Acidosis and Bone Disease in Chronic Renal Failure

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This thesis is dedicated to Professor B.E.C. Nordin and Dr Munro Peacock, without whose help the work would not have been possible.

Foreword

The observations that form the basis of this thesis were made mainly between 1969 and 1971 at the MRC Mineral Metabolism Unit, Leeds, under the directorship of Professor B.E.C. Nordin. The work led on naturally from a study of urine acidifying mechanisms in a group of patients, most of whom had renal stones. The patients generally had a substantial reduction in glomerular filtration rate, a urine acidifying defect, and a number had clinically apparent osteomalacia. However, the association of the osteomalacia with the chronic metabolic acidosis seemed at least as strong as with the degree of uraemia, raising again the old question of whether a causal connection existed between acidosis and renal bone disease.

Due especially to the beautiful work of David Fraser in Cambridge we now know that most creatures exposed to direct sunlight have evolved a metabolism that far from accumulating Vitamin D, tends rather to inactivate and excrete it, presumably to reduce the risk of toxic effects. Perhaps for similar reasons, the hydroxylating enzyme in the kidney seems to have a relatively limited capacity for increasing production of 1,25-dihydroxycholecalciferol. The material is so potent that the organism is probably better served by a system that is less, rather than more, able to generate it in large quantities when stimulated. A greater capacity for 1,25-dihydroxycholecalciferol production would require the existence of a stronger counter-regulatory mechanism than appears to be provided by calcitonin, for example. Thus if evolution has given any consideration to the patient with

chronic renal failure, it is to ensure protection against toxic hypercalcaemia, and it is clear that the effects of 1-hydroxylation failure of Vitamin D may appear in non-fatal uraemia. This does not mean that acidosis is irrelevant, however, because any adverse effect that it might have would be of critical importance in a situation where synthesis of the active metabolite of Vitamin D were already impaired.

In this study, two terms have been used in a general sense. The first is renal bone disease, synonymous with osteodystrophy, and simply implying the presence of a detectable bone abnormality related to the renal failure. The precise pathology has been considered when appropriate. The second term is Vitamin D, which may refer to any of the commonly prescribed analogues, although ergocalciferol has usually been used. Dihydrotachysterol has been distinguished in the text where necessary. In the figures, open circles always designate data from patients with normal plasma alkaline phosphatase levels, and closed circles refer to data from other patients. All patients in the study gave their informed consent.

Finally, it is a necessary and pleasant duty to acknowledge the enthusiastic help of many graduates and technicians at the MRC Unit in Leeds; the Department of Medicine, Sheffield Royal Hospital; and Strangeways Research Laboratory, Cambridge.

CONTENTS

CHAPTER 1

Introduction

When considering the disordered metabolism of bone that occurs in chronic renal failure, it is perhaps to Kodicek and his colleagues that we owe the greatest debt. In 1970 they showed that under normal conditions the calciferol molecule is altered by the body to successively more active compounds, and that the final stage in the process takes place in the kidney (Fraser & Kodicek, 1970). Furthermore, the synthesis of the final active metabolite, 1,25-dihydroxycholecalciferol, is subject to feed-back control probably with parathyroid hormone acting as mediator (Fraser & Kodicek, 1973). Earlier de Luca (1969) had suggested that a polar metabolite of Vitamin D, readily detectable in the serum of normal individuals, was absent from patients with chronic renal failure, and that this metabolite was more active in promoting gastro-intestinal transport of calcium than the parent substance; but the material, 25-hydroxy-cholecalciferol was not greatly more potent than calciferol itself (Blunt et al. 1968) and moreover was synthesized in the liver (Avioli et al. 1967), so that while this must rank as an extremely important piece of work, it failed to explain the Vitamin D resistance of chronic uraemia, nor did it involve the kidney in Vitamin D metabolism.

In spite of these advances the picture is not entirely clear. 1,25-Dihydroxycholecalciferol is a potent calcaemic hormone but there is no clear evidence that it is the major anti-rachitic agent, and there is some information suggesting the contrary (Omdahl et al. 1971). Furthermore, while malabsorption of calcium is invariably found in chronic renal failure when the glomerular filtration rate is less than 25 ml/minute, osteomalacic bone is not necessarily

present. It also appears that the synthetic compound 1-hydroxycholecalciferol, thought to be converted to 1,25-dihydroxycholecalciferol, is without any dramatic effect on renal osteomalacia or rickets, although calcium absorption is undoubtedly improved.

In the past the frequent co-existence of a severe metabolic acidosis and renal bone disease has led many to suspect a causal relationship and earlier authors (Holt et al. 1925; Parsons, 1927) suggested that the mechanism might lie in the sensitivity of the ionic product of calcium phosphate to change in the prevailing hydrogen ion concentration. Albright (1937) in particular emphasized the importance of acidosis, though he interpreted differently the way in which it might act. He believed that in a state of metabolic acidosis, calcium would be excreted as an available cation in place of ammonium, leading ultimately to a decreased rate of mineralisation. Albright's concept has been contradicted by observations in many individual cases (Stanbury, 1967), but its simplicity has proved attractive and has continued to focus attention on the possible central role of acidosis in renal bone disease. Stanbury, on the other hand, has frequently stated that bone disease and acidosis are independent consequences of uraemia, but are not causally related. There is no doubt, however, that acidosis may affect calcium metabolism (Litzow et al. 1967) and a number of reports suggest that healing of osteomalacia may be more easily achieved if the acidosis is corrected (Stanbury, 1962).

Hypocalcaemia is the other common biochemical feature of renal osteomalacia (Parsons, 1927; Stanbury, 1967), and is generally believed to be of importance in its development. However,

mineralisation of rachitic bone can occur with Vitamin D therapy prior to correction of the hypocalcaemia (Stanbury, 1967) and, while accepting its statistical association, Stanbury questions its role in the pathogenesis of bone disease.

A syndrome exists therefore where renal osteomalacia or rickets, a metabolic acidosis, and sustained hypocalcaemia frequently co-exist but their inter-relationships are obscure. This work was undertaken to establish, firstly, what part, if any, is played by acidosis in the development of renal bone disease - whether osteomalacic or parathyroid induced - and secondly, whether the acidosis in any way contributes to the development of hypocalcaemia. It remains possible, but not certain, that the hypocalcaemia may itself influence the bone disease and some consideration is also given to this question.

The development of the study

While studying a group of patients with chronic pyelonephritis it became clear that a number of them with a particularly severe degree of metabolic acidosis due to a urinary acidification defect acquired as a result of the infection (Cochran et al. 1968) also had clinical osteomalacia or rickets. This was rarely seen in patients with primary glomerular lesions whose acidosis was generally milder except in the terminal phase of their illness. This observation again emphasized the strong association between acidosis and renal osteomalacia and prompted the investigation of a group of fifty patients with stable chronic renal failure. The investigation was intended to determine the importance of various factors in the aetiology of the bone disease and it was therefore first of all necessary to

establish which patients had bone disease and which did not. The patients were therefore separated into those with normal and those with abnormal plasma alkaline phosphatase levels. The validity of this separation is discussed with respect to standard bone x-ray measurements and quantitative bone histology. It becomes apparent that the raised alkaline phosphatase level indicates an osteomalacic process whereas reduction in the standard bone x-ray measurements reflects parathyroid mediated bone resorption. Analysis of the relationship between the bone x-ray measurements and the various biochemical parameters suggests an association between reduction in cortical bone and hypocalcaemia, and between acidosis and both the presence of osteomalacia and hypocalcaemia. These data were further interpreted in the light of quantitative bone histology carried out on iliac crest biopsies. Calcium absorption was measured by a radiocalcium absorption test and the influence of acidosis on absorption is discussed. The kinetics of bone turnover in chronic renal failure were also investigated by means of metabolic balance studies and radioisotope techniques. In this way mineralisation and resorption rates were determined in a number of patients, and comparisons made between the acidotic state and non-acidotic state. Since hypocalcaemia seemed to lead to bone resorption, emphasis has been given to consideration to certain factors influencing plasma calcium levels in chronic renal failure, and in particular the mechanisms of action of acidosis and Vitamin D have been examined.

The causes of acidosis in chronic renal failure are well known, but the information presented here provides some explanation for the hypocalcaemia and it is possible to discuss the relative

contributions of acidosis and hypocalcaemia to the osteomalacia and hyperparathyroidism of renal bone disease.

CHAPTER 2

Review

The association of bone deformities with renal disease was first noted by Lucas who in 1883 published a paper entitled "A Form of Late Rickets Associated with Albuminuria". However, this author believed that both the rickets and the albuminuria were consequences of puberty, and assumed that the condition would stabilize or improve. At about the same time Davis-Colley described the case of thirteen year old girl with marked bone changes who subsequently died of renal failure (Davis-Colley, 1884). There followed three separate case reports of renal failure in young children with rickets but at the time the presence of bone disease in infancy was not considered in any way remarkable (Hellendall, 1897; Sequeira, 1901; Sawyer, 1903). In reporting a series of children with renal failure and hypertensive retinopathy, Nettleship (1903) mentioned the case of an older child with congenital renal disease and bowing of the tibiae.

In 1911 Fletcher described a six year old boy with marked genu valgum and renal disease as evidenced by polyuria, albuminuria and bilateral palpable kidneys. In the discussion, Fletcher did not particularly emphasize the association of bone and renal disease but he is generally credited by his own contemporaries for recognizing some aetiological connection, and about this time the term renal rickets began to appear. Miller and Parsons (1912) and Naish (1912) observed that rickets was a not uncommon complication of chronic renal disease in childhood. During the next 15 years a number of case reports on the subject were published, and Barber, in particular, clarified the clinical features of the disease in a series of papers (Barber, 1913, 1918, 1920, 1921, 1922 and 1926).

During this period there was considerable activity directed

towards understanding the nature of nutritional rickets and a number of hypotheses were proposed to explain the phenomenon. Firstly it had been shown that Vitamin D deficient rats would develop rickets when they were fed either a low calcium - high phosphorus or a low phosphorus - high calcium diet. As a result, it was suggested that there were two types of rickets: one form consequent upon a relative calcium deficiency, and the other due to a relative phosphorus deficiency. This theory was not easily reconciled with that of Howland (1923), who put forward the idea that serum and extracellular fluid formed a saturated solution in which the calcium phosphate of bone was in dynamic equilibrium with the dissolved salt.

$$3Ca^{++} + 2PO_4'' \rightleftharpoons Ca_3(PO_4)_2$$

It was further suggested that since bone was a relatively inactive tissue there would be low local concentrations of carbonic acid which would tend to favour the deposition of calcium phosphate.

$$3Ca^{++} + 2HPO_4'' + 2HCO'_3 \rightleftharpoons 2CaHPO_4 + Ca(HCO_3)_2$$

$$\rightleftharpoons Ca_3(PO_4)_2 + 2H_2CO_3$$

These ideas were put forward on purely theoretical grounds but Howland stated that they could be supported by clinical observations, and introduced the idea that a useful approximation could be made simply by considering the numerical calcium x phosphorus product when the concentration of each in serum was expressed in mg/100 ml. Thus

in the normal child products of 50 or 60 were obtained, but "when the product was below 30, rickets was invariably present. With products between 30 and 40 rickets was usually present and demonstrable healing occurred when the product was above 40". Howland must have met a number of cases which were not compatible with his physico-chemical theory for he later suggested that other factors might be involved (Shipley et al. 1926). He stated "the process is not one of simple precipitation; it depends upon the activity of living tissue". However, he went on to say that the mineralisation must depend upon the concentration of calcium and phosphorus exceeding a certain minimum value in the bone-forming cells. (These views were later echoed by Cochran and Nordin (1969) who published a small series of cases of renal osteomalacia and suggested that either the ionised calcium or phosphate, or both, were reduced in the serum in these patients). Another theory, which later gained a certain degree of favour was that of Robison (1926). He believed that by producing the enzyme alkaline phosphatase, the osteoblasts hydrolysed the phosphoric esters of the extracellular fluid and brought about a local increase in phosphate concentration, thereby leading to a local highly super-saturated solution of calcium phosphate with resulting precipitation of the salt. However, this hypothesis was quite impossible to substantiate and remained controversial. Although Howland had encountered difficulty in explaining the presence of nutritional rickets in certain cases, the problem appeared to be resolved by the suggestions of Holt et al. (1925). These authors pointed out that even in a super-saturated solution, calcium phosphate precipitates out relatively slowly and the speed with which this occurs decreases markedly as the

ion product falls toward the solubility product. Thus, although in active rickets an apparently supersaturated solution might occur they believed that calcification was so severely delayed that osteoid would gradually build up without proper mineralisation. Pathologically delayed mineralisation could only occur in this way in growing bone and they pointed out that in normal adult life, after growth had finished, the concentrations of calcium and phosphorus could be similar to those found in active rickets in infants.

As these concepts developed it was natural that most of the discussion and speculation regarding renal rickets was concerned with chemical changes in the blood. Halverson in 1917 showed that hypocalcaemia was a common accompaniment of chronic renal failure and this was abundantly confirmed in further studies by Denis et al. (1923), de Wesselow (1924), and Rabinowitch (1925). Greenwald (1915) had earlier reported an elevated plasma phosphate in chronic renal failure and his observation was confirmed and extended by Howland in 1916. This general recognition of a reduced or normal plasma calcium and hyperphosphataemia in renal failure led to controversy concerning the obviously defective mineralisation when considered in the light of Howland's hypothesis. These difficulties appeared to be resolved, at least in part, by considering the effect of the hydrogen ion concentration on physico-chemical equilibrium. Thus Freudenburg (1923) pointed out that the ion product of calcium phosphate would fall rapidly with any tendency to acidosis. Parsons (1927) in particular strongly emphasized the importance of the metabolic acidosis in the development of renal bone disease with a clear clinical, histological and biochemical account of a small

series of patients. However, Parsons also held the seemingly inconsistent view that the ratio of calcium to phosphorus in the blood was of importance in normal mineralisation, which he believed could not take place in the presence of a greatly elevated serum phosphate. This idea had arisen as a result of the experimental studies in animals and because of the widely held view that calcium would not be deposited in the skeleton when it was required in the serum to prevent the development of tetany. Commenting on the failure of anti-rachitic foodstuffs or ultra-violet light to cure renal rickets, Parsons agreed with Shipley (1926) that some "endogenous factor" must be present to account for this resistance to conventional therapy.

Some years later, Mitchell, in a long review of the subject (Mitchell, 1930) expressed doubts about Parsons' view that a reduced ion product existed in acidosis but this was clearly in order to advance an alternative theory, which was however based more on conjecture than the careful studies of the earlier workers. Nonetheless, Mitchell agreed that acidosis was of importance and this factor therefore continued to play a central role in the thinking of workers in this field. He quoted the experiments of Haldane (1923) and Nelson (1928) to show that acidosis led to mobilisation of tissue phosphate which was then normally excreted in the urine, but in renal failure he suggested that the phosphate could only be excreted via the gut where it would precipitate dietary calcium. In this way he attributed the development of bone disease entirely to the malabsorption of calcium.

An important concept was introduced in 1936 by Fanconi, who

pointed out that the renal tubule could be defective in secretion or resorption and thus allow wastage of essential materials. He described a syndrome of rickets associated with hypophosphataemia, metabolic acidosis and renal glycosuria without evidence of marked glomerular failure. The hypophosphataemia offered an apparently simple explanation for the failure of bone mineralisation and this relatively rare form of rickets unfortunately focussed attention away from the uraemic variety to such an extent that the existence of the latter began to be doubted. Thus Albright dogmatically affirmed that the disease in question was osteitis fibrosa (Albright & Reifenstein, 1948) and dismissed without comment the histological evidence of osteomalacia so clearly shown by Follis and Jackson (1943). According to Albright, three main types of renal bone disease were found:

1. That associated with chronic glomerular failure - regarded as osteitis fibrosa.
2. That associated with renal tubular acidosis without glomerular insufficiency - a true osteomalacia or rickets.
3. That associated with primary hypophosphataemia as a manifestation of the Fanconi syndrome - a true osteomalacia or rickets.

In all cases, the presence of acidosis was considered of prime importance for Albright believed that calcium was excreted in the urine as an available cation in place of ammonia. He postulated that this led to hypocalcaemia which was however compensated by a moderate degree of parathyroid hyperplasia, this secondary hyperparathyroidism in turn causing phosphaturia and hypophosphataemia

provided glomerular function was adequate. The calcium x phosphorus product thus decreased and mineralisation was delayed. In the presence of glomerular failure, hypophosphataemia could not occur, and the state of uncompensated hyperparathyroidism led to the development of osteitis fibrosa.

Nonetheless, there was general acceptance that osteomalacia could occur with general renal failure, and many believed that the cause was some accumulated metabolite, though McCune et al. (1943) denied this. This idea was revised by Yendt and Howard (1955) when they reported evidence of an inhibitor of calcification in uraemic serum, using a technique similar to that used by Howland thirty years before. This work was of great interest at the time but it has not been confirmed and its validity is consequently open to some doubt.

Stanbury has made careful observations correlating the clinical, histological and metabolic features of renal bone disease in several large series of patients (Stanbury & Lumb, 1962; Lumb & Stanbury, 1966; Stanbury, 1968a, b) and strongly criticised the views of Albright, pointing out that these have been contradicted by observations in many individual cases (Stanbury, 1962). Furthermore, Stanbury has frequently asserted that acidosis is merely a common accompaniment of renal bone disease but does not contribute to it.

A number of experiments have been carried out showing that bone contains a simple calcium carbonate fraction which may be eluted by ammonium chloride in vitro or by chronic acidosis in vivo (Pellegrino & Biltz, 1965). It has also been suggested that the mineral structure of bone is in the form of a lattice which may

become relatively calcium deficient in certain circumstances (Richelle et al. 1965). Yet other work has shown evidence of an amorphous calcium phosphate fraction which is considered to be more easily exchangeable than the mineral of structural bone (Watson & Robinson, 1953; Posner, 1969). It may be argued however that whether or not these observations are correct, the mineral phase under discussion is always a labile moeity which is structurally distinct from the bulk of the bone tissue; and a leeching out of mineral would in any case be most unlikely to give the morphological picture of osteomalacia. Litzow et al. (1967) demonstrated that the acidosis of uraemia leads to a chronic calcium loss both in the faeces and urine, and Lemann (1969) states that over a period of time this must contribute to the bone disease of chronic renal failure. Stanbury's own data show that correction of the metabolic acidosis in chronic renal failure results in a slight net positive shift in the calcium balance (Stanbury, 1962) but as he points out in another context (Stanbury, 1968a), this does not necessarily mean that correction of the abnormalities or mineralisation would follow. Nonetheless, it does show that acidosis affects calcium metabolism in a fundamental way and the work is amply supported by the studies of Lemann et al. (1967) and Litzow et al. (1967). Using semi-synthetic diets, they carried out metabolic balances before and after correction of the acidosis in uraemic patients, and examined the effect of induction of acidosis in normal subjects. Acidosis was associated with a decrease in gastro-intestinal absorption and renal tubular reabsorption of calcium. Further evidence implicating acidosis in the genesis of renal osteomalacia

has come from certain reports where rachitic lesions have apparently healed following correction of the acidosis alone (Foss et al. 1956; Green & Boyd, 1959; Hossain, 1970; Wrong, 1971; Bishop & Ledingham, 1972). Most, though not all, of these patients had a degree of acidosis that was out of proportion to the glomerular lesion as a result either of an acquired form of renal tubular acidosis or ureterosigmoidostomy. (It is possible to speculate that the renal tissue required for the synthesis of the active principal of Vitamin D is intact in such cases, but that this synthesis, or the end organ response, is inhibited by the acidosis).

As far as the plasma concentrations of calcium or phosphate are concerned, many clinicians have accepted the view that they determine the state of mineralisation. There are a number of studies where correction of the biochemical disorder alone without the use of Vitamin D appears to have improved nutritional or renal rickets (Fraser et al. 1958; Fraser et al. 1959; Cassimos et al. 1963; Snodgrass & de Wardener, 1969). However it is well known that nutritional rickets may begin to heal simply after hospital admission even when attempts are made to provide the patient with his usual diet and renal rickets may undergo spontaneous cure (Parsons, 1927). Fletcher et al. (1963) failed to improve the bone lesions of a group of patients with renal osteomalacia after correction of the hypocalcaemia by feeding calcium salts. It is also possible, though rare, for florid renal rickets to exist in the presence of hypercalcaemia without hypophosphataemia. In one such case (see appendix: case I) the bones were densely mineralised yet classical rachitic changes persisted at the diaphysis. In another case where severe osteomalacia

resulted from renal hypophosphataemia (see appendix: case II), after prolonged administration of phosphate, there was some slight improvement in mineralisation both radiologically and histologically but the latter showed no evidence that a real cure was taking place. Much of the mineral deposition was irregular rather than along a normal calcification front, and the general appearance of the trabeculae remained disorganised. Emphasising this point, a small series has been published showing that simple correction of the biochemical lesion in renal osteomalacia is associated with patchy, abnormal mineralisation (Eastwood et al. 1974).

It does seem possible that in certain circumstances rickets may be aggravated by low mineral concentrations in the plasma, and these two cases suggest that some mineral deposition may occur when correction of the biochemical lesion is achieved, but this is not to say that such mineralisation is necessarily normal or that it represents true healing.

CHAPTER 3

Concepts

ABBREVIATIONS, DEFINITIONS AND CONCEPTS

Abbreviations

GFR - Glomerular filtrate rate, ml/min.

GF - Glomerular filtrate.

ECF - Extracellular fluid.

Ca/Cr - Ratio of calcium concentration to creatinine concentration in any urine sample. The Ca/Cr is a measure of absolute calcium excretion in an individual since excretion of creatinine in the urine proceeds at a constant rate.

Ca_E - Urine calcium excretion, expressed as mg urine calcium derived from 100 ml of filtered plasma water, i.e. mg/100 ml GF.

P/Cr & P_E - Refer similarly to phosphorus excretion.

TmP - Maximum tubular reabsorptive capacity for phosphorus. When expressed in mg/100 ml of GF it is equal to the theoretical phosphate threshold.

α_1 - Calcium absorption expressed as the fraction of the dose of calcium presented to the gastrointestinal absorptive surface that is absorbed per hour. A tracer dose of radio-calcium is used in 20 mg of calcium as calcium chloride.

m - Bone mineralisation rate, measured isotopically and expressed as the amount of calcium entering the bone per unit body weight of the individual per day, i.e. mg/kg/day.

r	-	Bone resorption rate estimated indirectly by the difference between the mineralisation rate and the calcium balance of the individual and expressed in mg/Kg/day.
CA/TA	-	The ratio of the cross-sectional area of the cortex to the total cross-sectional area of the second metacarpal, mid-way along the shaft of the bone.
MTW	-	Total width of the second metacarpal at the mid-point, cm.
OHP	-	Urine total hydroxyproline, mg/24 hours.

Definition and Concepts

Filtered load

The filtered load is the unit quantity of material filtered at the glomerulus. If expressed per unit time, it equals the product of the glomerular filtration rate (GFR) and the concentration of the substance in question in the ultrafiltrate. Alternatively, since GFR is difficult to measure accurately, a unit volume of filtrate may be considered. The filtered load can then be expressed in mg/100 ml of filtrate, which is the same as the plasma water concentration. This has obvious advantages, provided the excretion and tubular reabsorption are related to that same standard volume of glomerular filtrate (GF). If the substance in the plasma is partly protein-bound, this has to be taken into account.

Calcium excretion

As with filtered load, calcium excretion can be expressed in more than one way. The absolute rate of excretion is usually expressed in mg/min. However, this leads to inaccuracies since exact timing of bladder emptying is required though these inaccuracies are of less significance when prolonged collections are made, for example, over a 24 hour period.

Another method of expressing the absolute rate of excretion is to relate the concentration of calcium to that of creatinine in the same urine sample. The justification for this is that in any individual, the rate of excretion of creatinine is relatively constant throughout the day and will only alter with change in muscle mass, the site of creatinine production. A fall in GFR will raise the plasma concentration of creatinine but the same amount will be excreted per day

in the urine. The urine calcium/creatinine ratio (Ca/Cr) is thus a measure of absolute calcium excretion which does not depend on timed urine samples. The objection to this way of expressing calcium excretion is that the rate of excretion of creatinine varies from individual to individual, so that a healthy man may excrete 1500 mg/24 hours, while a malnourished child may produce only 500 mg/24 hours. This would obviously affect comparison of the Ca/Cr in the two cases. However, most individuals produce 800 - 1200 mg/24 hours and in practice the Ca/Cr provides a very useful measure of instantaneous calcium excretion in absolute terms.

Calcium excretion may also be related to the glomerular filtrate. When a unit volume of plasma is filtered, materials which are of physiological importance to the body are largely reabsorbed, and a small amount excreted in the urine. Excretion and filtration must be considered in the same terms and it then becomes possible to compare the calcium excreted per 100 ml of GF - termed the Ca_E - directly with the plasma ultra-filtrate concentration. This is a relatively simple measurement, since creatinine provides a marker of glomerular filtrate. Thus the plasma creatinine concentration denotes 100 ml of glomerular filtrate and the volume of urine that contains the same amount of creatinine will be derived from that unit of filtrate. The calcium excreted per 100 ml of GF may be calculated knowing the urine calcium and creatinine concentrations in a random sample and the simultaneous plasma creatinine concentrations.

Thus: $\frac{V Cr_u}{Cr_p}$ = Volume of GF (ml)

Calcium (mg) excreted in $V = \frac{V.Ca_u}{100}$

∴ Calcium excreted/100 ml of GF,

$$Ca_E = V.Ca_u \cdot \frac{Cr_p}{V.Cr_u}$$

$$\text{i.e. } Ca_E = \frac{Ca_u Cr_p}{Cr_u}$$

when Cr_u = urine creatinine concentration, mg/100 ml

Cr_p = plasma creatinine concentration, mg/100 ml

Ca_u = urine calcium concentration, mg/100 ml

V = volume of urine, ml.

The relation between the filtered load of calcium and the calcium excretion per 100 ml of GF has been established in normal subjects (Peacock & Nordin, 1968). In practice it can be assumed that the ultrafiltrable calcium is directly proportional to the plasma concentration and it is therefore possible to consider urine calcium relative to GFR (Ca_E) as a function of plasma calcium concentration. The normal range for Ca/Cr is 0.05 - 0.15; and for Ca_E, 0.05 - 0.17 mg/100 ml GF.

Renal Threshold

Generally speaking, the urinary excretion of a substance is some direct function of the filtered load. However, below a certain value for filtered load, described as the threshold, the substance may virtually disappear from the urine owing to tubular reabsorption. The threshold level is therefore determined by the tubular handling of the substance.

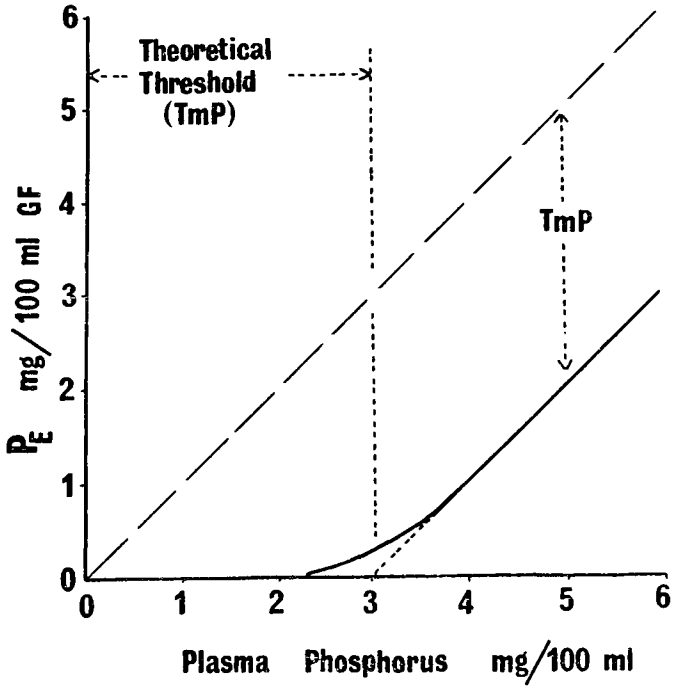

Fig. 1. The relation between filtered and excreted phosphate expressed per 100 ml of glomerular filtrate, showing the theoretical phosphate threshold, which is the same as the maximum tubular reasorption.

Phosphate excretion

Urine phosphate excretion may be considered in exactly the same way as calcium excretion, but it has been examined from a number of view-points by different workers (Anderson, 1955; Nordin & Fraser, 1960; Bijvoet, 1964; Nordin & Bulusu, 1968). When phosphate excretion relative to GFR (P_E) is related to the plasma phosphate concentration, it is found in normal subjects that as plasma phosphate is progressively elevated, urine excretion begins to rise at an increasing rate, until any further rise in the plasma level results in the same amount appearing in the urine. The phase of rising excretion rate represents splay, and the condition where each incremental rise in filtered load causes an equal rise in urine excretion is when the maximal tubular reabsorption rate has been achieved. Although the splay zone is a curve, it has been approximated to a linear function by Nordin and Bulusu (1968), who have established the relationship between phosphate excretion per 100 ml of GF for conditions where the state of maximal tubular reabsorption has not been reached. On the other hand, Bijvoet (1964) has examined by means of phosphate infusions the theoretical theshold, which is the same as the maximum tubular reabsorptive capacity relative to GFR (Fig. 1). Since most patients with renal failure are above the theoretical phosphate theshold due to a tendency to phosphate retention, the terminology of Bijvoet has been included in this study, and the state of tubular reabsorption of phosphate is therefore expressed as the theoretical phosphate threshold, TmP. It may be calculated knowing the plasma phosphate and the phosphate excretion per 100 ml GF (P_E).

Renal excretion and the steady state

The factors which influence the steady state in human physiology are frequently overlooked, and other factors, which may scarcely influence it, are sometimes considered of importance. There are two main ways in which the composition of plasma, and therefore extracellular fluid (ECF), is kept constant and both are concentration dependent. One mechanism is by the negative feed back control of endocrine release and the other is by means of a threshold which governs excretion, so that once the filtered load reaches a certain level, the surplus material is lost in the urine. The latter mechanism may be modified by the former. The kidney operates by filtering a relatively enormous amount of fluid per unit time and reabsorbing all but the surplus which is excreted, exact characteristics of reabsorption varying with different substances.

This method of retaining those materials that are of physiological importance applies to calcium and under normal conditions about 98% of filtered calcium has to be reabsorbed in order to sustain a normal plasma level. The corollary of this is that a small defect in the function of the tubule could allow a major fall in plasma calcium to occur.

If calcium begins to enter the plasma at an increased rate, the concentration will tend to rise. The output in the urine cannot remain unchanged, however, otherwise the plasma calcium concentration would increase indefinitely. Being concentration dependent, output rises until a new equilibrium is reached with input and output equal, but at a higher level. The exact change in concentration that occurs following a change in input is determined by the relation between

change in concentration and change in output, but there is always a tendency to achieve a steady state when intake and output become equal. This is not to say that the overall calcium balance, whether positive or negative, affects the plasma level, since in these situations calcium is simply passing via the blood into or out of the bone.

Calcium pool

The calcium pool is the mass of calcium in the body that is available for immediate exchange or alternatively the volume of fluid in which that mass of calcium is distributed. It is made up of ultrafiltrable and protein-bound calcium in the plasma, calcium in the ECF, and a certain amount of calcium in the bone. Although the intracellular calcium concentration is extremely low, the intracellular fluid volume is large, and cellular calcium probably contributes to some extent to the pool size.

Isotopic bone formation rate

The kinetics of bone formation using radioisotopes have been studied by a number of workers using conventional multiple analysis. There are a number of disadvantages to this form of calculation, particularly the difficulty in distinguishing between the calcium entering the skeleton as a simple exchange process and that being incorporated into newly formed bone. In seeking to develop a form of calculation which would satisfactorily allow for exchange processes, Burkinshaw et al. (1969) suggested that the progressive labelling of skeletal mineral by exchange processes could be likened to a continuously expanding exchangeable calcium pool, and that such a process might be represented by a power function. Thus when the logarithm of plasma specific activity of radio-calcium is plotted against the

logarithm of time, a linear decline in specific activity is found to occur during the first fifteen hours, with a more rapid fall during the next six days. Extrapolation of the linear part of the function, which represents the fall of plasma specific activity attributable to expansion of the exchangeable pool, onward to day 7 permits calculation of the pool size at that time. This value is the reciprocal of the extrapolated specific activity. This pool size is multiplied by the actual observed serum specific activity on day 7, to yield the amount of radioactivity present in the exchangeable pool at that time. The total activity retained in the body at day 7 is estimated by whole body counting or by measuring the difference between the original dose of radio-calcium and that lost in the faeces and urine. The amount of radio-calcium calculated to be present in the exchangeable pool is then deducted from the total radio-calcium retained in the body at day 7 to yield the amount of activity attributable to mineralisation. This value is then divided by the integrated serum specific activity over the seven day period to yield the bone mineralisation rate. Bone resorption is calculated indirectly by subtracting the calcium balance from the mineralisation rate.

The technique, though complex, has proved useful in practice and has been shown by Burkinshaw and Marshall (1971) to have an error of the order of 8%. Nordin and his colleague (Bullamore et al. 1971) have used this method to examine bone turnover in patients belonging to various diagnostic categories and the model appears to be a satisfactory one. The patients in the present study are included in their data.

Osteomalacia

Osteomalacia is generally defined as a histological entity. It is characterised by large areas of osteoid on the surface of a trabecular bone and these trabeculae are frequently thickened. Thus wide osteoid seams are taken to indicate advanced osteomalacia, whereas narrow osteoid seams imply a lesser degree of the same process. However, the work of Frost (1967) has shown that osteomalacia must also be considered in dynamic terms, and in this sense there is a decreased rate of osteoid production with a simultaneous, and often more severe, reduction in mineralisation rate. This means on the one hand that situations which cause high local rates of bone turnover cannot be considered osteomalacic in spite of the presence of numerous osteoid seams; and on the other, a situation where only narrow osteoid seams are present may be due to an extremely low rate of turnover, that is to say severe osteomalacia in the terms of Frost. Osteomalacia may also be defined radiologically by the presence of Looser zones which are pathognemonic, but this appearance is caused by the failure of localised cortical fractures to heal, the fractures themselves being caused by weakening of cortical bone by parathyroid induced resorption.

Quantitative Bone Histology

There are many ways of determining the volumes of bodies of irregular shape but the task becomes something of an enigma if the dimensions are microscopic and a further problem arises when it is impossible to isolate the object from the surrounding material. These difficulties were well known to geologists examining samples where one form of rock was embedded in another and it is the principles that they established which are now used for quantitative bone histology.

Volume Estimation

The volume of the material in question approximates to the sum of the areas of material seen in serial sections. The problem then becomes one of measuring an irregular microscopic area. Point counting (Glagoleff, 1933; Chayes, 1954) enables this to be done relatively easily. If a grid containing a certain number of points is placed in the eye-piece of the microscope, then the number of points overlying the material under examination is proportional to the area of the material. The area measured (a) may therefore be expressed as a fraction of the total area of the grid (A).

$$\text{Thus,} \quad \frac{a}{A} = \frac{n}{N}$$

where n is the number of points overlying the material, and N is the number of points on the grid.

The larger the number of points counted, the more accurate is the result and it has been found convenient to use a grid with twenty-five points, since this makes it easy to work in percentages. A fifty point graticule makes counting difficult.

The volume (v) of the material in question can also be determined either in absolute terms or relative to the total volume (V) examined.

$$\text{Thus,} \quad \frac{v}{V} = \frac{a}{A} = \frac{n}{N}$$

Surface Estimation

In bone, surfaces may be forming, resting or undergoing resorption. Forming surfaces are covered with osteoid and are therefore identified

as such using suitable stains. Resting surfaces are mineralised, and have more or less smooth contours. Resorbing surfaces are represented by Howship's lacunae. Quantitation of these surfaces is only partly of value in studying the dynamics of bone turnover, since no assumptions can be made regarding the rates of formation or resorption at a given site. It is known from the work of Frost (1967) that calcification of osteoid proceeds at markedly different rates in different clinical states, and therefore the mere presence of an osteoid surface is no indication of the degree of activity taking place. In order to understand the true dynamics of bone formation, a marker is required to identify the quantity of bone laid down during a given time interval. In practice tetracycline is regarded as satisfactory and two doses are given at the beginning and end of a suitable time period. Tetracycline combines with the mineral as it is deposited and an envelope of tetracycline stained material therefore encloses all bone laid down during the time interval. During this study in patients in chronic renal failure, however, it was not found possible to use tetracycline reliably in this way. Occasional bands were formed where osteoid was presumed to have mineralised correctly but frequently the tetracycline appeared as blotches or stippling within the osteoid mass. This may signify that the abnormal mineralisation of osteomalacia does not only proceed more slowly than normal at a given site, as Frost (1967) has suggested, but that it is also completely disorganised and occurs at random throughout the osteoid. It is also true that, at least in some patients, abnormally high blood levels of tetracycline occurred and this may have influenced its deposition.

For the purposes of this study, therefore, surfaces have simply

been identified as forming, resting or resorbing, and the proportion of each to the total surface has been estimated and expressed as a percentage. In addition, the number of osteoid surfaces with a distinct mineralisation front have been counted and expressed as a percentage of the total osteoid surface. The principles of surface counting are identical to those used for determining the volume, except that, since it is impractical to tell whether a grid-point overlies a line, which is how a surface appears in section, the graticule is made up of five parallel lines and the surface is counted if it intersects with any part of a line. A single graticule can be made of five lines, each divided by five equidistant points to serve both for volume and surface counting.

Validity of Method

The accuracy of any length, area or volume measurement can be increased to almost any degree if only enough measurements are made. According to the Gaussian concept of probability, every reduction in the deviation to one-half its original value requires four times as many measurements.

With the present technique, forty-eight serial sections are cut and every third section is used. Four fields from every section are examined, so that with a 25 point graticule, a total area corresponding to 1600 points is scanned. With volumes of bone tissue occupying between 5% and 50% of the total, statistical tables indicate that the mean error should be less than 1%. The actual accuracy of the method may be tested in two ways: either statistically, or by determination of the reproduceability. When surface counting is carried out on the sixty-four microscope fields that are viewed, a range of results is

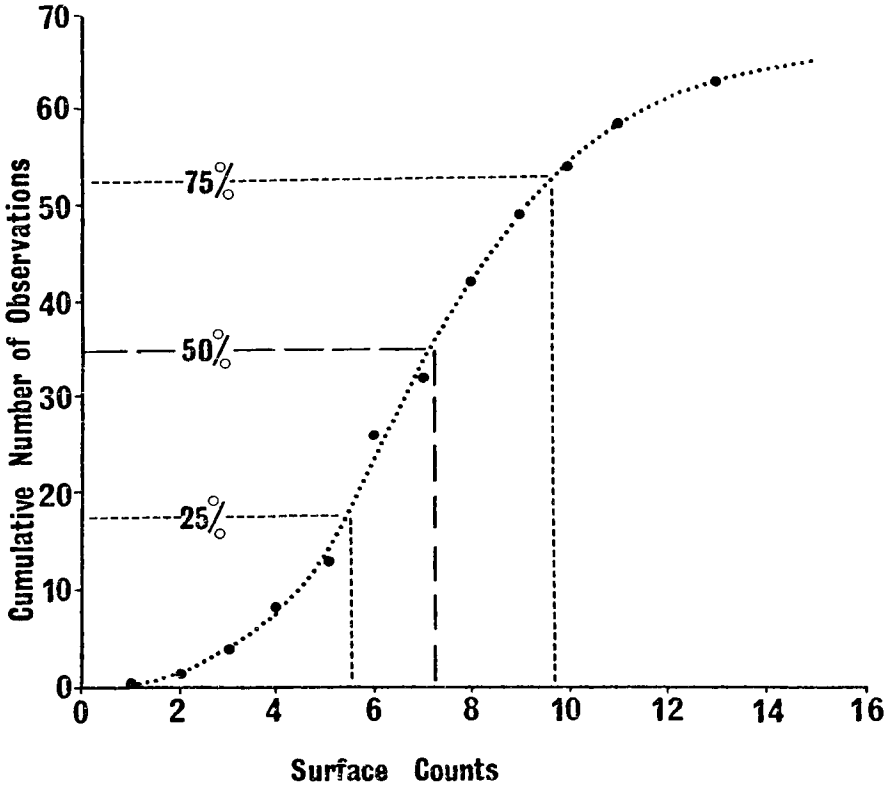

Fig. 2. Distribution of surface counts on a bone biopsy from a patient with chronic renal failure.

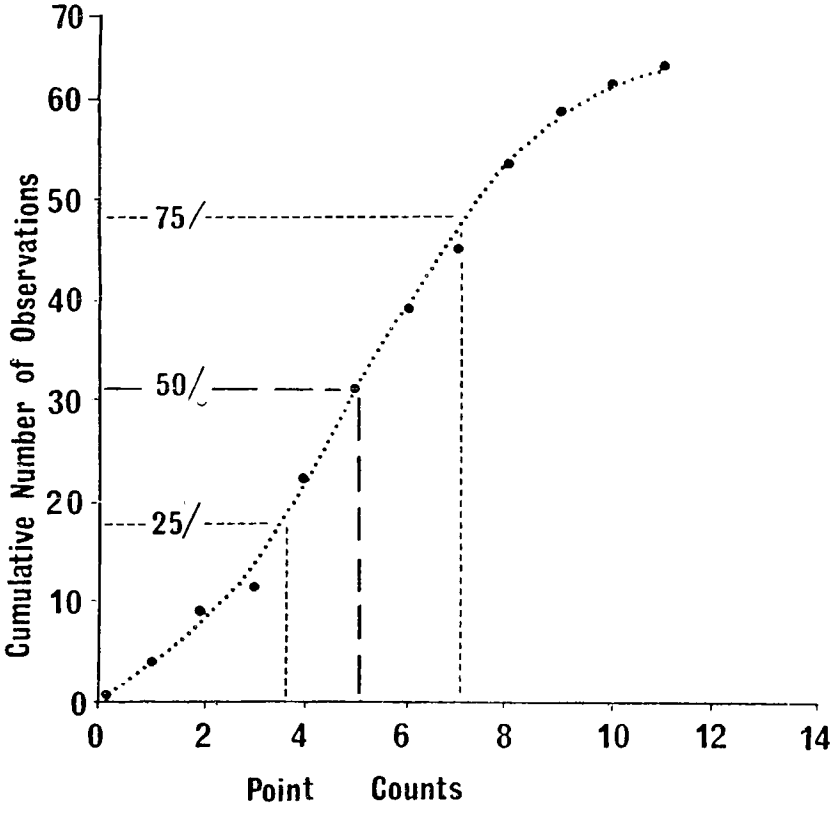

Fig. 3. Distribution of point counts (surface area) on a bone biopsy from a patient with chronic renal failure.

obtained which are distributed according to a normal probability curve. The data may be plotted as a continuous summation curve and the median one-half of all counts found by drawing in the 25% and 75% probability lines (Figs. 2 and 3). This gives the amplitude (a) about the mean, from which the deviation (d) may be calculated:

$$d = \frac{\pm\ a}{\sqrt{N}} \quad \text{where } N = \text{the number of tests}$$

In this way, it can be shown that the probable error of this method is 5-9%. Direct testing of the reliability of the method by repeating estimations yields the same error.

CHAPTER 4

Methods

Biochemistry

Extensive use has been made of the Technicon Auto-Analyser for biochemical determinations. Experience showed that this machine was not only convenient to use, but also gave reliable results of high reproduceability. In particular, it appeared to be considerably superior at estimating plasma calcium levels than any other device apart from the integrating atomic absorption spectrophotometer. The exact method used for both urine and plasma calcium estimations was that of Knowles (1968), and as far as plasma calcium is concerned, the variation of results was approximately $\pm$ 2%. The variation was somewhat greater for the estimation of urine calcium depending upon the concentration, but in general the method was extremely consistent. Plasma phosphorus and creatinine were measured by Auto-Analyser procedures N - 4a and N -11a respectively. The results for both varied approximately $\pm$ 6% in plasma and in urine. An example of estimations of serum calcium, phosphorus and creatinine that were carried out and then repeated later in the same day are shown in Table 1. Similarly, seven urine samples were chosen on the basis of their different concentrations and calcium, phosphorus and creatinine were measured in each on ten random occasions. The results are presented in Table 1 with the mean and standard error. For present purposes, where consistency of results is necessary to enable changes to be detected and comparisons to be made, the Auto-Analyser is an extremely satisfactory instrument.

Arterial blood pH was measured in certain cases using standard Radiometer micro-electrode equipment on samples obtained by brachial artery puncture. The reproduceability of the method was extremely

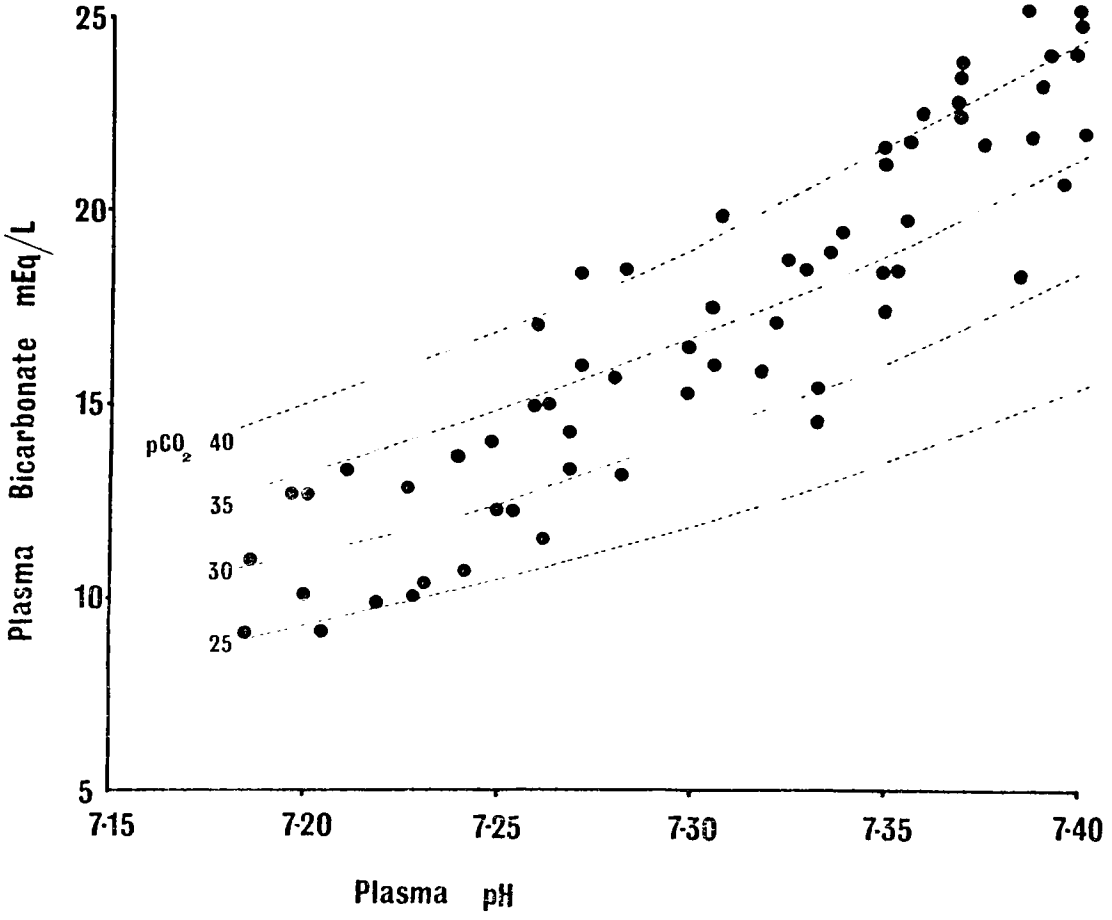

Fig. 4. The relation between plasma bicarbonate and pH in patients with chronic renal failure. The associated plasma pCO_2 is indicated.

high, results varying by not more than ± 0.004 pH units. The pCO_2 was measured on the same instrument. In general, however, the acid-base status of the patient has been expressed as the plasma bicarbonate measured by the Auto-Analyser procedure N - 21a. The main justification for the use of plasma bicarbonate rather than the blood pH is that it avoids arterial puncture as well as the necessity of immediate measurement of the sample. When the arterial blood pH is compared with the plasma bicarbonate measured on the same sample using multiple observations on 20 patients with varying degrees of acidosis, a predictable relationship is found (Fig. 4) though there is a tendency for a decreased plasma bicarbonate to over-estimate the degree of acidosis as a fall in pCO_2 due to compensatory hyperventilation offsets the reduction in plasma pH.

Urine total hydroxyproline was used as a measure of bone breakdown and was determined by the method of Grant (1964), adapted for use on the Auto-Analyser. The serum alkaline phosphatase was measured by Auto-Analyser method N 5, in which phenol liberated by hydrolysis of phenyl disodium phosphate is estimated colorimetrically. Chloride was measured by an E.E.L. chloride titrator.

Calcium absorption

Calcium absorption was measured in the basal stage using a tracer dose of ^{45}Ca in 20 mg of calcium given as calcium chloride, as described by Bullamore et al. (1970). The procedure was carried out with the patient fasting, when the calcium chloride with the tracer dose of ^{45}Ca was taken orally and washed down with 200 ml of distilled water. Blood samples were taken at fifteen, thirty, sixty, ninety and one hundred and twenty minutes after the administration of the dose

and the serum was counted in a Philips automatic liquid scintillation counter by the method described by Oxby and Kirby (1968). The purpose of using the small dose of calcium chloride was to avoid passive diffusion of calcium across the upper gastro-intestinal tract which might have occurred using larger doses. The distilled water was given in order to wash the calcium through the pylorus into the duodenum as soon as possible after the dose was given. Radiographic screening showed that most of the fluid in fact reached the duodenum within minutes of being taken. Carrier doses of 20 mg and 50 mg of calcium were tried and the method using 20 mg doses was found to be reasonably reproduceable and to correlate well with balance data (Wilkinson, 1971a). The serum counts were expressed as the percentage of the administered dose and a curve could be constructed showing a rising number of counts over the first one and a half hours with a gradual fall-off thereafter. Analysis of the curve permitted the calculation of the fraction of the dose that was absorbed in the first hour, and this was termed the α_1 value, the range in healthy adults being established as 0.35 - 0.90.

Metabolic balance studies

Each calcium balance study took two weeks to perform. The first week was the equilibration period and the second the balance proper. In order to improve the sensitivity of the technique the use of various non-absorbable faecal markers was investigated by Wilkinson (1971b), and polyethylene glycol 4000 (Peg) was found to be most suitable. This enabled the technique of daily balances to be evolved, using endogenous creatinine as a urine marker. Thus the patient was given 500 mg of Peg three times a day with each meal during the

equilibration and study weeks. Analysis of the faeces with correction according to the amount of Peg present enabled the daily estimation of absorption to be made. Similarly, by correction of the urine results to the average 24 hour creatinine output obtained during the whole week, the problem of the minor errors in urine calcium estimation due to the incomplete bladder emptying at the start or finish of the daily balance was overcome. The sum of the corrected amounts of daily calcium in the urine for the weekly period was not significantly different from the sum of the actual amounts excreted during the same time. Calcium and phosphorus were estimated in duplicate in a homogenized sample of the diet and on all faecal and urine samples, using ashed aliquots of faecal and diet homogenates taken up in hydrochloric acid and acidified samples of urine, the estimations being performed by the methods already described. The Peg content of the faeces was determined by the turbidimetric method of Malawer and Powell (1967). The advantage of this method of carrying out the calcium balance was that seven separate results could be obtained for each study and the mean calculated with its standard error.

Urine total hydroxyproline was measured on the urine collection, in order to complement the bone resorption rates calculated from the balance and isotopic bone formation rate studies. Since dietary gelatin would contribute to urine OHP, care was taken to ensure that the diets were gelatin free (Sjoerdsman et al. 1965)

Bone x-ray measurements

For the purposes of this study, two forms of x-ray measurement were investigated, both using the second metacarpal. The first of these, the ratio of the cross sectional area of cortical bone to the

total cross section area (Nordin & Smith, 1965) is a morphological measurement. The second, the estimated bone density of the cortex, is a photodensitometric method. A posteroanterior radiograph of the left hand was taken, the radiographic factors being FFD, 40"; current, 55 m. amps; time, 0.04 secs.; voltage, 52 kV; film type, Kodak Non-screen Auto-process. Processing was carried out by the Cronex auto-process for 3½ minutes. The x-ray was developed and the midpoint of the shaft of the bone located.

The total width of the bone at its mid-shaft position and the medullary width at the same site were each measured twice by one observer. The measurements were performed using needle-tipped Vernier calipers. From duplicate observations the experimental errors were evaluated and were found to be 0.010 cm for the total width and 0.015 cm for the medullary width, this larger error being attributed to the comparative irregularity of the endosteal margins. The purpose of the measurement is to obtain an index of the amount of cortical bone present and this index has been expressed as the ratio of the cortical cross-sectional area of the bone at that point, assuming the metacarpal mid-shaft to have a cylindrical configuration. The validity of this measurement has been fully investigated by Horsman and Kirby (1972) who carried out morphological measurements of 50 metacarpals and compared the results with the radiological values. The ratio of the cortical to the total cross-sectional area is calculated as follows:

$$CA/TA = 1 - (1-C/T)^2$$

where C is cortical width

and T is total width

Attempts were also made to evaluate the metacarpal cortical density at the mid-shaft using a densitometer. The method was an extension of that described by Anderson et al. (1966) and the exact procedure has been given in detail by Horsman (1971). It is indirect and the accuracy of the resulting value is open to doubt, but for purposes of comparison the technique might be expected to be of some value. In practice, however, the normal range was extremely wide and the measurement was subject to a relatively larger error. This probably occurred because of the assumption of a cylindrical model for the metacarpal. The bone may be roughly oval or triangular in cross-sectional area so that the x-ray is passing through unpredictable quantities of bone. This will not greatly influence the simple linear measurements on which the CA/TA depend, but it is likely to affect the x-ray absorption to a considerable degree. A bone of oval section viewed in its major axis will obviously appear radiographically denser than one with a circular section but whose cortex is otherwise identical. Moreover, the question arises as to what is implied by the term metacarpal cortical density. Under ideal conditions and using the techniques and assumptions as described, the metacarpal cortical density could be reduced by a decrease in the true mineralisation of the bone, or alternatively by a widening of the Haversian canals. In practice it is extremely unlikely that the former type of change occurs even in severe osteomalacia, although there may be some small amount of osteoid deposition at the surface of the Haversian canals. Any change in density is probably caused by the latter process. Bearing in mind these inherent disadvantages both in the measurement and the concept of the metacarpal cortical density, the measurement was tested

in 44 patients with varying degrees of chronic renal failure. No correlation could be found with any other parameter, in contrast to the CA/TA ratio which proved a useful measurement. The metacarpal cortical density was therefore abandoned in this study.

Iliac crest biopsies

Using full aseptic technique bone biopsies were taken under local anaesthetic from the anterior iliac crest, approximately 3 cm behind the anterior superior spine. The periosteal surface was exposed, scraped down to bone with a periosteal elevator and a cylindrical biopsy core removed with a 5 mm trephine. The deep fascia was closed over the biopsy site with interrupted cat-gut and the skin was then re-sutured in a separate layer. A moderate sized haematoma occurred in one patient, but otherwise there were no complications. Patients with malacic bone appeared to find the procedure more painful than those whose bone was normally mineralised.

Quantitative bone histology

The bone sample was placed in neutral 10% formal-saline for twenty four hours and dehydrated by passing through graded alcohols, finally being transferred to absolute alcohol for twelve hours.

The most satisfactory process of embedding the bone involved steeping the fixed samples for three weeks in methyl-butyl methacrylate monomer preparation, which included the polymerising agent. The mixture was made up of nine parts methyl methacrylate and one part butyl methacrylate, with 1 G benzoyl peroxide per 100 ml of monomer. This was normally stored over anhydrous copper sulphate at $4^{o}C$. The specimens placed in this mixture were also kept at $4^{o}C$ to allow maximum penetration without polymerisation occurring, using

small glass jars with air-tight caps to prevent evaporation. After three weeks the jars were left at room temperature to allow polymerisation to occur, which generally took three to seven days. The butyl methacrylate component gave a slight plastic quality to the polymer which made sections less brittle and more suitable for sectioning.

After polymerisation was complete the jar was broken and the sample removed. The surplus polymer was trimmed off with a saw and the embedded specimen mounted horizontally on a 5 mm thick alloy plate, using Araldite.

Forty eight serial sections, approximately 12 μ thickness, were cut using a Jung heavy sledge microtome with a K2 blade. Every third section was selected for staining with Toluidine Blue, and an alternative set of sections were made up and stained with Osteochrome.

Quantitative counting was carried out according to the principle of Frost (1967) using a Leitz microscope at a magnification of x 80 with a five line grid in the eye-piece.

Where possible, the thickness of the cortex of the iliac crest was measured by means of an eyepiece micrometer. A series of measurements approximately 100 μ apart were taken and the mean value calculated. The method was unsatisfactory owing to the gradual transition from cortex to medulla.

It has been stated by Frost (1967) that mineralisation normally takes place in the part of the osteoid seam adjacent to the already mineralised bone. Initially, however, there are certain features of this newly formed bone which distinguish it from mature bone. In particular, lipid material appears to be present and Matrajt has shown that during normal mineralisation, this line of active calcification

may be identified using a number of stains (Matrajt et al. 1967). When Toluidine Blue is used, a thin granular deep blue line shows up against the blue of mature bone and the turquoise of the osteoid. This zone has been designated the Mineralisation Front, and the fraction of osteoid seams with Mineralisation Fronts present may be estimated by grid counting.

Clinical material

The patients in the study came from four sources. A relatively small group with frank osteomalacia was referred by urological surgeons for metabolic assessment. The majority of these patients had anatomical abnormalities of the urinary tract. Another group of patients came from a renal stone clinic although not all of these patients had renal stones. This clinic was supplemented by patients known to have chronic pyelonephritis, frequently referred by the urologists, and by patients with other forms of renal disease, who were seen initially as out patients or general medical admissions. The third source of patients was a nephrology clinic, where patients were referred for assessment of renal function and, when appropriate, consideration for long term dialysis. Finally, a small number of patients were gathered from another nephrology clinic, but the purpose of including this group was to widen the spectrum of biochemical abnormality, with special reference to the effect on bone histology.

Investigation of the patients generally followed a standard pattern. The patient was seen initially as an out patient and routine biochemical and haematological screening tests carried out. The patient was then admitted and more detailed studies were undertaken, provided the patient's general condition was stable, taking the out patient results

into account. Treatment, where appropriate, was instituted following the investigations. Data from those patients who required urgent treatment, or whose metabolic state fluctuated, were not included in the study. The patients were on no drugs which were likely to affect the results; only antibiotics, antihypertensive drugs or occasional small doses of alkali were used. In no case were aluminium hydroxide, diuretics or anticonvulsants prescribed. Every attempt was made to ensure that metabolic measurements were made over a relatively short period of time, and the biochemical estimations were generally carried out on a single blood or urine sample collected in the fasting state. These biochemical tests were usually performed a few days after admission to hospital and generally seemed to be representative of the patient's metabolic state. It was noted when investigating biochemical relationships that tighter correlations emerged from data obtained from single samples than when the same comparisons were made using data that had been obtained a few days apart. All of the patients on whom iliac crest biopsies were carried out except one with renal tubular acidosis had sustained a blood urea level in excess of 100 mg/100 ml for at least a year. The majority of them had some degree of metabolic acidosis, but a small number who were closely observed had plasma bicarbonate levels persistently greater than 20 mEq/L. Most of these patients had slowly progressive glomerulonephritis and took alkali therapy. Another group of patients had bicarbonate levels which varied markedly around 20 mEq/L and these patients were therefore excluded from the study.

A diagnosis of pyelonephritis was made if at least three of the following criteria were fulfilled: a convincing history of recurrent

urinary tract infections, repeated pyuria with a positive bacterial culture in mid-stream specimens of urine, radiological changes on intravenous pyelography (Edwards, 1965), histological evidence (Cotran, 1965). Glomerular disease was diagnosed in most cases by percutaneous renal biopsy. The presence of polycystic disease was diagnosed radiologically, supplemented by a positive family history in many cases. Miscellaneous forms of renal failure were diagnosed by appropriate techniques.

CHAPTER 5

Results

SECTION 1

Cases of Florid Renal Osteomalacia

Eleven patients are described, representing the total number of cases with proven clinical osteomalacia with renal failure seen during the period of study. An outstanding feature of the study was severe metabolic acidosis. Five other patients were seen with a comparable degree of acidosis and in only one of these was osteomalacia excluded by thorough investigation. The other four presented some features of osteomalacia but were not included owing to the incompleteness of the data.

Clinical material

Details of the eleven cases are shown in Table 2. They comprise six women aged 16 to 65, two men aged 54 and 61 and three boys aged 4, 8 and 18. All except three had unequivocal evidence of chronic pyelonephritis with chronic pyuria and infection and radiological changes. Case GP had bilateral hypoplastic kidneys. No evidence of pyelonephritis was found in case RD but his underlying lesion was a congenital urethral obstruction, corrected at the age 2. Case HC gave a history of acute glomerulonephritis at the age 27, and had been asymptomatic until his presentation with muscle weakness and skeletal pain at the age 61.

Presentation and bone status

Case RD, the 4 year old boy with uretero-vesical obstruction, and case GP, a 16 year old girl with bilateral small kidneys, probably resulting from pyelonephritis or obstruction in childhood (Hodson, 1967),

both presented with genu valgum. Case GP also had a marked hip-girdle myopathy and an extensive Looser zone transversing most of the cortex of the left lower femoral shaft. Case HBr, an 8 year old boy with ureterostomy carried out for bladder neck obstruction and case LSl, a 60 year old woman with an asymptomatic stag-horn calculus and bilaterally scarred kidneys presented with spontaneous fractures of the tibia and femoral neck respectively. Hip-girdle weakness and bone pain were the major symptoms in cases PM, HC, HBa and IP. Case PM was an 18 year old boy who had had a bladder neck resection when 2 years of age, following investigation of polyuria. Some degree of incontinence persisted and a uretero-colic anastomosis was carried out at the age 7. Nine years later an ileal conduit was constructed, and at this time the patient reported that he was no longer able to run and had difficulty descending stairs. Case HC, a 61 year old man probably suffered an episode of acute glomerulonephritis with incomplete resolution. Case HBa gave a 20 year history of intermittent acute urinary tract infections, and was found to have evidence of active infection when she presented aged 61 with an obvious myopathy. Case IP, had a uretero-sigmoidostomy performed at the age of 45 along with excision of a carcinomatous bladder. She received intermittent courses of Vitamin D in therapeutic doses after hypocalcaemia was noted, but inspite of this, bone pain led to the discovery of Looser zones in both femora, confirming the diagnosis of osteomalacia.

Case MS had a uretero-colic anastomosis performed at the age of 43 following unsuccessful local resection of a bladder carcinoma. Eleven years later when uraemic symptoms developed he was referred for general assessment. Case LSy had bladder neck obstruction from birth, in

assocation with occult spina bifida, although the diagnosis was not made until age 7, when the patient was investigated for incontinence. A uretero-colic anastomosis was constructed, but this was converted to an ileal bladder at the age 15. Following this she was referred for assessment. Case WB originally presented aged 45, with general ill-health and haematuria. A diagnosis of tuberculous pyelonephritis with extensive bladder involvement was established, and after treatment, the scarred bladder was enlarged with a segment of colon. The patient was referred for assessment aged 50. The renal function in cases RD, HBr, LSy, LSl and HBa appeared to be reasonably stable, but both cases HBr (an 8 year old boy) and LSl (a 60 year old woman) died from cerebro-vascular haemorrhage in association with hypertension. The remaining patients showed a slow but steady decline in renal function down to terminal levels over a two to four year period of follow-up after their final presentation.

Radiographs of the bones showed fractures or pseudo-fractures and/or rachitic changes in every case except MS in whom the diagnosis of osteomalacia was confirmed by the reduced ash content (47%) of an iliac crest biopsy specimen (Morgan et al. 1968). Spinal density by visual inspection was obviously increased in eight of the cases. In all cases the metacarpal and femoral cortices tended to be thin, but frank periosteal resorption of bone was not seen.

Results

The blood urea levels ranged from 72 to 327 mg/100 ml. All the patients had a substantial metabolic acidosis (bicarbonate below 20 mEq/L) and there was hyperchloraemia in all but three cases (GP, MS, HC) with the most severe renal failure. Arterial blood pH values ranged

from 7.24 to 7.32. All the patients but one (RD) had hypocalcaemia (serum calcium below 9.0 mg/100 ml). The plasma phosphate levels were variable (2.8 to 6.9 mg/100 ml). All the plasma alkaline phosphatase values were high though in cases HBr and GP they were within the normal range, which in growing children is extremely wide (Sereny & McLaughlin, 1970). The serum calcium x phosphorus products ranged from 25 to 50.

Creatinine clearances were all below 20 ml/min. Twenty-four hour urine calcium was low in all cases (9 to 57 mg/24 hours), but when fasting calcium excretion was expressed in mg/100 ml of glomerular filtrate, it was raised - that is, over 0.14 - in all cases. Phosphate excretion expressed in mg/100 ml of glomerular filtrate was very high relative to the serum phosphate level in all cases indicating a reduced tubular reabsorption of phosphate. When the values are expressed as the maximum tubular reabsorption of phosphate, TmP, it is seen that all but one are below the normal range of 2.3 to 4.2 mg/100 ml (Bijvoet, 1969). Only two patients could achieve a urine pH below 5.8, despite the severe metabolic acidosis. Of these two, one had glomerulonephritis, in which the acidosis arises as a result of a diminished nephron population, and rarely runs the chronic course that occurred in this case. The other patient had a bicarbonate-losing state, which is an uncommon form of renal tubular acidosis (see Appendix: Case III).

Comment

The striking feature of this series is the metabolic acidosis, which characterised all the cases. The fact that this acidosis is associated with impairment of urinary acidification in all but one case implies that most patients had a tubular defect, which would have

led to a state of acidosis at a relatively early stage in the development of the renal disease. In the single patient with chronic glomerulonephritis, it is impossible to know the length of time for which acidosis had been present, but the overall history was 34 years. Of five other cases, not included here, but with a similar degree of acidosis, four probably had osteomalacia.

The series provides a further example of the close association that has been noted previously between renal acidosis and osteomalacia. The fact that renal osteomalacia was never seen in the absence of a severe metabolic acidosis adds emphasis to the association, and though it obviously does not necessarily indicate a causal relationship, it does seem to provide circumstantial evidence of one.

In view of these findings, it was appropriate to examine a group of patients with chronic renal failure of varying degrees, selected only on the basis of a raised plasma creatinine, in an attempt to evaluate the role of various biochemical abnormalities, in particular acidosis, in the bone disease of chronic renal failure.

SECTION II

Identification of the Presence of Bone Disease

In order to establish what factors influence bone disease in chronic renal failure a group of fifty uraemic patients (none of whom was on dialysis) has been examined. Details of the fifty patients, all of whom had a plasma creatinine of over 1.6 mg/100 ml, are given in Tables 3 and 4. All the patients were known to be in a relatively stable state of chronic renal failure except for one, ES, whose condition had deteriorated clinically shortly before the investigations were carried out.

It seemed necessary to determine some parameter that would serve as a useful indicator of bone disease. An elevated plasma alkaline phosphate level is generally accepted as characteristic of active osteomalacia or rickets in spite of the fact that practically nothing is known about its role. Familiarity with the enzyme associated with this complete lack of understanding of its function has led to its acceptance as a virtually non-specific marker of certain types of disease, but is hard to believe that it will not eventually prove to be of major importance in calcium metabolism. It was therefore decided to see whether the alkaline phosphatase levels of the patients could be used as a criterion of their bone status.

Standard radiographs of the hand were carried out in forty four cases and the CA/TA ratio was calculated from the caliper measurements on the second metacarpal as described by Nordin and Smith (1965). When the CA/TA and the plasma alkaline phosphatase are compared there is a

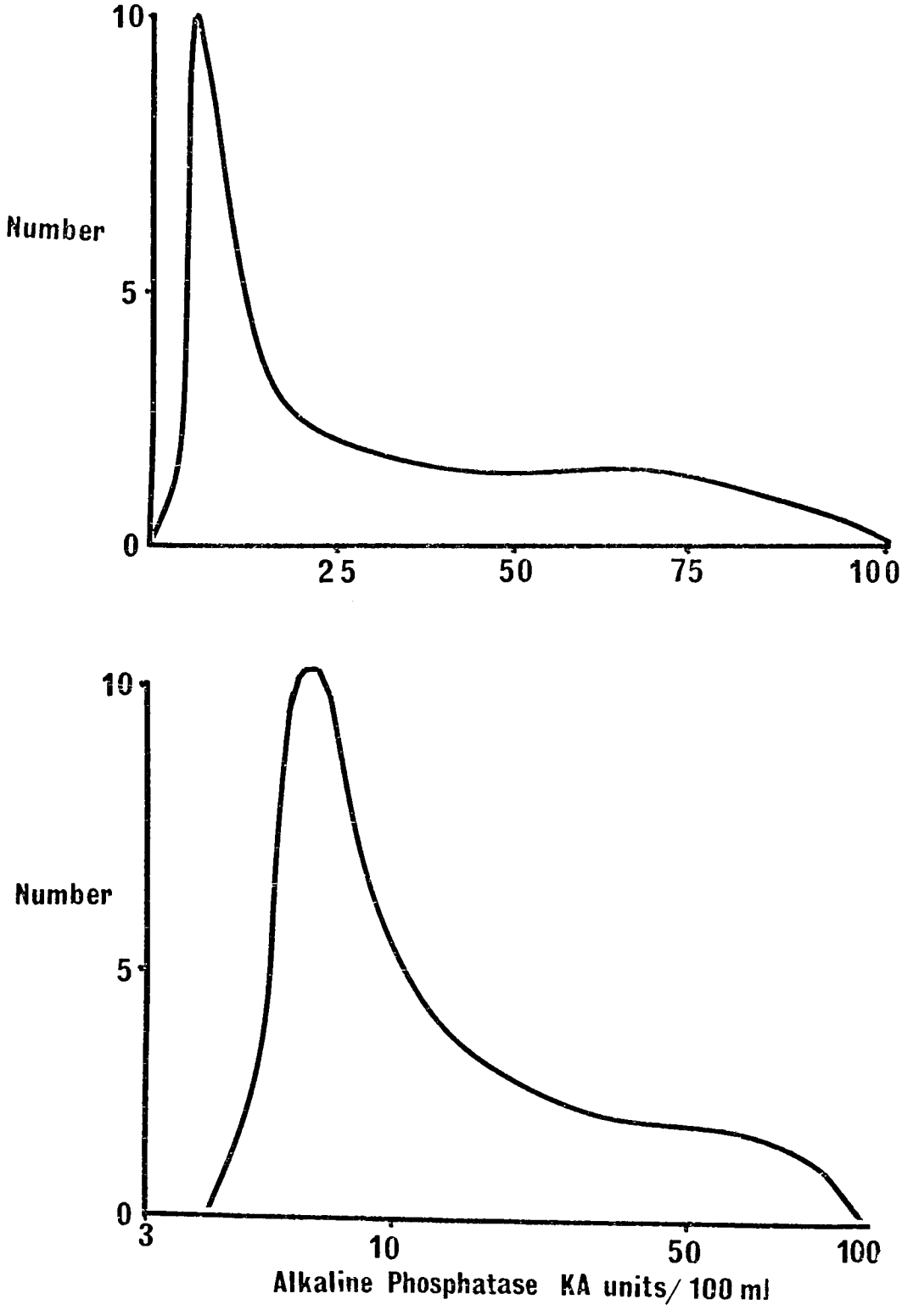

Fig. 5. Distribution of plasma alkaline phosphatase in patients with chronic renal failure, plotted arithmetically and logarithmically.

significant inverse correlation ($r = -0.38$, $p < 0.01$). However, when the distribution of the alkaline phosphatase level is examined it is apparent that there is a very marked positive skew which can be partly normalised by using a logarithmic scale (figure 5). It therefore seems appropriate to consider the logarithm of alkaline phosphatase for purposes of comparison with other biological parameters. When this is done a significant inverse correlation persists between CA/TA and logarithm of alkaline phosphatase, but the r value becomes $r = -0.50$ (Table 5).*

The normal ranges for both alkaline phosphatase and bone x-ray measurements vary with age and when this is taken into account the association between a reduced CA/TA (more than 1 standard deviation below the mean for the age) and a raised alkaline phosphatase is highly significant ($p < 0.001$). Thus a raised plasma alkaline phosphatase is generally associated with a reduction in the metacarpal cortical thickness and it may therefore be justifiably used to indicate the presence of bone disease without necessarily making any assumption as to the exact type. This being the case, it becomes possible to divide the patients into two groups according to the alkaline phosphatase, and the data of patients with normal alkaline phosphatase levels are shown in Table 3, and the data for the remaining patients are shown in Table 4.

Results

Plasma alkaline phosphatase and plasma calcium

Plasma calcium ranged from 5.3 to 10.7 mg/100 ml and was not

* Using a rank correlation procedure for non-normal distributions, $r = 0.51$.

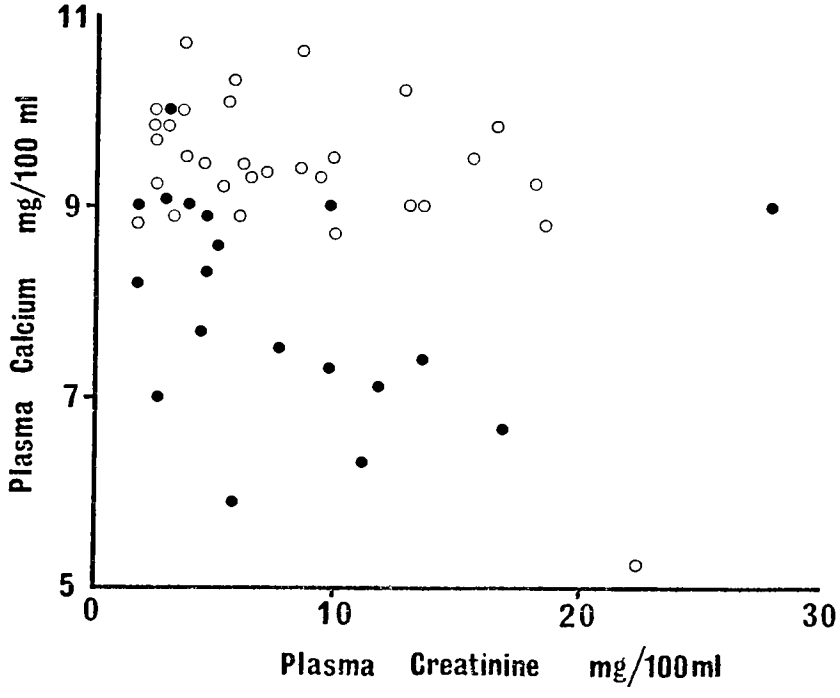

Fig. 6. The relation between plasma calcium and the degree of renal failure. Normal alkaline phosphatase ○, Raised alkaline phosphatase ●.

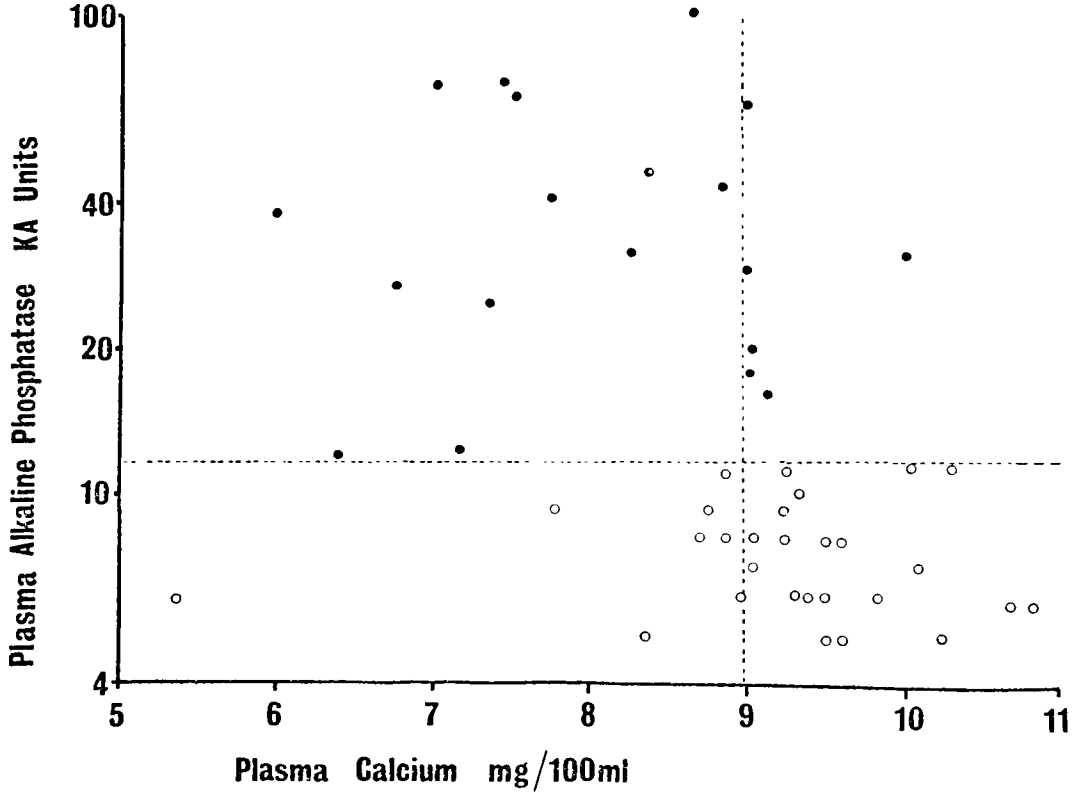

Fig. 7. The relation between plasma alkaline phosphatase (log scale) and plasma calcium in patients with chronic renal failure.

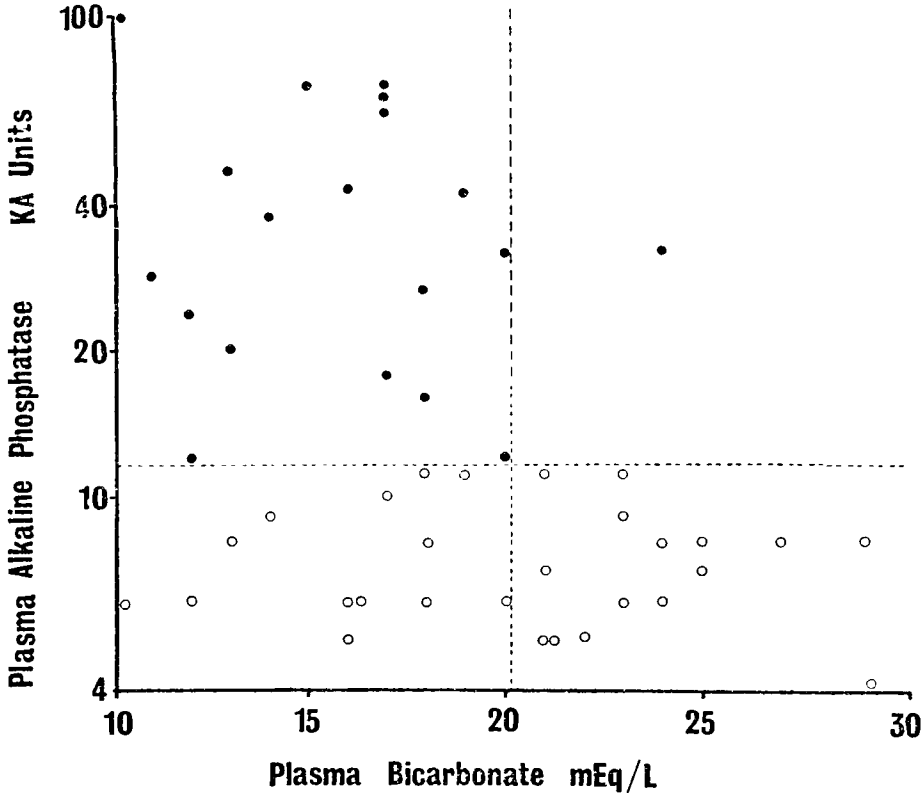

Fig. 8. Plasma alkaline phosphatase (log scale) and bicarbonate in chronic renal failure. There is no significant relationship.

significantly related to the degree of renal failure as judged by the plasma creatinine concentration (figure 6). There is a highly significant inverse correlation between the logarithm of plasma alkaline phosphatase and plasma calcium ($p < 0.005$; figure 7; Table 5). Patients RD, HBr and SC whose ages were 4, 8 and 16 years respectively, have not been included since their plasma alkaline phosphatase levels were probably normal for their age, although ouside the normal range for adults. Patient ES, whose clinical condition had deteriorated shortly before the results were obtained, shows an inappropriately low plasma calcium for her alkaline phosphatase level, as judged by the remainder of the data.

In spite of the overall correlation between plasma alkaline phosphatase and plasma calcium, six of the nineteen cases with a raised alkaline phosphatase had a plasma calcium that was definitely normal.

Plasma alkaline phosphatase and plasma bicarbonate

There is an inverse correlation of doubtful significance between the logarithm of plasma alkaline phosphatase and the plasma bicarbonate ($p \simeq 0.05$; figure 8; Table 5). Only one patient, JD, had a raised plasma alkaline phosphatase in the definite absence of acidosis. This man was suffering from retroperitoneal fibrosis, a condition which has certain systemic features, and it is possible that the excess alkaline phosphatase was derived from sources other than bone. However, enzyme studies to clarify this point were not performed, and there are no definite grounds to exclude his results.

Comment

The plasma alkaline phosphatase level correlates significantly both with the degree of hypocalcaemia and the degree of acidosis.

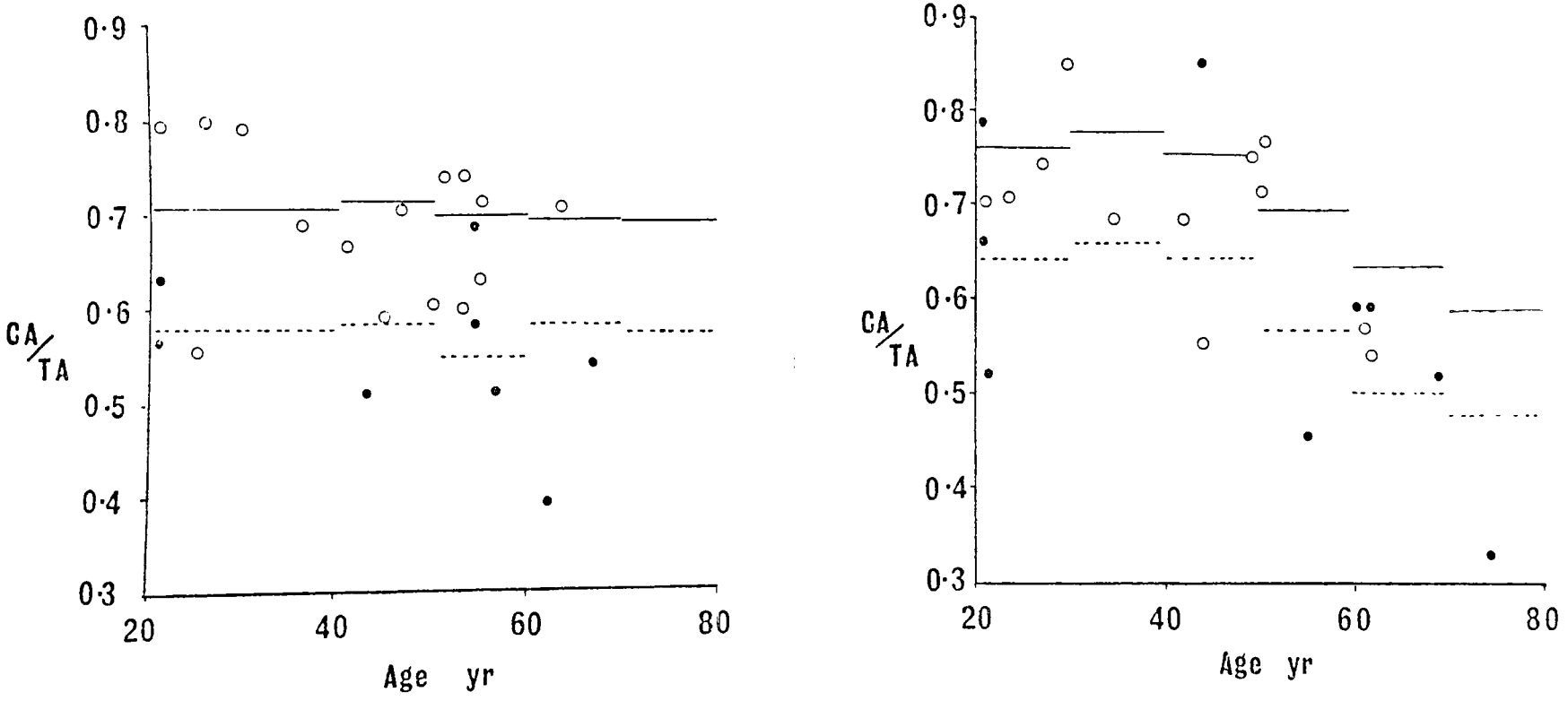

Fig. 9. Bone X-ray data on patients in chronic renal failure. The normal mean and standard deviation in relation to age are shown. Males left, females right.

However, a raised alkaline phosphatase value may occur in the presence of normocalcaemia, which means that the hypocalcaemia cannot be an important cause of the raised alkaline phosphatase, though the bone state which is associated with raised alkaline phosphatase levels could be contributing to the hypocalcaemia. As far as the relation between alkaline phosphatase and the bicarbonate level is concerned, the pattern of the distribution of the data is irregular. Nevertheless, when the normal physiological ranges for the parameters are taken into account it can be seen that while acidosis need not necessarily be associated with a raised alkaline phosphatase, raised levels virtually never occur in the absence of an acidosis (figure 8).

Bone X-ray measurements

The bone x-ray measurement used in this study was the ratio of the cortical to the total area of a cross-section of the mid-point of the second metacarpal (CA/TA). The normal value varies a small amount with age, and tends to be slightly greater in premenopausal women and slightly less in postmenopausal women than in men of the same age. In both sexes, the mean normal value at any age has a relatively large standard deviation.

The CA/TA value in the patients should therefore properly be considered in relation to age and sex, and it is possible to do this in at least two ways. The data may be expressed numerically as the difference from the mean value for the age and sex. Alternatively, since most of the points are scattered around one standard deviation below the mean (figure 9), they may be separated into two populations, depending on whether they fall above or below this value. Both modes of expression had disadvantages. In the first case, the difference

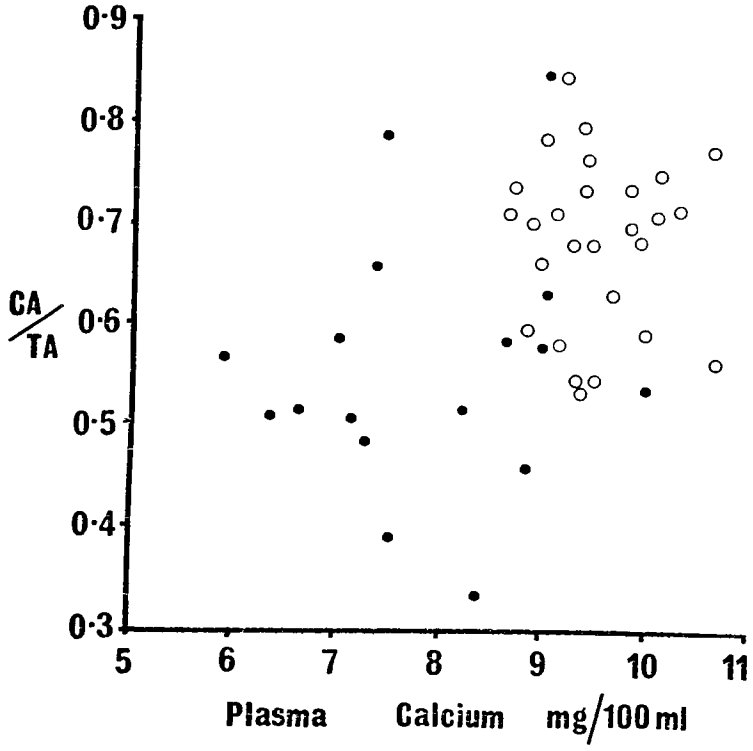

Fig. 10. The relation between CA/TA and plasma calcium in patients with chronic renal failure.

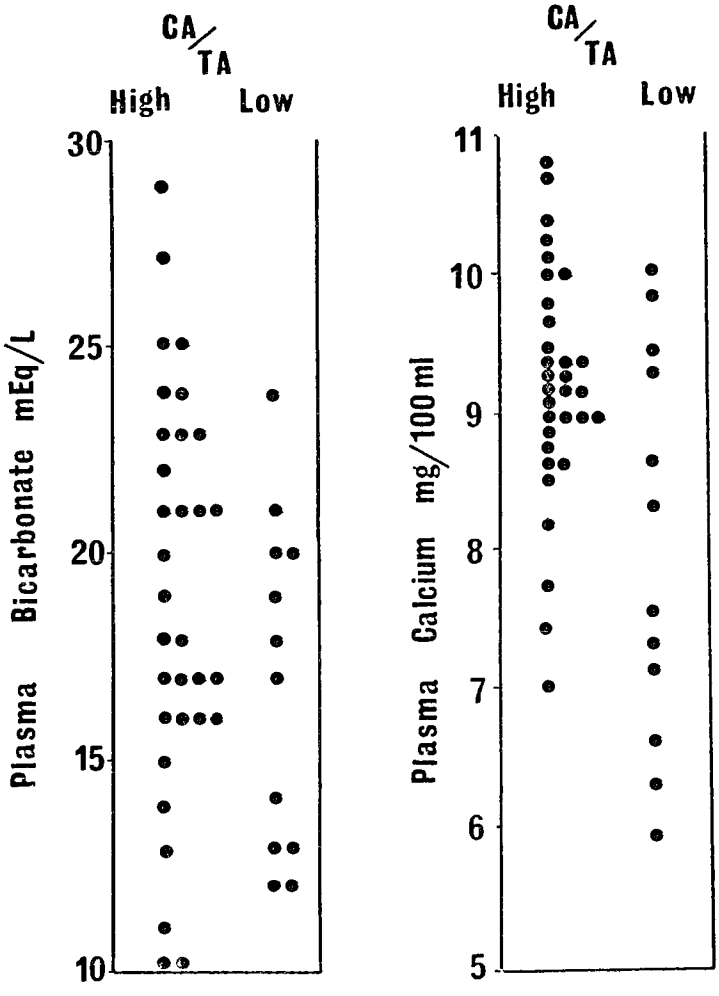

Fig. 11. The plasma bicarbonate and calcium in patients whose CA/TA ratio was greater or less than 1 S.D. below the mean (High and Low respectively). For plasma bicarbonate, $t = 1.29$, n.s. For plasma calcium $t = 3.19$, $p < 0.001$.

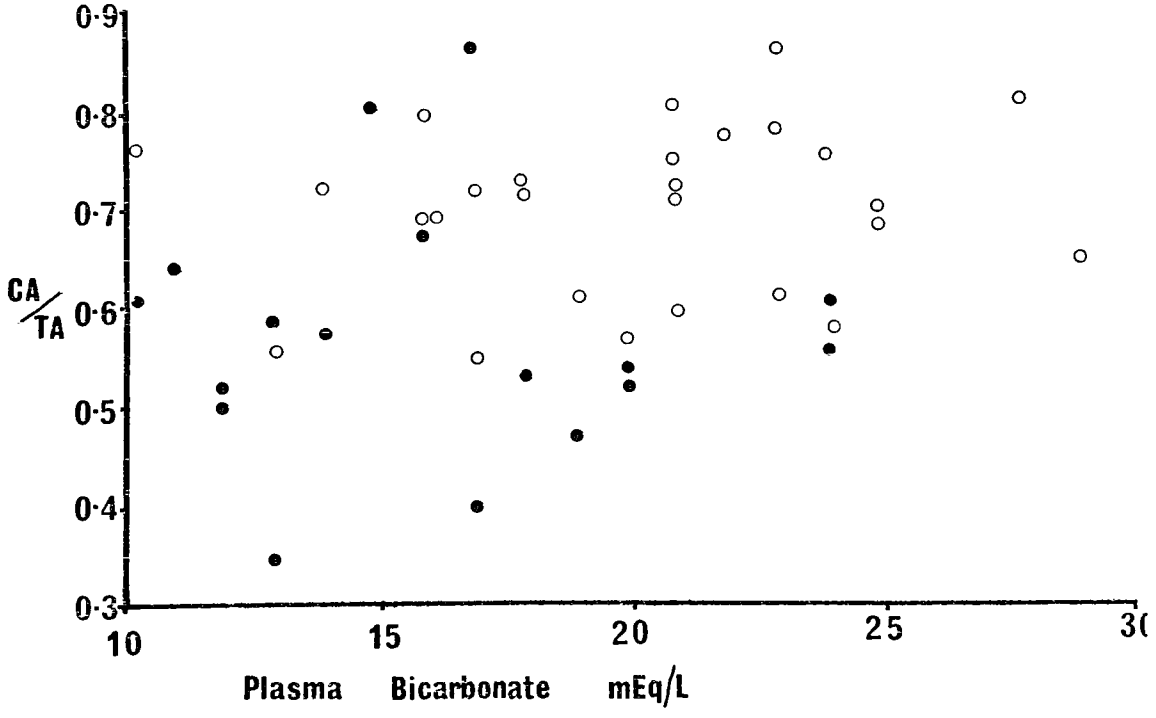

Fig. 12. The relation between CA/TA ratio and plasma bicarbonate in patients with chronic renal failure.

from the mean being small, the relative error of the resulting value is large; in the second case, the choice of one standard deviation below the mean to split the data is entirely arbitrary, though the problem of errors is reduced.

Results

Bone X-ray measurements and plasma calcium

The CA/TA ratio varied from 0.39 to 0.80 in the males, and from 0.33 to 0.85 in the females. The individual values with the mean and one standard deviation for the age and sex of the patient are shown in figure 9 and Table 6. There is a significant correlation when plasma calcium is related to the simple CA/TA ratio ($p < 0.01$; figure 10; Table 5) and a highly significant negative correlation is found when the comparison is made between the plasma calcium and the difference from the mean, ($p < 0.001$; Table 5). A highly significant association also exists between the plasma calcium concentration and the CA/TA ratio when the value of this is considered as greater or less than one standard deviation below the mean ($t = 3.19$, $p < 0.001$; figure 11).

Bone X-ray measurements and plasma bicarbonate

When the plasma bicarbonate is related to the CA/TA ratio considered in these three ways, a moderately significant association is found with the simple CA/TA value ($p = 0.05$, figure 12), and its difference from the mean ($p < 0.05$; Table 5). However, no significant difference is found between the plasma bicarbonate levels of groups of patients whose CA/TA values are either greater or less than one standard deviation below the mean ($t = 1.29$, $p = 0.10$; figure 11).

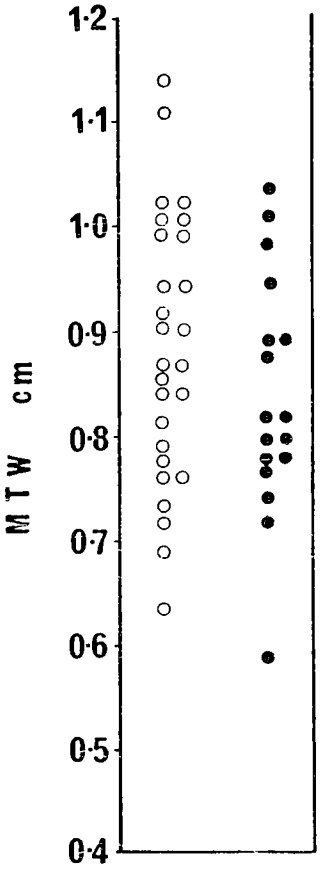

Fig. 13. Metacarpal total widths in patients with normal and raised alkaline phosphatase. There is no significant difference between the two groups.

Metacarpal cortical width

The CA/TA ratio could decrease as a result of either endosteal resorption or periosteal accretion. However, when the metacarpal total widths in the normal alkaline phosphatase group are compared with the corresponding values in the raised alkaline phosphatase group, there is no significant difference (figure 13; Table 6).

Comment

There are a number of difficulties in defining a group of bone x-ray values as normal or abnormal. The normal range, which varies with age and sex, is moreover so wide that an individual could undergo substantial bone loss as a result of a pathological process, and yet the CA/TA might remain within normal limits. For this reason, the CA/TA data have been considered in three ways: as absolute values; as the difference from the mean value for the age and sex of the patient; and according to whether they are greater or less than one standard deviation below the mean.

When any of these three modes of expression are used a significant positive association is found between the CA/TA value and the plasma calcium. There also appears to be some association between the amount of cortical bone and the plasma bicarbonate level, though the correlation is weaker than that seen with plasma calcium.

A low CA/TA value cannot be attributed to periosteal accretion and must therefore indicate endosteal resorption.

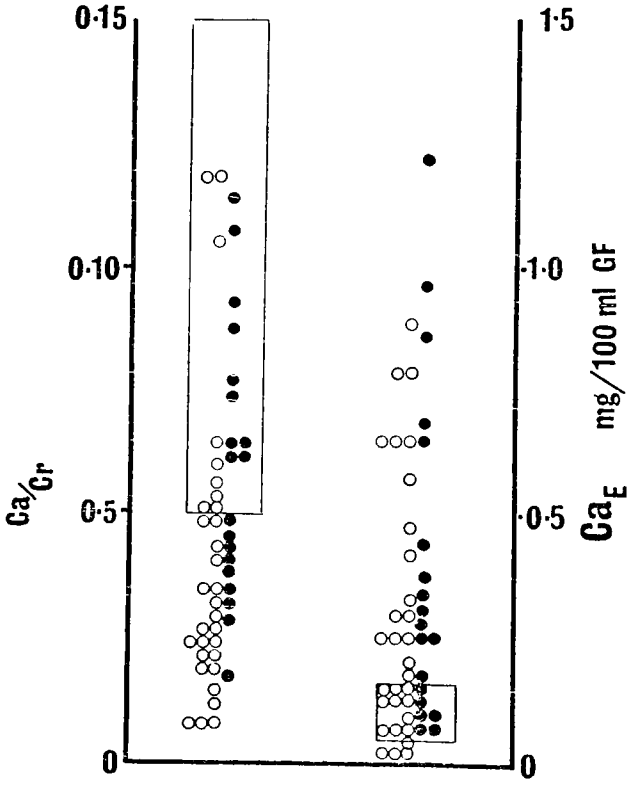

Fig. 14. Fasting calcium excretion relative to creatinine (Ca/Cr) and to GFR (Ca_E) in patients with chronic renal failure. The normal ranges are indicated.

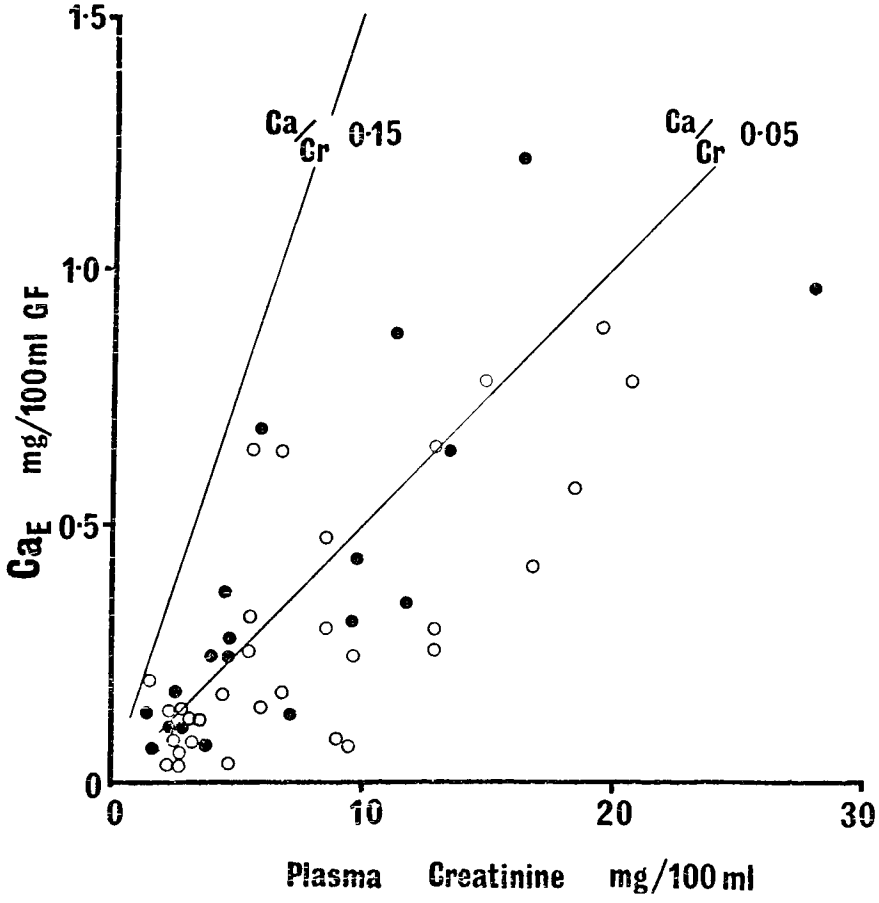

Fig. 15. Calcium excretion relative to GFR (Ca_E) at varying degrees of renal failure. The lines embrace the normal range of absolute calcium excretion.

SECTION III

Hypocalcaemia

Since the hypocalcaemia that commonly accompanies chronic renal failure is statistically related in these data both to a raised alkaline phosphatase level and a low metacarpal CA/TA value, it is important to test the possibility that acidosis might itself be influencing the plasma calcium level, if the relationships between acidosis, hypocalcaemia and bone disease are to be fully explored. The plasma calcium concentration is highly dependent upon renal tubular reabsorption and consideration is given first to this aspect of calcium homeostasis.

Results

Plasma and urine calcium

No significant relation is found between plasma calcium and the degree of renal failure as judged by plasma creatinine (figure 6; Table 5).

Fasting calcium excretion can be expressed in two ways: as the calcium/creatinine ratio (Ca/Cr) which is a function of the absolute rate of excretion; and in mg/100 ml of glomerular filtrate (Ca_E) which is excretion rate relative to GFR. The difference between these two modes of expression is illustrated in figure 14. Fasting Ca/Cr tends to be low in renal failure but may be within the normal range. Calcium excretion relative to GFR on the other hand is generally high. In both modes, calcium excretion tends to be high in the cases with raised alkaline phosphatase levels. When calcium excretion relative to GFR is related to plasma creatinine (figure 15), it is seen to rise

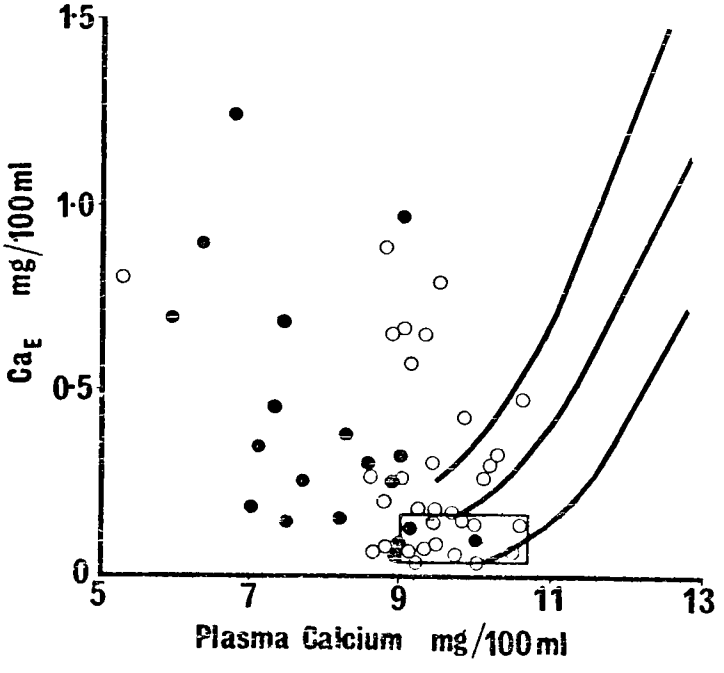

Fig. 16. Urine calcium excretion relative to GFR (Ca_E) and fasting plasma calcium in patients with chronic renal failure. The lines indicate the normal relationship.

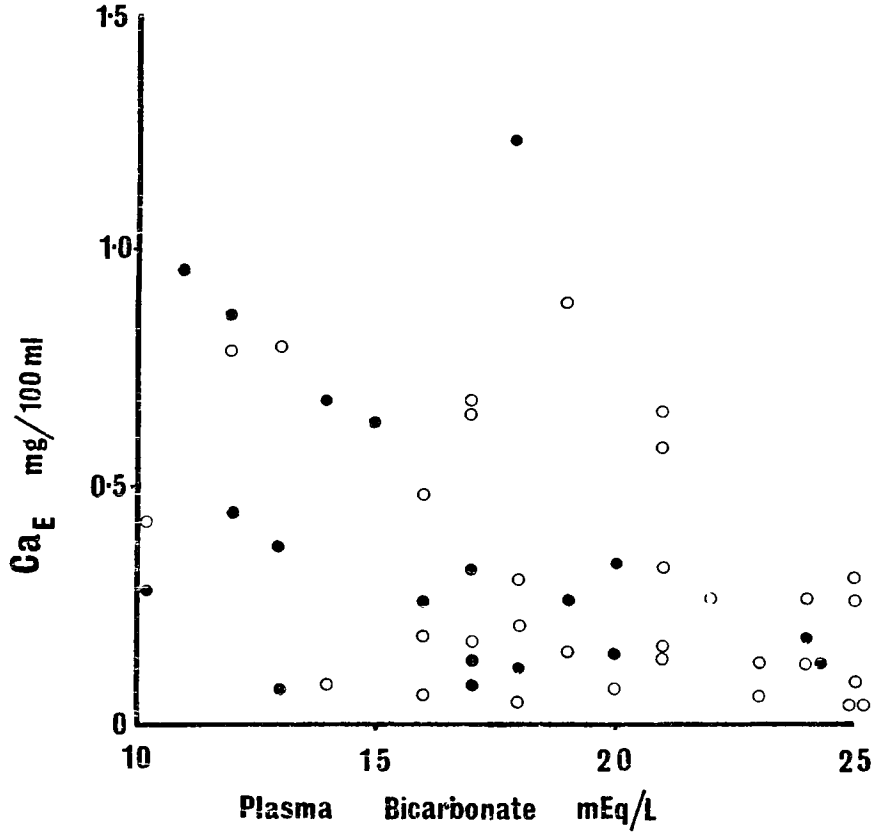

Fig. 17. The relation between Ca_E and plasma bicarbonate in patients with chronic renal failure.

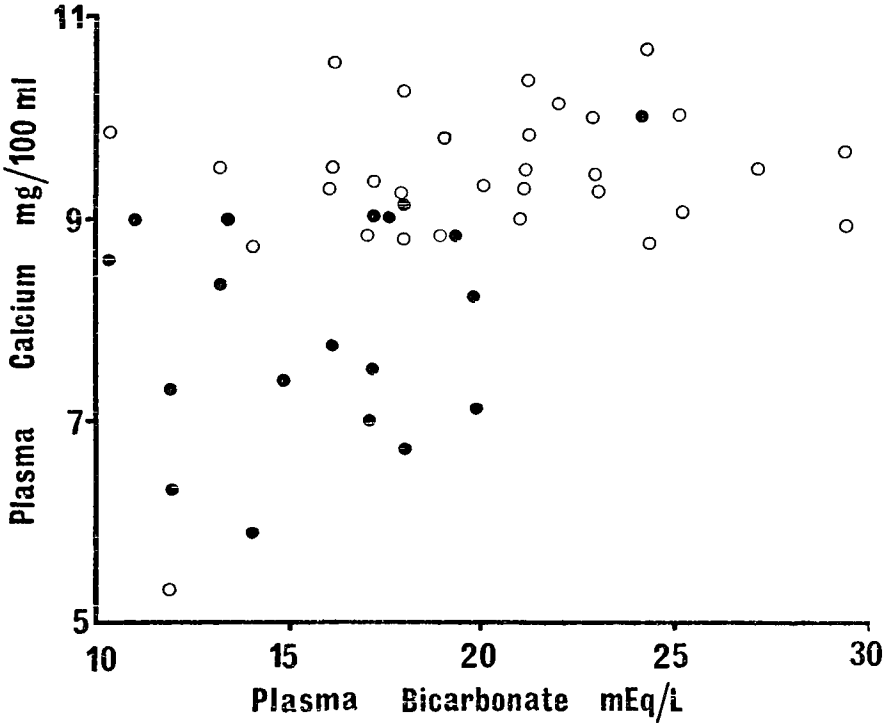

Fig. 18. The relation between plasma calcium and bicarbonate in patients with chronic renal failure.

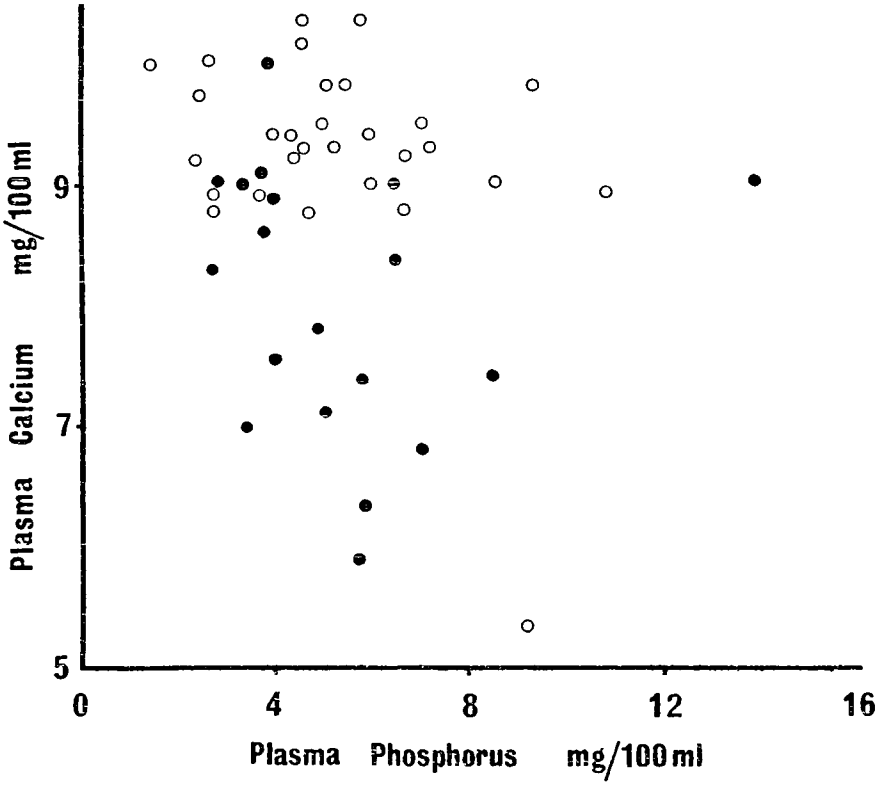

Fig. 19. Plasma calcium and phosphate in patients with chronic renal failure. There is no significant relationship.

with the degree of renal failure, though not sufficiently steeply to maintain a constant absolute rate of excretion in the face of declining renal function. Thus most of the points fall below the normal Ca/Cr limits as indicated in figure 15.

The relationship between fasting plasma calcium and calcium excretion per 100 ml of GF is shown in figure 16. The lines indicate the way in which urine calcium rises with the plasma calcium in normal subjects given calcium infusions (Nordin & Peacock, 1969). Values to the left of the normal range indicate reduced tubular reabsorption of calcium. There is a highly significant negative correlation between plasma calcium and calcium excretion relative to GFR ($p < 0.001$; Table 5), and cases with a raised plasma alkaline phosphatase tend to be those with the lowest plasma and highest urine calcium values, i.e. their tubular reabsorption of calcium is most severely reduced.

There is a highly significant inverse correlation between calcium excretion per 100 ml of GF and plasma bicarbonate ($p < 0.001$, figure 17; Table 5) and the patients with a raised plasma alkaline phosphatase tend to be the most acidotic and the most hypocalcaemic (Table 7). There is also a significant correlation between plasma calcium and plasma bicarbonate when both groups are considered together ($p < 0.01$, figure 18; Table 5), but it should be noted that the plasma calcium is independent of the degree of acidosis among the patients with normal alkaline phosphatase levels.

Plasma calcium and phosphate

The relation between plasma calcium and phosphate concentration is shown in figure 19. There is a weak inverse relation between these parameters which is not statistically significant (Table 5). Once

again it is apparent that the cases with raised plasma alkaline phosphatase tend to have hypocalcaemia, but it is clear that this hypocalcaemia cannot be directly attributed to hyperphosphataemia since many of the corresponding plasma phosphate values fall within or only slightly above the normal range.

Comment

There is a highly significant correlation between the calcium excretion relative to GFR and the degree of acidosis. It might be argued that acidosis could raise the urinary calcium excretion rate by increasing the proportion of plasma ultrafiltrable calcium. This is unlikely to be true. If the ultrafiltrable calcium were raised in this way while renal tubular handling remained unchanged, after an initial period of adjustment during which there would be a transitory rise in calcium excretion, both the plasma ultrafiltrable calcium and the urine calcium excretion rate would settle back to their previous levels. This would lead to a situation in which the total plasma calcium concentration was slightly reduced; but calcium excretion relative to GFR would be unaltered. These data show clearly that there is a raised urinary calcium excretion per unit GF. The hypocalcaemia, where present, cannot be attributed to any reduction in plasma proteins (Table 5), and the data in Table 8 indicate that no such reduction is present.

It is therefore apparent that hypocalcaemia is associated with a reduced tubular reabsorption of calcium in these cases, and is presumably secondary to it. A renal tubular leak, however small, will always lower the plasma concentration provided that the rate of input, and therefore output, remains constant. In these data obtained

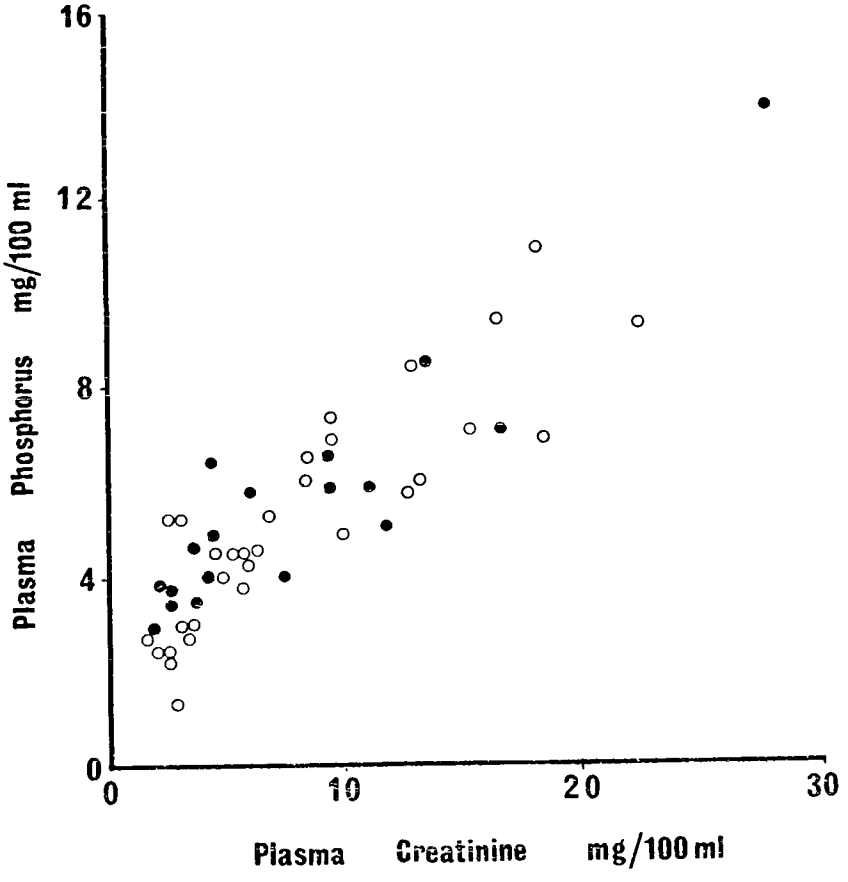

Fig. 20. The relation between plasma phosphate and creatinine in patients with chronic renal failure.

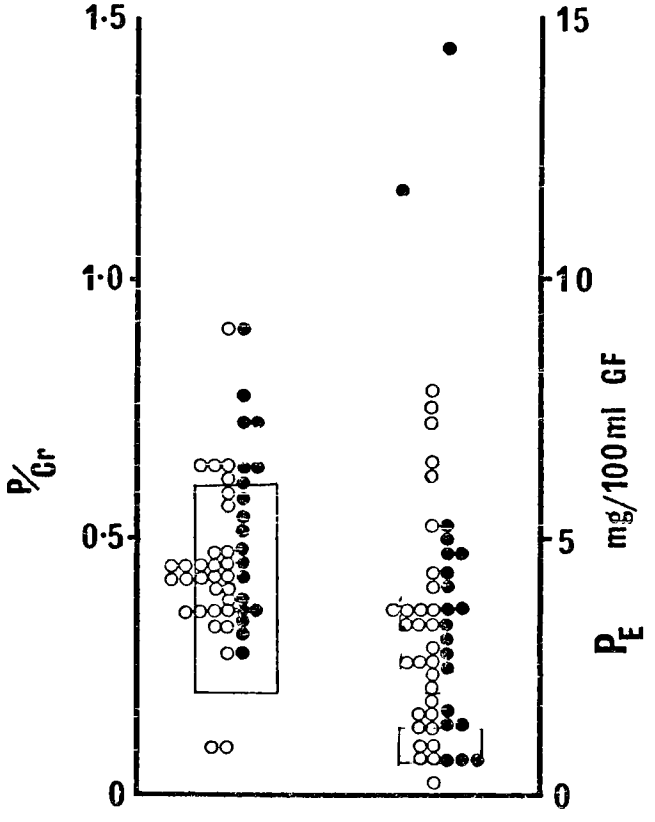

Fig. 21. The urine phosphate excretion expressed in absolute terms (relative to urine creatinine) and relative to GFR in patients with chronic renal failure. The normal ranges are indicated.

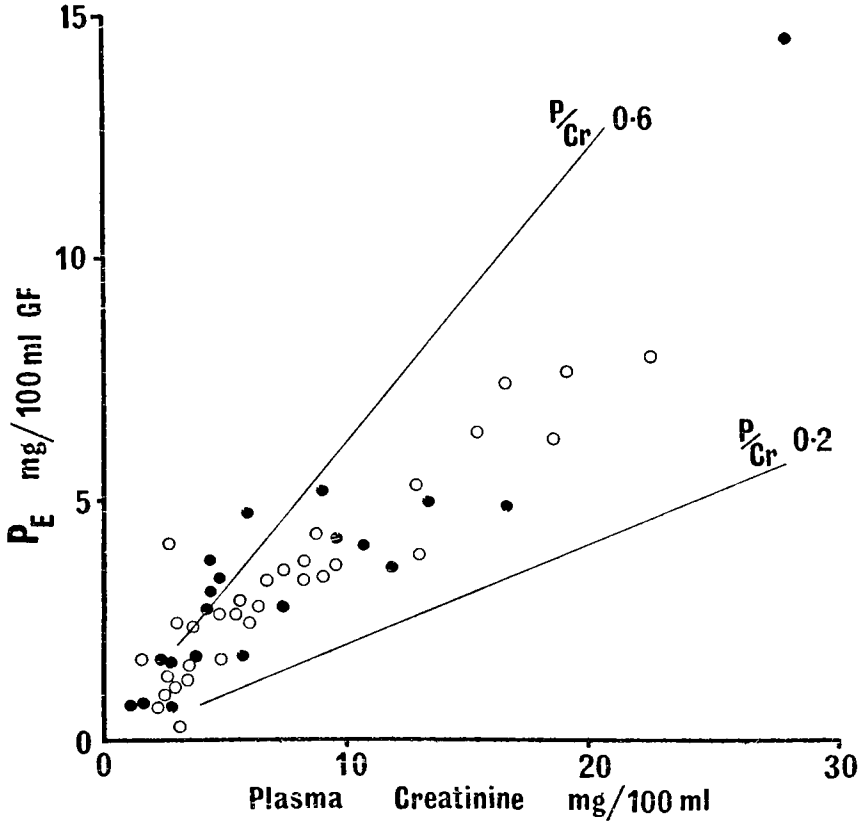

Fig. 22. Phosphate excretion relative to GFR (P_E) at varying degrees of renal failure. The lines embrace the normal range of absolute phosphate excretion.

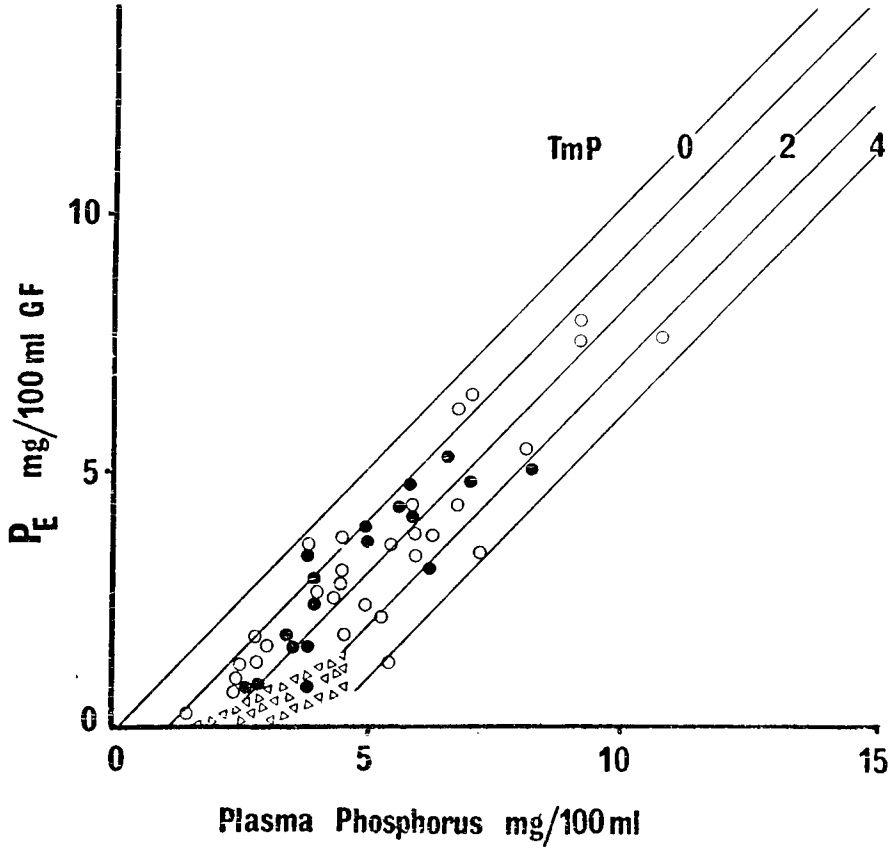

Fig. 23. The relation between phosphate excretion relative to GFR (P_E) and plasma phosphate in patients with chronic renal failure. The solid lines indicate the relationships at varying values for theoretical TmP, and the range in normal fasting subjects is shown (triangles).

from fasting subjects, the absolute excretion rate is frequently lower than normal (figure 14) and this will tend further to reduce the plasma calcium concentration, especially in cases with advanced renal failure.

It is of interest that patients with normal alkaline phosphatase levels appear to be protected in some way against the hypocalcaemia, which in the raised alkaline phosphatase group is associated with the metabolic acidosis (figure 18).

Plasma and urine phosphate

The relation between plasma creatinine and plasma phosphate is shown in figure 20. There is a highly significant positive correlation between these parameters ($p < 0.001$; figure 20; Table 5), plasma phosphate appearing to rise continuously as renal function declines.

Absolute fasting phosphate excretion (the ratio of phosphate to creatinine in the urine) is clearly maintained at a normal or even raised level despite renal failure, as shown in figure 21. It follows that phosphate excretion relative to GFR (P_E) is always raised. Thus, urine phosphate differs in this respect from urine calcium (compare figures 14 and 21).

The relation between phosphate excretion per 100 ml of GF and plasma creatinine is shown in figure 22. This again shows that fasting phosphate excretion is maintained at a relatively constant level within the normal range at all degrees of renal failure and phosphate excretion relative to GFR therefore rises as GFR falls.

The relation between phosphate excretion relative to GFR and plasma phosphate is shown in figure 23. The overall correlation is highly significant ($p < 0.001$; figure 23; Table 5). The normal relationship between these parameters is indicated and corresponds approximately to

the normal splay defined by Bijvoet (1969). Since virtually all the P_E values are over 1 mg/100 ml of GF it can be assumed that tubular reabsorption of phosphate is maximal, and the position of the points relative to the TmP lines therefore indicate the maximal reabsorptive capacity, this normally being about 3 mg/100 ml of GF (Bijvoet, 1969). It is clear that the TmP is reduced in nearly every case (Tables 3 and 4). There is no significant correlation between TmP and plasma calcium or bicarbonate (Table 5).

Comment

These data show that plasma phosphate rises in proportion to the degree of renal failure but the kidney clears normal quantities of phosphate in absolute terms, meaning that the tubular reabsorption of phosphate falls as renal function declines. There is much evidence that this effect is due to parathyroid activity (Slatapolsky et al. 1968), and the reduction in tubular reabsorption in these cases is interpreted as indicating secondary hyperparathyroidism.

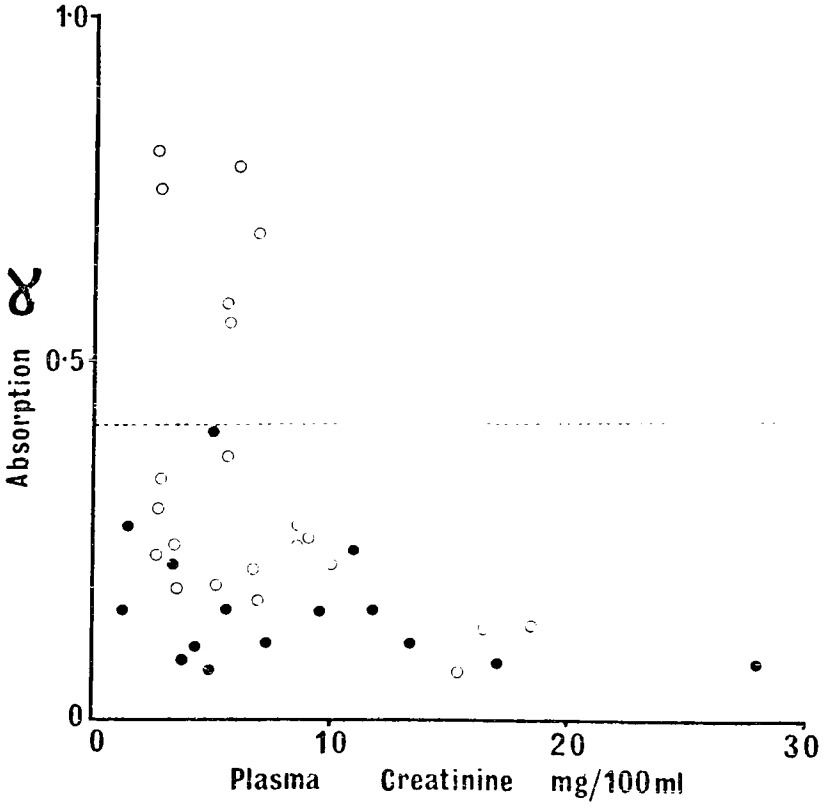

Fig. 24. Calcium absorption (fraction of tracer dose absorbed per hour) at varying degrees of renal failure. The lower limit for normal absorption is shown.

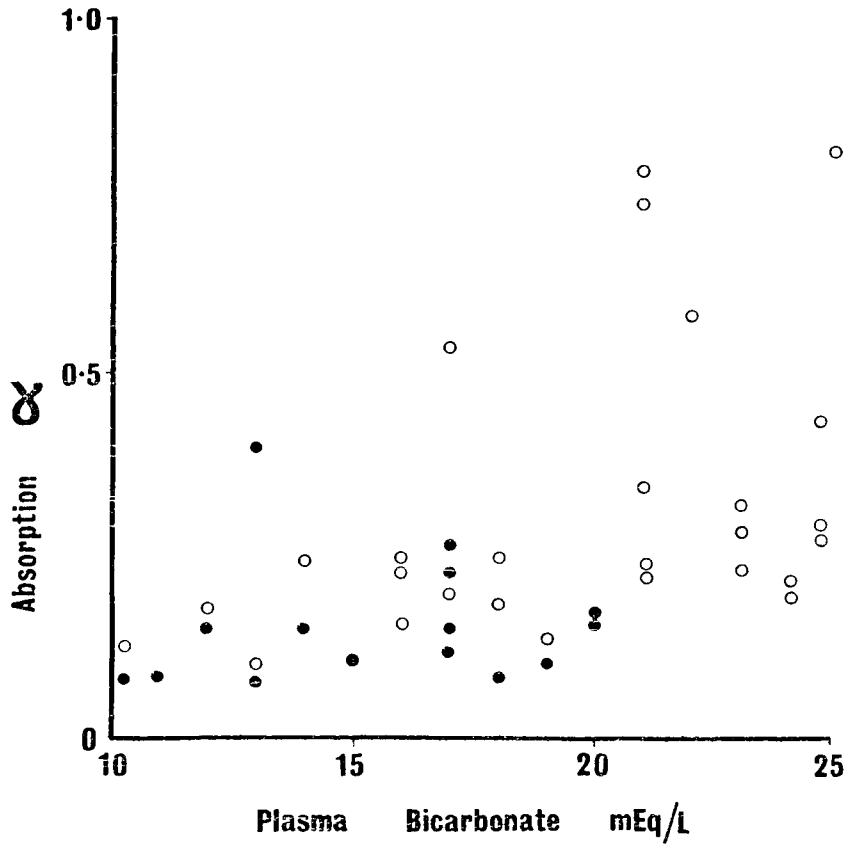

Fig. 25. The relation between calcium absorption (fraction of tracer dose absorbed per hour) and plasma bicarbonate in patients with chronic renal failure.

SECTION IV

Calcium Absorption

The malabsorption of calcium which accompanies chronic renal failure is often thought to be of importance in the pathogenesis of the bone disease. The logic behind this assumption is open to doubt as Stanbury (1968a) has pointed out, but it is conventional to consider absorption in any discussion of renal bone disease, and accordingly some consideration is given here to this aspect of calcium metabolism. The results presented are of some relevance to the final conclusions.

Absorption has been studied in the basal state by means of a standard test using radio-calcium, and on a daily basis by means of a modified version of the conventional metabolic balance technique.

Results

Calcium absorption test.

The relation between radio-calcium absorption and plasma creatinine is shown in figure 24. The fractional radio-calcium absorption is reduced below the lower limit of normal of 0.4 per hour in most of the cases, regardless of the degree of renal failure, the lowest absorption values being seen in the cases with a raised alkaline phosphatase. When the fractional absorption is related to the plasma bicarbonate, a highly significant correlation is found ($p < 0.005$; figure 25; Table 5).

The relative contribution of the degree of renal failure (i.e.

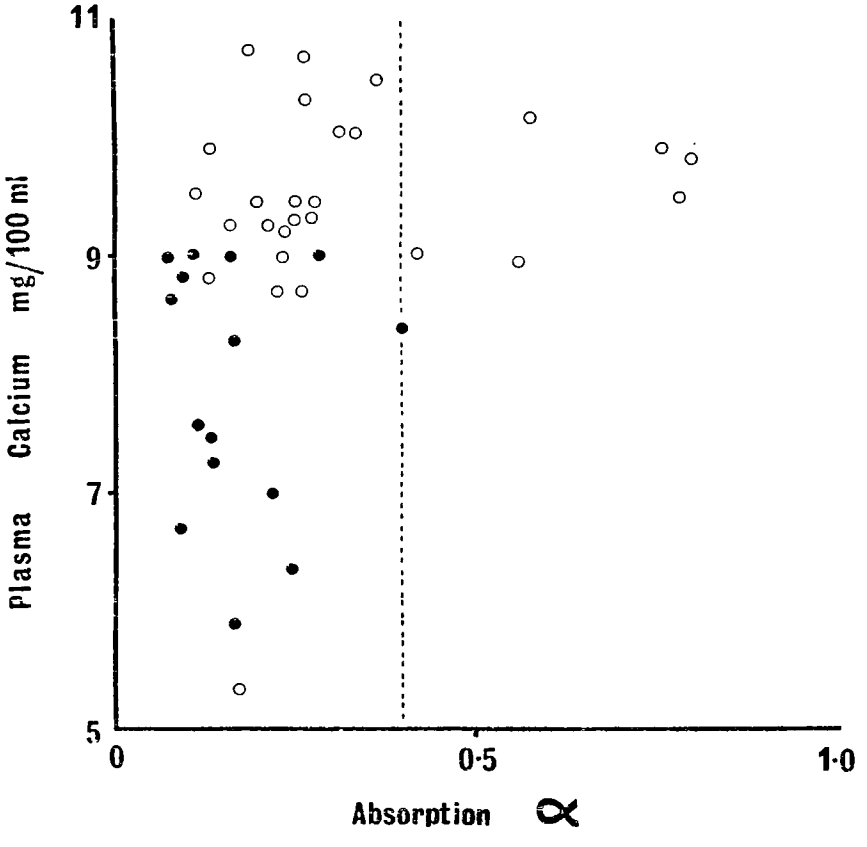

Fig. 26. Calcium absorption (fraction of tracer dose absorbed per hour) and plasma calcium in patients with chronic renal failure. The lower limit for normal absorption is shown.

plasma creatinine) and the degree of acidosis (i.e. plasma bicarbonate) to malabsorption of calcium was tested by the technique of multiple regression analysis. This shows that when the significant effect of acidosis on calcium absorption is allowed for, there is no further contribution from the degree of renal failure.

$$\alpha_1 = 0.0259 - 0.0063\ Cr^{*} + 0.0156\ HCO_3^{**}$$

*N.S.

**$p < 0.01$

The relation between calcium absorption and plasma calcium is shown in figure 26. The patients with the highest absorption values were normocalcaemic whereas all the hypocalcaemic cases had malabsorption. There is however a group of cases with normocalcaemia and malabsorption.

Metabolic balance studies.

Balance studies were carried out in seventeen patients, whose plasma creatinine levels ranged from 1.7 to 19.0 mg/100 ml. These patients were in a state of chronic renal failure and were not at the time of the study receiving treatment, other than small quantities of alkali, given as sodium bicarbonate, where necessary. A daily balance technique was used and the results are shown as the mean of a seven day period. Most of the patients were in essentially zero balance but four were in negative balance of more than -2.5 mg/Kg b.w./day and one, a case of severe osteomalacia with a myopathy, was in a positive balance of 4.7 mg/Kg b.w./day (figure 27).

No significant difference was found in either the plasma calcium level or degree of acidosis between the patients with or without a negative balance (Table 9). There was no indication that the dietary

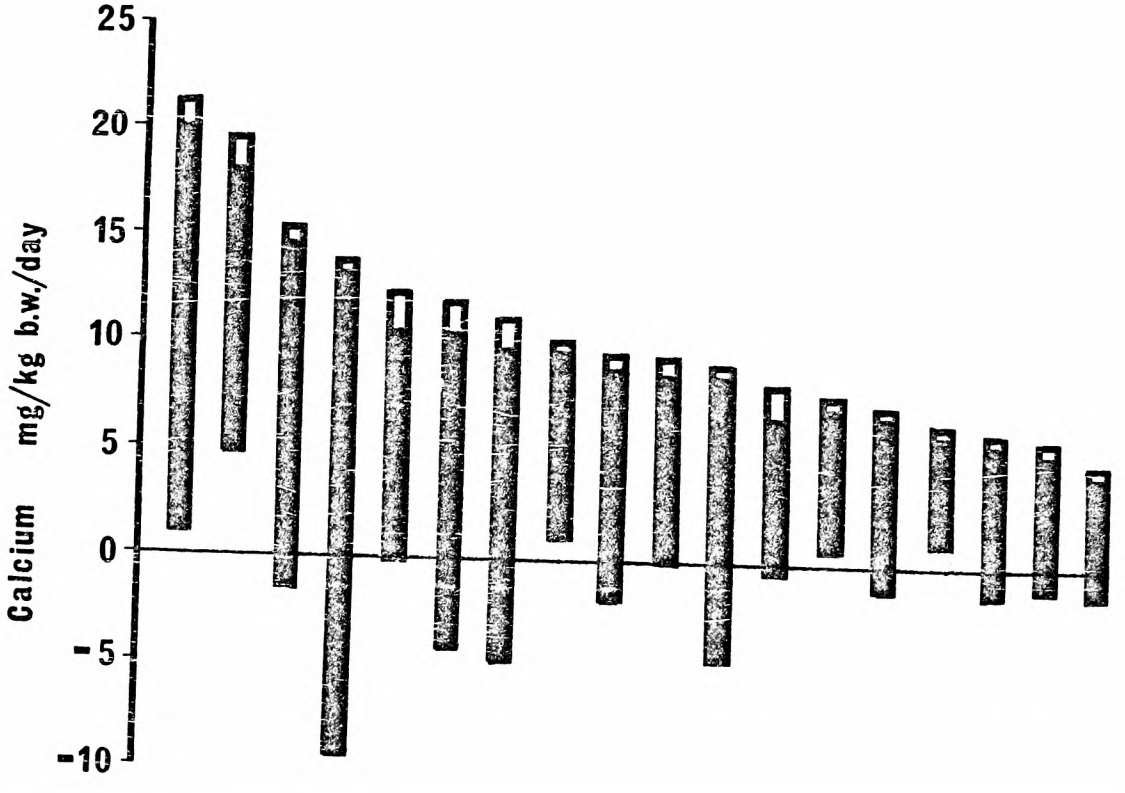

Fig. 27. Metabolic calcium balance data in seventeen patients with chronic renal failure.

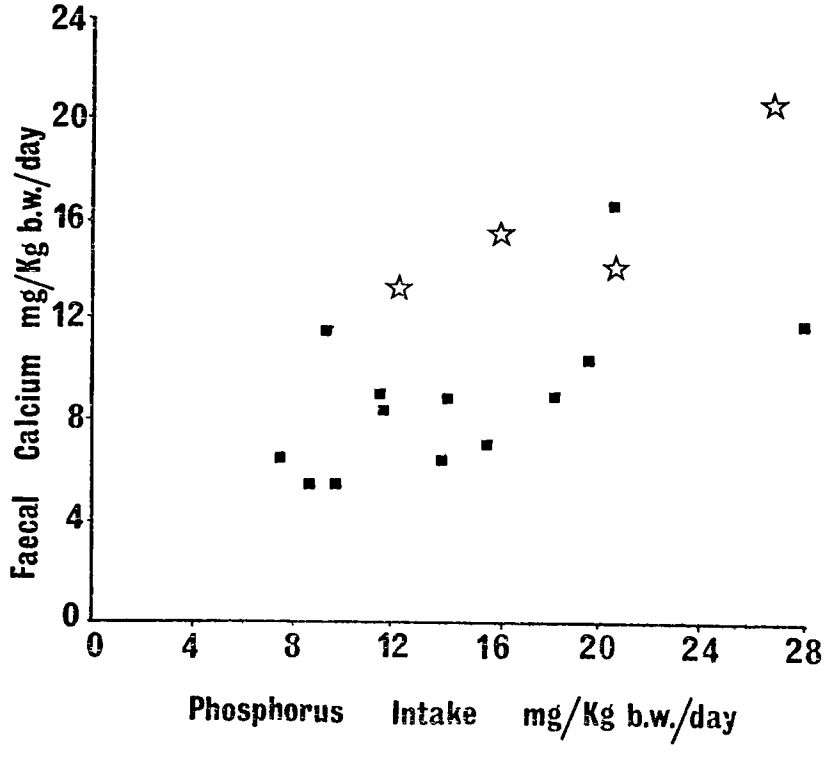

Fig. 28. Faecal calcium related to phosphorus intake in patients with chronic renal failure. Those in definite negative calcium balance are designated ☆.

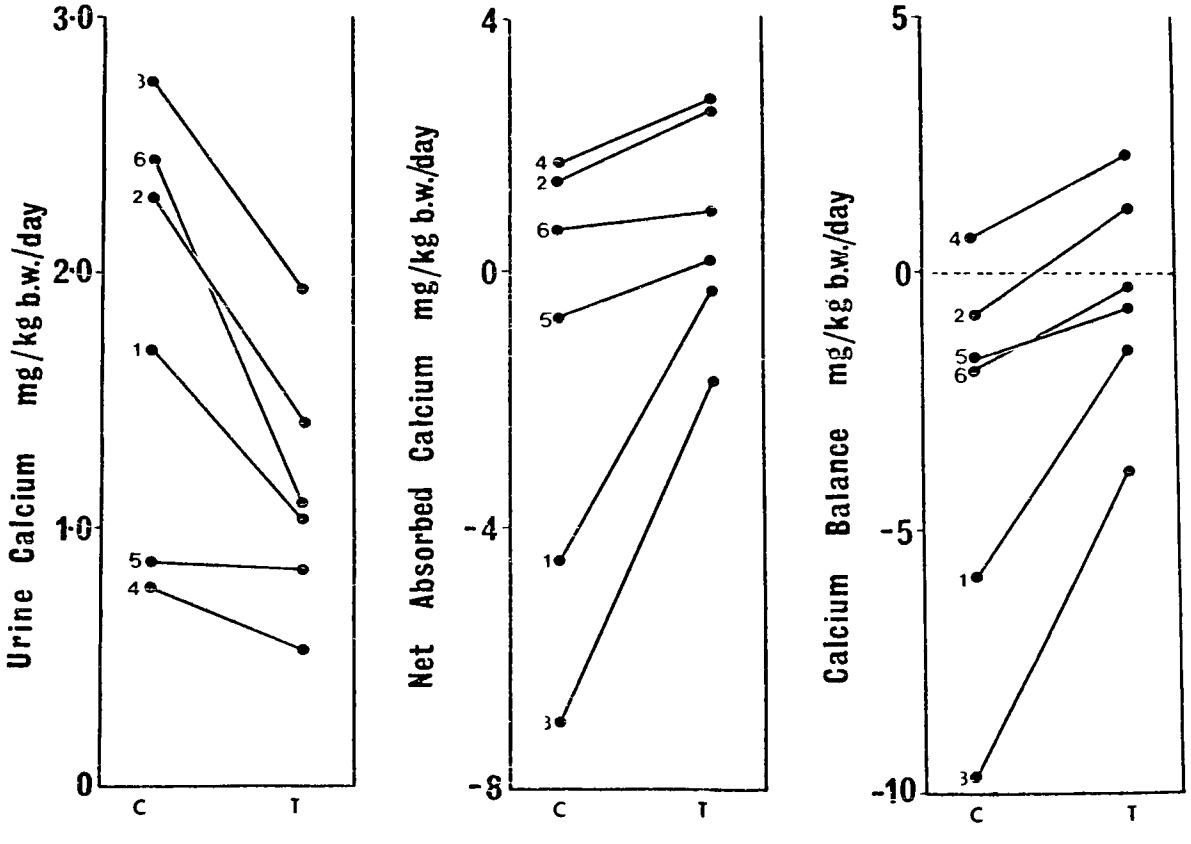

Fig. 29. The effect of correction of metabolic acidosis on the balance status of six patients with chronic renal failure. Control (C) and post-treatment (T) periods are shown.

calcium affected the balance state although the effect of very high calcium intakes was not examined. A low calcium intake was not necessarily associated with a negative balance state (figure 27).

A positive correlation exists between faecal calcium and phosphorus intake, but patients with a negative calcium balance were not those with a particularly high dietary phosphate (figure 28).

In order to test directly the effect of the acidosis on the balance state, in six cases two consequetive studies were carried out, each with one week's equilibration. After a loading dose of sodium bicarbonate, alkali (75-90 mEq/day) as sodium and potassium bicarbonate was administered during the second study, the blood pH rising in all cases and reaching a normal value in five patients. This correction of the metabolic acidosis was accompanied by a rise in net calcium absorption in all cases (paired $t = 2.58$, $p < 0.05$) and a simultaneous fall in urine calcium excretion (paired $t = 3.80$, $p < 0.02$) in all but two in whom it remained unchanged so that overall calcium balance became more positive (paired $t = 3.27$, $p < 0.05$; figure 29). There was no definite rise in the plasma calcium during the period of observation, and the creatinine clearance values remained almost constant (Table 10).

Comment.

These results suggest acidosis, rather than simply the degree of renal failure, is an important contributing factor in malabsorption of calcium that accompanies uraemia. Both plasma creatinine and a reduction in plasma bicarbonate appeared to influence calcium absorption adversely as judged by the radio-calcium absorption test, but multiple regression analysis implies that acidosis is the predominant factor.

In the balance studies there was no evidence that the degree of

renal failure, plasma bicarbonate or the diet itself influenced the balance state when the results were considered as a group. However, when the acidosis was corrected in five patients calcium absorption improved in every case, indicating clearly the importance of acid-base status in this respect.

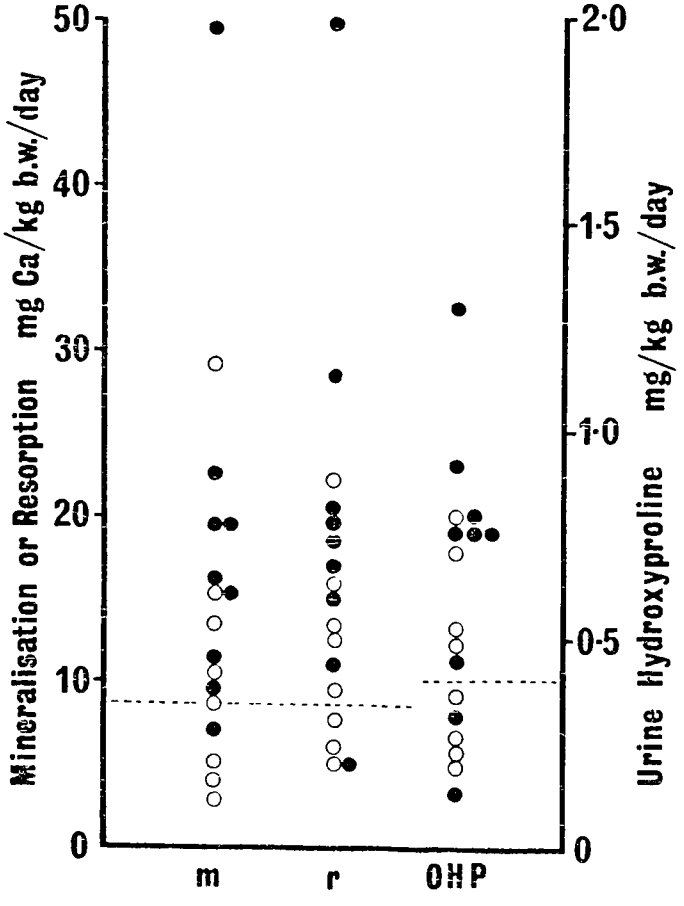

Fig. 30. Bone mineralisation and resorption rates and urine total hydroxyproline in patients with chronic renal failure. The upper limits of normal are shown.

SECTION V

Bone Kinetics

There is very little available information on bone kinetics in chronic renal failure and although it is generally assumed that bone resorption is increased in some patients, no attempt has been made to quantitate this increased resorption or to assess its frequency. Moreover since most patients in chronic renal failure are in approximately zero calcium balance, it follows that when the resorption rate is raised the formation rate must also be elevated. It is therefore of relevance to examine the bone mineralisation rate and in the present study turnover measurements were made in 17 patients, with metabolic balance studies being carried out during the same period. The formation rate was calculated from the rate of disappearance of radio-calcium into the skeleton, and resorption was determined indirectly from the difference between the mineralisation rate and the balance. Urine total hydroxyproline was also estimated as an independent measure of bone breakdown.

Bone turn-over rate.

It is clear that mineralisation rates are generally raised, especially in patients with raised plasma alkaline phosphatase levels (figure 30; Table 11). Only one such patient had a mineralisation rate within the normal range. Resorption rates were also raised in most cases and this is again a feature of those patients with raised alkaline phosphatase levels. When the urine hydroxyproline output is compared with the resorption rate, a highly significant correlation is found

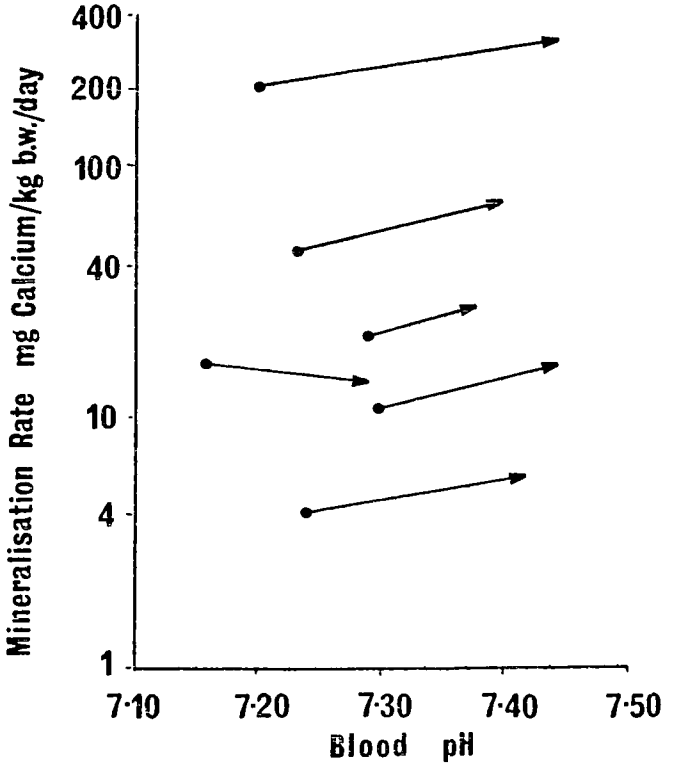

Fig. 31. The effect of correction of metabolic acidosis on the isotopic bone mineralisation rate in six patients with chronic renal failure. Note the logarithmic scales.

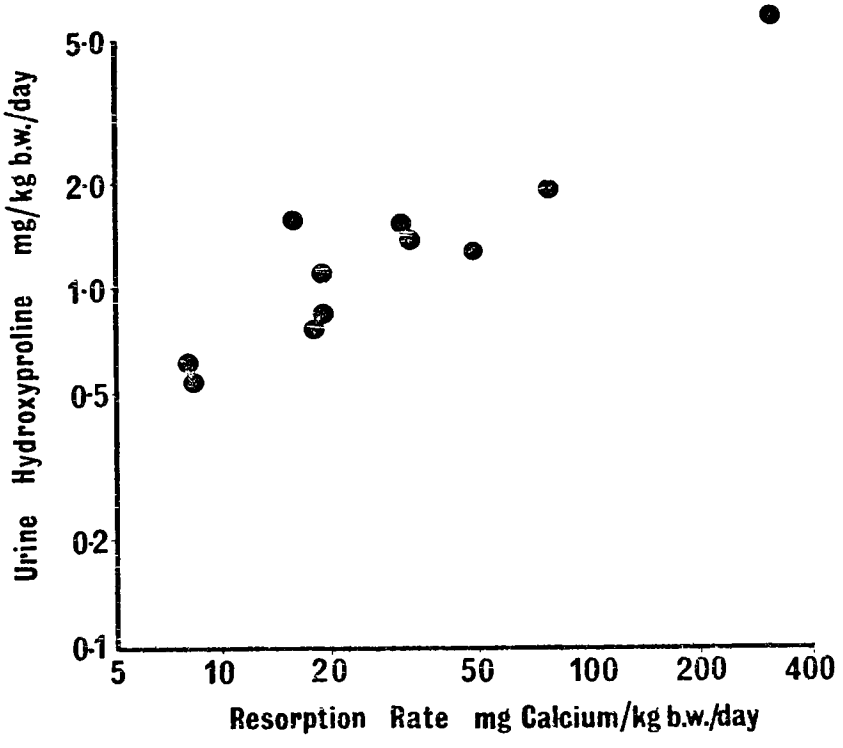

Fig. 32. The relation between urine total hydroxyproline and estimated bone resorption rate in six patients with chronic renal failure. Note the logarithmic scale.

($p < 0.001$, Table 5) supporting the validity of this indirect method of measurement.

In the six patients with a severe metabolic acidosis and moderately severe impairment of glomerular function (GFR 5 - 16 ml/min) on whom balance studies were carried out before and after administration of alkali, an attempt was made to see whether correction of the acidosis would alter the mineralisation rate. In five patients a normal blood pH was achieved but in the remaining patient the acidosis was only partially corrected. The results of the study are shown in figure 31 and Table 12. The mineralisation rates increased in all five patients in whom the acidosis was corrected (expressed as a percentage of the control value, paired $t = 2.85$, $p < 0.05$) but appeared to fall slightly in the patient in whom correction was not achieved. The rise in mineralisation rate exceeded or was not significantly different from the positive shift in calcium balance (Table 13). These events were unassociated with any alteration in plasma calcium or alkaline phosphatase or with a change in creatinine clearance (Table 10). However, plasma phosphorus fell significantly with alkali (paired $t = 3.76$, $p < 0.02$) and this was reflected in a decreased urine phosphorus excretion (paired $t = 6.47$, $p < 0.01$). The maximal tubular reabsorption of phosphate, TmP, did not change significantly (paired $t = 1.62$, n.s.). The effect on bone resorption rate was unpredictable. It remained unchanged in four patients but rose in two in whom a large change in mineralisation rate took place. Urine hydroxyproline rose variably with alkali treatment, but again correlates closely with the calculated resorption rate (figure 32).

Comment.

When the skeleton as a whole is considered, both bone mineralisation and bone resorption rates are frequently elevated in chronic renal failure and on occasions bone turnover is taking place at many times the normal rate.

Osteomalacia implies a sluggish rate of mineralisation at the bone surface but the apparently contradictory observation that these patients have a high bone formation rate can be explained when the total number of mineralising sites in the skeleton is considered, for these may be so many that the overall rate of calcium deposition is also increased (Frost, 1967). Normal bone turnover rates are occasionally seen, but rarely in patients with raised alkaline phosphatase levels. It is important to note that the indirectly calculated resorption rate correlates significantly with the urine hydroxyproline output which was used as an independent indicator of bone breakdown.

The results of the balance and bone turnover study before and after treatment with alkali extend the known effects of acidosis to include a direct action on skeletal metabolism. The positive trend in the balance with correction of the acidosis might have been reflected in an elevation of the plasma calcium concentration and the fact that this did not occur suggests that the calcium was cleared into some other pool. The rise in the mineralisation rate was sufficient to account for this retention of calcium in all cases (Table 13).

It may be objected that the estimated rise in mineralisation rate does not represent mineralisation at all, but is the result of deposition of alkaline calcium salts, such as calcium carbonate, in the tissues or skeleton. Certainly a moeity of calcium exists physiologically in this form (Pellegrino & Biltz, 1965) but simple precipitation

is unlikely to be the explanation for at least two reasons. Precipitations of calcium salts with correction of the acidosis would take place fairly quickly and then cease, whereas the isotope studies show a continual removal of calcium by a steady process during the second week after this correction was achieved. Secondly, the amount of calcium precipitated in a given time would be a function of the change in pH, whereas the observed increase in mineralisation rate is an approximate function of the initial rate (figure 31). Also the observed fall in plasma phosphorus that occurred with alkali therapy, though open to several interpretations, is consistent with the idea that true mineralisation has taken place. The fall in urine phosphorus was presumably the result of the decreased plasma level, since tubular reabsorption remained unchanged. It therefore appears that the slowing of mineralisation at individual bone forming sites which leads eventually to histological osteomalacia is aggravated by metabolic acidosis, since correction of this leads to an increased mineralisation rate. Although there are probably many factors which contribute to the development of renal osteomalacia, the present study strongly suggests that acidosis is one of them. The bone resorption rate can only be measured indirectly but appeared to increase in the two patients whose mineralisation rates rose markedly, and was unchanged in the other cases. This variation in resorption rate cannot be explained from the data, but had resorption not risen in the two patients in whom the mineralisation rate exceeded the increase in calcium balance, then the plasma calcium would almost certainly have fallen.

SECTION VI

Bone Histology

The enzyme alkaline phosphatase may be derived from a number of sources though when of bony origin its exact relationship with bone turnover is not clear. It is usually thought that bone alkaline phosphatase results from osteoblastic activity though histo-chemical evidence is hard to find. However, while it is true that the plasma alkaline phosphatase is known to be elevated in most states where excessive osteoid is present, as in the bone of the growing child or in osteomalacia, it is in precisely these states that a rapid rate of bone break-down also occurs. Furthermore, it is a well known clinical fact that in chronic renal failure complicated by osteitis fibrosa, where bone destruction is such a major feature, the alkaline phosphatase levels are frequently very high indeed. In the present work, the concentration of the enzyme is used as a simple indication of the presence of bone pathology, and it is necessary to determine which facet of turnover - formation or resorption - the alkaline phosphatase level reflects.

Secondly, it is important to establish what connections exist between the acid-base status and the plasma calcium level of the individual, and the fraction of bone surface that is coated with osteoid or undergoing resorption.

Results.

Bone histology and plasma alkaline phosphatase

A raised plasma alkaline phosphatase appears to reflect the

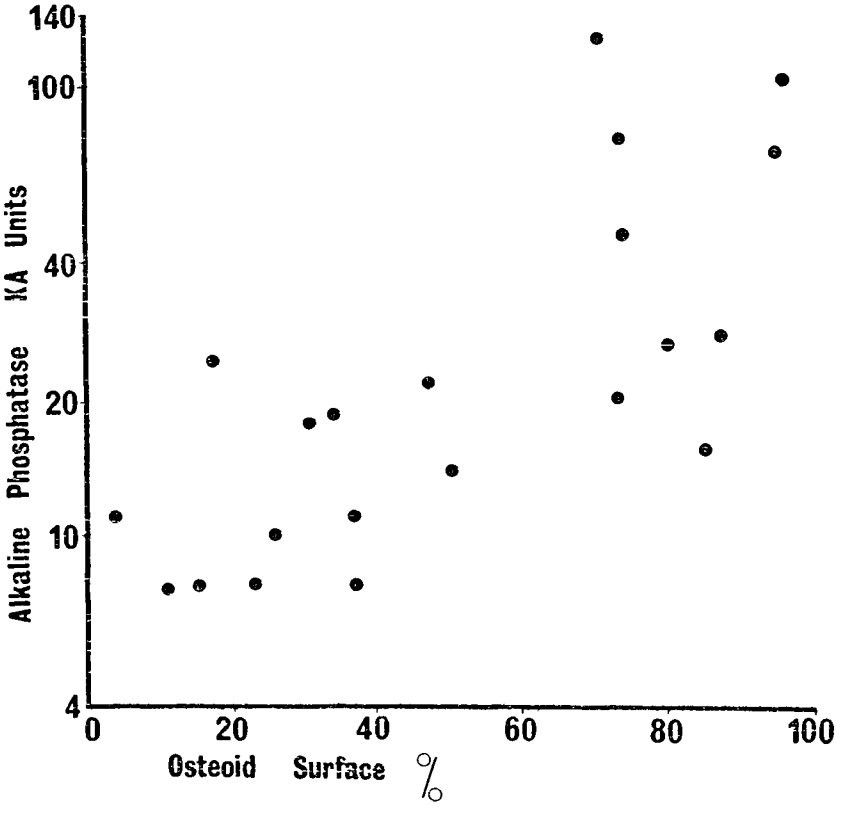

Fig. 33. The relation between plasma alkaline phosphatase and osteoid surface of iliac crest biopsies.

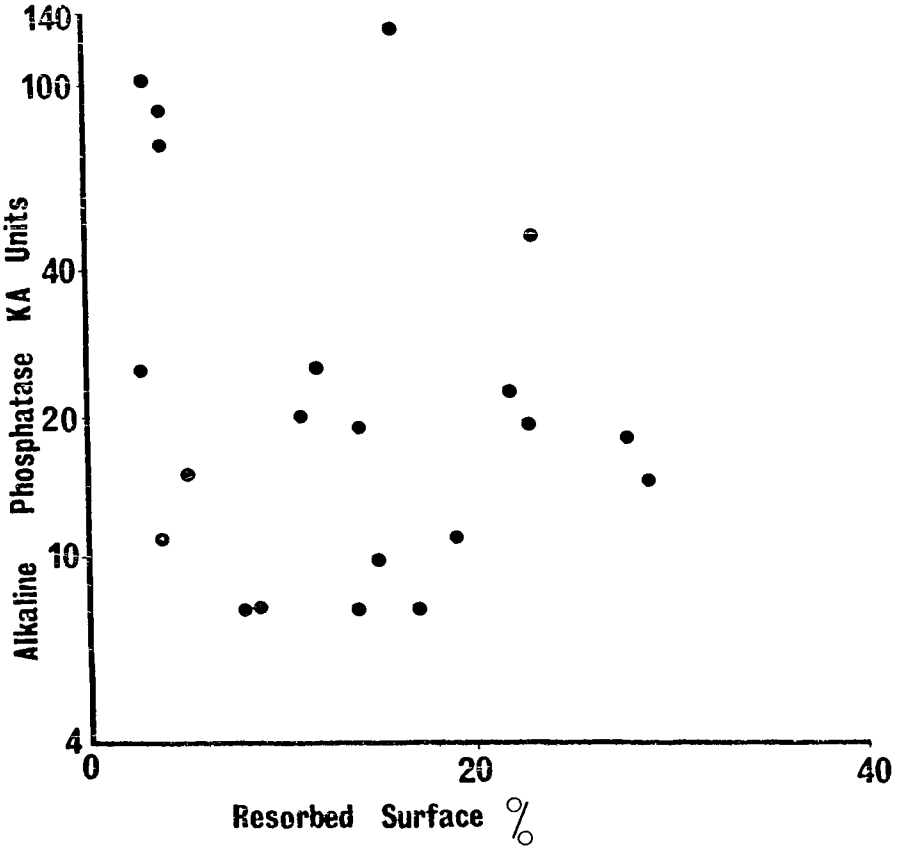

Fig. 34. Plasma alkaline phosphatase and percentage resorbed surface of trabecular bone. There is no relationship.

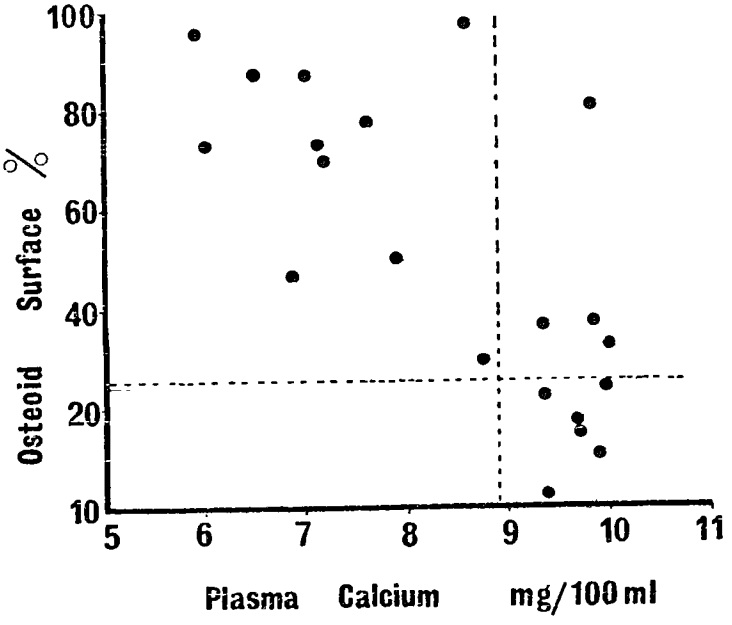

Fig. 35. The relation between osteoid surface on trabecular bone (upper limit shown) and plasma calcium

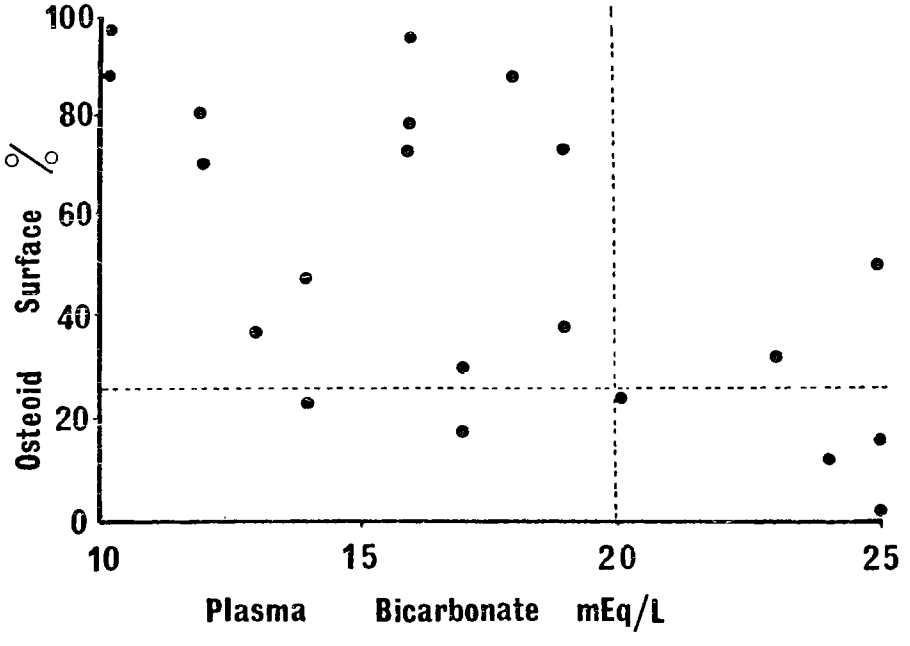

Fig. 36. The relation between osteoid surface on trabecular bone (upper limit shown) and plasma bicarbonate.

proportion of surface coated with osteoid (i.e. the degree of osteomalacia). This is shown in figure 33. The plasma alkaline phosphatase was raised in fourteen of the twenty-one cases, in all of whom the proportion of trabecular surfaces coated with osteoid was abnormally high (over 25%). The proportion of osteoid surfaces showing a normal calcification front was estimated in eight of the biopsies and was abnormally low (less than 50%) in all these cases, including one with a normal alkaline phosphatase (Table 14). While the percentage of osteoid coated surface is significantly related to the logarithm of the plasma alkaline phosphatase, ($p < 0.001$, Table 15) the percentage of surfaces undergoing resorption is not (figure 34).

Osteoid and plasma calcium and plasma bicarbonate.

The fraction of trabecular surface covered by osteoid ranged from 11-96 per cent. A highly significant inverse relationship exists between the fraction of surface undergoing formation and the plasma calcium concentration ($p < 0.001$; figure 35; Table 15), but in two patients, both with renal tubular acidosis, a particularly severe degree of osteomalacia was apparent in spite of a relatively normal plasma calcium. In none of the patients was hypophosphataemia a feature (Table 14). In four patients in whom the plasma calcium was definitely normal, osteomalacia was present as judged by a raised level of osteoid coated bone surface. All patients with hypocalcaemia had osteomalacia.

When the degree of osteomalacia is considered in relation to the prevailing plasma bicarbonate level, a highly significant inverse correlation is found ($p < 0.001$; figure 36; Table 15). Two patients had definite osteomalacia with a minor degree of metabolic acidosis, and of the five patients that were not acidotic only one had well established osteomalacia.

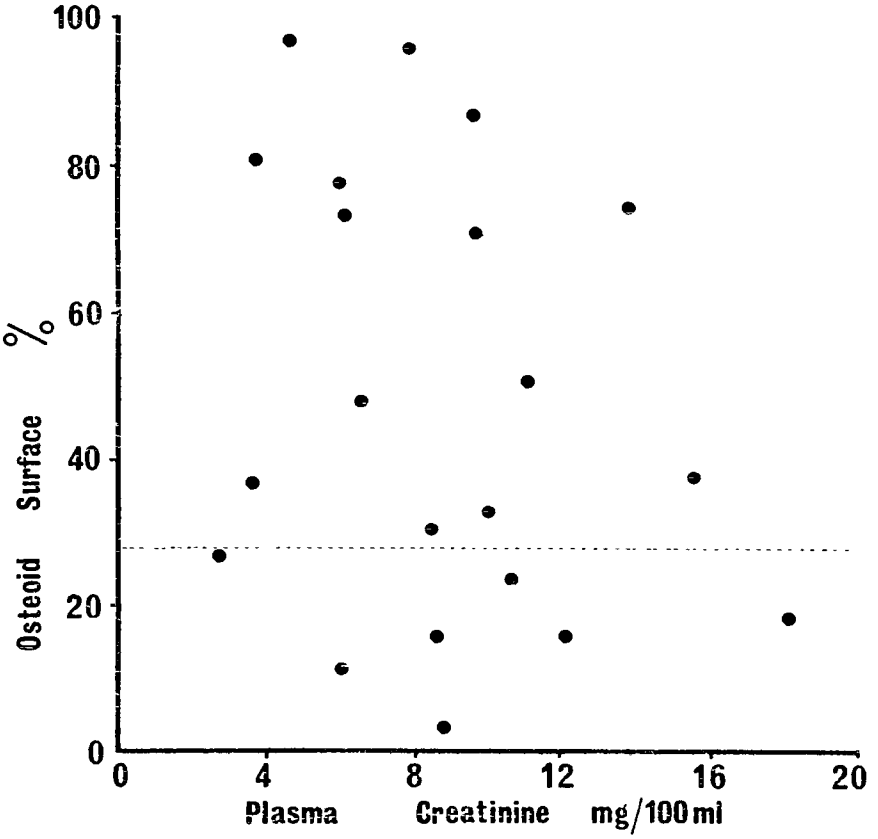

Fig. 37. The fraction of trabecular surface coated by osteoid (upper limit shown) and the degree of renal failure. There is no relationship.

When the percentage of osteoid surface is considered in relation to the plasma creatinine concentration, there is no correlation but the data split into two groups (figure 37). The patients in one group had severe osteomalacia with a fractional osteoid surface of at least 70 percent while the remainder had osteomalacia of mild degree or none at all. The plasma bicarbonate level in the former group ranged from 10-19 mEq/L, with a mean of 14.1 mEq/L. The corresponding range for plasma bicarbonate in the latter group was 13-25 mEq/L with a mean of 19.6 mEq/L. These differences are significant ($p = 0.01$). Similarly, when the relative influence of the degree of renal failure and of acidosis are considered with respect to the severity of the osteomalacia using multiple regression analysis, the significant relationship with acidosis persists, but the degree of renal failure does not contribute.

$$F_{os} = 128.01 - 1.25\ Cr^{*} - 3.82\ HCO_3^{**}$$

where F_{os} is percent surface covered by osteoid

* N.S.

** $p < 0.001$

There is no significant relation between the plasma bicarbonate and creatinine.

Comment.

This study clearly shows that a raised alkaline phosphatase level reflects the osteomalacic state and is not directly indicative of bone resorption.

There is an overall correlation between the degree of osteomalacia and the presence of hypocalcaemia, though it is evident that osteomalacia, sometimes of severe degree, can exist with little or no abnormality of

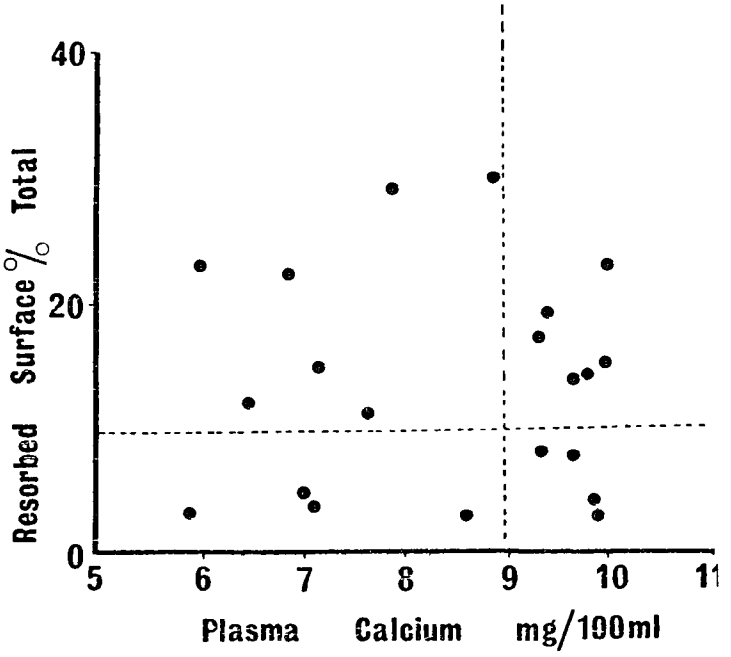

Fig. 38. The fraction of trabecular bone undergoing resorption (upper limit shown) and plasma calcium. There is no relationship.

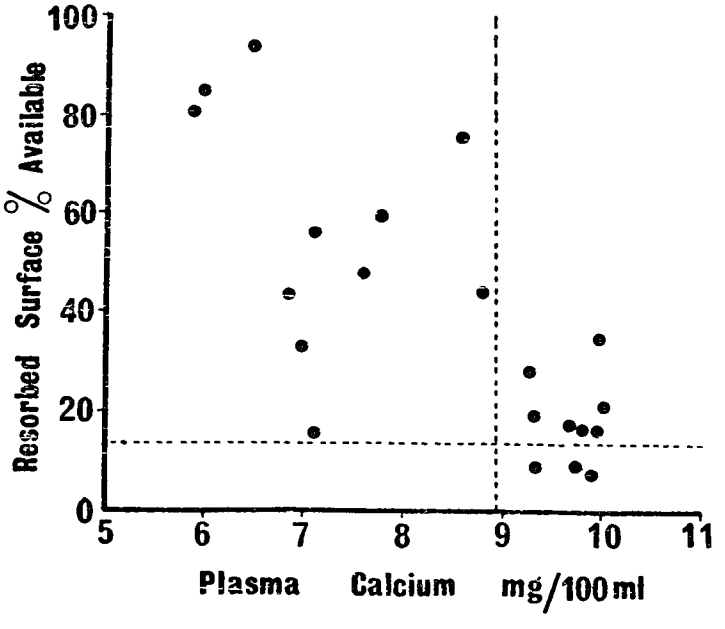

Fig. 39. The relation between the fraction of available surface undergoing resorption (upper limit shown) and plasma calcium.

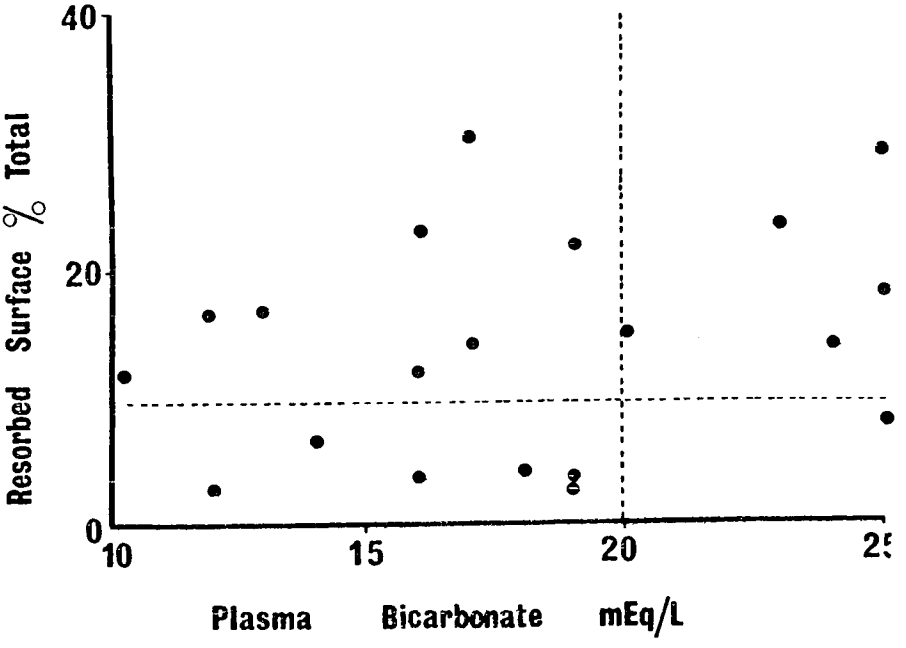

Fig. 40. The fraction of trabecular surface undergoing resorption (upper limit shown) and plasma bicarbonate. There is no relationship.

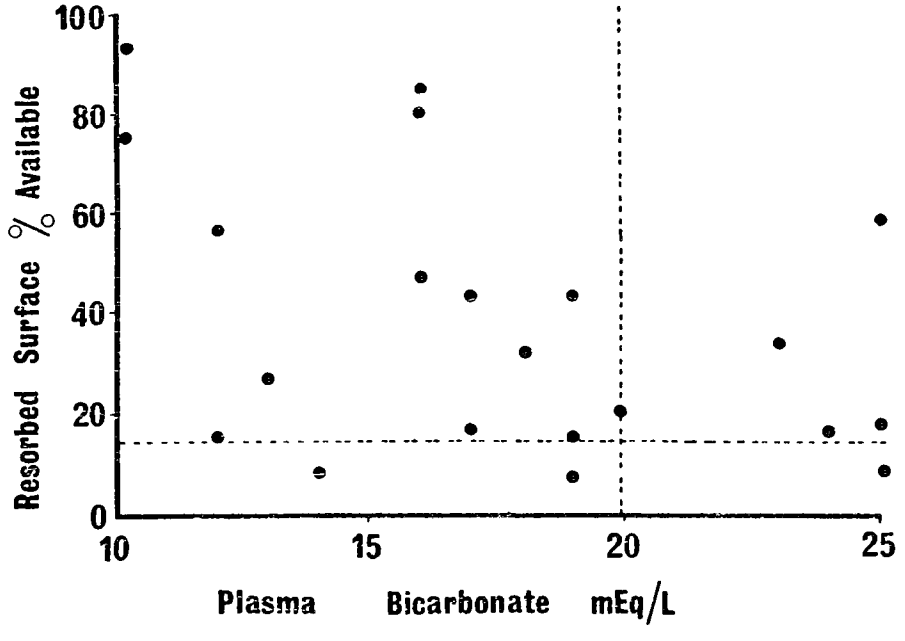

Fig. 41. The relation between the fraction of available surface undergoing resorption (upper limit shown) and the plasma bicarbonate.

plasma calcium concentration.

A highly significant correlation exists between the percentage surface covered with osteoid and the degree of acidosis, although in one case osteomalacia is fairly marked in spite of a mild degree of acidosis and in another definite osteomalacia is present in the absence of acidosis. It may be argued that the osteomalacia is a consequence of co-existing uraemia and is unrelated to the metabolic acidosis. However, in these data there is no good association between the metabolic acidosis and the degree of renal failure as judged by the plasma creatinine concentration and it is clear that the presence of excessive amounts of osteoid is related to the acidosis rather than simply the degree of renal failure.

Resorbed surface and plasma calcium and plasma bicarbonate.

When the percentage of total trabecular surface undergoing resorption is related to the plasma calcium concentration no correlation is found (figure 38; Table 15). There is an inverse relation between the percentage of available surface undergoing resorption and the plasma calcium concentration ($p < 0.001$; figure 39; Table 15). Similarly, when the fraction of total surface undergoing resorption is related to the degree of acidosis, no association is apparent (figure 40; Table 15), but when the percentage of surface available for resorption is considered a moderately significant negative correlation is found ($p < 0.05$; figure 41; Table 15).

Comment.

These data show that the overall rate of resorption taking place in trabecular bone is neither related to the degree of hypocalcaemia nor the degree of acidosis and it is, in the final analysis, this absolute

rate of bone destruction that would determine the rapidity with which bone disease developed. On the other hand, there is a significant correlation between the degree of hypocalcaemia and the fraction of available surface undergoing resorption, although the scatter is very wide. If bone resorption is mediated by parathyroid hormone, these results are consistent with the concept of hypocalcaemia leading to increased parathyroid hormone secretion, which would, in turn, result in more active bone resorption at surfaces not covered by osteoid. There is clearly a great deal of variability in this process. With regard to the association between fraction of available surface undergoing resorption and the degree of metabolic acidosis, the significant relationship may simply reflect the correlation already noted between metabolic acidosis and hypocalcaemia, or alternatively, might be due to some other, more direct effect of metabolic acidosis on the resorbing process.

The mineralised bone mass.

In chronic renal failure the bone resorbing process that is known to occur does not usually lead to a generalised osteoporosis, but cystic lesions or areas of sub-periosteal erosion may be seen radiologically. In a proportion of cases however certain bones, especially those of the vertebral column, actually look sclerotic. This can be due to large increases in non-mineralised osteoid, as Garner and Ball (1966) pointed out, or even to an increase in mineralised trabecular bone. The amount of mineralised tissue per unit volume of bone has therefore been estimated in the present series.

Results.

Of the twenty-one biopsies that were examined, the proportion of

bone (including mineralised tissue, osteoid tissue and marrow space) that was occupied by mineralised material was measured. In only two cases did the mineralised fraction exceed the normal upper limit of 30% but in eight others it was close to this value (> 26%).

Comment.

The osteosclerosis of chronic renal failure has been discussed by Stanbury (1967) who states that the amount of mineralised cancellous bone is not usually increased, but when the frequently thick osteoid coating is taken into account, the radiological density is readily explained. The biopsies in the present series suggest that the trabecular bone is well mineralised as a rule, regardless of the osteoid covering, and occasionally the amount of mineralised tissue is substantially increased. In conjunction with the observations of Pellegrino and Biltz (1965) who noted increasing cortical porosity with time in chronic renal failure, these results imply that a shift of mineral from cortical to trabecular bone takes place in this situation.

Cortical thickness of the iliac crest.

The structural strength of bone is in most situations dependent upon the cortical thickness and it therefore seemed necessary to examine this in the iliac crest specimens. This was possible in ten of the renal failure patients in whom the cortex was intact. For comparison, the cortical thickness was also measured in sixteen normal adults, aged 20 to 50 years, and in four patients with primary hyperparathyroidism.

Results.

The cortical thickness varied from 0.02 to 2.13 mm in the controls, from 0.52 to 1.31 mm in the patients with primary hyperparathyroidism, and from 0.16 to 1.39 mm in the renal failure cases. There was no

correlation with either the percentage bony tissue in the trabecular zone or the fractions of available surface undergoing resorption. Comment.

This apparent failure of the cortex of the iliac crest to serve as a useful parameter of the structural status of the skeleton may be due to a number of reasons. Firstly, it is possible that in some cases oblique sections were being obtained, giving false high readings. However, because of the method of obtaining the biopsy and embedding it, which automatically resulted in sections being taken at right angles to cortex, this seems unlikely. A second possible explanation is that the exact site for obtaining strictly comparable specimens from the iliac crest is critical, for when two biopsies were taken simultaneously about 1 cm apart, there often appeared on naked eye examination to be somewhat thicker cortical bone in the posterior sample. Finally, it is conceivable that the cortex of the iliac crest, never being subjected to large compressive forces, shows natural greater variability in its development than the cortices of cylindrical bones.

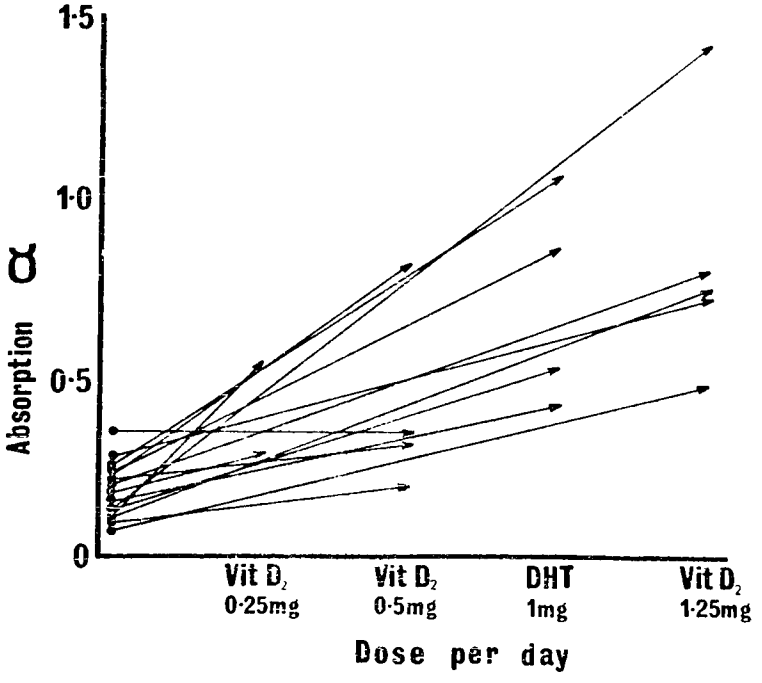

Fig. 42. The effect on calcium absorption of oral treatment with Vitamin D for six weeks in patients with chronic renal failure.

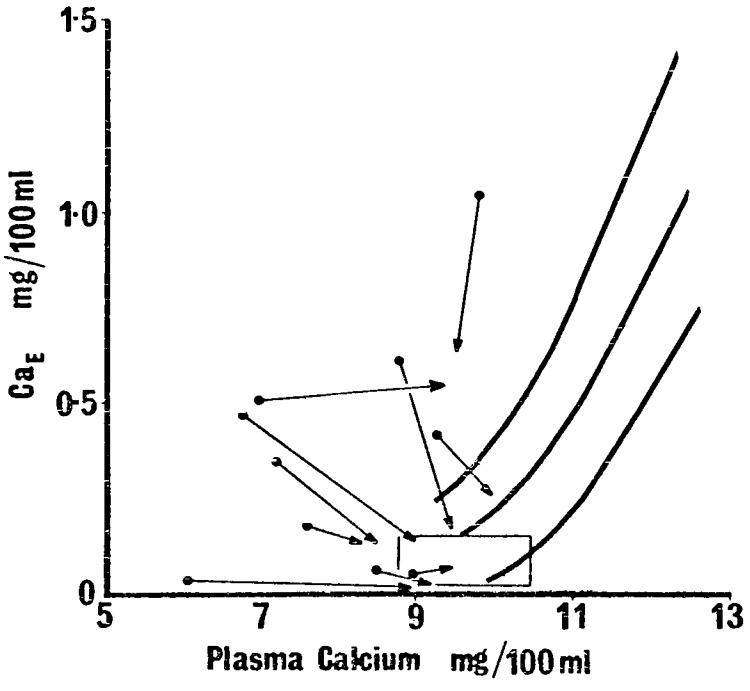

Fig. 43. The effect of Vitamin D on the relation between plasma and urine calcium in patients with chronic renal failure.

SECTION VII

The Calcaemic Action of Vitamin D

The apparent resistance to Vitamin D in chronic renal failure is not fully understood but must be due in part to the disturbance of the intermediate metabolism of the vitamin (Mawer et al. 1973). The hypocalcaemia which is a feature of renal osteomalacia can be corrected if sufficiently large doses of Vitamin D are given, and this effect is usually ascribed to increased intestinal absorption and bone resorption in response to high concentrations of partially hydroxylated derivatives like 25-hydroxycholecalciferol (Brumbaugh & Haussler, 1974; Reynolds et al. 1974)

In this study absorption has been assessed by the radio-calcium test and some indication of the state of bone resorption can be inferred from the fasting urine calcium excretion, whose source is probably the skeleton.

Results.

The effect on the radio-calcium absorption test of oral Vitamin D given daily in varying amounts for six weeks is shown in figure 42. There is a clear upward trend in absorption with increasing dosage, though the effect of moderately small doses was not examined.

The effect of Vitamin D on fasting plasma and urine calcium in 10 patients is shown (figure 43) and though in all but one the plasma calcium rose, the urine calcium remained unchanged or even declined. This means that tubular reabsorption has increased, but not necessarily that the renal threshold for calcium has changed.

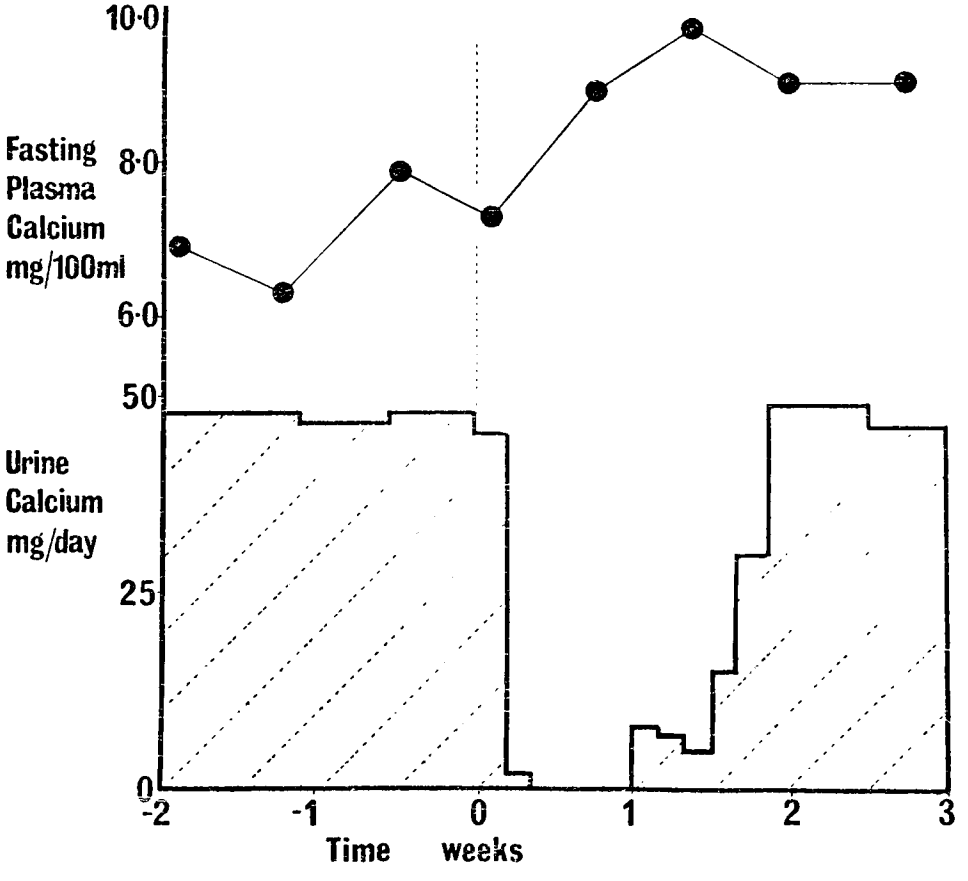

Fig. 44. The effect on plasma and urine calcium of Vitamin D 50,000 I.U. daily, given at time 0 to a patient with renal osteomalacia.

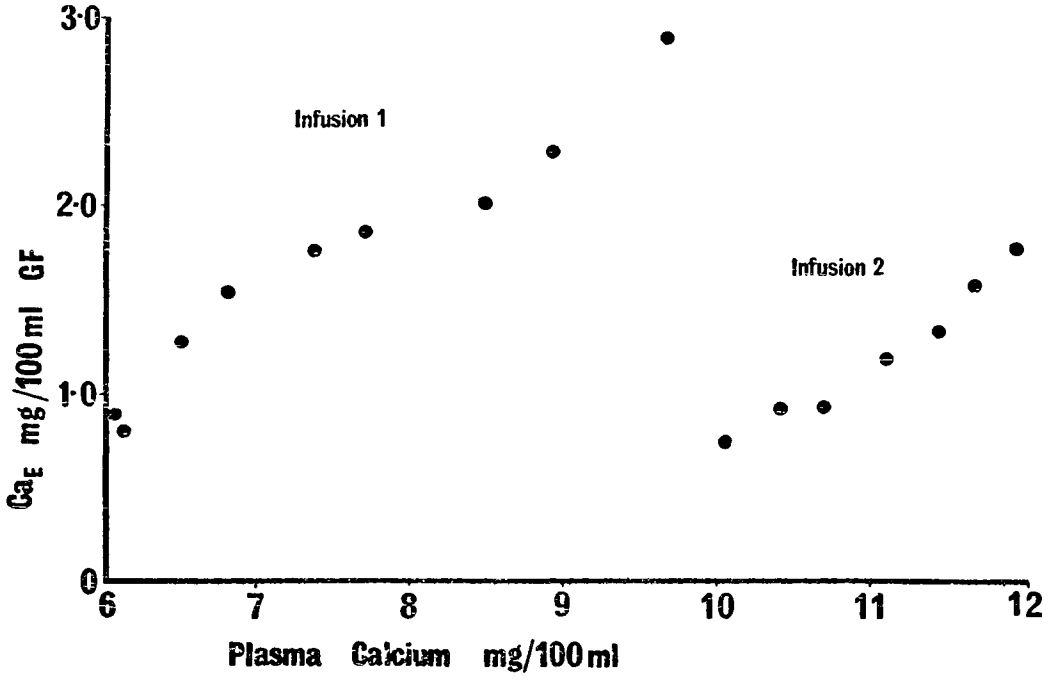

Fig. 45. The effect of oral Vitamin D for six weeks on the relation between plasma and urine calcium established by calcium infusions in a patient with renal osteomalacia. The GFR was 8 ml/min at which level the Ca_E 3.0 mg/100 ml GF corresponds to an absolute urine calcium excretion of 350 mg/24 h.

In a number of patients with renal osteomalacia, the effect of treatment with pharmacological amounts of Vitamin D, the first dose being given by intra-muscular injection, was studied by examining plasma and urine calcium at intervals during therapy. The results for one such patient (Appendix: Case IV) are shown (figure 44). The complete disappearance from the urine of measurable amounts of calcium while the plasma level rose indicates a major change in renal tubular handling of calcium. The effect however was not maintained, for during the second week of therapy the urine calcium returned to its original value though a higher plasma calcium concentration was sustained. The onset of these effects was never observed within thirty hours of the initial dose of Vitamin D in careful observations on six patients.

In order to see whether a change in renal threshold occurred to account for the correction of hypocalcaemia with Vitamin D, in some patients calcium infusions were carried out before and after therapy. The results of one such study are shown (figure 45). It is clear that entry of calcium into the plasma from the infusion - though this might equally well have been from the gut or bone - resulted in an immediate elevation of both plasma and urine calcium. In order to sustain a normal plasma calcium level without Vitamin D treatment, in the steady state, calcium would have had to enter the plasma and leave in the urine at a rate equivalent to 350 mg/24 hours (Infusion 1). Correction of the hypocalcaemia with Vitamin D was not achieved in this way at all, but took place with very little change in urine calcium excretion by a marked shift in renal threshold (Infusion 2).

Comment.

These observations indicate clearly that Vitamin D improves or corrects the reduced tubular reabsorption of calcium in renal hypocalcaemia, and this largely or wholly accounts for the calcaemic action of the vitamin in this condition. The transient total disappearance of calcium from the urine that occurred initially with therapy probably does not represent entirely a true trans-tubular reabsorption but may be due in part to uptake of calcium by intracellular organelles prior to the enhancement of trans-cellular movement (Borle, 1970).

Vitamin D also corrects the malabsorption of calcium but while this may make some contribution to plasma calcium homeostasis, it cannot be decisive factor since plasma calcium is raised without a corresponding elevation of urine calcium, and tubular reabsorption must therefore be the dominant factor. The results of the infusion study also emphasise the fundamental nature of the change in renal handling of calcium in response to Vitamin D.

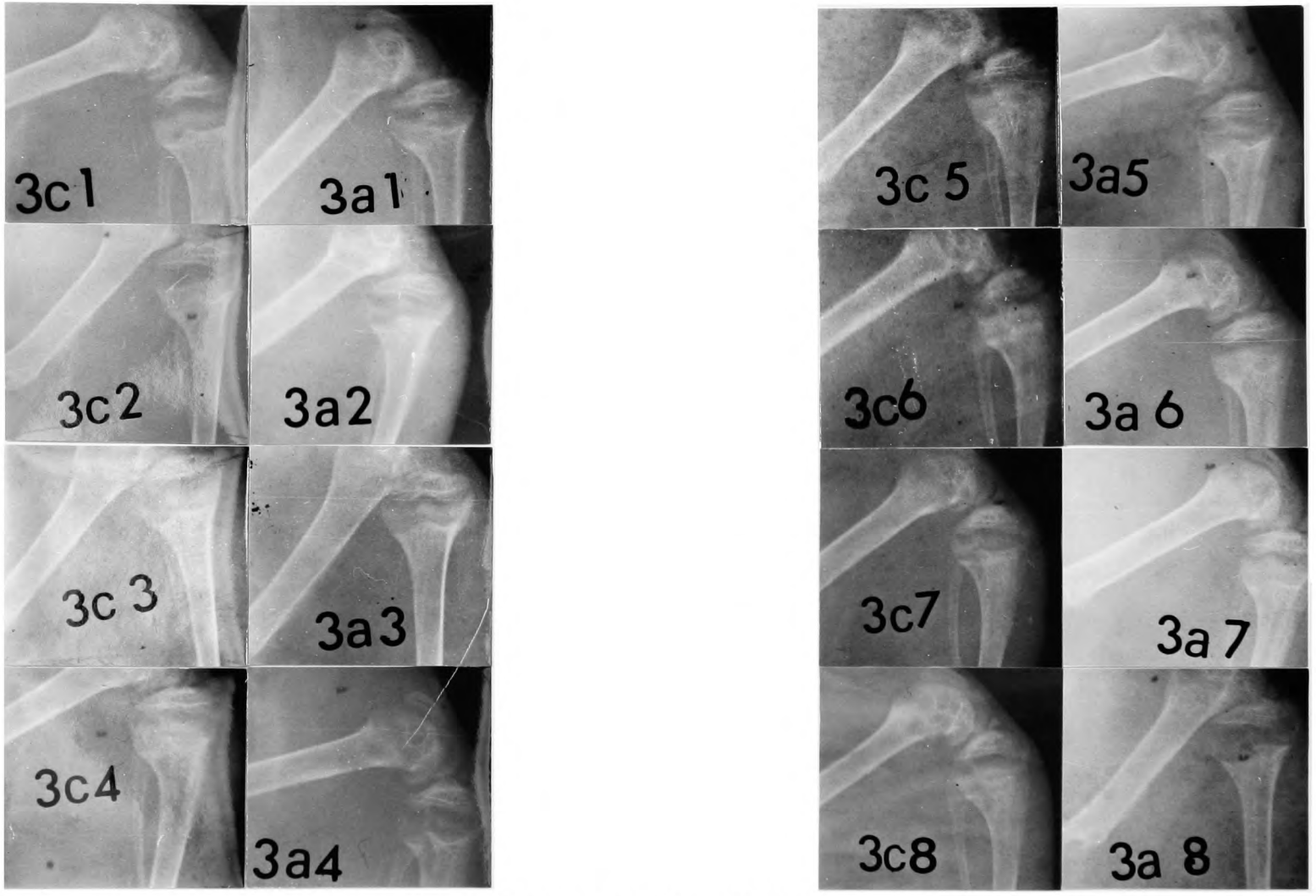

Fig. 46. The knee joints of non-acidotic (group C) and acidotic (group a) rats given 0.05 µg 25-hydroxycholecalciferol 10 days previously.

SECTION VIII

Acidosis and Experimental Rickets

The influence of acidosis on the healing of rickets induced experimentally in rats was tested in the following way. Male Wistar rats were raised by mothers fed a low Vitamin D diet. After weaning at twenty days, the animals were kept for a further four weeks on a modified Steenbock diet (Ca/P, 7:1) by which time rickets was well established. Twenty animals of similar weight (55-75 G) were then selected and divided randomly into two groups. Group C continued with the diet and distilled water while Group A received the diet but drank 0.15 M NH_4Cl. After one week, blood was taken from each animal by cardiac puncture under ether anaesthetic and plasma calcium, phosphorus, alkaline phosphatase and pH were measured. The hind limbs were then x-rayed. Following this, each animal received by subcutaneous injection 50 ng (125 picomole) of 25-hydroxycholecalciferol and after four and ten days further blood samples and x-rays were taken.

The x-rays were presented twice in random order to an independent experienced worker (Dr E. Kodicek) who assessed the severity of the rickets using the Bourdillon scale.

Results.

The results are shown in Table 16 and figure 46. After one week on NH_4Cl the Group C animals had a mean blood pH 7.35, and Group A animals had a mean blood pH 7.21, these differences being highly significant ($p < 0.005$). The Group C mean plasma calcium and phosphorus levels were

10.4 mg/100 ml and 6.9 mg/100 ml respectively, and in Group A the respective values were 10.1 mg/100 ml and 6.5 mg/100 ml. The differences between the two groups are not significant. The plasma alkaline phosphatase level was greater than 60 KA units/100 ml in all cases.

The mean values on the Bourdillon scale were 1.18 for Group C and 0.50 for Group A, and these differences are not significant.

Four days after the 25-hydroxycholecalciferol there were already signs of healing, but the effect was more marked at ten days. At this time, the mean plasma calcium and phosphorus levels in Group C were 10.8 mg/100 ml and 10.5 mg/100 ml respectively and in Group A were 10.5 mg/100 ml and 10.6 mg/100 ml respectively, the groups not being significantly different with regard to these parameters. However, when the x-ray values were obtained, healing of the rickets was significantly delayed in the acidotic animals (figure 46), the mean Bourdillon scores being 4.9 in Group C and 2.6 in Group A ($p << 0.01$; Table 16).

Comment

The Vitamin D metabolite 25-hydroxycholecalciferol has been shown to have potent anti-rachitic activity in the rat (Omdahl et al. 197) and the present study confirms this. The animals were severely rachitic when the material was administered and both groups showed evidence of healing four days after a single small dose. However, partial inhibition of the healing process occurred in the acidotic animals, indicating that acidosis can interfere with normal bone formation and predispose to the rachitic state. This experimental model strongly suggests that the relationships already noted between acidosis and osteomalacia are cause and effect rather than simply independent consequences of renal disease.

CHAPTER 6

Discussion and General Summary

Osteomalacia and Osteoclasis

The contribution of metabolic acidosis to bone disease in chronic renal failure remains controversial. It is clear that the vast majority of patients with overt bone disease are severely acidotic, and the various explanations for the association have inevitably helped to entrench, falsely or otherwise, the idea of a causal relationship. It is however the experience of many nephrologists that marked acidosis can be tolerated by some patients without the development of clinical bone disease. Furthermore, certain of the arguments used to explain the part played by acidosis in bone disease when it does occur can be dismissed as only qualitatively consistent with the clinical and biochemical evidence.

An additional difficulty arises from the fact that renal bone disease consists of two quite distinct components, osteomalacia and osteoclastic resorption. Neither process is fully understood, but in the former there is a breakdown in the orderly production of bone tissue with a slow but persistent deposition of osteoid and, in most cases, an even slower rate of mineralisation. The bone resorption component is usually regarded as being entirely parathyroid hormone mediated. These two elements commonly coexist, sometimes one or other predominating. Clinical osteomalacia takes years to develop, not surprisingly, since it involves a reduced rate of bone formation which is a slow process under normal circumstances. Severe parathyroid induced bone resorption on the other hand seems able to take place rapidly by comparison, sometimes appearing over months, and it may therefore exist either with little sign of osteomalacia or superimposed

on osteomalacia of severe degree.

In general, bone fractures, whether partial (Looser zones) or complete are a feature of osteomalacia rather than osteitis fibrosa. This is probably due to the fact that in osteomalacia, where bone formation is defective, any break, however small, cannot heal but continues to weaken the adjacent structure with eventual extension of the fracture. When bone resorption predominates, these incomplete fractures are not seen though the areas of erosion and cyst formation must weaken the bone. This strongly suggests that healing can take place, and this may be due to the action of parathyroid hormone which is able to promote bone formation in certain circumstances (Talmage, 1966; Parsons et al. 1973).

In osteomalacia, bone resorption is usually increased above normal levels, presumably as an indirect result of the hypocalcaemia, though the osteoid surface which coats the trabeculae is curiously resistant to resorption and protects the bone to some extent (Wolbach, 1947). It has also been suggested that parathyroid activity is only moderately increased in osteomalacia in spite of the prolonged hypocalcaemic stimulus (Stanbury, 1967; Ellis, 1973). The cause of this is not clear: it cannot be explained in terms of the plasma phosphate level which is usually lower in osteomalacia than in osteitis fibrosa (Stanbury & Lumb, 1966) since it has been shown that phosphate affects the parathyroids entirely by its action on ionised calcium (Reiss & Cantebury, 1971). It may be that osteomalacia cannot persist in the presence of severe secondary hyperparathyroidism.

Bone resorption.

In the present series the reduction in CA/TA ratio indicates an

endosteal resorption process, since it is not due to periosteal accretion (figure 13). In an independent study using identical radiological techniques Hossain et al. (1970) showed that in primary hyperparathyroidism, the CA/TA ratio tended to be low for the age of the patient. It has also been shown that in chronic renal failure the degree of resorbing activity measured histologically correlates with serum immuno-assayable parathyroid hormone levels (Ellis, 1973). The low CA/TA values in this renal failure series are almost certainly also due to parathyroid activity, which is in line with their strong association with the degree of hypocalcaemia.

Similarly, the histological examination of trabecular bone from iliac crest showed a highly significant correlation between the fraction of available surface undergoing resorption and the degree of hypocalcaemia. Measurement of iliac crest cortical thickness however gave so wide a range of results as to be of no value. This was also the experience of Meunier (1967).

The state of secondary hyperparathyroidism that is a consequence of the hypocalcaemia should to some extent be mirrored by a reduced tubular reabsorption of phosphate. This is found in virtually all cases but what is perhaps surprising is that there is no relation between TmP and the degree of hypocalcaemia. There is normally a reciprocal relationship between serum calcium level and the concentration of immuno-assayable parathyroid hormone (Berson & Yalow, 1966) and it might therefore be expected that the plasma calcium concentration would determine the TmP. That this is not the case may simply indicate that TmP is not an adequate indicator of parathyroid hormone levels or

that circulating parathyroid hormone concentration in chronic renal failure is maximal for the degree of parathyroid hyperplasia in each case and does not directly reflect the degree of hypocalcaemia. Moreover, although on the one hand hypocalcaemia increases the drive to the parathyroid glands, it also reflects the parathyroid response, and the relation between plasma calcium and circulating parathyroid hormone levels would therefore represent a mixture of calcium-driven parathyroid hormone concentration and parathyroid hormone-driven calcium concentration. For this and other reasons (Berson & Yalow, 1971), no attempt was made to measure plasma parathyroid hormone level in this study.

There is almost certainly a causal relationship between the reduction in metacarpal cortical thickness and the hypocalcaemia, but weaker relationships also exist between the CA/TA ratio and the plasma alkaline phosphatase and bicarbonate levels. With regard to the alkaline phosphatase, the quantitative bone histology clearly shows that the plasma concentration of this enzyme is a function of the bone forming surfaces, but not of resorbing sites. There cannot therefore be a direct connection between a resorbing process as measured by the CA/TA ratio and the alkaline phosphatase level, but an indirect one operating through the hypocalcaemia which is a feature of osteomalacia. It is likely that the association between the CA/TA and the degree of acidosis is similarly indirect and due to the relation of the latter to hypocalcaemia, since in the bone histology there is an inverse relation between the fraction of available surface undergoing resorption and plasma calcium but a weaker correlation was found between histological resorption and the plasma bicarbonate level. Using the technique of microradiography,

Barzel and Jowsey (1969) suggested that acidosis created conditions in which bone resorption was increased, though the mechanism by which the effect might be operating was not discussed. The hypocalcaemia is therefore the most important single determinant of bone resorption, and it is necessary to consider some of the factors that determine plasma calcium level.

Plasma calcium homeostasis.

The sources of plasma calcium are net absorbed calcium from the gut and net resorbed calcium from the bone. Calcium absorption is low in chronic renal failure and net absorption is generally about zero (Stanbury, 1968b) or may be negative (Table 7). There is a loose relation between absorption and plasma calcium (figure 26) but many of the low absorption cases were normocalcaemic. Furthermore, all plasma calcium estimations were made in the fasting state, so that in fact the plasma calcium is being maintained by bone resorption, and absorption cannot be the decisive factor in causing the hypocalcaemia when it occurs.

Net resorbed calcium is the difference between the rates of mineralisation and resorption. It may be argued that renal hypocalcaemia is the result of a failure to resorb bone at an adequate rate, and while this is true in a sense, it is an unsatisfactory explanation when the data on bone kinetics are considered. These data (figure 30) indicate that most patients with hypocalcaemia have high rates of bone resorption as determined by the combination of balance and isotope procedures and from the rate of urine hydroxyproline output. Moreover, since the patients all have a low GFR, the resorption rate relative to GFR is very high indeed. It is therefore impossible to argue that impaired

bone resorption is the cause of hypocalcaemia. Why then is the plasma calcium low? One reason is that although the resorption is high, so is bone mineralisation and the calcium resorbed from some sites is therefore being redeposited in others. Accordingly, net resorption is not as high as might be expected. This is indicated by the fasting Ca/Cr values which reflect net bone resorption and which are never raised (figure 14). Nonetheless, net resorption should be sufficient to maintain normocalcaemia since the Ca/Cr is frequently in the normal range and the fasting Ca_E, which is in fact net bone resorption relative to GFR, is in fact highest in the patients with hypocalcaemia.

The other regulator of plasma calcium is the renal tubular reabsorption of calcium (Peacock & Nordin, 1969). When calcium is infused into normal individuals or patients with renal failure there is a positive correlation between plasma and urine calcium (figure 45), but it is a striking feature of this series of 50 patients in varying degrees of renal failure that there is a highly significant inverse correlation between fasting plasma and urine calcium when the latter is related to GFR (figure 16). The patients with hypocalcaemia tended to have a high rate of calcium excretion relative to GFR, and the normocalcaemic individuals generally excreted calcium at a normal rate relative to GFR. If there is a causal relation between these two phenomena, it can only mean that the hypocalcaemia is the result of a reduced tubular reabsorption of calcium since it is impossible to conceive of any mechanism whereby hypocalcaemia could itself raise urine calcium. The fact that the absolute rate of calcium excretion is low in renal failure is of no relevance in this context. The absolute rate must govern the time taken to achieve the steady state, but it cannot determine the steady

state itself. Plasma calcium is a function of the filtered load relative to GFR, and the Ca_E is calcium excreted relative to GFR. Using this mode of expression means that filtered load and excreted calcium are considered in the same terms and clearly illustrates the reduced tubular reabsorption of calcium that characterises the hypocalcaemic cases. A change in ultra-filtrable calcium by acidosis or decreased plasma proteins would have no lasting effect on the Ca_E and could not explain these data. There is therefore a tubular leak of calcium in these hypocalcaemic patients and this is the most important reason for the hypocalcaemia.

It is known that metabolic acidosis can reduce the tubular reabsorption of calcium (Lemann et al. 1967) and the present data are consistent with this: correction of the acidosis in the patients studied by metabolic balances led to a fall in urine calcium in the fact of increased absorption; the patients with hypocalcaemia were more acidotic than those with normocalcaemia, and the plasma bicarbonate is inversely related to calcium excretion relative to GFR. To this extent, acidosis would seem to be contributing to the hypocalcaemia. However, it was not found possible to alter significantly the plasma calcium by changing the acid-base status of the balance patients, making careful observations over a two week period. This anomaly is accounted for by the effect of correction of the acidosis on the bone mineralisation rates, which rose significantly to absorb all the extra calcium that was retained.

The relationships between acidosis and plasma or urine calcium are more definite in the patients with raised alkaline phosphatase levels than in those without. This may simply mean that there is a group

with metabolic acidosis who have not yet developed its complications, but this would be to presuppose that these complications take time to develop, which is unlikely to be true, for example, of the effect on the renal tubule. Such an effect might take days or weeks to appear but these patients had been in stable chronic renal failure for many months. It seems more likely that as the osteomalacic state develops, indicated by a rise in plasma alkaline phosphatase levels and reflecting an insufficiency of Vitamin D activity, the patient is more vulnerable to the effects of acidosis which in turn tend to aggravate the osteomalacic syndrome. Conversely, while Vitamin D metabolism remains adequate, the patient is generally protected against the effect of acidosis. A deficiency of active Vitamin D metabolites may be more critical if parathyroid gland sensitivity is reduced in this situation as Oldham et al. (1973) have suggested.

There is no doubt of course that Vitamin D alone, if given in sufficiently large doses, will overcome the hypocalcaemia and the data in this study offer some explanation of the mechanism by which this takes place. Vitamin D obviously corrects the malabsorption of calcium but although this may make some contribution to calcium homeostasis improved absorption cannot be the most important factor since plasma calcium rises without simultaneous elevation of urine calcium, which may actually fall. This indicates an increased tubular reabsorption. Furthermore, since the urine calcium excretion is unchanged in the fasting state, increased net bone resorption cannot account for the correction of the hypocalcaemia. The results of the calcium infusions show that in the hypocalcaemic state there is an abnormally low renal threshold for calcium and this is corrected after

administration of suitable doses of Vitamin D. Were it not for this change in threshold, the total rate of entry of calcium into the plasma in order to sustain a normal serum level might have to be extremely high, and this would be reflected in a similarly high urine calcium excretion.

Alkaline phosphatase.

Osteomalacia, as evidenced by increased osteoid coating of trabeculae and an abnormally low proportion of calcification fronts, is strongly associated with raised plasma alkaline phosphatase concentrations. The high isotopic bone mineralisation rates in these cases are due to the large number of mineralising sites in the skeleton as a whole, though activity at each individual site is decreased (Frost, 1967). Garner and Ball (1966) have shown that while the bone in renal osteomalacia is undermineralised, the total amount of bony tissue is frequently so great that the absolute quantity of mineralised trabecular bone may sometimes be at the upper limit of or greater than normal. This is true of many of the biopsies in the present series (Table 14). Since the patients are seldom in positive overall balance, this gain in mineral by the trabeculae must take place at the expense of compact bone. Furthermore, the trabecular bone of the spine and iliac crest is resistant to resorption because of its osteoid cover, which in turn reflects its high intrinsic rate of turnover. The apparently dense radiological appearance of the spine in many of the osteomalacic cases may be explained on these grounds. Cortical bone, which turns over more slowly (Fletcher et al. 1965) developing less osteoid, is more available for resorption and is mobilised in response to hypocalcaemia. Thus the overall process is a transfer of mineral from cortical to trabecular

bone at a continuing low plasma calcium concentration determined by renal tubular function.

What are the factors that influence the development of osteomalacia? It has been commonly thought that a low calcium-phosphate product is at least in part responsible and this concept continues to find support in one form or another. The emphasis on the bone resorbing activity of the metabolites of Vitamin D and the failure to find a direct anabolic effect (Raisz et al. 1972; Reynolds, 1973) has again led to the suggestion that mineralisation is essentially a passive process. Thus Anderson and Reynolds (1973) state that microcrystals extruded from the osteoblast act as nucleating sites for appatite provided the ECF is saturated with respect to the salt, and it is believed that Vitamin D simply ensures that the calcium and phosphorus are made available at the bone forming surface. The general concept is supported by the work of Baylink (Stauffer et al. 1973) who showed that histological osteomalacia could result from hypocalcaemia alone, and while Vitamin D increased the osteoclastic population there was no detectable effect on the degree of osteomalacia. However, any theory which depends in the final analysis upon the ionic product of the bathing fluid determining calcification is difficult to accept in the light of clinical experience (Stanbury, 1967) even when the prevailing pH is taken into account.

An alternative hypothesis maintains that all material in contact with the bone has to pass across a cell membrane (Baud, 1968; Canas et al. 1969) in which case a moderate fall in the concentration of calcium in the ECF would be unlikely to have any significant effect on the rate of transport across the cell. This is because the

intracellular calcium concentration is kept extremely low, in the region of 10^{-4} mM (Talmage, 1969) so that the calcium gradient into the cell is always very large. Using electron microscopy, however, Reynolds doubts the universal existance of this cell envelope to bone (Reynolds, 1974).

A third concept, based upon sound experimental data and entirely consistent with the observations of many workers, goes further toward explaining the process of bone mineralisation than any of the earlier suggestions. According to this theory, intracellular vesicles appear in the neighbourhood of the Golgi apparatus of the bone forming cell, (Aaron & Pautard, 1973a,b; Aaron, 1973) and begin to accumulate amorphous calcium phosphate. These vesicles are subsequently extruded from the cell into the surrounding osteoid (Anderson, 1969; Bonucci, 1971) and may continue to take up mineral from the bathing fluid (Ali et al. 1970). There is wide agreement on these views but Aaron and Pautard believe that the bone forming cell undergoes a cycle of activity in which it loads up with mineral, packages it in the vesicles and these are then jettisoned into the surrounding matrix (Aaron & Pautard, 1973a). These authors believe that mineralisation takes place as more and more vesicles are extruded into the loosely arranged osteoid. There is also evidence that these young osteocytes can accumulate and distribute calcium without phosphorus, and possibly phosphorus without calcium, into the adjacent matrix. The concepts of Pautard and Aaron as based upon the use of high resolution light microscopy and time-lapse cinephotography in living systems and probably avoid a number of artefacts introduced by conventional fixation techniques, and moreover require no assumptions about the effects of the mineral concentration of the bathing fluid.

At what stage, if any, is Vitamin D involved in these processes? It seems reasonable to suppose that Vitamin D affects calcium transport into bone in a way analagous to the one by which it regulates transport at other sites and yet experimental evidence has tended to suggest that it is without a direct action on calcification of osteoid (Stauffer et al. 1973; Reynolds, 1973). Nonetheless de Luca (Omdahl et al. 1971) has stated that 25-hydroxycholecalciferol may have more anti-rachitic activity than the 1,25-dihydroxy metabolite in spite of the latter's greater calcaemic action, implying that there may be an unidentified metabolite with a trophic effect on bone. Moreover, Anderson (1974) reported that matrix vesicles appeared to contain relatively little mineral in the rachitic state, though it is impossible to tell whether this was due directly to the absence of the vitamin or to the associated hypophosphataemia. Experience with bone histology (Frost, 1963; Rasmussen & Bordier, 1973) has led to the suggestion that bone resorption at any particular site precedes, and is followed by, formation , and Baylink(1970) has shown that Vitamin D induces differentiation of resting cells into osteoclasts, so that is possible that the whole process of bone turnover is under the control of this hormone.

None of these views involve the acid-base status of the tissue fluid, and yet the data in the present work imply that acidosis can contribute to the osteomalacia. A highly significant relation exists between the fraction of surface covered by osteoid and the degree of acidosis, and histological osteomalacia is rarely seen in patients protected against acidosis. An important feature of the treatment of acidosis in cases of renal osteomalacia was the increase in isotopic bone mineralisation rate, suggesting that acidosis can slow the process

of calcification. In this procedure, administration of bicarbonate resulted in a rise in blood pH and, to a variable extent, the pCO_2. Barzel (1971), in an *in vitro* experiment, cultured rat bone explants and varied the pH of the medium by altering the pCO_2. It was noted that a mild degree of acidosis (pH 7.2) was sufficient to reduce bone accretion in this system. Though the experiment should obviously be repeated with the pH varied at a constant pCO_2, it is broadly in line with the observations in the present study. In acidotic rachitic rats given a small single dose of 25-hydroxycholecalciferol there was significantly reduced healing compared with non-acidotic controls treated the same way. By what mechanism could acidosis be affecting bone formation? A direct effect on Vitamin D metabolism seems unlikely (see p. 84) and Norman *et al.* (1973) have investigated the hydroxylation step leading to synthesis of 1,25-dihydroxycholecalciferol. This takes place at an optimum pH 7.4, but the reaction is only slightly impaired by degrees of acidosis which would be regarded as clinically severe, and the contribution of this effect cannot be considered important in the present context.

It is highly relevant however that matrix vesicles have been isolated and shown to accumulate calcium *in vitro* by an energy dependent process, and this effect is critically sensitive to pH in the physiological range with marked uptake of calcium at pH 7.4, but almost no activity at pH 7.2 (Ali & Evans, 1973). These values correspond closely with those of the metabolic balance patients before and after correction of their acidosis. The relation between calcium uptake by the vesicles and pH is strikingly similar to the variation of alkaline phosphatase

activity with pH, and the vesicles contain this enzyme in large quantities (Ali et al. 1970). If alkaline phosphatase is important in bone mineralisation, it might be expected that this would be enhanced by physiologically slightly alkaline conditions, and in the experiment of Barzel (1971) this is what was found. Holdsworth et al. (1970) and Russell et al. (1972) made the interesting suggestion that the calcium ATPase in gut might be an alkaline phosphatase. This species of enzyme is difficult to work with due to its heterogeneity, its instability when isolated, and its lack of antigenicity (Reynolds, 1974). Furthermore, its catalytic properties require it to be in a dimeric form (Chappelet-Tordo et al. 1974). Nonetheless, in a clear and detailed series of experiments Birge and Gilbert (1974) succeeded in showing that certain of the intestinal alkaline phosphatases are highly specific calcium ATPases. Such enzymes are likely to constitute a pH-sensitive step in energy-dependent calcium translocation in the gut, and if this is the case at other sites of calcium transport it would provide a simple explanation for the association between metabolic acidosis and the inhibition of bone mineralisation which characterises rickets and osteomalacia.

TABLES

Figure 47. X-rays on Case 1.

APPENDIX

Case I

A.S. Male 1½ years

Patient investigated at 1 year because of failure to thrive, thirst and polyuria. Food was rejected and only milk taken. Examination showed a pale, hypotonic child with mental retardation. Radiological examination showed dense cortical bone with irregular diaphyses (figure 47) and periosteal calcification. Investigations were:

Plasma urea	161 mg/100 ml
Plasma calcium	11.2 mg/100 ml
Plasma phosphate	6.2 mg/100 ml
Plasma Alkaline phosphatase	11 KA units/100 ml

Blood pH 7.31

Parathyroid hormone level - not detectable.

Urine phosphoethanolamine - not detectable.

Renal biopsy: microscopic interstitial calcification.

Progress: the patient was given a low calcium diet and the hypercalcaemia cleared and the urea fell to 51 mg/100 ml. There was no benefit from vitamin supplements until ergocalciferol 1,000 i.u./day was introduced.

Case II

L.C. Male 43 years.

Patient gave a 12 year history of fracture with mild trauma,

episodes of spontaneous pain in the back and loss of about 12 cm height. Over a 3 year period, severe hip-muscle weakness had developed. No family history of bone disease. Examination showed evidence of thoracic and lumbar vertebral body collapse. There was a proximal myopathy (hips and shoulders). X-rays showed multiple vertebral body fractures and Looser zones in the femora and pubic rami. Investigations were:

Normal haematological picture, urea, electrolytes and plasma proteins.

Plasma calcium	9.9 mg/100 ml
Plasma phosphate	0.9 mg/100 ml
Plasma Alkaline phosphatase	20 K.A. units/100 ml
Urine calcium	176 mg/24 h
Urine phosphorus	722 mg/24 h
Urine hydroxyproline	68 mg/24 h

TmP 1.2 mg/100 ml GF

There was poor calcium absorption and negative calcium balance, but phosphate absorption was normal despite the low renal threshold. Iliac crest biopsy showed thick irregular undermineralised bone covered with wide osteoid borders. Fraction of bony tissue: 36%. Fraction of surface covered by osteoid: 96%. No calcification fronts and no tetracycline bands.

Progress: the patient was given an 7 day phosphate infusion and oral supplements for 5 weeks during which time the plasma phosphate ranged from 1.6 to 3.2 mg/100 ml. Following this, iliac crest biopsy showed areas of patchy mineralisation within the osteoid but essentially

little change. Calcifications fronts were absent and tetracycline labelling was patchy or diffuse. X-rays suggested some mineralisation around Looser zones but this was not incontrovertible.

Case III

R.D. Male 3 years.

Patient investigated at 2 years because of thirst and polyuria and found to have a congenital absence of the left kidney and an aperistaltic segment in the right ureter with proximal obstruction. Examination showed an active child without muscle weakness. Rickets was present (skull, costo-chondral junction, wrists) as confirmed by x-ray.

Investigations shows:

Plasma urea	125 mg/100 ml
Plasma bicarbonate	13 mg/100 ml
Plasma calcium	9.3 mg/100 ml
Plasma phosphate	6.9 mg/100 ml
Alkaline phosphatase	22 K.A. units/100 ml

Progress: the ureteric obstruction was corrected and the plasma urea and phosphate decreased slightly (105 mg/100 ml and 5.0 mg/100 ml respectively). Plasma calcium and electrolytes were unchanged, hypocalcaemia never being seen. Though the patient was acidotic, the urine could reach pH 5.4. However, alkali supplements (50 mEq/day) failed to correct completely the acidosis. A bicarbonate infusion showed the presence of a reduced bicarbonate threshold suggesting a predominently proximal tubular defect.

The rickets was successfully treated with ergocalciferol 10,000 i.u./day and alkali 40 mEq/day.

Case IV

HBa Female 61 years.

Patient investigated at 52 years for recurrent attacks of acute pyelonephritis, with right nephrectomy carried out at 53 years. At 60 years patient developed severe hip-girdle weakness, the only abnormality noted clinically. X-rays showed osteoporosis but not osteomalacia.

Investigations were:

Plasma urea	90 mg/100 ml
Plasma bicarbonate	17 mEq/L
Plasma calcium	7.0 mg/100 ml
Plasma phosphate	3.6 mg/100 ml
Plasma alkaline phosphatase	45 K.A. units/100 ml
Blood pH	7.25
M.S.U.	Persistent pyuria. Sterile.
Urine calcium	45 mg/24 h
Lowest urine pH	5.6
Urine amino-acids	- normal

Progress: a balance study showed zero calcium balance, and no evidence of malabsorption syndrome. The acidosis was corrected and slight improvement of the balance occurred entirely due to absorption. When ergocalciferol 50,000 i.u./day was given in addition, absorption increased further, and urine calcium fell. The plasma calcium rose

to normal levels, and plasma phosphorus rose slightly. A bicarbonate infusion showed the presence of a slightly reduced bicarbonate threshold.

APPENDIX

Specimen number	Number of estimations	Mean Calcium mg/100 ml	S.E.M.	Mean phosphorus mg/100 ml	S.E.M.	Mean creatinine mg/100 ml	S.E.M.
Urine 1	10	17.87	0.21	58.66	0.50	100.22	1.57
2	10	14.15	0.18	47.20	0.32	70.40	0.85
3	10	26.66	0.33	49.72	0.40	80.90	1.05
4	10	11.22	0.14	50.60	0.56	64.90	0.82
5	10	7.70	0.08	16.90	0.23	45.80	0.53
6	10	4.90	0.03	50.11	0.53	89.90	1.23
7	10	4.40	0.03	64.40	0.56	43.40	0.52
Plasma 1	2	7.60	0.00	2.45	0.05	2.05	0.05
2	2	8.25	0.05	2.70	0.00	2.05	0.05
3	2	10.20	0.10	6.45	0.05	13.25	0.05

TABLE 1. Reproducibility of Auto-Analyser methods.

Samples were introduced at random during routine work

Clinical						Plasma								Urine					
Patient	Age	Sex	Presentation	Renal lesion	Bone lesion	Urea mg/100 ml	Bicarbonate mEq/L	Chloride mEq/L	pH	Calcium mg/100 ml	Phosphorus mg/100 ml	Ca x P	Alkaline phosphatase KA units	Creatinine clearance ml/min	Lowest urine pH	Calcium mg/24 hr.	Ca_E mg/100 ml G.F.	P_E mg/100 ml G.F.	TmP mg/100 ml G.F.
RD	4	M	Rickets	Megaureter	Rickets	150	19	115	7.24	9.8	5.1	50	40	9	5.4	126	0.15	2.18	2.9
HBr	8	M	Fracture Myopathy	Ureterostomy	Rickets Tibial fracture	98	17	109	7.29	8.8	2.8	25	20	13	6.5	50	0.20	1.90	0.9
PM	18	M	Myopathy	Ileal bladder	Looser zones	198	14	110	7.27	5.9	5.9	35	38	8	6.5	57	0.69	4.67	1.2
MS	54	M	Renal failure	Colonic bladder	Normal	200	13	105	7.29	7.3	5.8	43	24	5	7.7	52	0.45	4.23	1.6
HC	61	M	Myopathy	Chronic G.N.	Looser zones	147	17	107	7.27	7.6	3.9	31	67	8	5.1	9	0.50	2.84	1.1
LSy	16	F	Renal failure	Ileal bladder	Looser zones	184	16	112	7.29	7.7	4.9	38	53	8	6.8	27	0.25	3.70	1.2
GP	16	F	Myopathy	Renal Hypoplasia	Looser zones rickets	327	16	105	7.30	6.7	6.9	46	27	4	6.8	45	1.21	4.80	2.1
WB	52	F	Renal failure	Pyelonephritis	Looser zones	120	14	120	7.32	8.9	4.0	36	40	9	6.7	28	0.25	2.60	1.4

TABLE 2. Data of patients with renal osteomalacia

Clinical						Plasma								Urine					
Patient	Age	Sex	Presentation	Renal lesion	Bone lesion	Urea mg/100 ml	Bicarbonate mEq/L	Chloride mEq/L	pH	Calcium mg/100 ml	Phosphorus mg/100 ml	Ca x P	Alkaline phosphatase KA units	Creatinine clearance ml/min	Lowest urine pH	Calcium mg/24 hr.	Ca_E mg/100 ml G.F.	P_E mg/100 ml G.F.	TmP mg/100 ml G.F.
LS	60	F	Fracture	Pyelonephritis	Femoral fracture	140	17	110	7.30	8.2	3.4	28	15	10	6.9	20	0.21	3.10	0.3
HBa	61	F	Myopathy	Pyelonephritis	Looser zones	90	16	113	7.27	7.0	3.6	27	85	11	5.9	14	0.18	1.70	1.9
IP	65	F	Myopathy	Colonic bladder	Looser zones	72	17	126	7.29	7.5	4.1	31	49	19	6.9	23	0.96	1.95	2.2

TABLE 2 continued. Data of patients with renal osteomalacia

Patient				Plasma					Urine						
case	age, years	sex	diagnosis	Length of history, years	calcium, mg/100 ml	phosphorus, mg/100 ml	creatinine, mg/100 ml	bicarbonate, mM/l	Alkaline phosphatase, KA units/100 ml	Ca/Cr	Ca_E, mg/100 ml GF	P/Cr	P_E, mg/100 ml GF	Bone X-ray, CA/TA	Absorption test, fraction of dose/h	TmP, mg/100 ml GF
R.D.	4	M	hydro-nephrosis	4	9.8	5.1	2.4	19	23	0.063	0.15	0.917	2.2	–	–	2.9
H.Br.	8	M	hydro-nephrosis	8	8.8	2.8	1.7	18	20	0.118	0.20	0.112	1.9	–	–	0.9
J.R.	21	M	Primary hyper-oxaluria	11	9.0	6.0	13.3	21	7	0.049	0.65	0.278	3.7	0.79	0.24	2.3
P.H.	25	M	hereditary nephritis	1	9.3	7.3	9.1	20	6	0.008	0.07	0.374	3.4	0.56	–	3.9
B.C.	26	M	chronic GN	4	9.4	6.0	8.4	27	8	0.036	0.30	0.405	3.4	0.80	0.27	2.6
J.Ba.	29	M	? chronic GN	2	10.6	6.3	8.4	16	6	0.057	0.48	0.440	3.7	0.78	0.25	2.5
J.G.	37	M	polycystic	3	10.0	2.5	2.5	25	7	0.012	0.03	0.400	1.0	0.69	0.29	1.5
P.H.	42	M	chronic GN	–	9.0	8.2	13.0	25	8	0.020	0.26	0.400	5.2	0.67	0.43	3.0
J.P.	44	M	chronic GN	10	9.2	6.8	18.4	21	11	0.032	0.58	0.332	6.1	0.58	0.24	0.7
F.C.	46	M	polycystic	10	10.3	4.5	5.7	21	5	0.054	0.31	0.474	2.7	0.71	0.35	1.8
E.A.	50	M	chronic GN	1	8.8	10.9	18.5	19	11	0.048	0.89	0.411	7.6	0.60	0.12	3.3
J.Bo.	51		polycystic	3	9.4	4.3	6.0	21	5	0.027	0.16	0.417	2.5	0.74	0.78	1.8
A.V.	53	M	essential hypertension	2	8.9	2.8	3.6	29	8	0.025	0.09	0.361	1.3	–	–	1.5
N.S.	53	M	polycystic	–	8.7	4.9	9.9	24	8	0.026	0.26	0.374	3.7	0.74	0.21	1.2
F.B.	53	M	hydro-nephrosis	6	10.0	1.5	3.0	23	11	0.040	0.12	0.100	0.3	0.60	0.31	1.2
H.H.	54	M	chronic GN	6	9.7	2.5	2.1	29	4	0.014	0.03	0.429	0.9	0.63	0.80	1.6
R.S.	55	M	? chronic GN	2	9.2	4.5	4.6	18	8	0.007	0.03	0.391	1.8	0.72	0.18	2.7
E.Br.	63	M	chronic GN	5	8.7	6.7	9.5	14	9	0.008	0.08	0.442	4.2	0.71	0.24	2.5
Normal range					9.0 –10.8	2.5 –4.0	0.7 –1.3	25 –29	4 –11	0.05 –0.15	0.05 –0.15	0.2 –0.6	0.2 –0.6	–	0.4 –1.0	2.2 –4.2

GN = Glomerulonephritis.

Table 3

Clinical and biochemical data on patients

Patient					Plasma						Urine						
case	age, years	sex	diagnosis	Length of history, years	calcium, mg/100 ml	phosphorus, mg/100 ml	creatinine, mg/100 ml	bicarbonate, mM/l	Alkaline phosphatase, KA units/100 ml		Ca/Cr	Ca_E, mg/100 ml GF	P/Cr	P_E, mg/100 ml GF	Bone X-ray, CA/TA	Absorption test, fraction of dose/h	TmP, mg/100 ml GF
S.C.	15	F	interstitial nephritis	4	9.8	5.3	2.3	21	18		0.065	0.15	0.609	1.4	0.70	0.75	3.9
B.B.	23	F	chronic pyelo-nephritis	6	8.9	3.8	5.8	17	6		0.112	0.65	0.621	3.6	0.71	0.55	9.2
M.H.	27	F	? chronic pyelo-nephritis	–	9.8	9.4	16.5	10	6		0.025	0.41	0.442	7.3	0.74	0.12	2.1
J.H.	29	F	chronic GN	8	9.2	2.5	2.5	23	9		0.020	0.05	0.440	1.1	0.85	0.23	1.4
E.Su.	33	F	chronic pyelo-nephritis, stones	14	5.3	9.1	22.2	12	6		0.036	0.79	0.356	7.9	0.78	0.17	1.2
T.T.	34	F	chronic GN	15	9.3	5.3	7.0	16	6		0.027	0.19	0.486	3.4	0.68	0.15	1.9
M.Sm.	42	F	polycystic	10	9.5	5.0	3.7	16	5		0.022	0.08	0.622	2.3	0.68	0.24	2.7
J.Wi.	44	F	? chronic GN	–	9.5	7.0	15.5	13	8		0.051	0.79	0.406	6.3	0.55	0.10	0.7
I.P.	48	F	polycystic	5	10.1	4.5	5.1	22	5		0.051	0.26	0.578	2.9	0.76	0.58	1.6
E.Ba.	51	F	chronic GN	14	9.4	4.0	4.3	23	6		0.042	0.18	0.628	2.7	0.77	0.28	1.3
J.We.	51	F	? chronic GN	8	10.2	5.8	12.6	18	11		0.024	0.30	0.333	4.2	0.71	0.26	1.6
K.C.	61	F	primary hyperpara-thyroidism (operated)	9	10.7	3.0	3.7	24	6		0.032	0.12	0.432	1.6	0.57	0.18	1.4
L.A.	62	F	chronic pyelo-nephritis	2	9.3	4.5	6.2	17	10		0.105	0.65	0.597	3.7	0.54	0.20	0.8
Normal range					9.0 –10.8	2.5 –4.0	0.7 –1.3	25 –29	4 –11		0.05 –0.15	0.05 –0.15	0.2 –0.6	0.2 –0.6	– –	0.4 –1.0	2.2 –4.2

GN = Glomerulonephritis.

Table 3 (cont.)

Clinical and biochemical data on patients

Patient					Plasma					Urine						
case	age, years	sex	diagnosis	Length of history, years	calcium, mg/100 ml	phosphorus, mg/100 ml	creatinine, mg/100 ml	bicarbonate, mM/l	Alkaline phosphatase, K/A units/100 ml	Ca/Cr	Ca_E, mg/100 ml GF	P/Cr	P_E, mg/100 ml GF	Bone X-ray, CA/TA	Absorption test, fraction of dose/h	TmP, mg/100 ml GF
P.M.	18	M	ileal bladder, chronic pyelo-nephritis	11	5.9	5.9	6.0	14	13	0.115	0.69	0.783	4.7	0.57	0.14	1.2
J.M.	18	M	hydro-nephrosis	10	9.0	13.8	28.0	11	29	0.034	0.96	0.511	14.4	0.63	0.09	–
S.K.	43	M	polycystic	2	7.1	5.0	11.9	20	12	0.029	0.34	0.303	3.6	0.51	0.14	1.4
L.B.	54	M	chronic pyelo-nephritis, stones	29	9.0	3.4	3.9	13	20	0.092	0.08	0.462	1.8	0.58	0.08	1.6
M.S.	54	M	uretero colic, anastomosis, chronic pyelo-nephritis	11	7.3	5.8	9.8	12	24	0.045	0.44	0.429	4.2	0.49	–	1.6
H.G.	57	M	hydro-nephrosis	–	6.3	5.9	11.1	12	12	0.078	0.87	0.360	4.0	0.51	0.24	1.9
H.C.	61	M	chronic GN	30	7.5	4.0	7.7	17	67	0.018	0.14	0.377	2.9	0.39	0.11	1.1
J.D.	67	M	retro-peritoneal fibrosis	2	10.0	3.9	2.5	24	32	0.044	0.11	0.336	0.84	0.54	–	3.1
L.D.	15	F	ileal bladder, chronic pyelo-nephritis, stones	3	7.7	4.9	4.1	16	42	0.061	0.25	0.902	3.7	0.66	–	1.2
G.P.	17	F	? chronic pyelo-nephritis	5	6.7	7.0	16.6	18	27	0.073	1.21	0.289	4.8	0.52	0.08	2.2
Normal range					9.0 –10.8	2.5 –4.0	0.7 –1.3	25 –29	4 –11	0.05 –0.15	0.05 –0.15	0.2 –0.6	0.2 –0.6	–	0.4 –1.0	2.2 –4.2

GN = Glomerulonephritis.

Table 4

Clinical and biochemical data on patients

Patient					Plasma					Urine						
case	age, years	sex	diagnosis	Length of history, years	calcium, mg/100 ml	phosphorus, mg/100 ml	creatinine, mg/100 ml	bicarbonate, mM/l	Alkaline phosphatase, KA units/100 ml	Ca/Cr	Ca_E, mg/100 ml GF	P/Cr	P_E, mg/100 ml GF	Bone X-ray, CA/TA	Absorption test, fraction of dose/h	TmP, mg/100 ml GF
C.W.	18	F	chronic pyelo-nephritis	10	7.4	8.4	13.5	15	70	0.047	0.64	0.370	5.0	0.79	0.11	3.4
M.G.	44	F	chronic pyelo-nephritis	–	9.0	2.8	1.8	17	18	0.039	0.07	0.483	0.87	0.85	0.27	1.9
R.B.	51	F	chronic GN	15	9.0	6.5	9.7	17	64	0.032	0.31	0.526	5.1	–	0.13	1.4
T.B.	55	F	chronic pyelo-nephritis	10	8.9	4.0	4.1	19	42	0.061	0.25	0.634	2.6	0.46	0.10	1.4
G.C.	55	F	chronic pyelo-nephritis	5	9.1	3.8	2.7	18	16	0.041	0.11	0.630	1.7	–	–	2.1
M.St.	61	F	chronic pyelo-nephritis	4	8.6	3.8	4.6	10	103	0.063	0.29	0.722	3.3	0.59	0.08	0.5
H.Ba.	62	F	chronic pyelo-nephritis	10	7.0	3.6	2.8	17	70	0.064	0.18	0.607	1.7	0.59	0.22	1.9
E.Sa.	69	F	chronic pyelo-nephritis, stones	2	8.2	2.7	1.4	20	32	0.107	0.15	0.571	0.8	0.52	0.15	1.9
V.S.	73	F	? chronic pyelo-nephritis	2	8.3	6.3	4.3	13	47	0.088	0.38	0.721	3.1	0.33	0.40	3.2
Normal range					9.0 –10.8	2.5 –4.0	0.7 –1.3	25 –29	4 –11	0.05 –0.15	0.05 –0.15	0.2 –0.6	0.2 –0.6	–	0.4 –1.0	2.2 –4.2

GN = Glomerulonephritis.

Table 4 (cont.)

Clinical and biochemical data on patients

Variables	Number of Cases	r	p
CA/TA and log alkaline phosphatase	45	-0.50	<0.001
Plasma Ca and log alkaline phosphatase	48	-0.45	=0.001
Plasma HCO_3 and log alkaline phosphatase	48	-0.23	n.s.
CA/TA and plasma Ca	45	0.41	<0.01
Δ CA/TA and plasma Ca	45	-0.51	<0.001
CA/TA and plasma HCO_3	45	0.29	=0.05
Δ CA/TA and plasma HCO_3	45	-0.34	<0.05
Plasma Ca and plasma Cr	50	-0.26	n.s.
Ca_E and plasma Ca	50	-0.46	<0.001
Ca_E and plasma HCO_3	50	-0.45	<0.001
Plasma Ca and plasma HCO_3	50	0.38	<0.01
Plasma Ca and plasma P	50	-0.22	n.s.
Plasma Ca and plasma albumin	36	-0.10	n.s.
Plasma Ca and plasma proteins	36	0.23	n.s.
Plasma P and plasma Cr	50	0.83	<0.001
P_E and plasma P	50	0.92	<0.001
TmP and plasma Ca	50	0.11	n.s.
TmP and plasma HCO_3	50	0.17	n.s.
α_1 and plasma HCO_3	41	0.47	<0.005
OHP and resorption rate (all data)	17	0.79	<0.001
OHP and resorption rate (alkali treatment)	11	0.96	<0.001

TABLE 5. Correlation coefficients for biological data of patients.

MALES					FEMALES				
Patient	Age yr.	MTW cm.	CA/TA	Normal mean S.D.	Patient	Age yr.	MTW cm.	CA/TA	Normal mean S.D.
JR	21	0.84	0.79	0.706	SC	15	0.76	0.70	0.760
Pha	25	1.02	0.56	0.125	BB	23	0.64	0.71	0.122
BC	26	0.99	0.80		MH	27	0.71	0.74	
JBa	29	0.82	0.78		JH	29	0.69	0.85	
JG	37	0.99	0.69						
					TT	34	0.86	0.68	0.778
PH	42	1.14	0.67	0.710					0.118
JP	44	0.94	0.58	0.126					
FC	46	0.91	0.71		MSm	42	0.78	0.68	0.757
					JW	44	0.83	0.55	0.108
EA	50	1.10	0.60	0.697	IP	48	0.79	0.76	
JBo	51	1.02	0.74	0.148					
NS	53	0.91	0.74						
FB	53	1.01	0.60		EBa	51	0.87	0.77	0.697
HH	54	0.94	0.63		JWe	51	0.76	0.71	0.130
RS	55	1.00	0.72						
					KC	61	0.86	0.57	0.633
EBr	63	0.91	0.71	0.693	LA	62	0.73	0.54	0.133
				0.111					

TABLE 6. Bone X-ray data of the patients, with the normal values for the corresponding age and sex. Data from patients with normal alkaline phosphatases.

MALES					FEMALES				
Patient	Age yr.	MTW cm.	CA/TA	Normal mean S.D.	Patient	Age yr.	MTW cm.	CA/TA	Normal mean S.D.
PM	18	0.77	0.57	0.706	LD	15	0.58	0.66	0.760
JM	18	0.82	0.63	0.125	GP	17	0.72	0.52	0.122
					CW	18	0.75	0.79	
SK	43	0.89	0.51	0.710					
				0.126	MG	44	0.78	0.85	0.757
									0.108
LB	54	1.01	0.58	0.697					
MS	54	1.03	0.49	0.148	TB	55	0.89	0.46	0.697
									0.130
HC	61	0.97	0.39	0.693	MS	61	0.80	0.59	0.633
JD	67	0.94	0.54	0.111	HBa	62	0.78	0.59	0.133
					ESa	69	0.82	0.52	
					VS	73	0.72	0.33	0.587
									0.114

TABLE 6 continued. Bone X-ray data of the patients, with the normal values for the corresponding age and sex. Data from patient with raised alkaline phosphatases

	Normal alkaline phosphatase group (31)	Raised alkaline phosphatase group (19)	t	p
Plasma bicarbonate	20.1	16.3	2.89	<0.01
Plasma calcium	9.35	8.00	4.62	<0.001

TABLE 7. Plasma bicarbonate and calcium in patients with normal and raised alkaline phosphtase levels

Normal Alkaline Phosphatase				Raised Alkaline Phosphatase			
	Plasma				Plasma		
Patient	Calcium mg/100 ml	Albumin G/100 ml	Total protein G/100 ml	Patient	Calcium mg/100 ml	Albumin G/100 ml	Total protein G/100 ml
RD	9.8	4.3	8.1	PM	5.9	3.9	6.1
PHm	9.3	3.8	5.6	JM	9.0	3.2	6.2
JG	10.0	4.3	7.3	SK	7.1	4.4	7.6
JP	9.2	4.7	7.3	LB	9.0	4.7	7.8
FC	10.3	4.1	7.7	HG	6.3	4.8	7.5
JBo	9.4	3.2	6.5	HC	7.5	4.2	6.0
NS	8.7	4.3	6.7	JD	10.0	4.0	7.6
FB	10.0	4.6	7.5	LD	7.7	4.5	8.3
HH	9.7	3.1	5.2	CW	7.4	4.3	6.2
RS	9.2	3.6	6.3	RB	9.0	4.0	7.1
EBr	8.7	3.3	7.7	TB	8.9	4.5	6.5
SC	9.8	4.6	8.4	MSt	8.6	4.5	7.2
BB	8.9	4.0	6.8	HBa	7.0	4.3	7.3
MH	9.8	3.9	6.9	ESa	8.2	4.2	8.0
TT	9.3	4.2	6.5				
MSm	9.5	4.6	7.5				
JWi	9.5	4.2	6.4				
IP	10.1	4.0	7.5				
EB	9.4	3.8	6.6				
JWe	10.2	4.3	7.3				
KC	10.7	3.7	7.8				
LA	9.3	4.1	6.8				
Mean		4.03	7.06			4.24	7.10
S.D.		0.45	0.69			0.43	0.76

TABLE 8. Plasma calcium and protein concentrations of the patients

Patient	Calcium balance mg/kg bw/day	Plasma calcium mg/100 ml	Plasma bicarbonate mEq/L	Plasma creatinine mg/100 ml
HBa	+0.9	7.2	19	2.8
WB	+4.7	8.8	16	4.1
MSt	-1.7	8.6	10	4.6
LD	-9.6	7.0	17	4.1
JW	-0.1	9.3	15	15.5
ESa	-4.3	8.3	19	1.4
PM	-5.0	6.0	16	6.0
CW	+0.8	7.1	12	13.5
TF	-2.0	9.9	18	8.0
LA	-1.7	9.5	12	6.2
GP	-5.0	7.0	14	16.6
HC	-0.5	5.9	16	7.7
IP	+0.2	9.1	23	5.1
MH	+0.6	10.0	19	16.5
RS	-1.5	9.2	18	4.6
JP	-1.3	9.2	21	18.4
AC	+0.6	9.7	20	11.3

TABLE 9. Balance state of patients showing biochemical data

Patient	Blood pH	Blood pCO_2 mm Hg	Plasma Ca mg%	Plasma P mg%	TmP mg/100ml G.F.	Plasma Alk. Phosphatase K.A. units/ 100ml	Creatinine Clearance ml/min	Calcium Balance mg/kg bw/day
				PRE-TREATMENT				
PM	7.30	27	6.0	4.8	0.6	38	6	-5.97
DH	7.19	32	8.2	10.0	1.0	128	11	-0.75
LD	7.29	34	7.4	4.6	0.9	40	9	-9.60
CW	7.24	29	7.1	8.9	1.2	82	5	0.85
MSt	7.15	27	8.6	3.9	0.9	103	10	-1.61
LA	7.25	26	9.5	4.9	1.0	8	8	-1.74
				POST-TREATMENT				
PM	7.44	36	6.5	3.6	0.4	29	7	-1.41
DH	7.43	36	8.3	6.6	0.0	116	13	1.25
LD	7.36	39	7.6	4.1	1.2	46	10	-3.58
CW	7.40	42	6.1	8.2	1.1	79	7	0.22
MSt	7.29	34	8.3	3.3	0.6	114	9	-0.75
LA	7.38	34	9.3	4.2	0.6	9	7	-0.22
Normal Range	7.37-7.40	35-42	8.9-10.4	2.5-4.0	2.2-4.2	4-11	70-120	-2.0-2.5

TABLE 10. The effect of treatment with alkali on biochemical and balance status of six patients with renal acidosis.

Patient	Mineralisation rate, mg Ca^{++}/kg/day	Resorption rate, mg Ca^{++}/kg/day	Urine hydroxyproline mg/kg/day	Plasma calcium, mg/100 ml	Alkaline phosphatase, KA units
WB	29.40	22.30	0.80	10.0	6
MH	13.40	13.43	0.22	9.8	7
RS	5.01	7.16	0.37	9.2	8
JWi	3.22	6.98	0.24	9.5	8
LA	4.82	6.47	0.55	9.3	8
AC	8.40	9.78	0.20	9.9	9
TF	15.07	16.54	0.50	9.9	9
TP	10.69	12.15	0.71	9.2	10
IP	19.43	19.50	0.93	9.2	17
HBa	7.05	5.59	0.80	7.0	25
FS	9.30	11.47	0.32	8.2	34
HC	19.70	20.14	0.76	7.5	38
GP	15.54	18.31	0.46	6.7	39
CD	22.77	28.91	1.30	7.7	40
PM	11.50	15.52	0.75	5.9	41
CW	49.80	51.20	0.12	7.4	82
MSt	16.50	16.97	0.76	8.6	103
Normal range	3-7	1-9	0.10-0.40	9.0-10.8	<12

TABLE 11. Bone turnover and urine hydroxyproline in patients with chronic renal failure.

Patient	PRE-TREATMENT			POST-TREATMENT		
	Mineralisation rate (mg/kg bw/day)	Resorption rate (mg/kg bw/day)	24-hr. total urine hydroxyproline (mg/kg bw/day)	Mineralisation rate (mg/kg bw/day)	Resorption rate (mg/kg bw/day)	24-hr. total urine hydroxyproline (mg/kg bw/day)
PM	11.5	17.5	0.75	17.0	18.4	1.08
DH	208	209	-	336	335	7.40
LD	22.8	32.4	1.30	27.3	30.9	1.40
CW	49.8	49.0	1.20	75.5	79.3	1.79
MSt	16.5	18.1	0.76	14.0	14.8	1.54
LA	4.8	6.6	0.55	6.2	6.4	0.63
Normal Range	3-7	1-9	0.10-0.40	3-7	1-9	0.10-0.40

TABLE 12. The effect of alkali on bone mineralisation and resorption rates and urine hydroxyproline.

Patient	Rise in mineralisation rate (mg/kg b.w/day)	Rise in calcium balance (mg/kg b.w/day)
PH	5.5	4.6
DH	126	2.0
LD	4.5	6.0
CW	25.7	1.6
MSt	-2.5	0.8
LA	1.4	1.5

TABLE 13. The change in bone mineralisation rate and calcium balance after treatment with alkali

tient	Osteoid Surface %	Resorbed Surface % Total	Resorbed Surface % Available	Calcification Front % Osteoid	Volume Bone Tissue % Total	Plasma Calcium mg/100ml	Plasma Bicarbonate mEq/L	Plasma Creatinine mg/100ml	Plasma Phosphorus mg/100ml	Plasma Alkaline Phosphatas KA units
BCa	15	8	9	-	23	9.7	25	8.4	6.0	8
JBo	11	14	16	-	15	9.8	24	6.0	4.3	8
JWi	37	17	27	-	21	9.3	13	15.5	7.0	8
SWa	23	8	9	-	21	9.3	14	10.8	10.0	8
SLe	26	15	20	55	28	10.0	20	2.5	3.9	10
RLa	37	4	7	-	30	9.9	19	3.7	4.1	11
JWh	2	19	19	-	21	9.3	28	8.8	4.2	11
DPa	50	29	58	-	28	7.8	25	11.0	5.3	14
MKy	86	5	33	-	29	7.0	18	9.8	5.6	14
TBe	30	30	43	21	22	8.8	17	8.1	4.0	18
JCo	33	23	33	-	27	10.0	23	10.0	6.8	19
SDa	77	11	47	-	28	7.6	16	6.0	4.7	20
MSa	47	22	42	-	24	6.8	14	5.2	7.2	21
MMu	18	14	17	-	36	9.7	17	19.0	12.0	24
HSp	80	3	15	-	23	9.8	12	3.7	3.8	25
JBu	87	12	93	13	24	6.5	10	10.0	8.3	27
PMa	73	23	85	10	26	6.0	16	6.0	5.9	48
HCh	95	4	80	19	43	5.9	16	7.7	4.0	60
CWa	73	4	15	11	27	7.1	19	13.5	8.4	83
MSt	96	3	75	14	17	8.6	10	4.6	3.8	105
DHe	70	16	56	40	28	7.1	12	9.8	9.8	128
Normal Range	2-26	1-10%	1-14%	>60	15-30	9.0-10.8	23-28	0.7-1.3	2.5-4.0	<12

TABLE 14. Bone histology data on patients in chronic renal failure

Variables	Number of cases	r	p
Osteoid surface (% total) and plasma Ca	21	0.71	<0.001
Osteoid surface (% total) and plasma HCO_3	21	0.64	<0.001
Resorbed surface (% total) and plasma Ca	21	-0.02	n.s.
Resorbed surface (% total) and plasma HCO_3	21	0.29	n.s.
Resorbed surface (% available) and plasma Ca	21	-0.75	<0.001
Resorbed surface (% available) and plasma HCO_3	21	-0.44	<0.05

TABLE 15 Correlation coefficients for bone histology data and plasma biochemistry in patients with renal failure

PRE-TREATMENT									POST-TREATMENT					
Group C					Group A				Group C			Group A		
	Blood pH	Plasma calcium mg/100 ml	Plasma phosphorus mg/100 ml	Bourdillon score	Blood pH	Plasma calcium mg/100 ml	Plasma phosphorus mg/100 ml	Bourdillon score	Plasma calcium mg/100 ml	Plasma phosphorus mg/100 ml	Bourdillon score	Plasma calcium mg/100 ml	Plasma phosphorus mg/100 ml	Bourdillon score
	7.36	10.9	8.7	0	7.24	10.4	6.2	0	11.5	11.4	4.0	10.6	11.9	2.5
	7.36	10.4	5.9	1.5	7.26	10.3	7.4	0	11.5	11.5	4.5	9.6	10.5	2.0
	7.35	10.1	6.1	2.5	7.25	10.7	5.1	1.0	10.0	10.3	5.0	9.8	9.5	3.0
	7.34	10.0	7.3	0	7.00	9.5	4.9	0	10.1	11.6	4.5	10.6	9.3	1.5
	7.33	10.5	6.8	2.0	7.32	9.6	7.5	0	10.2	9.4	6.0	11.6	11.5	0
	7.37	10.0	6.7	0	7.19	9.8	8.0	0	11.4	11.1	5.5	10.4	12.9	1.5
	7.34	10.7	7.9	1.5	-	-	-	1.0	11.3	9.5	6.0	10.0	9.3	6.5
	7.33	10.4	5.7	1.0	-	-	-	0.5	10.8	10.3	4.0	11.3	9.6	3.5
	-	-	-	1.0	-	-	-	2.0	10.1	9.6	-	10.5	11.3	-
Mean	7.35	10.5	6.9	1.2	7.21	10.1	6.5	0.5	10.8	10.5	4.9	10.5	10.6	2.6
S.D.	0.02	0.3	1.0	0.9	0.11	0.5	1.3	0.7	0.7	0.9	0.8	0.7	1.3	1.9

TABLE 16. The influence of acidosis on the healing of experimental rickets in the rat.

Significance of difference between Groups C and A with respect to

	ν	t	p
Blood pH	12	3.53	<0.005
Bourdillon Score 1	16	1.78	n.s.
Plasma Calcium 1	12	1.50	n.s.
Plasma Phosphorus 1	12	0.59	n.s.
Bourdillon Score 2	14	3.22	<0.01
Plasma Calcium 2	16	0.89	n.s.
Plasma Phosphorus 2	16	0.22	n.s.

TABLE 17. Influence of acidosis on the healing of experimental rickets in the rat: analysis of data

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