not possible to ascertain its source, i.e. liver or bone. To date there is no evidence of significant liver involvement occurring in this disease and it is tempting to postulate that the increase in this enzyme might be related to possible increased osteoblastic activity associated with extensive ligamental ossification. However this is purely speculative, and must remain so until isoenzyme investigation can be carried out.

Rheumatoid arthritis differs from the other arthritides in that it is associated with profound disturbances in both humoral and cellular immunity (Anderson, Buchanan and Goudie, 1967; Froebel, Sturrock, Dick and MacSween, 1975).

Logically the next step in investigation of the phenomenon would seem to be the study of the effect of rheumatoid sera on bone in an *in vitro* situation and such a study forms the final section of this Chapter.

SECTION 3

In Vitro Studies on the Effects of Rheumatoid Serum on a Bone-Culture Model

Despite the fact that erosive changes in articular bone and generalised osteoporosis are so common in patients with rheumatoid arthritis it is surprising that no-one has published studies on the effects of rheumatoid sera on bone in tissue culture. In the investigation of this phenomenon I was privileged to have the able direction and assistance of Dr. R. Lindsay of the Dept. of Medicine Unit at the Western Infirmary, Glasgow.

Methods and Materials

The sera studied was obtained from 14 of the 23 patients included in the biochemical and hormonal profile reported in the earlier section of this Chapter. The sera were obtained at the same time of the ionised calcium determinations.

Methods

Bone Culture Model

The bone culture model employed in this study is a modification of those described by Raisz and Niemann (1969) and Reynolds and Minkin (1970).

Female Sprague-Dawley rats were injected with 100 μCi of $^{45}CaCl_2$ on the seventeenth day of pregnancy. Not less than 24 hours and not more than 48 hours later the rats were sacrificed, and under aseptic conditions, the calvariae of the foetuses were

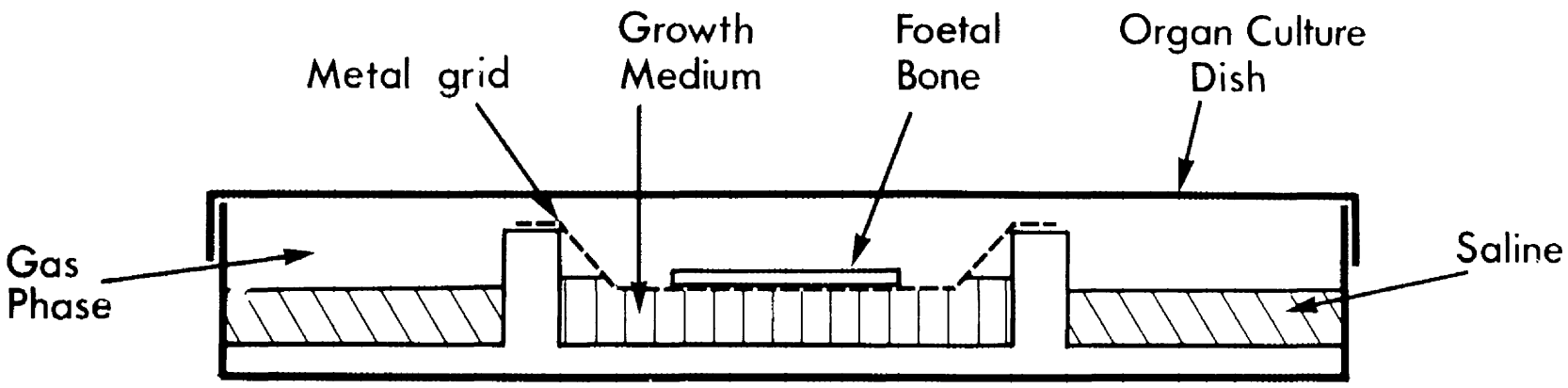
dissected out. The paired half calvariae were subsequently washed in medium No. 199 and placed on a metal grid whose lower surface was immersed in the growth medium (No. 199) contained in an organ culture dish (Fig II: 13). One of each pair of half calvariae served as a control with pooled human serum added (0.1 ml.) and the serum to be tested was added to the other half (0.1 ml.). The organ culture dishes were kept at a constant temperature of 37^{o}C for 48 hours.

Resorption was measured in terms of the percentage of ^{45}Ca released into the medium, determined by a liquid scintillation well counter at 48 hours after the test sera were added. Serum from each patient was always tested in triplicate and the mean of the three readings gave the final result. Two culture dishes with 0.1 ml. each of a solution containing 0.5 and one unit of PTH per ml. were added during each experimental run to act as controls.

Results were expressed as the ratio of ^{45}Ca released by treated and control members of each bone pair (test/control ^{45}Ca ratio). Cell mediated bone resorption was calculated by subtracting the ^{45}Ca released from a dead explant (killed by freezing and subsequently thawing three times) from that of the living paired bone (Raisz and Niemann, 1969; Reynolds and Minkin, 1970).

Fig II:13

Diagrammatic representation of the *in vitro* bone culture model employed in the study of bone resorption activity in rheumatoid sera.



Rat Hemi-calvarium Organ Culture

Results

Table II: XVII shows the results of ^{45}Ca released from the bones cultured with sera from the rheumatoid patients. In each case the results have been calculated as the ratio of the ^{45}Ca released from the bone treated with rheumatoid sera to that released from its control treated with normal sera.

The patient number corresponds to the number given to each patient in Table II: IX of the previous study. The serum ionised calcium concentration for each patient is the result obtained during the study described previously (Table II: IX) and are laid out in descending order of ionised calcium concentration. It was not possible to measure the ionised calcium concentration of the sera samples at the time of this bone resorption study because of the small volumes of sera which were available.

The results lying above the interrupted line belong to the patients who were hypercalcaemic, and those below to the patients who were normocalcaemic. A correlation coefficient was carried between the serum ionised calcium concentration and the corresponding ratio of bone resorption (Fig. II: 14). It can be seen that a highly significant positive correlation exists between these indices ($r = 0.76$; $p < 0.001$).

The results were then divided into two groups, hypercalcaemic (6) and normocalcaemic (8) and the mean of the results of ^{45}Ca resorption for each group compared with each other and with the responses to the two concentrations of parathyroid hormone (PTH)

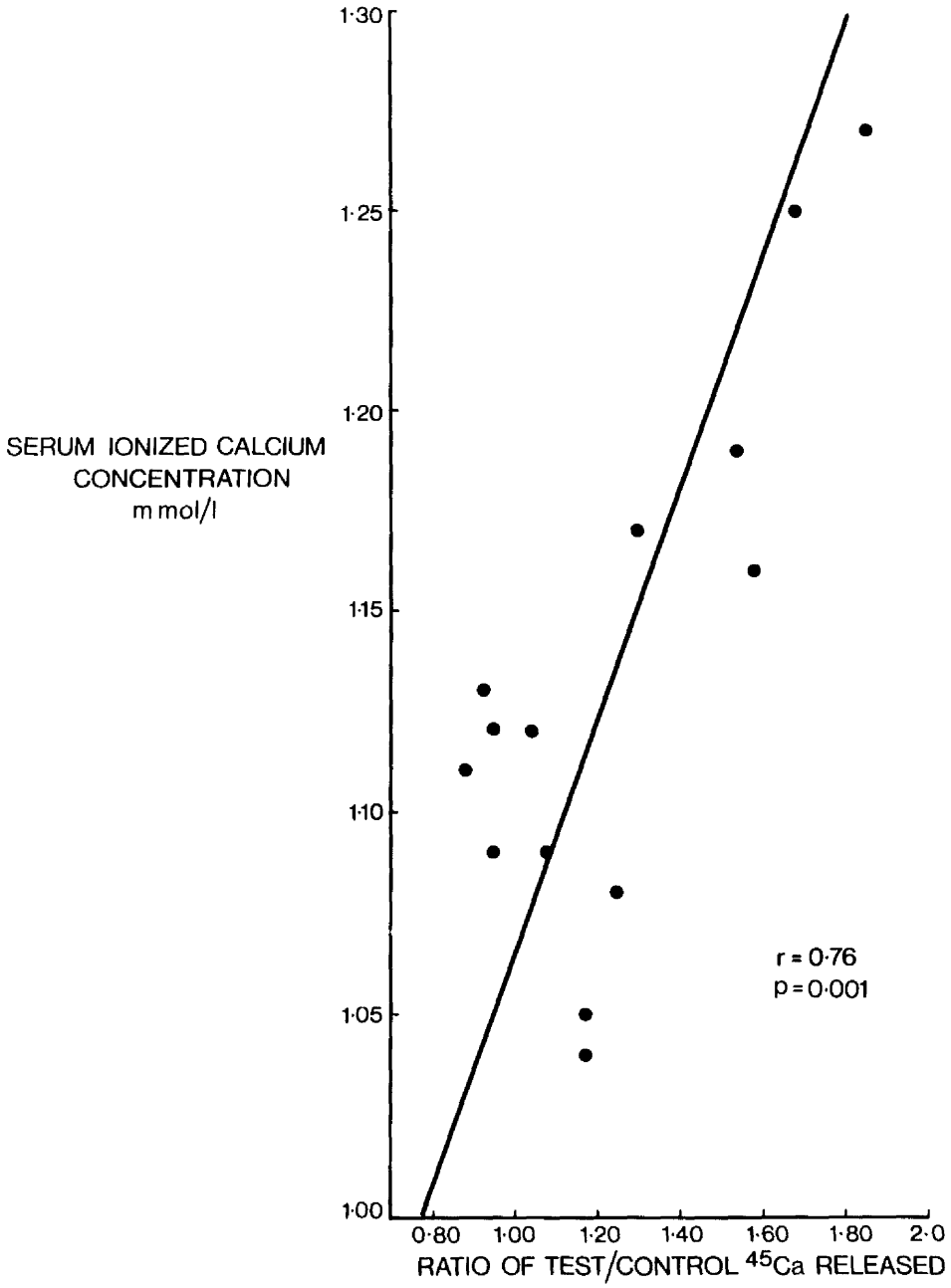
Patient Number	Serum Ionised Calcium Concentration mmols/l	Test/Control ^{45}Ca ratio
11	1.27	1.86
16	1.25	1.68
7	1.19	1.54
14	1.17	1.30
12	1.16	1.58
3	1.13	0.91
6	1.12	0.95
4	1.12	1.04
13	1.11	0.93
18	1.09	1.08
19	1.09	0.95
9	1.08	1.25
8	1.05	1.17
1	1.04	1.18

Table II: XVII

The individual results of the serum ionised calcium concentration and test/control ^{45}Ca ratios in the 14 patients studied. The patient numbers correspond to those in tableIIIX and the interrupted line divides the patients hyper and normocalcaemic groups.

Fig II:14

The correlation between ^{45}Ca released from bone culture by rheumatoid sera and the ionised calcium concentrations of the same sera samples.


SERUM IONIZED CALCIUM CONCENTRATION m mol/l
1·30
1·25
1·20
1·15
1·10
1·05
1·00
0·80
1·00
1·20
1·40
1·60
1·80
2·0
RATIO OF TEST/CONTROL 45Ca RELEASED
r = 0·76
p = 0·001

in the same system (Table II: XVIII). It can be seen that there was a significant increase in ^{45}Ca resorption in the hypercalcaemic group corresponding approximately to the resorption induced by 0.1 ml. of a solution of PTH containing 0.5 units/ml. When 0.1. ml. of a solution containing 4 m units/ml. of salmon calcitonin was added to the bone culture at the same time as sera from all the patients who were hypercalcaemic the resorbing effect of these sera was inhibited (Table II: XVIII).

Experiments with dead bone by the technique of Reynolds and Minkin (1970) showed no evidence of ^{45}Ca resorption when test sera and PTH were added confirming that the increased ^{45}Ca resorption noted in the live bone situation was cell mediated.

Assessment of the Action of Other Factors on the Bone Resorption induced by the Hypercalcaemic Rheumatoid Sera

The apparent anti resorption effect exerted by the salmon calcitonin on the hypercalcaemic sera was of interest and it was decided to test other relevant factors and substances in this model to determine their effects on the bone resorption induced by the hypercalcaemic sera.

Corticosteroids The results of the effect of hydrocortisone in varying molar concentrations is shown in Figure II: 15. It can be seen that as the concentration of hydrocortisone increases from $10^{-9}M$ to $10^{-6}M$ there is a progressive inhibition of ^{45}Ca release, until at the latter concentration there is no evidence of resorption present.

	Rheumatoid sera			Parathyroid hormone	
	Normal Ca (n=8)	High Ca (n=6)	High Ca +calcitonin (n=6)	0.5 unit/ml (n=10)	1.0 unit/ml (n=10)
Test/control ^{45}Ca ratio	1.06±0.26	1.47±0.2	1.09±0.16	1.50±0.14	2.21±0.28
Significance	P<0.01		P<0.01		

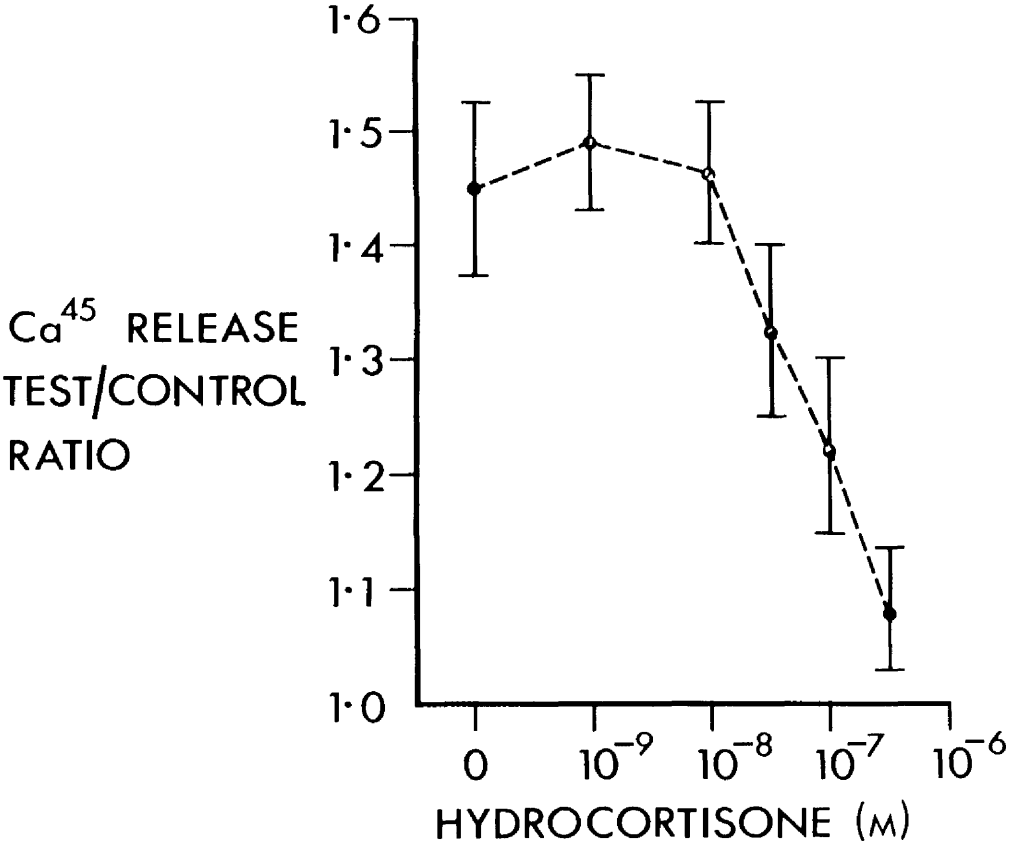
Table II: XVIII

Bone-resorbing activity of rheumatoid sera

Results are expressed as the ratio of ^{45}Ca resorbed from hemi-calvaria treated with rheumatoid sera to the ^{45}Ca resorbed from its pair incubated with control pooled human serum M ± I.S.D. Calcitonin inhibits the resorbing power of the sera with high calcium concentrations. Responses to two concentrations of parathyroid hormone are shown for comparison. Significance values were obtained by Wilcoxon's rank test.

Fig II:15

The effect of increasing molar concentration of hydrocortisone on the capacity of rheumatoid sera to resorb bone *in vitro*.


1·6
1·5
1·4
1·3
1·2
1·1
1·0
Ca45 RELEASE
TEST/CONTROL
RATIO
0
10−9
10−8
10−7
10−6
HYDROCORTISONE (M)

Indomethacin - Since PGE_2 had been incriminated as a possible bone resorptive agent in rheumatoid arthritis (Robinson, et al, 1975) it was decided to test an anti-prostaglandin (synthetase inhibitor), viz. Indomethacin on the resorption effect of the rheumatoid sera. To this end the hypercalcaemic sera was pre-incubated for 6 hours with 0.1 ml. of a solution containing 1 mg. indomethacin in 1 ml. of absolute alcohol. The control sera were pre-incubated with 0.1 ml. of absolute alcohol.

No inhibitory effect of indomethacin on ^{45}Ca resorption induced by the hypercalcaemic sera was observed.

Heat The hypercalcaemic sera was heated in a water bath to 60°C for 20 minutes and subsequently re-tested in the bone culture model. All ^{45}Ca resorption activity was lost.

Acid Following addition of HCl to the hypercalcaemic sera, the pH was reduced to 1, and subsequently converted back to 7.4 by alkali. All ^{45}Ca resorption activity was lost when the treated sera was re-tested.

Finally incubation with a solution of trypsin destroyed the ^{45}Ca resorption activity of the sera whereas incubation with papain did not.

DISCUSSION

Recent study of the development of erosive bone disease in rheumatoid arthritis has swung towards potential local biochemical and immunological events occurring in or around the inflamed joints. Robinson, Tashjian and Levine (1975) demonstrated that synovial cells, from patients with rheumatoid arthritis, maintained in organ culture secreted into the media a substance which released calcium from cultured mouse calvaria. They postulated that this substance was PGE_2 citing as proof the facts that the presence of indomethacin (a potent prostaglandin synthetase inhibitor) in the synovial culture abolished the bone resorption effect, that this activity could be quantitatively extracted into ether and that it could be accounted for by measuring the concentration of PGE_2 in the synovial culture medium.

In a not dissimilar experiment Krane (1974) also demonstrated bone resorption activity in rheumatoid synovial culture media. However, in his experiments, unlike those of Robinson and his colleagues, incubation of the culture in the presence of indomethacin did not inhibit this bone resorption activity. Krane therefore concluded that the observed resorptive effect was unlikely to be due to the presence or production of prostaglandins. In comparing such differing results it is an important fact that the majority of patients with rheumatoid arthritis receive non-steroidal anti-inflammatory agents and, to date, these drugs, many of which are potent inhibitors of prostaglandin synthetase,

have not been shown to alter significantly the progression of bone destruction in this disease.

Horton and his colleagues (1972) demonstrated that, when normal human peripheral blood leucocytes in culture were stimulated by either antigen or a mitogen, they produced a polypeptide, osteoclast activating factor (OAF), which initiated bone resorption in vitro. Similar activity has been found in the supernatants of cultures of marrow cells from patients with myeloma, in which massive loss of bone often occurs (Mundy, et al, 1974), and it could be postulated that such OAF activity, its production stimulated locally at the joint, could account for Krane's results. OAF not only stimulates bone resorption in vitro but also inhibits bone collagen synthesis and its effects are not inhibited by prostaglandin synthetase inhibitors (Raisz, Dietrich and Conalis, 1976).

The biochemical findings indicating abnormal calcium metabolic status in some patients with rheumatoid arthritis described previously suggested further circumstantial evidence of an active process of bone loss at the clinical level. It therefore seemed appropriate to test this sera for any evidence of bone resorbing activity. The bone culture model provided an ideal in vitro method of assessing this possibility.

The results of the study indicate that sera from some patients with rheumatoid arthritis can indeed induce bone resorption in vitro. The significant positive correlation (Fig.II-14) noted between the degree of ^{45}Ca resorption and the serum ionised calcium concentration demands some explanation and consideration.

The ionised calcium results were those measured at the time of the initial biochemical, hormonal profile described previously. It was not possible to measure the ionised calcium concentration of the sera actually used in this current study because sufficient volumes were not available. Thus the concentration of the ionised calcium in the sera sample at the time of this study is unknown. However, it is unlikely that they would be the same as the original values because they were not handled in the same way at the time of venesection, i.e. no care was taken to exclude oxygen and they were stored at $-20^{o}C$ before use. In addition the time of the experiment (48 hours) would conspire together with these factors to change the original ionised calcium levels because of changes in CO_2 and pH of the sample. Thus it seems most unlikely that the original serum ionised calcium values would be the same as those in the test sera used in the bone culture model. This is of importance because it might be assumed that the ^{45}Ca resorption was a function of the serum ionised calcium concentration itself. Further evidence against this possibility was noted in a study by Raisz and Niemann (1969) using a similar bone culture model. They showed that when the calcium

concentration in the test media was increased calcium loss from the culture bone was decreased. Thus the actual level of calcium in the test sera is unlikely to account for the degree of ^{45}Ca resorbed. The alternative possibility is that some substance/s was/were present in the hypercalcaemic sera which had the capacity to induce resorption of ^{45} calcium. That this is an active process seems to be confirmed by the fact that there was no effect by the same sera on dead bone, suggesting that the resorbing factor acted through live bone cells.

It is conceivable that enzymes in the serum, such as lysosomal enzymes and neutral proteases, could stimulate osteoclastic activity and cause bone resorption. However, these enzymes have not been found to be elevated in rheumatoid sera (Jasani, Katori and Lewis, 1969; Wood, Pryce-Jones, White and Nuki, 1971), and plasma inhibitors such as α_2 macroglobulin are also present in abundance to block their action. There is no literature on the effects of such enzymes on either bone or ionised calcium in rheumatoid arthritis, and although possible it seems unlikely that such enzymes could explain the phenomenon.

The bone resorbing effect of the hypercalcaemic sera was not inhibited by freezing and thawing the sera several times. Similarly the presence of a prostaglandin synthetase inhibitor in the test situation did not prevent ^{45}Ca resorption. This suggests that production of prostaglandin is not involved, however it does not exclude the possibility that pre-formed prostaglandin might be

responsible for the ^{45}Ca resorption. When salmon calcitonin (4m units/ml.) was added to the culture medium all bone resorption activity was totally abolished. The fact that the resorption activity of the sera is destroyed by acid and heat and is digested by trypsin but unaffected by papain suggests that the resorptive substance may well be similar to osteoclast activating factor (OAF) (Raisz et al, 1975). The inhibitory effect of hydrocortisone in high concentrations (Fig. II: 15) is also a factor common to this study and to the study of the activity of OAF.

Although these various studies have apparently conflicting results, it is entirely possible that local bone resorption occurs through both mechanisms, i.e. an immunological effect (OAF) and a biochemical one (PGE_2) and that these mechanisms are interrelated. Both are potent stimulators of bone resorption in vitro (Horton et al, 1972; Klein and Raisz, 1970) and it has been suggested that in some situations stimulation of prostaglandin synthesis may be complement dependent (Raisz et al, 1975), and this will occur within and around the diseased synovium. The relationship between OAF and prostaglandins therefore remains to be clarified further and both may be synthesized around actively inflamed joints as part of a humoral immune response. Whether or not the substance identified in this current study is related to OAF and to a local immune response in this context requires further proof. It is of particular interest that bone resorption was

stimulated only with sera from patients with hypercalcaemia and it should be noted that prostaglandins can produce hypercalcaemia when infused into animals but only in toxic doses (Klein and Raisz, 1970), while OAF apparently does not affect serum calcium when injected intravenously (Raisz, et al, 1975).

The nature of the bone resorbing substances present in rheumatoid sera therefore remains obscure, but its detection and indentification may improve further the understanding of the pathogenesis of bone loss occurring in rheumatoid arthritis.

SUMMARY

The results of the studies in this chapter demonstrate the following findings:-

1. Serum ionised calcium concentrations were found to be elevated in eight of 23 patients with rheumatoid arthritis studied. The correlation coefficient between serum ionised calcium and total serum calcium corrected for albumin was found to be more significant than the correlation coefficient between serum ionised calcium and total serum calcium not corrected for albumin. This together with the acceptable value for ultrafiltrable calcium at 0 albumin concentration obtained from this correction formula suggests that the correction factor employed in the study reasonably reflects the status of serum ionised calcium concentrations.

2. Associated biochemical abnormalities in some of these patients such as increased alkaline phosphatase activity reduced tubular reabsorption of phosphate, increased tubular reabsorption of calcium, increased urinary excretion of calcium and hydroxyproline and evidence of possible increased calcium absorption in a separate study were also found.

3. The possibility of a trend towards primary hyperparathyroidism accounting for the above features was disproved by the immuno-assay results from two separate techniques which convincingly demonstrated no elevation in serum parathyroid hormone levels.

4. Plasma levels of 25 - OH vitamin D were normal.

5. Two patients had increased plasma levels of calcitonin and one of these also had an elevated serum gastrin level.

6. The prevalence of hypercalcaemia was assessed in a study of 364 patients with rheumatoid arthritis, employing the correction factor for serum calcium concentration. Approximately 50 per cent of these patients were found to be hypercalcaemic when due correction was made for hypoalbuminaemia.

7. In a study of other arthritides, namely, osteoarthritis, ankylosing spondylitis, psoriatic arthritis and gout, no trend towards hypercalcaemia was evident. However, approximately 40 per cent of patients with ankylosing spondylitis were noted to have elevated serum alkaline phosphatase levels.

8. Increased bone resorption was noted in an in vitro bone culture preparation when sera from patients with rheumatoid arthritis who were hypercalcaemic was added. This effect was abolished by administering thyrocalcitonin, increasing molar concentrations of hydrocortisone and incubating the sera with trypsin. It was unaffected by incubating the sera with indomethacin or papain.

9. Finally, the index of bone resorption was found to correlate well with the ionised calcium concentration of the test sera.

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDIES

This thesis has clearly shown that osteoporosis is a generalised phenomenon in patients with rheumatoid arthritis. The most important variables in determining bone loss were: age, duration of arthritis, and corticosteroid therapy. Bone loss is particularly marked in post-menopausal females.

Perhaps the most surprising finding was that a high proportion of ambulant patients with rheumatoid arthritis have hypercalcaemia. In view of the high prevalence of this disease in the community it seems that rheumatoid arthritis should now be recognised as a not uncommon cause of hypercalcaemia.

The fact that this is the first time hypercalcaemia has been recognised in rheumatoid arthritis is probably due to it being masked by hypoalbuminaemia.

The cause of the hypercalcaemia was not ascertained and clearly deserves further study. It is not due to primary hyperparathyroidism as the serum immunoreactive parathyroid hormone levels are not elevated. However, there were some biochemical features consistent with a state of primary hyperparathyroidism, which raise interesting parallels with some forms of hypercalcaemia of malignancy. It is possible that a polypeptide is being produced by one or more of the cells involved in synovial inflammation which has parathyroid hormone-like activity but which is not detected by standard radioimmunoassay techniques. The fact that some rheumatoid sera have an osteolytic effect on bone in tissue culture, which can be blocked by calcitonin and increasing molar concentrations of hydrocortisone is not inconsistent

with this hypothesis. However, it will be necessary to carry out studies to determine the role, if any, of prostaglandin E_2 in causing bone erosion in this disease.

Perhaps the most exciting prospect is the fact that a means may be found for arresting bone erosions in rheumatoid arthritis. For example, it might be possible to prevent or minimise bone erosions in rheumatoid arthritis by treatment with calcitonin. In addition, a new in vitro model may be derived from the tissue culture experiments to ascertain the potential for inhibiting structural joint damage by slow acting drugs such as gold, D-penicillamine, and levamisole.

Adamski, A. and Smarz, C. (1969) Calcium binding capacity of plasma proteins in certain diseases. Polish Medical Journal, 8, 304-311.

Albright, F., Burnett, C.H., Cope, O. and Parsons, W. (1941) Acute atrophy of bone (osteoporosis) simulating hyperparathyroidism. Journal of Clinical Endocrinology, 1, 711-716.

Albright, F. and Reifenstein, E.C. (1948) Parathyroid Glands and Metabolic Bone Disease. Williams and Wilkins, Baltimore.

Aldred, J.P., Kleszynski, R.R. and Bastian, J.W. (1970) Effects of acute administration of porcine and salmon calcitonin on urine electrolyte secretion in rats. Proceedings of the Society for Experimental Biology in Medicine, 134, 1175-1180.

Anderson, J.B., Shimmins, J. and Smith, D.A. (1966) A new technique for the measurement of metacarpal density. British Journal of Radiology, 39, 443-450.

Anderson, J.D.W., Dent, C.E., Harrer, C. and Philpot, G.R. (1954) Effect of cortisone on calcium metabolism in sarcoidosis with hypercalcaemia. Lancet, 11, 720-724.

Anderson, J.R., Buchanan, W.W. and Goudie, R.B. (1967) in Autoimmunity, Clinical and Experimental. Charles C. Thomas, Springfield, Ill.

Anton, H.C. (1969) Width of clavicular cortex in osteoporosis. British Medical Journal, 1, 409-411.

Ardaillou, R., Vuoguant, P., Milhaud, G. and Richet, G. (1967) Effets de la thyrocalcitonine sur l'excretion rénale des phosphates du calcium et des ions H^+ chez l'homme. Nephron, 4, 298-314.

Arnaud, C.D., Littledike, T. and Tsao, H.S. (1970) Calcium homeostasis and the simultaneous measurement of calcitonin and parathyroid hormone in the pig. In Calcitonin: the Proceedings of the Second International Symposium on Calcitonin 1969, 95-101. S. Taylor and G. Foster, Heineman, London.

Arnaud, C.D., Tsao, H.S. and Littledike, T. (1971) Radioimmunoassay of human parathyroid hormone in serum. Journal of Clinical Investigation, 50, 21-34.

Arnold, D.E., Stansell, M.J. and Malvin, H.H. (1968) Measurement of serum ionic calcium using a specific ion electrode. American Journal of Clinical Pathology, 49, 627-634.

Arnold, J.S., Bartley, M.H., Tont, S.A. and Jenkins, D.P. (1966) Skeletal changes in ageing and disease. Clinical Orthopaedics, 49, 17-38.

Atkinson, M., Nordin, B.E.C. and Sherlock, S. (1956) Malabsorption and bone disease in prolonged obstructive jaundice. Quarterly Journal of Medicine, 25, 299-312.

Atkinson, P.J. (1965) Changes in resorption spaces in femoral cortical bone with age. Journal of Pathology and Bacteriology, 89, 173-178.

Avioli, L., McDonald, J.E. and Lee, S.W. (1965) The influence of age on the intestinal absorption of 47-Ca absorption in post menopausal osteoporosis. Journal of Clinical Investigation, 44, 1960-1967.

Axelson, H., Ekman, B. and Knutsson, D. (1965) Technician Symposia on Automation in Analytical Chemistry, 603-611. Mediac Inc., N.Y.

Baker, P.T., Schraer, H. and Yalman, R.G. (1959) Photogrammetric Engineering, 455-460.

Balfour, W. (1816) Observations with cases illustrative of a new simple and expeditious mode of curing rheumatism and strains. M.D. Edinburgh.

Ball, J. (1952) Sheep cell agglutination test for rheumatoid arthritis. A clinico-pathological study. Annals of Rheumatic Diseases, 11, 97-111.

Bannatyne, G.A. (1896) Rheumatoid Arthritis: Its pathology, morbid anatomy and treatment. Wright, Bristol.

Barling, P.M., Hendy, G.N., Evans, M.C. and O'Riordan, J.L.H. (1975) Region specific immunoassays for parathyroid hormone. Journal of Endocrinology, 66, 307-318.

Barnett, E. and Nordin, B.E.C. (1960) The radiological diagnosis of osteoporosis - a new approach. Clinical Radiology, 11, 166-174.

Barreras, R.F. (1973) Calcium and Gastric secretion. Gastroenterology, 64, 1168-1184.

Barwell, R. (1865) Diseases of the joints. Hardwicke, London.

Bell, N.H., Barret, R.J. and Patterson, R. (1966) Effects of porcine calcitonin on serum calcium, phosphorus and magnesium in the monkey and in man. Proceedings of the Society for Experimental Biology in Medicine, 123, 114-118.

Berens, D.L., Lockie, L.M., Lin, R.K. and Norcross, B.M. (1964) Roentgen changes in early rheumatoid arthritis. Radiology, 82, 645-654.

Berry, E.M., Gupta, M.M., Turner, S.J. and Burns, R.R. (1973) Variation in plasma calcium with induced changes in plasma specific gravity, total protein and albumin. British Medical Journal, 4, 640-643.
Berry, E.M., Dent, C.E., Flynn, F.V., Gupta, M.M. and Turner, S. (1974) Correction of plasma calcium measurements. British Medical Journal, 2, 53-54.

Berson, S.A. and Yalow, R.S. (1958) Isotopic tracers in the study of diabetes. In Advances in Biological and Medical Physics, p. 350. Tobias, C.A. and Lawrence, J.N., Academic Press Inc., New York.
Berson, S.A., Yalow, R.S., Aurbach, G.D. and Potts, J.T. Jr. (1963) Immunoassay of bovine and human parathyroid hormone. Proceedings of the National Academy of Sciences U.S.A., 49, 613-617.
Berson, S.A. and Yalow, R.S. (1968) Immunochemical heterogeneity of parathyroid hormone in plasma. Journal of Clinical Endocrinology and Metabolism, 28, 1037-1047.

Bijvoet, O.L. (1969) Relation of plasma phosphate concentration to renal tubular reabsorption of phosphate. Clinical Science, 37, 23-36.
Bijvoet, O.L., Morgan, D.B. and Fourman, P. (1969) The assessment of phosphate reabsorption. Clinica Chimica Acta, 26, 15-24.
Bijvoet, O.L., Veer, S.V.D., de Vries, H.R. and Van Koppen, L.T. (1971) Natriuretic effect of calcitonin in man. New England Journal of Medicine, 284, 681-688.

Bjelle, A.O. and Nilsson, B.E. (1970) Osteoporosis in rheumatoid arthritis. Calcified Tissue Research, 5, 327-332.
Bjelle, A.O. and Nilsson, B.E. (1971) The relationship between radiologic changes and osteoporosis of the hand in rheumatoid arthritis. Arthritis and Rheumatism, 14, 646-649.

Boyle, J.A. and Buchanan, W.W. (1971) Clinical Rheumatology, Blackwell, Oxford and Edinburgh.

Brewer, H.B. Jr. and Ronan, R. (1970) Bovine parathyroid hormone, amino acid sequence. Proceedings of the National Academy of Science U.S.A., 67, 1862-1869.

Buchanan, K.D. and McCarroll, A.M. (1971) Comparison of methods of separation of free from bound hormone in the radioimmunoassay of insulin and glucagon. Radioimmunoassay Methods, 136-142, Livingstone, Edinburgh.
Buchanan, K.D. (1973) Ph.D thesis on Studies on the pancreatic enteric hormones, 47-87. Queens University, Belfast.

Buckle, R. (1974) Measurement of parathyroid hormone. Clinics in Endocrinology and Metabolism, 3, 345-387.

Bulusu, L., Hodgkinson, A., Nordin, B.E.C. and Peacock, M. (1970) Urinary excretion of calcium and creatinine in relation to age and body weight in normal subjects and patients with renal calculus. Clinical Science, 38, 601-612.

Bywaters, E.G. (1960) The early radiological signs of rheumatoid arthritis. Bulletin of the Rheumatic Diseases, 11, 231-234.

Cameron, D.A. (1961) Erosion of the epiphysis of the rat tibia by capillaries. Journal of Bone and Joint Surgery, 43B, 590-594.

Cameron, J.R. and Sorenson, J. (1963) Measurement of bone mineral in vivo; an improved method. Science, 142, 230-232.

Caniggia, A., Gennari, C. and Cesari, L. (1965) Intestinal absorption of ^{45}Ca in stone forming patients. British Medical Journal, 1, 427-429.

Castillo, B.A., El Sallab, R.A., Scott, J.T. (1965) Physical activity, cystic erosions and osteoporosis in rheumatoid arthritis. Annals of Rheumatic Diseases, 24, 522-527.

Cham, B.E. (1972) A semi-automated method for the estimation of ionic calcium activity and concentration in biological solutions. Clinica Chimica Acta, 37, 5-14.

Chiaje, S. Della (1853) Arthritis Deformans from Pompeii. Osteologia Pompeiana, ii, 555.

Cochran, M. and Nordin, B.E.C. (1971) The causes of hypocalcaemia in chronic renal failure. Clinical Science, 40, 305-315.

Cockel, R., Kendall, M.J., Becker, J.F. and Hawkins, C.F. (1971) Serum biochemical values in rheumatoid disease. Annals of the Rheumatic Diseases, 30, 166-170.

Collins, D.H. (1966) Pathology of Bone, 120-128, Butterworths, London.

Conney, A.H. (1967) Pharmacological implications of microsomal enzyme induction. Pharmacological Reviews, 19, 317-366.

Cook, M.G., Levell, M.J. and Payne, R.B. (1970) A method for deriving normal ranges from laboratory specimens applied to uric acid in males. Journal of Clincial Pathology, 23, 778-780.

Coombs, R.C., Hillyard, C., Greenberg, P.B. and McIntyre, I. (1974) Plasma immunoreactive calcitonin in patients with non-thyroid tumour. Lancet, 1, 1080-1083.

Copeman, W.S.C. (1978) Textbook of the Rheumatic Diseases, pp. 511, 537, 597, 656. Ed. J.T. Scott, Churchill Livingstone, Edinburgh, London and New York.

Copp, D.H., Cameron, E.C., Cheney, B.A., Davidson, A.G. and Henze, K.G. (1962) Evidence for calcitonin - A new hormone from the parathyroid that lowers blood calcium. Endocrinology, 70, 638-649.

Dale, N.E. and Kellerman, G.M. (1967) The binding of calcium by the plasma proteins in hyperparathyroidism. Clinical Science, 32, 433-442.

Danowski, T.S., Weigand, F.A., Greenman, L., Gailani, S., Greenberg, W.V. and Mateer, F.N. Corticotrophin (ACTH) therapy of nephrotic syndrome in children II Laboratory findings in one hundred and six instances. American Journal of Diseases of Children, 93, 604-614.

Davis, R.H., Morgan, D.B. and Rivlin, R.S. (1970) The excretion of calcium in the urine and its relation to calcium intake, sex and age. Clinical Science, 39, 1-12.

Deftos, L.J., Murray, T.M., Powell, D., Habener, J.F., Singer, F.R., Mayer, G.P., Potts, J.T. Jr. (1972) Radioimmunoassays for parathyroid hormones and calcitonins. In Calcium, Parathyroid Hormone and the Calcitonins, p. 140. Eds. Talmage, R.V. and Munson, P.L., Excerpta Medica, Amsterdam.

Demartini, F., Grokoest, A.W. and Regan, C. (1952) Pathological fractures in patients with rheumatoid arthritis treated with cortisone. Journal of the American Medical Association, 149, 750-754.

Dent, C.E. (1962) Some problems of hyperparathyroidism. British Medical Journal, 2, 1419-1425.

Dent, C.E. and Friedman, M. (1965) Idiopathic juvenile osteoporosis. Quarterly Journal of Medicine, 34, 177-210.

Dent, C.E. and Lyal Watson (1966) Osteoporosis. Postgraduate Medical Journal (Suppl.), 583-608.

Dent, C.E. (1969) Ageing of connective and skeletal tissue. Osteoporosis: a Thule International Symposium, 261. Nordiska Bokhandelns, Forlag, Stockholm.

Doyle, F.H. (1961) Ulnar bone mineral concentration in metabolic bone disease. British Journal of Radiology, 34, 698-712.

Drinker, N., Green, A.A. and Hastings, A.B. (1939) Equilibria between calcium and purified globulins. Journal of Biological Chemistry, 131, 641-647.

Duncan, H., Frost, H.M., Villanueva, A.R., Sigler, J.N. (1965) The osteoporosis of rheumatoid arthritis. Arthritis and Rheumatism, 8, 943-954.

Dymling, J.F. (1964) Bone and Tooth, p. 151. Eds. H.J.J. Blackwood. Pergammon Press, Oxford.

Eisenman, G., Rudin, D.O. and Casby, J.U. (1957) Glass electrode for measuring sodium ion. Science (Washington), 126, 831-834.

Ellman, P. and Ball, R.E. (1948) Rheumatoid disease with joint and pulmonary manifestations. British Medical Journal, 2, 816-820.

Epker, B.N., Kelin, M. and Frost, H.M. (1965) Magnitude and location of cortical bone loss in human rib with ageing. Clinical Orthopaedics, 41, 198-203.

Epker, B.N. and Frost, H.M. (1965) A histological study of remodelling at the periosteal haversian canal, cortical endosteal and trabecular endosteal surfaces in human rib. Antatomical Record, 152, 129-135.

Epker, B.N. and Frost, H.M. (1966) Periosteal appositional bone growth from age two to age seventy in man. A tetracycline investigation. Anatomical Record, 154, 573-577.

Ettori, J. and Scoggan, S.M. (1961) A determination of ionised calcium in serum by two wavelength spectrophotometry of a metal indicator. Clinica Chimica Acta, 6, 861-865.

Eve, J. (1890) British Medical Journal, 1, 423.

Exton-Smith, A.N., Millard, P.H., Payne, P.R. and Wheeler, E.F. (1969) Pattern of development and loss of bone with age. Lancet, 2, 1154-1157.

Fairney, A., Jackson, D. and Clayton, B.E. (1973) Measurement of serum parathyroid hormone with particular reference to some infants with hypocalcaemia. Archives of Diseases of Children, 48, 419-424.

Fell, G.S. and Peaston, R. (1973) Atomic absorption techniques in clinical chemistry. Proceedings of the Society of Analytical Chemists, 10, 256-257.

Flannagan, R.W.J., Buchanan, K.D. and Murphy, R.F. (1974) Specificity of antibodies in the radioimmunoassay of glucagon. Diabetologia, 10, 365-369.

Follis, R.H. (1953) Skeletal changes associated with hyperthyroidism. Bulletin of Johns Hopkins Hospital, 92, 405-421.

Fourman, P. and Royer, P. (1968) Calcium Metabolism and the Bone. Blackwell Scientific, Oxford.

Frame, B. and Nixon, R.K. (1968) Bone marrow mast cells in osteoporosis of ageing. New England Journal of Medicine, 279, 626-631.

Fraser, R., Harrison, M. and Jones, E. (1960) Radioaktive Isotope in Klinik und Forschung. Eds. K. Fellinger and R. Hofer, Urban und Schwartzenberg, Munchen-Berlin.

Freaney, R., O'Donohoe, P., Bergin, R., Bernard, J., Maughan, G. and Muldowney, F.P. (1974) Serum ionised calcium determination by ion specific electrode and its value in clinical metabolic disorders. Irish Journal of Medical Science, 143, 286-295.

Friedman, J. and Raisz, L.G. (1965) Thyrocalcitonin: Inhibitor of bone resorption in tissue cultures. Science, 150, 1465-1467.

Froebel, K., Sturrock, R.D., Dick, W.C. and MacSween, R.N.M. (1975) Cell mediated immunity in the rheumatic diseases 1. Skin testing and mitogenic responses in sero-negative arthritides. Clinical and Experimental Immunology, 22, 446-452.

Frost, H.M. (1963) Mean formation time of human osteons. Canadian Journal of Biochemistry, 41, 1307-1310.

Fuchs, C., Paschen, K., Spieckermann, P.G. and Westburg, C. (1972) Bestimmung des Ionisierten Calciums im Serum mit einer Ionenselectiven Durchflusselektrode: Methodik und Normalwerte. Klinische Wochenschrift, 50, 824-832.

Gallagher, J.C., Aaron, J., Horsman, A., Wilkinson, R. and Nordin, B.E.C.. (1973) Corticosteroid Osteoporosis. Clinics in Endocrinology and Metabolism, 2, 355-368.

Gaillard, P.J. and Thesingh, C.W. (1968) Bone culture studies with thyrocalcitonin. Calcitonin; Symposium on Thyrocalcitonin and the C. Cells, 238-245. Ed. S. Taylor. Heinemann, London.

Garn, S.M., Rohmann, C.G., Wagner, B. and Ascoli, W. (1967) Continuing bone growth throughout life; a general phenomenon. American Journal of Physiology and Anthropology, 26, 313-317.

Garrod, A.B. (1859) Nature and treatment of gout and rheumatic gout, Walton and Maberly, London.

Gitelman, H.J., Kukolj, S. and Welt, L.G. (1965) Effect of thyrocalcitonin on the partition of calcium. Clinical Research, 13, 323.

Gitman, L. and Kamholtz, T. (1965) Incidence of radiographic osteoporosis in a large series of aged individuals. Journal of Gerontology, 20, 32-33.

Glueck, H.I., Wayne, L. and Goldsmith, R. (1962) Abnormal calcium binding associated with hyperglobulinaemia clotting defects and osteoporosis, a study of this relationship. Journal of Laboratory and Clinical Medicine, 59, 40-64.

Goldhaber, P. (1965) Bone-resorption factors and co-factors and giant vacuole osteoclasts in tissue culture. In The Parathyroid Glands, 153-171. Eds. P.J. Gaillard, R.V. Talmage,and A.M. Budy, Chicago University Chicago Press.

Gosling, P., Robinson, B.H.D., Sammons, H.G. (1975) Changes in protein bound calcium in the serum of haemodialysis patients. Clinical Science and Molecular Medicine, 48, 521-528.

Goverde, B.C. and Veenkamp, F.J. (1972) Routine assay of total hydroxyproline based on resin catalysed hydrolysis. Clinica Chimica Acta, 41, 29-40.

Gray, T.K., and Munson, P.L. (1969) Thyrocalcitonin; Evidence for physiological function. Science, 166, 512-513.

Greenberg, D.M. and Greenberg, L.D. (1932) Is there an unknown compound of the nature of calcium citrate in the blood. Journal of Biological Chemistry, 99, 1-15.

Gutman, A.B. and Gutman, E.B. (1937) Relation of serum calcium to serum albumin and globulins. Journal of Clinical Investigation, 16, 903-919.

Habener, J.F., Kemper, B., Ernst, M., Rich, A., and Potts, J.T. (1975) Pre-proparathyroid hormone. Clinical Research, 23, 321A.
Habener, J.F., Mayer, G.B., Dee, P.C. and Potts, J.T. Jr. (1976) Metabolism of amino and carboxyl sequence immunoreactive parathyroid hormone in the bovine; Evidence for peripheral cleavage of hormone. Metabolism, 25, 385-395.
Habener, J.F. and Potts, J.T. Jr. (1976) In Hormones in human blood detection and assay. Ed. H.N. Antoniades, Harvard University Press, Cambridge, Mass and London.

Haddad, J.G. and Chyu, K.G. (1971) Competitive protein binding radioassay for 25 hydroxycholecalciferol. Journal of Clinical Endocrinology and Metabolism, 33, 992-995.

Hahn, T.J., Birge, S.J., Scharp, C.R. and Avioli, L.V. (1972) Phenobarbital induced alterations in vitamin D metabolism. Journal of Clinical Investigation, 51, 741-748.

Hansen, S.O. and Theodorsen, L. (1971) The usefulness of an improved calcium electrode in the measurement of ionized calcium in serum. Clinica Chimica Acta, 31, 119-122.

Hartmann, F., Rohde, J., Schmidt, A. (1969) Aktivitatsdiagnostik bei der primar - chronischen polyarthritis. Zeitschrift for Rheumatologie, 28, 263-266.

Hattner, R.S., Johnson, J.W., Bernstein, D.S., Wachman, A. and Brackman, J. (1970) Electrochemical determination of apparent ionized serum calcium using a calcium selective electrode: The method and values in normal humans and a comparison to total serum calcium. Clinica Chimica Acta, 28, 67-75.

Haymovits, A. and Rosen, J.F. (1967) Human Thyrocalcitonin. Endocrinology, 81, 993-1000.

Heath, D.A. (1976) Hypercalcaemia and malignancy. Annals of Clinical Biochemistry, 13, 555-561.

Held, I.R. and Freeman, S.J. (1964) Binding of calcium by human plasma proteins under simulated physiologic conditions. Journal of Applied Physiology, 19, 292-296.

Henneman, P.H., Dempsey, E.F., Carrol, E.L. and Albright, F. (1956) The cause of hypercalciuria in sarcoid and its treatment with cortisone and sodium phytate. Journal of Clinical Investigation, 35, 1229-1242.
Henneman, P.H., Benedict, P.H., Forbes, A.P. and Dudy, H.R. (1958) Idiopathic hypercalciuria: importance of dietary calcium in the definition of hypercalciuria. New England Journal of Medicine, 259, 802-807.

Hennessy, J.F., Gray, T.K., Cooper, C.W. and Ontjes, D.A. (1973) Stimulation of thyrocalcitonin secretion by pentagastrin and calcium in 2 patients with medullary carcinoma of the thyroid. Journal of Clinical Endocrinology and Metabolism, 36, 200-203.

Higgs, G.A., Vane, J.R., Hart, F.O. and Wojulewski, J.A. (1974) The effects of anti-inflammatory drugs on prostaglandin concentrations in synovial fluid from patients with rheumatoid arthritis. In Prostaglandin Synthetase pp 165-171. Eds. Robinson, H.J., & Vane, J.R. Raven Press N.Y.C.

Hioco, D., Bordier, P., Miravet, L., Denys, H. and Tun-Chot, S. (1969) Prolonged administration of calcitonin in man: Biological isotopic and morphological effects. Calcitonin 1969: Proceedings of the Second International Symposium, 514-522. Ed. S. Taylor and G.V. Foster, Heinemann, London.

Hirsch, P.F., Voelkel, E.F. and Munson, P.L. (1964) Thyrocalcitonin: hypocalcaemic hypophosphataemic principle of the thyroid gland. Science, 146, 412-413.

Hodgkinson, A. (1963) Biochemical aspects of primary hyperparathyroidism: an analysis of 50 cases. Clinical Science, 25, 231-242.
Hodgkinson, A. and Knowles, C.F. (1976) Laboratory methods. In Calcium phosphate and magnesium metabolism, 542. Ed. B.E.C. Nordin, Churchill Livingstone, Edinburgh, London and New York.

Hodkinson, H.M. (1976) "Corrected" calcium concept. British Medical Journal, 1, 219-220.

Hodkinson, H.M. (1974) Correction of plasma calcium measurements. British Medical Journal, 2, 223.

Horton, J.E., Raisz, L.G., Simmons, H.A., Oppenheim, J.T. and Mergenhagen, S.E. (1972) Bone resorbing activity in supernatant fluid cultured from human peripheral blood leucocytes. Science, 177, 793-795.

Howard, J.E., Parson, W. and Bingham, R.S. (1945) Studies on patients convalescent from fractures. Bulletin of Johns Hopkins Hospital, 77, 291-313.

Howell, D.S. (1966) Arthritis and Allied Conditions: A textbook of Rheumatology, 984. Ed. J.L. Hollander. Kimpton, London.

Hunter, W.M. and Greenwood, F.C. (1962) Preparation of iodine 131 labelled human growth hormone of high specific activity. Nature, 194, 495-496.

Husdan, H., Rapoport, A. and Locke, S. (1973) Influence of posture on the serum concentration of calcium. Metabolism, 22, 787-797.

Husdan, H., Rapoport, A., Locke, S. and Oreopoulos, D. (1976) Adjustment of serum calcium for protein. Clinical Chemistry, 22, 282-283.

Instruction Manual, model 99-20 serum calcium flow-thru system (1972) Orion Research Inc., Cambridge, Mass 02139, U.S.A.

Jasani, M.K., Katori, M. and Lewis, G.P. (1969) Intracellular enzymes and kinin enzymes in synovial fluids in joint diseases. Annals of Rheumatic Diseases, 28, 497-513.

Jones, J.H., Peters, D.K., Morgan, D.B., Coles, G.A. and Mallick, N.P. (1967) Observations on calcium metabolism in the nephrotic syndrome. Quarterly Journal of Medicine, 36, 301-320.

Jowsey, J. (1966) Quantitative microradiography. A new approach in the evaluation of metabolic bone disease. American Journal of Medicine, 40, 485-491.

Keane, B.E., Speigler, G. and Davis, R. (1959) Quantitative evaluation of bone mineral by a radiographic method. British Journal of Radiology, 32, 162-167.

Keutmann, H.T., Dawson, B.F., Aurbach, G.D. and Potts, J.T. Jr. (1972) A biologically active amino-terminal fragment of bovine parathyroid hormone prepared by dilute acid hydrolysis. Biochemistry, 11, 1973-1979.
Keutmann, H.T., Barling, P.M., Hendy, G.N., Segre, G.V., Niall, H.D., Aurbach, G.D., Potts, J.T. Jr., and O'Riordan, J.L.H. (1974) Isolation of human parathyroid hormone. Biochemistry, 13, 1646-1652.

Klein, D.C. and Talmage, R.V. (1968) Thyrocalcitonin suppression of hydroxyproline release from bone. Proceedings of the Society for Experimental and Biological Medicine, 127, 95-99.
Klein, D.C. and Raisz, L.G. (1970) Prostaglandins: stimulation of bone resorption in tissue culture. Endocrinology, 86, 1436-1440.

Kind, P.R.N. and King, E.J. (1954) Estimation of plasma phosphatase by determination of hydrolysed phenol with amino antipyrine. Journal of Clinical Pathology, 7, 322-326.

Knaggs, R. Lawford (1926) The Inflammatory and Toxic Diseases of Bone, Wright, Bristol.

Knowelden, J., Buhr, A.J. and Dunbar, O. (1964) Incidence of fractures in persons of over 35 years of age. A report to the M.R.C. Working Party on fractures in the elderly. British Journal of Preventative and Social Medicine, 18, 130-141.

Knowlton, A.I. (1953) Cushings Syndrome. Bulletin of the New York Academy of Medicine, 29, 441-465.

Krane, S.M. (1974) Degradation of collagen in connective tissue diseases. Rheumatoid arthritis. In Dynamics of Connective Tissue Macromolecules, 7th Edition. McGraw Hill, New York.

Ladenson, J.H. and Bowers, G.N. (1973) Free calcium in serum II. Rigor of homeostatic control correlations with total serum calcium and review of data on patients with disturbed calcium metabolism. Clinical Chemistry, 19, 575-582.

Lafferty, F.W., Spencer, G.E. and Pearson, O.H. (1964) Dynamic Studies of Metabolic Bone Disease, 101. Eds. O.H. Pearson and G.F. Joplin. Blackwell Scientific Publiscations, Oxford.

Landre Beauvais, A.J. (1800) Doit on admettre une nouvelle espece de goutte sous la denomination de goutte asthenique primitif, Paris.

Last, R.J. (1963) Anatomy - Regional and Applied Section I - 1-27. J & A Churchill, London.

Leading article (1977) correcting the calcium. British Medical Journal, 1, 598.

Lebert, R. (1859) Handbuch der Pract. Med., 2, 874.

Leme, C.E., Coslovsky, R. and Wajchenberg, B.L. (1973) Ionised calcium in normal human serum; Determination with specific electrode and by tetramethylmurexide. Biomedicine, 19, 431-436

Lequin, R.M., Hackeng, W.H.L. and Schopman, W. (1970) A radioimmunoassay for parathyroid hormone in man II. Measurement of parathyroid hormone concentrations in human plasma by means of a radioimmunoassay for bovine hormone. Acta Endocrinologica Scandinavica,63, 655-666.

Liberman, U.A., Sperling, O., Atsmon, O., Frank, M., Modan, M. and De Vries, A. (1968) Metabolic and calcium kinetic studies in idiopathic hypercalciuria. Journal of Clinical Investigation, 47, 2580-2590.

Lingarde, F. (1972) Potentiometric determination of serum ionized calcium in a normal human population. Clinica Chimica Acta, 40, 477-484.
Lingarde, F. and Zetterval, O. (1973) Hypercalcaemia and normal ionized serum calcium in a case of myelomatosis. Annals of Internal Medicine, 78, 396-399.

Loken, H.F., Havel, R.J., Gordon, G.S. and Whittington, S.L. (1960) Ultracentrifugal analysis of protein bound and free calcium in human serum. Journal of Biological Chemistry, 235, 3654-3658.

McConkey, B., Frazer, G. and Bligh, A. (1965) Transparent skin and osteoporosis - a study in patients with rheumatoid disease. Annals of Rheumatic Diseases, 24, 219-223.

McLaughlin, M., Fairney, A., Lester, E., Raggatt, P.R., Brown, D.J. and Wills, M.R. (1974) Seasonal variations in serum 25-hydroxycholecalciferol in healthy people. Lancet, 1, 536.

McLean, F.C. and Hastings, A.B. (1934) A biological method for the estimation of calcium ion concentration. Journal of Biological Chemistry, 107, 337-350.
McLean, F.C. and Hastings, A.B. (1935a) The state of calcium in fluids of the body. I. The conditions affecting the ionization of calcium. Journal of Biological Chemistry, 108, 285-322.
McLean, F.C. and Hastings, A.B. (1935b) Clinical estimation and significance of calcium ion concentration in the blood. American Journal of Medical Science, 189, 601-613.

Maddison, P.J. and Bacon, P.A. (1974) Vitamin D deficiency, spontaneous fractures and osteopenia in rheumatoid arthritis. British Medical Journal, 4, 433-435.

Marsh, C.A. (1963) Metabolism of D-glucuronolactone in mammalian systems. Identification of D-glucaric acid as a normal constituent of urine. Biochemical Journal, 86, 77-86.

Marshall, D.H., Peacock, M. and Nordin, B.E.C. (1973) Plasma calcium homeostasis. In Rein et calcium. Ed. D. Hioco. 15-25. Rueil-Malmaison Sandoz Editions.

Marshall, R.W. and Nordin, B.E.C. (1974) Adjustment of plasma calcium measurements. British Medical Journal, 2, 729-730.
Marshall, R.W. (1976) Plasma Fractions. In Calcium, Phosphate and Magnesium Metabolism, 162-186. Ed. B.E.C. Nordin. Churchill Livingstone, Edinburgh, London and New York.

Martel, W. (1968) Radiological manifestations of rheumatoid arthritis with particular reference to the hand, wrist and foot. Medical Clinics in North America, 52, 655-665.

Meema, H.E. (1963) Cortical bone atrophy and osteoporosis as a manifestation of ageing. American Journal of Roentgenology, 89, 1287-1295.

Melvin, K.E.W., Hepner, G.W., Bordier, P., Neale, G. and Joplin, G.F. (1970) Calcium metabolism and bone pathology in adult coeliac disease. Quarterly Journal of Medicine, 39, 83-113.

Meunier, P., Bernard, J. and Vignon, G. (1971) The measurement of periosteocytic enlargement in primary and secondary hyperparathyroidism. Israeli Journal of Medical Science, 7, 482-485.

Meyers, A.M., Laidley, L., Levine, E., Milne, F.J. Salant, D. and Kennedy, A.C. (1976) Ionized calciums in renal and other metabolic osteodystrophies. The Association of Physicians of South Africa held at The University of the Orange Free State, Bloemfontein, S.A.

Milhaud, G., Perault-Staub, A.M. and Moukhtar, M.S. (1965) Etude du mecanisme de l'action hypocalcemiante de la thyrocalcitonine. Comptes rendues hebdomadaires des seances de l'Academic des sciences, 261, 813-816.
Milhaud, G. and Moukhtar, M.S. (1966) Antagonistic and synergistic actions of thyrocalcitonin and parathyroid hormone on the levels of calcium and phosphate in the rat. Nature, 211, 1186-1187.

Miravet, L., Hioco, D., Debeyre, N., Dryll, A., Ryckewaert, A. and de Seze, S. (1966) La cinetique du calcium au cours de l'osteoporose cortisonique. Semaine des Hopitaux de Paris, Nov., 60-65.

Moore, E.W. (1970) Ionized calcium in normal serum ultrafiltrates and whole blood determined by ion exchange electrodes. Journal of Clinical Investigation, 49, 318-334.
Moore, E.W. (1971) Studies with ion exchange calcium electrodes III the state of serum calcium in patients with cirrhosis. Gastroenterology, 60, 43-54.

Moore, N. (1885) Pathological Society Transaction, XXXIV, 226.

Morgan, D.B., Spiers, F.W., Pulvertaft, C.N. and Fourman, P. (1967) The amount of bone in the metacarpal and phalanx according to age and sex. Clinical Radiology, 18, 101-108.
Morgan, D.B. (1973a) The metacarpal bone: A comparison of the various indices for the assessment of the amount of bone and for the detection of loss of bone. Clinical Radiology, 24, 77-82.
Morgan, D.B., (1973b) Osteomalacia, Renal Osteodystropy and Osteoporosis. C.C. Thomas. Springfield, Illinois.

Mueller, M.N. and Jurist, J.M. (1973) Skeletal status in rheumatoid arthritis. A preliminary report. Arthritis and Rheumatism, 16, 66-70.

Munday, K.A. and Mahy, B.W.J. (1964) Determination of ultrafiltrable calcium and magnesium in small quantities of plasma. Clinica Chimica Acta, 10, 144-151.

Munday, G.R., Raisz, L.G., Cooper, R.A., Schechter, G.P. and Salmon, S.E. (1974) Evidence for the secretion of an osteoclast activating stimulating factor in myeloma. New England Journal of Medicine, 291, 1041-1046.

Murray, R.O. (1960) Radiological bone changes in Cushing's syndrome and steroid therapy. British Journal of Radiology, 33, 1-19.

National Food Survey Committee (1973) Household Food Consumption and Expenditure 1970 and 1971. London, HMSO.

Neuhausen, B.S. and Marshall, E.K. (1922) An electrochemical study of the condition of several electrolytes in the blood. Journal of Biological Chemistry, 53, 365-372.

Newton-John, H.F. and Morgan, D.B. (1968) Osteoporosis; Disease or Senescence. Lancet, 1, 232-233.

Niall, H.D., Keutmann, H.T., Sauer, R., Hogan, M.L., Dawson, B.F., Aurbach, G.D., and Potts, J.T. Jr. (1970) The amino-acid sequence of bovine parathyroid hormone. Hoppe-Seyler's Zeitschrift fur Physiologische Chemie, 351, 1586-1588.

Nordin, B.E.C. (1961) Pathogenesis of osteoporosis. Lancet, 1, 1011-1015.
Nordin, B.E.C., Barnett, E., McGregor, J. and Nisbett, J. (1962) Lumbar spine densitometry. British Medical Journal, 1, 1793-1796.
Nordin, B.E.C., Smith, D.A. and Glass, H.I. (1964) Bone and Tooth, 145. HJJ Blackwood, Pergammon Press, London.
Nordin, B.E.C., McGregor, J. and Smith, D.A. (1966) The incidence of osteoporosis in normal women, its relation to age and the menopause. Quarterly Journal of Medicine, 35, 25-38.
Nordin, B.E.C. (1967) Diagnostic uses of radioisotopes - the study of calcium metabolism. Hospital Medicine, 2, 211.
Nordin, B.E.C., Hodgkinson, A. and Peacock, M. (1967) The measurement and meaning of urinary calcium. Clinical Orthopaedics and Related Research, 52, 293-322.
Nordin, B.E.C. and Bulusu, L. (1968) A modified index of phosphate excretion. Postgraduate Medical Journal, 44, 93-97.
Nordin, B.E.C., Young, M.M., Bentley, B., Ormondroyd, P. and Sykes, J. (1968) Lumbar spine densitometry methodology and results in relation to the menopause. Clinical Radiology, 19, 459-464.
Nordin, B.E.C., Peacock, M. and Wilkinson, R. (1972) Hypercalciuria in calcium stone disease. Clinics in Endocrinology and Metabolism, 1, 169-183.

Nordin, B.E.C. (1973) Metabolic Bone and Stone Disease. Churchill Livingstone, Edinburgh and London.
Nordin, B.E.C. (1976) In Calcium Phosphate and Magnesium Metabolism, 3. Ed. B.E.C. Nordin. Churchill Livingstone, Edinburgh, London and New York.
Nordin, B.E.C., Horsman, A. and Aaron, J. (1976) Diagnostic precedures. In Calcium, Phosphate and Magnesium Metabolism, 469-525. Ed. B.E.C. Nordin. Churchill Livingstone. Edinburgh, London and New York.

Northam, B.E. and Widdowson, G.M. (1965) Associated Clinical and Biochemical Technical Bulletin No. 11.

Northover, B.J. (1973) Effect of anti-inflammatory drugs on the binding of calcium to cellular membranes in various human and guinea-pig tissues. British Journal of Pharmacology, 48, 496-504.

Odery-Symes, J. (1905) The Rheumatic Diseases. John Lane, The Bodley Head, London and New York.

Oreskes, I., Hirsch, G., Douglas, K.S. and Kupfer, S. (1968) Measurement of ionised calcium in human plasma with a calcium selective electrode. Clinica Chimica Acta, 21, 303-313.

Orrell, D.H. (1971) Albumin as an aid to the interpretation of serum calcium. Clinica Chimica Acta, 35, 483-489.

Pain, R.W., Rowland, K.M., Phillips, P.J. and Duncan, B.McL. (1975) Current "corrected" calcium concept challenged. British Medical Journal, 4, 617-619.
Pain, R.W. and Phillips, P.J. (1976) Adjustment of serum calcium for protein. Clinical Chemistry, 22, 282.

Pak, C.Y., Ruskin, B. and Casper, A. (1969) Renal Effects of Thyrocalcitonin. Calcitonin 1969: Proceedings of the Second International Symposium, 154-160. Eds. S. Taylor and G.V. Foster. Heinemann, London.

Papapoulos, S.E., Hendy, G.N., Tomlinson, S., Lewin, I.G. and O'Riordan, J.L.H. (1977) Clearance of exogenous parathyroid hormone in normal and uraemic man. Clinical Endocrinology, 7, 211-225.

Parfitt, A.M. (1969) Chlorothiazide induced hypercalcaemia in juvenile osteoporosis and hyperparathyroidism. New England Journal of Medicine, 281, 55-59.
Parfitt, A.M. (1974) Correction of plasma calcium measurements. British Medical Journal, 1, 520.

Parsons, J.A. (1976) Endocrine Pharmacology. In Peptide Hormones, 67-83. Ed. J.A. Parsons. Baltimore Univ. Park Press.

Paterson, C.R. (1973) Tests of phosphate reabsorption. Lancet, 1, 1251.

Paupe, J. (1955) Utilisation du coeur de crapaud pour le dosage biologique du calcium ionise dans le serum. Comptes Rendus des Seances de la Societe de Biologie et des Filiales (Paris), 149, 2103-2105.

Payne, R.B., Little, A.J., Williams, R.B. and Milner, J.R. (1973) Interpretation of serum calcium in patients with abnormal serum proteins. British Medical Journal, 4, 643-646.
Payne, R.B., Little, A.J., Williams, R.B. and Milner, J.R. (1974a) Adjustment of plasma calcium measurements. British Medical Journal, 2, 504-505.

Payne, R.B., Little, A.J., Williams, R.B. and Milner, J.R. (1974b) Adjustment of plasma calcium measurements. British Medical Journal, 3, 345.
Payne, R.B., Little, A.J., Williams, R.B. and Milner, J.R. (1976) "Corrected" calcium concept. British Medical Journal, 1, 153-154.
Payne, R.B. and Little, A.J. (1976) More on adjusting serum calcium for protein. Clinical Chemistry, 22, 933-934.

Peacock, M. and Nordin, B.E.C. (1968) Tubular reabsorption of calcium in normal and hypercalciuric subjects. Journal of Clinical Pathology, 21, 353-358.
Peacock, M., Robertson, W.G. and Nordin, B.E.C. (1969) Relation between serum and urinary calcium with particular reference to parathyroid hormone. Lancet, 1, 384-386.

Pearse, A.G. and Carvalheira, A.F. (1967) Cytochemical evidence for an ultimobranchial origin of rodent thyroid C cells. Nature, 214, 929-930.

Pedersen, K.O. (1969) Determination of calcium fractions of serum. I. The separation of protein-bound and protein-free fractions by means of a simplified ultrafiltration technique. Scandinavian Journal of Clinical and Laboratory Investigations, 24, 69-81.
Pedersen, K.O. (1970) Determination of calcium fractions of serum. II. Ionized calcium in ultrafiltrates of normal serum and examination of pertinant variables. Scandinavian Journal of Clinical and Laboratory Investigations, 25, 223-230.
Pedersen, K.O. (1971a) The effect of bicarbonate $_pCo_2$ and pH on serum calcium fractions. Scandinavian Journal of Clinical and Laboratory Investigations, 27, 145-150.
Pedersen, K.O. (1971b) Binding of calcium to serum albumin. I. Stoichiometry and intrinsic association constant at physiological pH, ionic strength and temperature. Scandinavian Journal of Clinical and Laboratory Investigations, 28, 459-469.
Pedersen, K.O. (1972a) Binding of calcium to serum albumin. II. Effect of pH via competitive hydrogen and calcium ion binding to imidazole groups of albumin. Scandinavian Journal of Clinical and Laboratory Investigations, 29, 75-83.
Pedersen, K.O. (1972b) Binding of calcium to serum albumin. III. Influence of ionic strength and ionic medium. Scandinavian Journal of Clinical and Laboratory Investigations, 29, 427-432.
Pedersen, K.O. (1972c) Binding of calcium to serum albumin. IV. Effect of temperature and thermodynamics of calcium-albumin interaction. Scandinavian Journal of Clinical and Laboratory Investigations, 30, 89-94.
Pedersen, K.O. (1972d) On the cause and degree of intraindividual serum calcium variability. Scandinavian Journal of Clinical and Laboratory Investigations, 30, 191-199.
Pedersen, K.O. (1972e) Protein-bound calcium in human serum, quantitative examination of binding and its variables by a molecular binding model and clinical chemical implications for measurement of ionized calcium. Scandinavian Journal of Clinical and Laboratory Investigations, 30, 321-329.

Petrie, F. (1890) A Scientific Report. British Medical Journal, 1, 423.

Pfordte, K. and Ponsold, W. (1971) Uber die calcium/serumprotein-binding. Endokrinologie, 57, 230-236.

Pittinger, C., Chang, P.M. and Faulkner, W. (1971) Serum ionized calcium; some factors influencing its level. Southern Medical Journal (Bgham, Ala.) 64, 1211-1215.

Pommer, G.A. (1885) Rachitis and Osteomalacia. A Thesis, Leipzig.

Potts, J.T. Jr., Murray, T.M., Peacock, M., Niall, H.D., Tregear, G.W., Keutmann, H.T., Powell, D. and Deftos, L.J. (1971) Parathyroid hormone; sequence, synthesis, immunoassay studies. American Journal of Medicine, 50, 639-649.
Potts, J.T. Jr., Tregear, G.W., and Keutmann, H.T. (1971) Synthesis of a biologically active N-terminal tetratricontapeptide of parathyroid hormone. Proceedings of the National Academy of Science, U.S.A., 68, 63-67.
Potts, J.T. Jr. and Deftos, L.J. (1974) Parathyroid hormone, calcitonin, vitamin D, bone and bone mineral metabolism. In Duncan's Disease of Metabolism. Eds. Bondy, P.K. and Rosenberg, L.E. Seventh Edition, 1225. Philadelphia, Saunders.

Prasad, A.S. and Flink, E.B. (1957) Effect of carbon dioxide on concentration of calcium in an ultrafiltrate of serum obtained by centrifugation. Journal of Applied Physiology, 10, 103-107.
Prasad, A.S. and Flink, E.B. (1958) The base binding property of the serum proteins with respect to calcium. Journal of Laboratory and Clinical Medicine, 51, 345-350.
Prasad, A.S. (1960) Studies on ultrafiltrable calcium. American Medical Association's Archives of Internal Medicine, 105, 560-573.

Pribam, R. (1871) Bericht Kgl. Sachs, 23, 279-286. Ges. d. Wiss, Leipzig.

Putman, J.M. (1972) A routine method for determining plasma ionized calcium and its application to the study of congenital heart disease in children. Clinica Chimica Acta, 32, 33-41.

Raisz, L.G. and Niemann, I. (1969) Effect of phosphate, calcium and magnesium on bone resorption and hormonal responses in tissue culture. Endocrinology, 85, 446-452.
Raisz, L.G., Trummel, C.L., Mundy, G.R. and Luben, R.A. (1975) Immunological factors influencing bone resorption, role of osteoclast activating factor from human lymphocytes and complement mediated prostaglandin synthesis. Calcium Regulating Hormones. Proceedings of the Fifth Parathyroid Conference. Eds. R.V. Talmage, M. Owen and J.A. Parsons. Excerpta Medica, Amsterdam.
Raisz, L.G., Dietrich, J.W. and Conalis, E.M. (1976) Factors influencing bone formation in organ culture. Israel Journal of Medical Sciences, 12, 108-114.

Raman, A. (1970) Determination of ionized calcium in serum with a calcium electrode. Biochemical Medicine, 3, 369-375.
Raman, A. (1971) The calcium fractions of normal serum. Clinical Biochemistry, 4, 141-146.
Raman, A. (1972) A comparison of two methods for the determination of serum ionic calcium. Clinical Biochemistry, 5, 208-213.

Ramsey, L.E. and Shelton, J.R. (1976) "Corrected" calcium concept. British Medical Journal, 1, 219.

Rasmussen, H., Anast, C.S. and Arnaud, C.D. (1967) Thyrocalcitonin, EGTA and urinary electrolyte excretion. Journal of Clinical Investigation, 46, 746-752.

Rawson, A.J. and Sunderman, F.W. (1948) Studies in serum electrolytes; calcium binding property of serum proteins (multiple myeloma, lymphogranuloma venereum and sarcoidosis.) Journal of Clinical Investigation, 27, 82-90.

Reifenstein, E.C. Jr. (1958) Control of corticoid induced protein depletion and osteoporosis by anabolic steroid therapy. Metabolism, 7, 78-89.

Reiner, M., Sigurdsson, G., Nunziata, V., Malik, M.A., Poole, G.W. and Joplin, G.F. (1976) Abnormal calcium metabolism in normocalcaemic sarcoidosis. British Medical Journal, 4, 1473-1476.

Reiss, E. and Canterbury, J.M. (1971) Genesis of hyperparathyroidism. American Journal of Medicine, 50, 679-685.

Reiss, E. and Canterbury, J.M. (1973) Blood levels of parathyroid hormone in disorders of calcium metabolism. Annual Review of Medicine, 24, 217-232.

Reynolds, J.J. and Minkin, C. (1970) Bone studies in vitro; use of calcitonin as a specific inhibitor of bone resorption. In Calcitonin 1969 Proceedings II International Symposium, 168. Eds. S. Taylor and G.V. Foster. Heinemann, London.

Robbins, J.J. and Saville, P.D. (1971) Corticoids in osteoporosis. Annals of Internal Medicine, 74, 1013-1014.

Robertson, W.G. and Peacock, M. (1968) New techniques for the separation and measurement of the calcium fractions of normal human serum. Clinica Chimica Acta, 20, 315-326.

Robertson, W.G. (1969) Measurement of ionized calcium in biological fluids. Clinica Chimica Acta, 24, 149-157.

Robertson, W.G. (1976) cellular calcium and calcium transport in Calcium, Phosphate and Magnesium Metabolism, 230-257. Ed. B.E.C. Nordin. Churchill Livingstone. Edinburgh, London and New York.

Robinson, C.J., Martin, T.J. and McIntyre, I. (1966) Phosphaturic effect of thyrocalcitonin. Lancet, 2, 83-84.

Robinson, D.R., Tashjian, A.H. Jr. and Levine, L. (1975) A possible mechanism for bone destruction in rheumatoid arthritis. Journal of Clinical Investigation, 56, 1181-1188.

Rona, P. and Takahashi (1911) Uber das Verhalten des calciums in serum mid uber den gehalt der blutkorperchen an calcium. Biochemie Zeitschrift, 31, 336-344.

Rooney, P.J., Kennedy, A.C., Gray, G.H., Sturrock, R.D., Buchanan, K.D. and Dick, W.C. (1976) Serum immunoreactive gastrin (IRG) in rheumatoid arthritis; further observations on its identity, clinical specificity and relationship to gastric function. Annals of the Rheumatic Diseases, 35, 246-251.

Ropes, M.W., Bennet, G.A., Cobb, S., Jacox, R. and Jessar, A.R. (1959) Proposed diagnostic criteria for rheumatoid arthritis. Annals of Rheumatic Diseases, 18, 49.

Rose, G.A. (1965) A study of treatment of osteoporosis with fluoride therapy and high calcium intake. Proceedings of the Royal Society of Medicine, 58, 436-440.
Rose, G.A. (1966) Immobilization osteoporosis. A study of the extent, severity and treatment with bendrofluazide. British Journal of Surgery, 53, 769-774.
Rose, G.A. (1967) Some thoughts on osteoporosis and Osteomalacia. Scientific Basis of Medicine, Athlone Press, University of London.
Rose, G.A. (1972) A simple and rapid method for the measurement of plasma ultrafiltrable and ionized calcium. Clinica Chimica Acta, 37, 343-349.

Ross, J.W. Jr. (1967) Calcium-selective electrode with liquid ion exchanger. Science, 156, 1378-1379.

Rowe, D.J. and Stamp, T.C. (1974) Anticonvulsant osteomalacia and vitamin D. British Medical Journal, 1, 392.

Rushton, M.L., Sammons, H.G., Gosling, P. and Robinson, B.H.B. (1973) Measurement of total, ultrafiltrable and ionized serum calcium. Clinical Biochemistry, 10, 63-71.

Russell, R.G.G. (1976) Regulation of calcium metabolism. Annals of Clinical Biochemistry, 13, 518-539.

Ryden, S.E., Kirkish, L.S., McCann, D.S. (1976) Evaluation of serum ionic calcium measurement in a general hospital population. American Journal of Clinical Pathology, 66, 634-638.

Sachs, C., Bourdeau, A.M. and Balsan, C. (1969) Determination du calcium ionisé dans le serum avec une électrode spécifique à membrane liquide. Annales de Biologie Clinique (Paris), 27, 487-510.

Sanderson, C. (1974) Correction of serum calcium measurements. British Medical Journal, 1, 245.

Saville, P.D. (1965) Changes in bone mass with age and alcoholism. Journal of Bone and Joint Surgery, 47, 492-499.
Saville, P.D. and Kharmosh, D. (1967) Osteoporosis of rheumatoid arthritis; Influence of age, sex and corticosteroids. Arthritis and Rheumatism, 10, 423-430.
Saville, P.D. (1970) Osteoporosis and corticoid drugs. Annals of Internal Medicine, 73, 1038.
Saville, P.D. (1973) The syndrome of spinal osteoporosis. Clinics in Endocrinology and Metabolism, 2, 177-185.

Schneider, A.B. and Sherwood, L.M. (1974) Calcium homeostasis and the pathogenesis and management of hypercalcaemic disorders. Metabolism, 23, 975-1007.

Schwartz, H.D., McConville, B.C., Christopherson, E.F. (1971) Serum ionized calcium by specific ion electrode. Clinica Chimica Acta, 31, 97-107.

Schwille, P.O. and Ernstberger, W. (1972) Ultrafiltrables Calcium und Magnesium im Serum von Gesunden und Nierensteinkranken. Clinica Chimica Acta, 38, 379-384.

Seamonds, B., Towfighi, J. and Arvan, D.A. (1972) Determination of ionized calcium in serum by use of an ion selective electrode. I. - Determination of normal values under physiologic conditions with comments on the effects of food ingestion and hyperventilation. Clinical Chemistry, 18, 155-160.

Segre, G.V., Habener, J.F., Powell, D., Tregear, G.W. and Potts, J.T. Jr. (1972) Parathyroid hormone in human plasma; immunochemical characterization and biological implications. Journal of Clinical Investigation, 51, 3163-3172.
Segre, G.V., Tregear, G.W., and Potts, J.T. Jr. (1975) Development and application of sequence specific radioimmunoassays for analysis of the metabolism of parathyroid hormone. Methods in Enzymology, 37, Part B, 38-66.

Sharp, J.T., Lidsky, M.D., Collins, L.C. and Moreland, J. (1971) Methods of scoring the progression of radiologic changes in rheumatoid arthritis. Arthritis and Rheumatism, 14, 706-720.

Shimmins, J., Anderson, J.B., Smith, D.A. and Aitken, M. (1972) The accuracy and reproducibility of bone mineral measurements in vivo a) The measurement of metacarpal mineralization using an X-ray generator. Clinical Radiology, 23, 42-46.
Shimmins, J., Smith, D.A., Aitken, M., Anderson, J.B. and Gillespie, F.C. (1972) The accuracy and reproducibility of bone mineral measurements in vivo b) Methods using sealed isotope sources. Clinical Radiology, 23, 47-51.

Silverman, R. and Yalow, R.S. (1973) Heterogeneity of parathyroid hormone; clinical and physiologic implications. Journal of Clinical Investigation, 52, 1958-1971.

Simmons, C.J., Davis, M., Dordoni, B. and Williams, R. (1974) Urinary D-glucaric acid assay by an improved enzymatic procedure. Clinica Chimica Acta, 51, 47-51.

Sissons, H.A. (1952) Osteoporosis and epiphyseal arrest in joint tuberculosis. Journal of Bone and Joint Surgery, 34B, 275.
Sissons, H.A. (1956) Osteoporosis of Cushing's Syndrome. Journal of Bone and Joint Surgery, 38B, 418-433.
Sissons, H.A., Holley, K.J. and Heighway, J. (1967) L'Osteomalacie, D.C. Hioco, Masson, Paris.

Sjoberg, H.E. (1970) Retention of ^{47}calcium administered orally in patients with primary hyperparathyroidism. Hormone and Metabolic Research, 1, 32-34.

Smith, D.A., Anderson, J.B., Shimmins, J., Speirs, C.G. and Barnett, E. (1968) Progress in Methods of Bone Mineral Measurements, 177. Eds. G.D. Whedon and J.R. Cameron. U.S. Dept. of Health, Education and Welfare, U.S. Gov't., Printing Office, Washington D.C. 20402 U.S.A.
Smith, D.A., Anderson, J.B., Shimmins, J., Speirs, C.G. and Barnett, E. (1969). Changes in metacarpal mineral content and density in normal male and female subjects with age. Clinical Radiology, 20, 23-31.
Smith, D.A. (1970) M.D. Thesis on Osteoporosis, University of Glasgow.

Smith, G. Elliot and Jones, F. Wood. (1910) Archaeological Survey of Nubia. Report for 1907-1908, Vol. 2. Cairo National Printing Dept.

Smith, R.W. Jr., and Rizek, J. (1966) Epidemiological studies of osteoporosis in women of Puerto Rico and Southeastern Michigan with special reference to age, national origin and to other related or associated findings. Clinical Orthopaedics, 45, 31-48.

Soila, P. (1958) Roentgen manifestations of adult rheumatoid arthritis, with special regard to the early changes. Acta Rheumatologica Scandinavica Suppl. 1, 1-144.

Soila, P. (1963) The causal relations of rheumatoid disintegration of juxta-articular bone trabeculae. Acta Rheumatologica Scandinavica, 9, 231-236.

Soria, J., Soria, C., Dao, C., James, J.M., Bousser, J. and Bilski-Pasquier, G. (1976) Immunoglobulin bound calcium and ultrafiltrable serum calcium in myeloma. British Journal of Haematology, 34, 343-344.

Soulier, J.P. and Crosnier, J. (1958) Nouvelle methode de dosage du calcium ionise utilisant un systeme coagulant. La Press Medicine, 66, 617-619.

Spencer, H.V., Menczel, J., Lewin, I. and Samachson, J. (1964) Absorption of calcium in osteoporosis. American Journal of Medicine, 37, 223-234.

Sprague, R.G., Randall, R.V., Salassa, R.M., Scholz, D.A., Priestley, J.T., Walters, W. and Bulbulian, A.H. (1956) Cushing's Syndrome. Archives of Internal Medicine, 98, 389-397.

Stanbury, S.W. (1968) The intestinal absorption of calcium in normal adults, primary hyperparathyroidism and renal failure. In Nutrition in Renal Failure. Ed. G.M. Berlyne. 118-132. E. & S. Livingstone, Edinburgh.

Steinbrocker, O., Traeger, C.H. and Batterman, R.C. (1949) Therapeutic criteria in rheumatoid arthritis. Journal of the American Medical Association, 140, 659-662.

Steiner, D.F., Clark, J.L., Nolan, C., Rubinstein, A.H., Margoliash, E., Aten, B. and Oyer, P.E. (1969) Proinsulin and the biosynthesis of insulin. Recent Progress in Hormone Research, 25, 207-282.

Stevenson, F.H. (1952) The osteoporosis of immobilization in recumbency. Journal of Bone and Joint Surgery, 34-B, 256-265.

Tashjian, A.H. Jr., Melvin, K.E.W., Voelkel, E.F., Howland, B.G., Zuckerman, J.E. and Minkin, C. (1972) Calcitonin in blood, urine and tissue: Immunological and biological studies. In Calcium, Parathyroid Hormone and the Calcitonins, 97. Eds. Talmage, R.V. and Munson, P.L. Excerpta Medica, Amsterdam.

Teicher, R. and Nelson, C.T. (1952) Osteoporosis and pathological fractures following treatment with ACTH and cortisone. Journal of Investigative Dermatology, 19, 205-210.

Tendeloo, H.J.C. (1936) A new and easy method for the potentiometric determination of calcium concentration in solution. Journal of Biological Chemistry, 113, 333-339.

Tenenhouse, A., Arnaud, C.D. and Rasmussen, H. (1965) The isolation and characterization of thyrocalcitonin. Proceedings of the National Academy of Science, 53, 818-822.

Thomson, D.L. and Collip, J.B. (1932) Parathyroid Glands. Physiological Review, 12, 309-383.

Toribara, T.Y., Terepka, A.R. and Dewey, P.A. (1957) The ultrafiltrable calcium of human serum. I. Ultrafiltration methods and normal values. Journal of Clinical Investigation, 36, 738-748.

Trotter, M., Broman, G.E. and Peterson, R.R. (1960) Densities of bones of white and negro skeletons. The Journal of Bone Joint Surgery, 42-A, 50-58.

Truesdell, A.H. and Christ, C.L. (1967) Glass electrodes for calcium and other divalent cations. In Glass Electrodes for Hydrogen and other Cations: Principle and Practice. Ed. G. Eisenman. Marcel Dekker Inc. New York.

Turbey, W.J. and Passaro, E. Jr. (1972) Hyperparathyroidism in the Zollinger-Ellison Syndrome. Archives of Surgery, 105, 62-66.

Turek, J. and Goverde, B.C. (1973) The significance of hydroxyproline assay in urine. Organon Teknika Oss Holland.

Urist, M.R. (1960) Bone as a Tissue, Chapter 2. McGraw-Hill, New York.

Van Slyke, D.D., Hastings, A.B., Hiller, A. and Sendroy, J. (1928) Studies of gas and electrolyte equilibria in blood. XIV. The amounts of alkali bound by serum albumin and globulin. Journal of Biological Chemistry, 79, 769-780.

Varghese, Z. (1973) Determination of Plasma Calcium Fractions. Annals of Clinical Biochemistry, 10, 120-129.

Villanueva, A.R., Klein, M. and Frost, H.M. (1964) Effect of adrenal-corticoid hormones on human lamellar bone: Quantitative histological measurements of resorption and formation indices. Henry Ford Hospital Medical Bulletin, 12, 63-73.

Virchow, R. (1869) Virchows Archives, 47, 298.

Virtama, P., Helela, T. and Kalliomaki, J.L. (1968) Osteoporosis in rheumatoid arthritis: A follow-up study. Acta Rheumatologica Scandinavica, 14, 276-284.

Walser, M. (1961) Ion Association VI. Interactions between calcium, magnesium, inorganic phosphate, citrate and protein in normal human plasma. Journal of Clinical Investigation, 40, 723-730.

Walser, M. (1962) The separate effects of hyperparathyroidism, hypercalcaemia of malignancy, renal failure and acidosis on the state of calcium phosphate and other ions in plasma. Journal of Clinical Investigation, 41, 1454-1471.

Wang, C.C. and Robbins, L.L. (1956) Cushing's Disease: its roentgenographic findings. Radiology, 67, 17-24.

Watson, M. (1975) Ph.D. Thesis. University of Glasgow.

Webb, J., Whaley, K., MacSween, R.N., Nuki, G., Dick, W.C. and Buchanan, W.W. (1975) Liver disease in rheumatoid arthritis and Sjøgren's Syndrome. Prospective study using biochemical and serological markers of hepatic dysfunction. Annals Rheumatic Diseases, 34, 70-81.

Whedon, G.D. and Shorr, E. (1957) Metabolic studies in paralytic acute anterior poliomyelitis. II. Alterations in calcium and phosphorus metabolism. Journal of Clinical Investigation, 36, 966-981.
Whedon, G.D. (1959) Effects of high calcium intake on bones, blood and soft tissue - relationship of calcium intake to balance in osteoporosis. Federal Proceedings, 18, 1112-1118.

Wills, M.R., Fairney, A., Varghese, Z., Tatler, G.L., Baillod, R.A. and Moorehead, J.F. (1974) Serum parathyroid hormone concentration in chronic renal failure patients on maintenance haemodialysis. Clinica Chimica Acta, 57, 83-89.

Wood, G.C., Pryce-Jones, R.H. White, D.D. and Nuki, G. (1971) Chondromucoprotein-degrading neutral protease activity in rheumatoid synovial fluid. Annals of Rheumatic Diseases, 30, 73-77.

Woodard, H.Q. (1964) The composition of human cortical bone. Clinical Orthopaedics and Related Research, 37, 187-193.

Woodhead, J.S. and Walker, D.A. (1976) Assay of parathyroid hormone in human serum and its use. Annals of Clinical Biochemistry, 13, 549-555.

Yalow, R.S. and Berson, S.A. (1970) Radioimmunoassay of gastrin. Gastroenterology, 58, 1-14.

Yee, H.Y. (1968) A simplified method for automated phosphorus analysis of serum and urine. Clinica Chimica Acta, 14, 898-903.

Yendt, E.R., Connor, T.B. and Howard, J.E. (1955) In vitro calcification of rachitic rat cartilage in normal and pathological human sera, with some observations of the pathogenesis of renal rickets. Bulletin of Johns Hopkins Hospital, 96, 1-12.

Zanelli, J. and Rafferty, B. (1976) Current state of radioimmunoassay of parathyroid hormone and calcitonin. At International Atomic Energy Agency Research Co-ordination Meeting on in vitro assay procedures and Techniques. London.

Ziff, M., Kibrick, A., Dresner, E. and Giribetz, H.L. (1956) Excretion of hydroxyproline in patients with rheumatic and non-rheumatic diseases. Journal of Clinical Investigation, 35, 579-583.