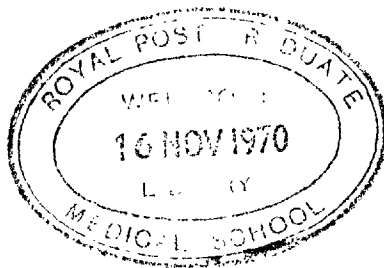


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QUANTITATIVE HISTOPATHOLOGICAL STUDIES IN METABOLIC BONE DISEASE

by

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ABSTRACT

A technique for the histoquantitation of undecalcified sections of iliac bone biopsies has been developed and the amount of calcified trabecular bone, osteoid and resorption measured. A control series of 50 cases has been studied and the mean and normal range for each variable defined. The amount of calcified trabecular bone declines significantly with age, particularly in males; the amount of osteoid and of resorption rises slightly with age. Bone biopsies from a variety of other conditions have been studied and compared with age and sex matched controls. In 10 patients with the clinical diagnosis of osteoporosis, the amounts of calcified trabecular bone were in the lower half of the normal range. Resorption in these patients was, if anything, reduced and the amount of osteoid was in general normal. Biopsies from 12 patients with a clinical diagnosis of osteomalacia have been studied. A correlation was shown between the serum alkaline phosphatase, 24 hour strontium space and the amount of osteoid and between calcium and phosphorus product and the amount of osteoid. Biopsies from 15 cases of endocrine bone disease have been studied and a close relationship shown between the phosphate excretion index and the amount of resorption. In addition, resorption was shown to be normal in two patients with hypercalcaemia, and considerably increased in Cushing's Syndrome. In biopsies from 60 patients with renal failure nearly all showed an increase in the amount of osteoid or resorption, or both. Haemodialysis led to a significant increase in the amount of osteoid, and a drop in cortical bone thickness. Renal transplant led to a relatively slow change in osteoid but a quick return to normal of resorption; parathyroidectomy led to dramatic changes.

The biopsy results are also compared and contrasted with clinical and radiological observations, and the value of biopsy quantitation as a diagnostic and research technique established.

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When you can measure what you are speaking about and express it in numbers you know something about it, but when you cannot express it in numbers your knowledge is of meagre and unsatisfactory kind, it may be the beginning of knowledge, but you have scarcely in your thoughts advanced to the stage of science.

Lord Kelvin, 1883.

CHAPTER 1

FOREWORD

1.1. GENERAL INTRODUCTION

The quotation from Lord Kelvin has probably headed many theses over the past 86 years, but it has lost nothing of its original force. It is particularly apt for histopathology which has been the least numerate of the sub-division of pathology. The gradual introduction of quantitative methods into histology has been one of the changes that led to this project, with the additional stimulus of the development of satisfactory techniques for the preparation of undecalcified bone sections and the increasing sophistication of modern techniques for the study of metabolic bone disease. The developments that have taken place in these separate fields have now made it possible to attempt to correlate the quantitative histological changes in bone with clinical and radiological studies of the patient. There have been few publications on correlative studies between the results of morphometric measurements in bone and detailed metabolic studies. The work presented here includes quantitative histopathological studies on undecalcified iliac bone samples from patients with metabolic bone disease, in particular, osteoporosis, osteomalacia and renal osteodystrophy. The results obtained from the morphometric measurements have been correlated with the clinical state of the patient, with the biochemical investigations and kinetic studies and also with the various parameters of radiological examinations.

Due to the complex nature and diverse aetiology of metabolic bone disease, each major disease is made the subject of a separate chapter, for the sake of simplicity and lucid clinico-pathological correlations.

1.2. HISTORICAL REVIEW

Bone, which is one of the connective tissues in the body, shares a major role in various metabolic activities; the importance of which became widely appreciated at about the turn of the century.

In the early days many research workers studied bone diseases in a static way, either in autopsy materials or in experimental animals, with few clinical applications. In recent years, with the introduction of various laboratory investigations, the study of metabolic bone disease has become more dynamic in nature. In this context I have made an attempt to review the numerous methods of bone analysis, mainly from the quantitative aspects.

The first study that could be considered bone analysis was made in 1863 by Bischoff where he determined the skeletal weight in relation to the total body weight in a young female corpse and found that it was 16%. 30 years later, Gabriel (1894) studied the composition of bone minerals, measured the amount of the major constituents, calcium and phosphorus and found that other elements were also present in smaller amount, for example, magnesium, sodium, potassium, chlorine and fluorine.

Allison and Brooks (1921) studied the composition of organic and inorganic substances present in humeri of a young dog - where the animal had been subjected to section of a brachial plexus. After 24 days of complete paralysis, the animal was sacrificed and the humeri of both used and non-used were removed. The bones were weighed in a fresh state, after drying at 100°C and after incineration. They found that nerve section led to a loss of mineral mass of about 27%.

Mechanik in 1928 (cited by Casuccio, 1962) studied the weight of bone from human subjects and stated that in young people the weight of bone substance was more than double that of bone marrow. 8

years later Lachman and Whelan (1936) subjected a human bone to increasing decalcification by means of nitric acid and simultaneously carried out roentgenographies of the bone at each stage. The amount of calcium salts thus removed at various stages was estimated and expressed as a percent of the total amount. They then compared the radiological findings with the known reduction in the amount of bone mineral and concluded that before rarefaction could be detected roentgenologically, the amount of calcium loss should be in the region of 30-50%. They also applied this study to an osteomalacic rib and found loss of calcium salts in the region of 60-70%.

Broman et al (1958) studied the gross density of lumbar vertebra and femur in a population of white and negro races and concluded that there was a progressive decline with age in both sexes and in both races. Caldwell and Collins (1961) measured the calcium content of lumbar vertebra and stated that the calcium content gave an estimate of the salt content, since the proportion of calcium in bone salt was 36%. Lindahl and Lindgren (1962) determined the apparent density of spongy bone in tibia and vertebra of autopsy materials and later applied this technique to assess the degree of osteoporosis on bone biopsy materials obtained from the epiphysis of upper tibia (Lindahl, 1966). Doyle (1961, 1969) basing his work on microdensitometry of radiographs determined the amount of mineral present in the ulnar bone of normal subjects with a view to extending its applications to various forms of 'metabolic bone diseases'.

Bone analysis has been made not only by biochemical and radiological means but also by the application of biophysical methods. The inorganic fraction of bone has been studied by means of x-ray microscopy or microradiography (Engfeldt et al, 1954). Jowsey et al (1965) also applied the technique of quantitative microradiography to the study of normal and osteoporotic bones.

The structure of bone has been studied both in terms of qualitative and quantitative means either based on decalcified or undecalcified sections. In the past, the histological structures were mainly studied after various forms of decalcification of the tissue (Richman et al, 1947, Dotti et al, 1951, and Hilleman and Lee, 1953), but in recent years, with special reference to the study of metabolic bone disease; the use of tetracycline labelling and fluorescent microscopy, autoradiography and microradiography necessitates preparation of undecalcified section (Arnold and Jee, 1954, Woodruff and Norris, 1955, Villanueva and Santoro, 1962). There are various techniques of preparing undecalcified sections, the former method of grinding a section with free abrasives between two glass plates was tedious and has been supplemented in part by various additional methods which combine embedding in plastics with sawing, (Kropp, 1956) milling, (Kelly et al, 1957) and grinding (Beals and Guard, 1958). Gettner and Ornstein (1956) gave a general review of embedding media and suggested advantages of using plastic materials; as a result of which a number of materials including methacrylate, araldite, resin and polyesters, have been introduced as embedding media. Plastic embedding was advocated, because there is a better preservation of bony architecture although the cellular details are less distinct. Moreover, with appropriate stains, the undecalcified bone or osteoid tissue could easily be seen and hence mensuration of the various components of bone could be carried out with ease and accuracy.

During this decade many quantitative studies of bone structure in relation to 'metabolic bone disease' have been carried out. Beck and Nordin (1960) introduced a 9 point photomicrography visual scale based on decalcified iliac crest bone sections for the assessment of osteoporosis. Van de Huel et al (1964) also estimated the amount of trabecular bone in iliac crest biopsy sections both in normal and osteoporotic subjects. The tissue was formalin fixed, decalcified and

paraffin embedded, and a planimeter consisting of 100 small squares was used to measure the different components. Bordier et al (1964) studied the amount of trabecular bone in normal and osteoporotic subjects using plastic embedded iliac crest bone biopsy samples and Zeiss I and II integrating eyepieces. Garner and Ball (1966) also made quantitative observations on mineralised and unmineralised bone in chronic renal azotaemia and intestinal malabsorption syndrome, using undecalcified iliac crest sections prepared as advocated by Ball (1957, 1963), and a 16 point graticule for the quantitation. Sissons et al (1967) carried out quantitative assessment of the normal structure of bone using ground sections embedded in methyl methacrylate and Zeiss I and II integrating eyepieces. Woods et al (1968) also made measurements of osteoid in iliac crest bone biopsies from osteomalacic cases using the double embedding technique as described by Ball.

Much work has been done in the past on bone and quantitation has advanced rapidly over the last ten years. No large series of bone biopsies from carefully studied patients with a variety of different disease has been quantified and compared with age and sex matched controls. This gap, the present thesis attempts to fill.

CHAPTER 2

METHODS AND MATERIALS

2.1. BONE BIOPSY TECHNIQUE

An adequate bone and marrow biopsy which is usually obtained by means of open surgical methods or by the use of bone biopsy needle is often necessary in the diagnosis of various forms of metabolic bone disease - infiltrative disease of the bone marrow and in a variety of blood dyscrasias.

The first deliberate bone biopsy seems to have been performed by Ghedini in 1908 (cited by Padilla et al, 1967), to study the marrow in a patient with a haematologic disorder. 15 years later Seyfarth (1923) performed the first sternal trephine biopsy for a marrow specimen. Since then various workers have invented different forms of needles and performed biopsies from different sites of which iliac crest is the most popular site, due to easy accessibility, lack of hazards, easy performance and provision of reliable confirmatory diagnoses. Sacker and Nordin (1954) described a simple biopsy needle and later a modified form was introduced by Williams and Nicholson (1963). Hartman and Phalen (1967) reviewed a series of bone biopsies performed with the Craig needle for neoplastic bony disorders. Padilla et al (1967) studied the metabolic and infiltrative diseases of bone from iliac crest samples.

Beck and Nordin (1960) advocated the use of bone biopsies from the anterior iliac crest between the two tables, in contrast Bordier et al (1964) introduced a new method of 'through and through' biopsy, which included both inner and outer cortices.

In reviewing the various techniques of bone biopsy there seems to be no doubt that the basic technique of Bordier et al (1964) is superior to the other methods. The disadvantages of those techniques that involve penetrating the crest from above and taking a core of trabecular bone by passing downwards between the two cortices are several.

The cortex obtained is curved and difficult to study, the trabecular bone may be taken from very close to or some distance from the cortices. In contrast the Bordier technique provides a method by which the distance of any part of the trabecular bone from the cortex is always known, and the exact point on the iliac bone from which the biopsy is taken can be located reproducibly. The method is also safe, the only difficulty encountered has been some local pain, this can largely be overcome by local anaesthesia. For these reasons a modified Bordier technique has been used.

Most of the specimens used in this investigation were obtained by the modified form of biopsy needle described by Sacker and Nordin (1954) (See Figure 1), those from the renal failure patients were nearly all obtained by different trephine needle which is devoid of a cross-bar, but fits into the socket of the hand operated 'Black and Decker' electric drill (Figure 2).

Biopsies are obtained from the iliac bone approximately 2.5 cm below and behind the anterior superior iliac spine (Figure 3). A small 5 mm incision is made under local anaesthesia (2% Lignocaine) up to the periosteum using a scalpel. A toothed trocar is inserted to grip the periosteum. Then the stainless steel trephine is introduced held at right angles to the cortex and rotated manually. The external cortex and the bone marrow are pierced with ease, but there is a slight difficulty for the inner cortex and periosteum. After obtaining the full thickness of the iliac bone with two cortices (Figure 4), the trephine needle is withdrawn and the specimen is ejected by means of the centre pin into a specimen jar containing 70% buffered formol saline. The skin incision is dressed with sterile gauze, ^{often} no sutures are needed and the wound heals by first intention. This technique of performing 'through and through' iliac bone biopsy has proved easy to use and also

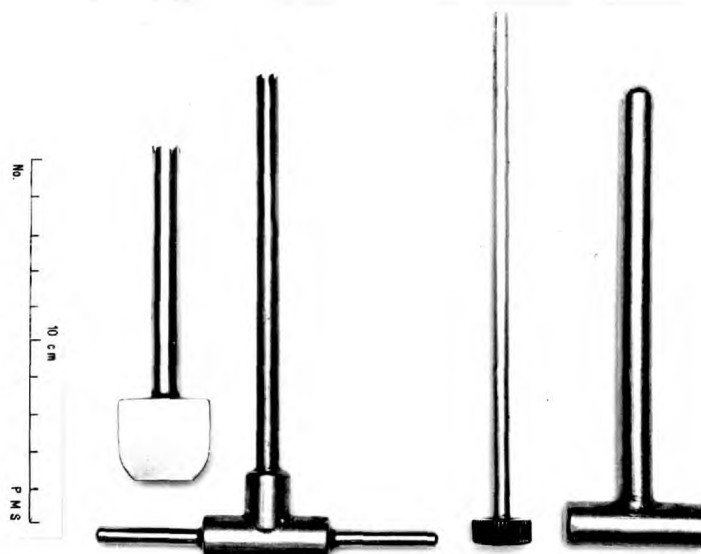


Fig. 1
Bone biopsy instruments showing a trocar, a crossed-bar trephine needle, a cannula and a hammer.

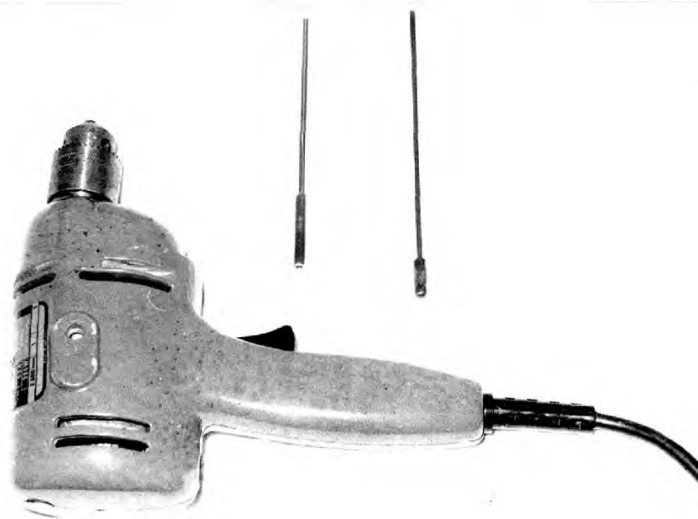


Fig. 2
A modified trephine bone biopsy needle, a cannula and a "Black & Decker" electric drill. (By courtesy of Professor R. Shackman).

Fig. 3

—DIAGRAMMATIC REPRESENTATION OF THE PELVIS TO SHOW
THE SITE OF "THROUGH & THROUGH" TREPHINE BONE BIOPSY—

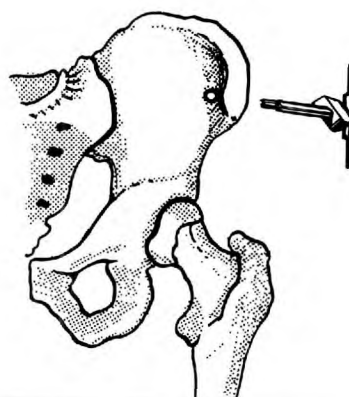




Fig. 4

A bone biopsy specimen showing two cortices and a core of cancellous bone.

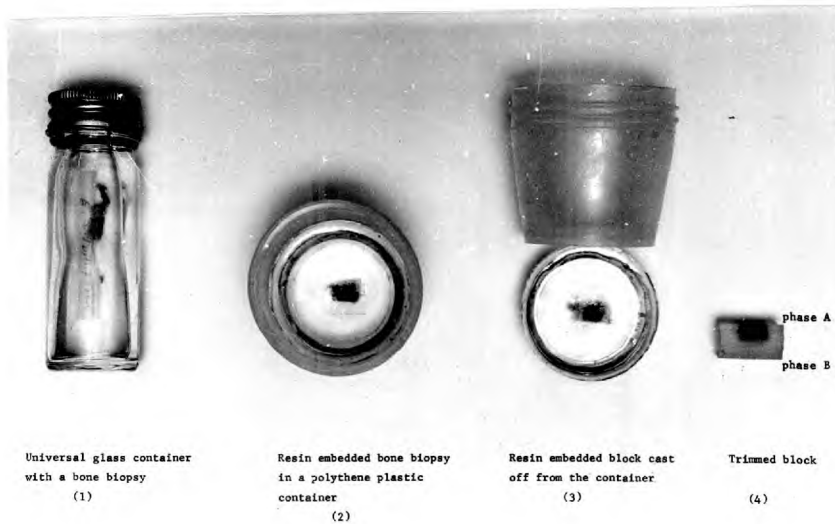


Fig. 5

Materials used in embedding procedure

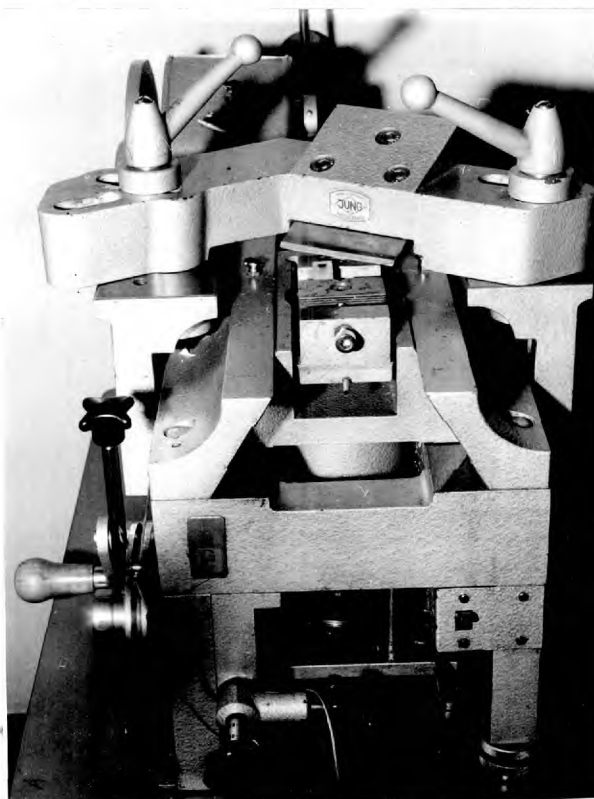


Fig. 6 Jung K Microtome for cutting undecalcified sections

free from hazardous problems.

The samples obtained by means of the hand operated electric drill give a better preservation of bony architecture. The diameter of the steel trephine is 3 mm, one mm smaller than the Sacker and Nordin needle used in the Metabolic Unit. There is no charring of the tissues, because the motor is driven at a very low speed. The actual area of section quantified is very similar in the two series of biopsies because of the slightly greater artefact incidence and breakage of biopsies when the Sacker and Nordin needle is used.

2.2. SECTION PREPARATION

A wide variety of methods have been used for the preparation of undecalcified sections. No embedding medium need be used, as in frozen sections of bone. A microtome need not be used, a thin slice of bone can be cut with a fine saw followed by manual grinding on a flat abrasive surface such as a hone or a fine carborundum paper or between sheets of plate glass previously 'sharpened' with silicon carbide abrasive (Jowsey, 1955, Frost, 1958). Paraffin wax of a harder than normal consistency has been used and Duthie (1954) and Ball (1957) improved the standard technique by double embedding with low viscosity nitrocellulose or celloidin and placing a strip of transparent adhesive tape (sellotape) on the surface of the block immediately before cutting. Synthetic resin embedding techniques have been used for preparation of sections from hard objects and any calcified tissues (bone, teeth, etc.) and for cutting thin sections suitable for electron microscopy work. Numerous articles have been published on the embedding procedures and many plastics have been suggested including methacrylate, araldite, resins, polyesters and resins. Many have claimed that plastic embedding does not produce entirely satisfactory sections for undecalcified bone on account of shattering

and fragmentation of the trabecular and cortical bone. In my experience these hazards could be overcome by using vacuum impregnation (Figure 7) and paying great attention to the sharpness of the knife.

Recently new embedding techniques for undecalcified bone biopsies have been introduced by Matrajt et al (1967). This method has been used in my research work, but every step in the tissue processing has been modified to give ideal sections. This technique, which will be described in detail in Appendix, was chosen because it gave excellent undecalcified sections which were flatter than comparable methacrylate embedded sections, and thinner and easier to prepare than ground sections. Buffered formol saline (pH 6.8-7.0) was used as a fixative to avoid leaching out of calcium.

A Jung K microtome is used (Fig.6). It is heavy and rigid; both features are essential for good undecalcified section cutting, and it can be operated with or without an electric motor drive. The knife in use is No. 4. This is preferred rather than a tungsten tipped knife, because the latter cannot be sharpened manually. In cutting sections, I used two knives, one just for trimming down the tissues to the surface of the bone 'Trimmer' and the other for cutting sections 'Cutter'. In this way the need for sharpening the knife has been minimised.

Various stains have been advocated for undecalcified sections. Frost (1959) recommended a variety of chemicals, e.g., basic fuchsin, metallic sulfides, Alizarin Red S and Potassium permanganate. Hyman and Poulding (1961) suggested the use of solochrome cyanin - iron alum for rapid stain of frozen sections of bone. Villanueva et al (1964) used a tetrachrome stain for fresh unfixed, mineralised, unembedded thin bone sections. Matrajt and Hioco (1966) used solochrome cyanin R as an indicator dye of bone morphology to demonstrate osteoid tissue and calcification fronts. Solochrome cyanin R dye (Edward Gurr) has been used in this work to stain undecalcified sections of bone, because in

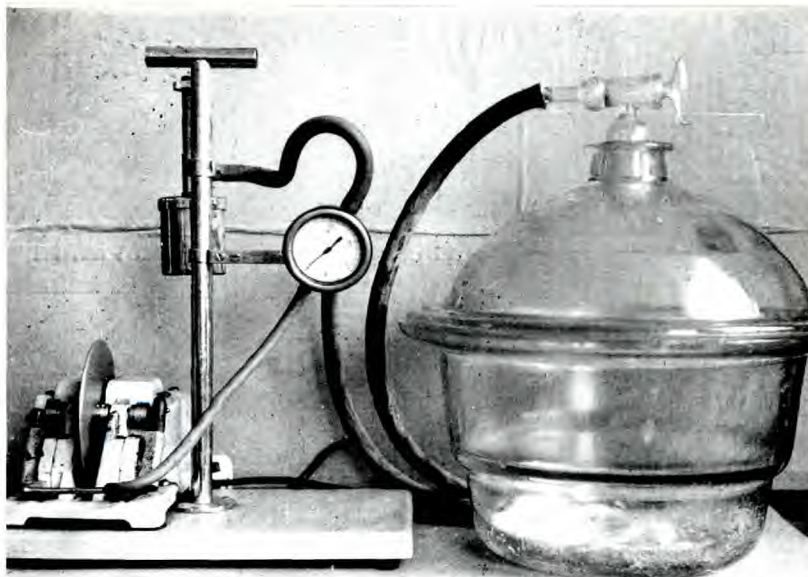


Fig. 7
The vacuum equipment used during embedding for the final stage of impregnation with pure resin.

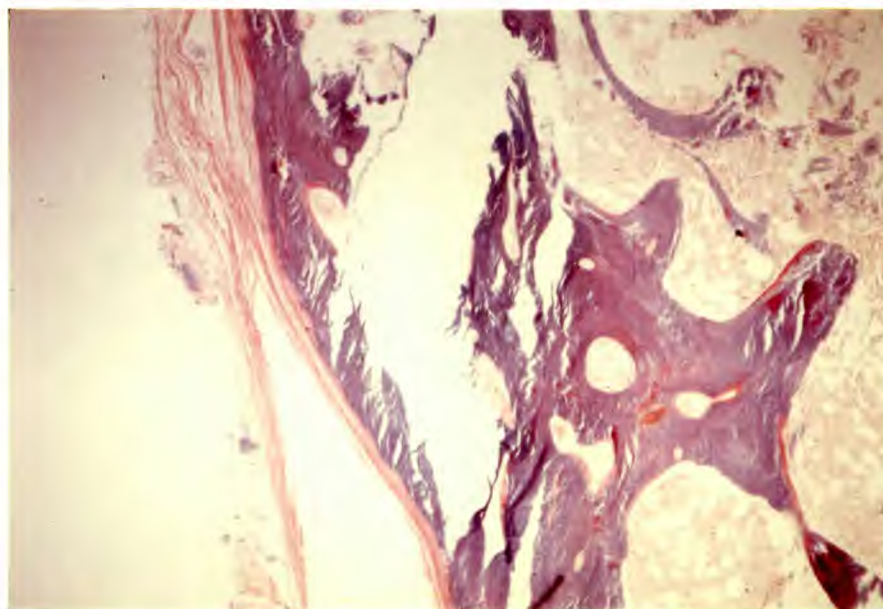


Fig. 8
An undecalcified section to show shattering and stain precipitation. The faults are due to poor embedding and the use of a blunt knife. (Solochrome cyanin x 30).

addition to excellent demonstration of osteoid in calcified bone it also offers the opportunity of visualizing the osteocytes and calcification fronts. Marrow elements are not in general well shown. Stain must be used with great care for reliability and reproducibility. Otherwise many technical errors may result. The stain must be freshly prepared with a correct pH (2.8-3.0) in order to avoid stain precipitates (Figure 8). The differentiation time too, is very critical, if prolonged, the osteoid tissue and calcification fronts are indistinct. However, when correctly performed excellent results are obtained.

2.3. MICROSCOPIC INTERPRETATION OF STAINED SECTIONS OF UNDECALCIFIED BONE

The ideal undecalcified section from the iliac bone biopsy shows two cortices and a medulla. The former consists of compact bone and the latter of trabecular or cancellous bone (Fig.9). The structures of these are essentially similar; the compact bone is dense with few narrow channels and the trabecular bone is represented by anastomosing bony spicules intermingled with wide marrow spaces. Both the cortices are covered externally by a vascular fibro-collagenous connective tissue, which is known as the periosteum.

Bone is composed of lamellae or layers of fine collagen fibres situated in a calcified matrix. The lamellae structures are more easily seen when viewed with polarized light and an analyser. Between the lamellae are bone cells or osteocytes lying in cell spaces known as lacunae. In the cortical bone, the Haversian canals are surrounded by concentrically arranged lamellae, whereas in the trabecular bone, the lamellae tend to be parallel and are devoid of Haversian systems.

Solochrome cyanin R (E. Gurr's Michrome No. 750) is a good indicator dye for demonstration of osteoid and calcification fronts in undecalcified plastic embedded sections (Matrajt and Hioco, 1966). It is used in acid solution as a progressive stain for acid and basic proteins. This stain is also recommended in demonstration of myelin in



Fig. 9

An undecalcified section of control bone showing normal cortical thickness and trabecular pattern. (C.9. Solochrome cyanin stain x 30).

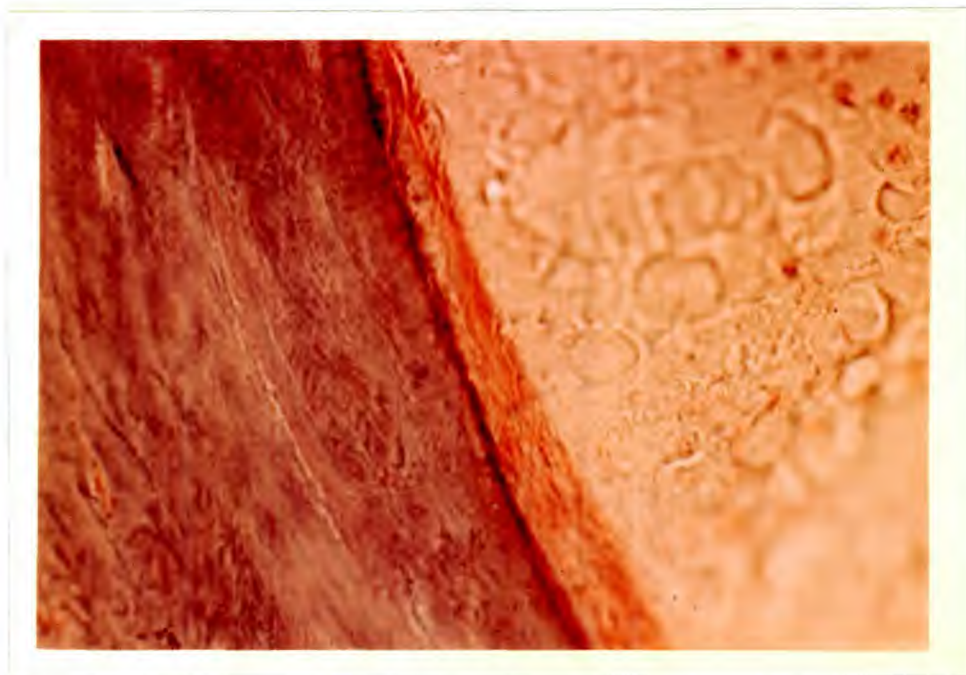


Fig. 10

An undecalcified section showing calcified bone and osteoid with a dark purplish granular layer at their junction the so-called calcification front. (C.9. Solochrome cyanin x 480).

peripheral nerves (Page, 1965). The mode ^{of} action of this stain on undecalcified bone is still unknown, although it might depend on the reaction with some components of the matrix, rather than on the mineral itself. The nature of this action is very similar with that of haematoxylin (Cameron, 1930).

2.3.1. Calcified Bone

When the undecalcified section of bone is stained with Solochrome cyanin R, the calcified bone appears as a deep blue or sky blue in colour (Fig.10). The trabeculae are covered in areas by thin, orange red zones which are areas of osteoid or uncalcified bone tissue.

2.3.2. Osteoid

It is a term denoting the layer of unmineralised bone matrix on the surface of calcified bone, where new lamellae bone formation has been in progress. The fact that osteoid represents uncalcified bone tissue has been confirmed by microradiographic techniques (Meyer, 1956). It has long been known that the presence of increased number and thickness of osteoid seams characterise the osteomalacic skeleton. The thickness of osteoid seams has been measured in human beings from fresh, undecalcified 40 u sections of long bones (e.g., femurs, humerus, rib, etc.) stained with basic fuchsin. Measurements are made with calibrated micrometer eyepiece, the normal thickness of osteoid seams is found to be 9 u (Frost, 1962).

The osteoid tissue is also stained red when the von Kossa-van Gieson technique is used (Fig.41). In histoquantitation all the blue stained areas are counted as calcified bone and orange red zones as osteoid. The fibro-collagenous connective tissue lying external to the cortices which has the same colour as osteoid is not included; all other red staining collagenous material lying apposed to the bone surface, or forming masses with the morphology of trabecular bone is included. In cases of doubt polarising microscopy was used to aid identification.

2.3.3. Calcification Front

At the junction of the calcified bone and osteoid, dark purple granular linear areas may occur (Fig.10), these are known as calcification fronts. (Matrajt and Hioco, 1966). This is the first zone of mineralisation which is named differently by various authors; borderline by Lacroix and Ponlot (1956), transition area by Loe (1959), calcification fronts by Thomas (1961) and area of demarcation by Frost (1962).

During investigations for the staining properties of the calcification front, Perl's Prussian blue reaction for iron was carried out. A positive reaction was seen in some cases (Fig. 12) corresponding to the distribution of the calcification front stained by Solochrome cyanin (Fig.11). A similar reaction with Prussian blue is also noted in weanling rat bones by Thomas (1961). The presence of iron in the calcification front was confirmed by using an Electron Probe Microanalyser (McIndoe et al, 1970) which showed a definite peak for iron (Fig.13). Becker et al (1968) have shown that iron is present in trace amount in human bone ranging from 4-60 p.p.m. The significance of this finding lies in the possible role of iron in the normal process of calcification. It is known that iron is often associated with calcification in abnormal situations and it is possible that it forms an essential component for the deposition of hydroxyapatite crystals on collagen.

The calcification fronts were noted to be very prominent in the sections from autopsy material. This raises the interesting speculation that ferric irons from the marrow spaces might become incorporated at the sites of active bone formation. Past (1967) has shown that frozen sections of mouse foetal bone accumulated iron on their surfaces when immersed in an iron containing phosphate buffer. The bone biopsies from living patients have been fixed in buffered formol saline, the iron content of this, estimated by means of the Technicon Auto analyser method, (Iron is reduced by ascorbic acid and a blue



Fig. 11

An undecalcified section from an autopsy specimen showing prominent 'calcification' fronts. (Solochrome cyanin x 30).

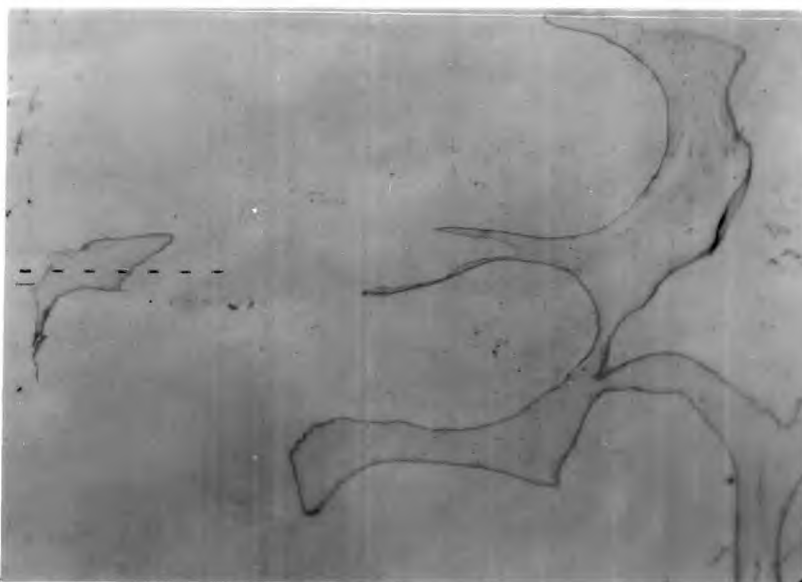


Fig. 12

An adjacent undecalcified section from the previous autopsy specimen showing prominent iron deposition corresponding to the 'calcification fronts'. (Perl's prussian blue x 30). Note particularly the left hand trabeculum.

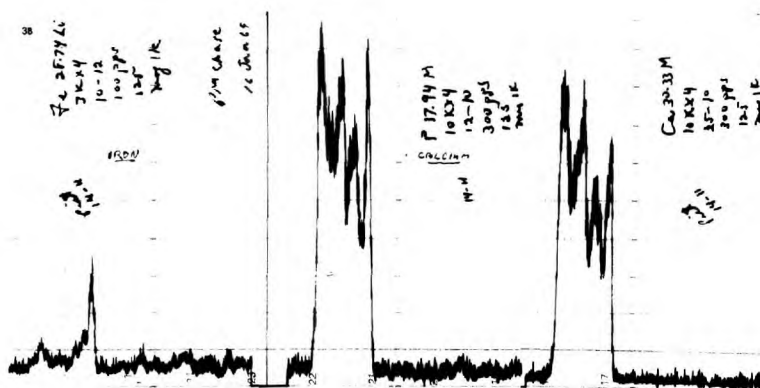


Fig. 13

An electron probe microanalysis tracing from an undecalcified section from the previous autopsy specimen. Note the definite peak for iron. The scan performed in the area as shown in Fig. 12 indicated by interrupted lines.

complex is formed with 2,4,6 trypridyl - 1,3,5 triazine which is measured calorimetrically) is in the range of 10-15 ug/100 ml, and is not considered significant.

Many of the sections under study show calcification fronts, but these are much more prominent in bone biopsies obtained from autopsy specimens. Iron is likewise more prominent in staining autopsy biopsies than in surgical biopsies. It is therefore possible that the iron is an artefact, due to local release from haemoglobin or its precursors after death. The fact that iron staining and calcification fronts are more prominent in autopsies, and that they coincide spatially, suggests that some of the calcification front staining may be due to the iron deposition.

In the group of renal osteodystrophies, calcification fronts were in most cases found to be absent. In a small group of 9 cases, calcification fronts were prominent, despite the presence of excess osteoid, and the absence of any vitamin D therapy (Fig.14). In all 9 cases tetracycline had been administered in the dose of 2 gm a day for three days, followed by a similar course after 10 days, and followed immediately by bone biopsy. None of the other renal failure cases had had recent tetracycline. The incorporation of tetracyclines at the sites of active bone formation (Milch et al, 1958) could possibly have changed the isoelectric point within this zone and shown prominent fronts when stained with Solochrome cyanin. This finding has been confirmed by Bordier, Ph. (Personal Communication).

2.3.4. Resorption Surface

These are seen as irregular, scalloped margins of the bone surfaces. These correspond to areas of osteoclastic resorption, but in plastic embedded material cellular detail is obscured, and osteoclasts are not well seen. The irregular scalloped edges are easy to identify and quantify and are clearly evidence of resorption. However, it is not certain how long a scalloped area remains scalloped after



Fig. 14

An undecalcified section from a patient with chronic renal failure before haemodialysis who had two doses of 500 mg oxytetracycline with a ten day interval. The last dose was 3 days prior to the date of bone biopsy. Note prominent dark, purplish, granular, linear areas, the so-called 'calcification fronts' with the overlying pale pink osteoid tissue (1). Note also at (2) fronts appearing on the scalloped irregular resorption surfaces with a very thin layer of osteoid and also at (3) fronts appearing even in the relative absence of osteoid tissue. (Patient R.14, L.G. Solochrome cyanin x 120).

active resorption has ceased. The measurement of resorption surface quantified here, thus may represent both present and ^{recent} past resorption. At times the resorption surfaces are covered by varying amounts of osteoid, this combination is often prominent in renal osteodystrophy and in cases of dietary osteomalacia with hyperparathyroid activity (Fig. 36). This combination of resorption surface covered by osteoid is included as a separate parameter in the quantitation studies. The figures for osteoid covered resorption surface are also included in the total for osteoid, but are not included in the total for resorption.

In my experience Solochrome cyanin R staining procedure is not ideal for the demonstration of calcification fronts. There are many factors involved, firstly the differentiation period in water is very critical; secondly the pH and freshness of the staining solution is very important. Any alteration could easily give rise to false negative results. In order to avoid these hazards, staining must be done very meticulously and dubious fronts restained together with a control section which shows prominent fronts.

In view of the extreme care required for accurate and reproducible demonstration of calcification fronts with solochrome cyanin and the findings which suggest (a) that the preparation administration of tetracycline may make them more prominent and (b) that the prominence of fronts in post mortem material may be due to local absorption of iron, it was decided not to measure the amount of bone surface showing fronts. Too many uncontrolled variables could affect the amount of fronts so that no conclusions could be drawn from these measurements.

2.4. HISTOQUANTITATION PROCEDURE

Haematologists, bacteriologists and biochemists have been expressing their results in a quantitative manner for centuries, but histopathologists have in general been expressing opinions based on descriptive, qualitative observations. It is to be understood that morphometric measurements cannot be used for all aspects of histopathology

but from the point of view of a scientific approach, quantitative studies should be extended to histopathology whenever possible.

The quantitative procedures have been popular in the field of geological science and Delesse the famous French geologist and mathematician in 1848, enunciated a theorem which states that :- 'In a rock, composed of a number of minerals, the area occupied by any given mineral on a surface of a section of the rock is proportional to the volume of the mineral in the rock '..... Based on this theory, various methods have been developed to carry out morphometric studies on biological tissues. As a result of these, many workers have attempted quantitative procedures on various organs, notably lung, placenta, kidney and bone, etc.

The original techniques employed were manual and tedious, as indeed most modern techniques still are. Chalkey in 1943 introduced a point counting technique, using randomly distributed pointers in the eyepiece, and scoring the 'hits' made by these pointers on the different tissue components in a succession of fields. Chalkey applied his technique to a number of tissues including liver and kidney, in order to determine the ratio between the volumes or areas particularly the nucleocytoplasmic ratios or the volume ratios of active and inactive gland cells. The mean linear intercept principle, based on the demonstration that the sum of the length of all the intercepts lying over one component as a fraction of the total length of the line is equivalent to the proportional volume of that component (Rosiwal, 1898) has also frequently been used. This, as well as the point counting technique /ordinarily as used does depend on the assumption that the component is randomly distributed. Refinements and combinations of these techniques have been introduced, one of the recent ones being the Weibel grid (1963), where a series of short lines are used, the ends recording as points, and the lines giving numbers of intercepts across surfaces. From these

intercepts the surface area of the component can be calculated.

These morphometric techniques have been applied to a number of biological tissues, in pathology the lung has been particularly well studied, for example the study of centrilobular emphysema (Leopold and Gough, 1957) and the quantitative assessment of chronic bronchitis (Reid, 1960). Morphometric studies have also been applied to the study of bone, the mathematics being based both on the principles of point counting systems (Chalkey, 1943, Hennig, 1959) and the principles of mean linear intercepts (Short, 1950, Hennig, 1956). Bordier et al (1964) measured the specific osseous volume and the degree of resorption in iliac crest biopsies from osteoporotic cases. Garner and Ball (1966) have studied samples of undecalcified sections of iliac crest bone from cases of chronic renal azotaemia and intestinal malabsorption syndrome. Recently Dunnill et al (1967) using a point counting system estimated the volume of bone, haemopoietic and fatty marrow in the body of second lumbar vertebra from a series of autopsies. Paterson et al (1968) measured the amount of osteoid in undecalcified sections of iliac crest samples from cases of metabolic bone disease.

Up to the present moment very little work has been done on the measurement of the various components of bone from cases of metabolic bone disease and the correlation of these with clinical, biochemical and radiological studies. The histoquantitation studies needed for this work were obviously going to be lengthy, and to make the collection of data from these undecalcified sections less tedious, an instrument has been devised for semi automatic histoquantitation. The histoquantitation procedure will be discussed in detail in the appendix. Briefly the technique used is an adaption of that of Lazarow and Carpenter (1962), devised for the study of pancreatic islets. The basic principle is that of moving the section across a reference spot; the stage holding the section being motor driven. The motor is auto-

mated by any one of 5 different buttons, all of which produce the same movement, but each of which is connected to a different recorder. The instrument built for this work (Figs. 15 and 16) uses a projected image; the micrometer stage on the optical bench is moved by a stepping electrical motor (m). Each button is represented by two electromagnetic counters (c 1 and 2), the former records the distance (in microns) the stage moves while the button is depressed, the latter the number of times the buttons is depressed. When a section is projected, each component to be measured is allocated to one button, and that button is depressed as the component in question marks the reference spot. At the end of a traverse across the section, the 'line' drawn by the spot will have been broken down into its constituent components, and these will have been summed for each component. The number of intercepts for each component will also be known. The accuracy of functioning of the instrument is checked by measuring the total distance moved on the micrometer stage, and showing that this agrees with the sum of the intercept measurements.

This technique of histoquantitation not only allows the derivation of the composition of the amounts of calcified bone, osteoid and marrow, but also gives additional information with regard to the nature of bone surfaces, i.e., the percent total bone surface showing resorption, percent total bone surface covered with osteoid and percent total bone surface showing resorption which is covered with osteoid. In the past the amount of osteoid was determined either from the percent volume of osteoid in the cancellous area or from the percent of total bone surface covered with osteoid. Garner and Ball (1966) determined the percent volume osteoid in trabecular bone in quantifying sections. Morgan et al (1968) stated that the determination of the absolute amount of osteoid on the basis of measuring the width of osteoid seams might not reveal the true amount of osteoid present, because the osteoid seams might have an apparent increase in width if the sections are cut obliquely,

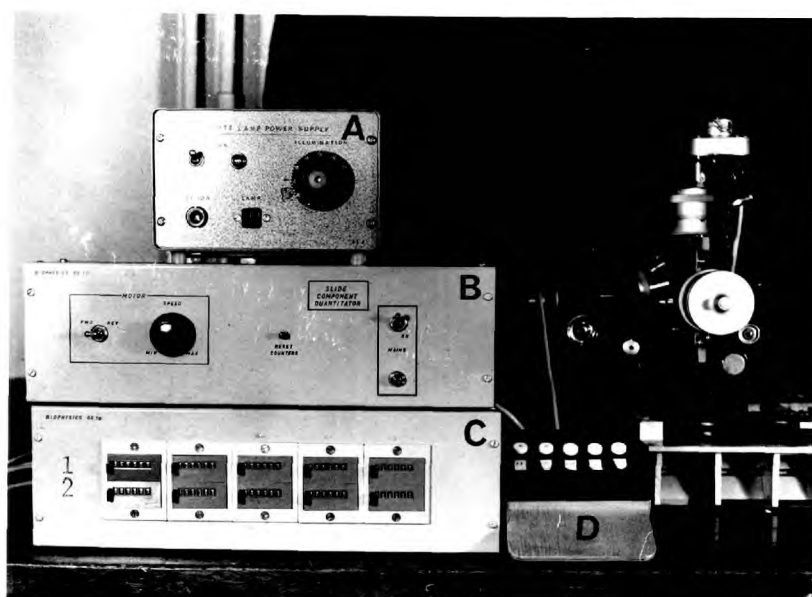


Fig. 15 Histoquantitation Equipment

- A. Quartz iodine lamp power supply.
- B. Slide component quantitator electronic control equipment, with variable speed control, forward and reverse control and counter rest.
- C. Slide component quantitator electromagnetic counters.
- D. Finger control buttons; one for each of 5 channels reading on separate counters.

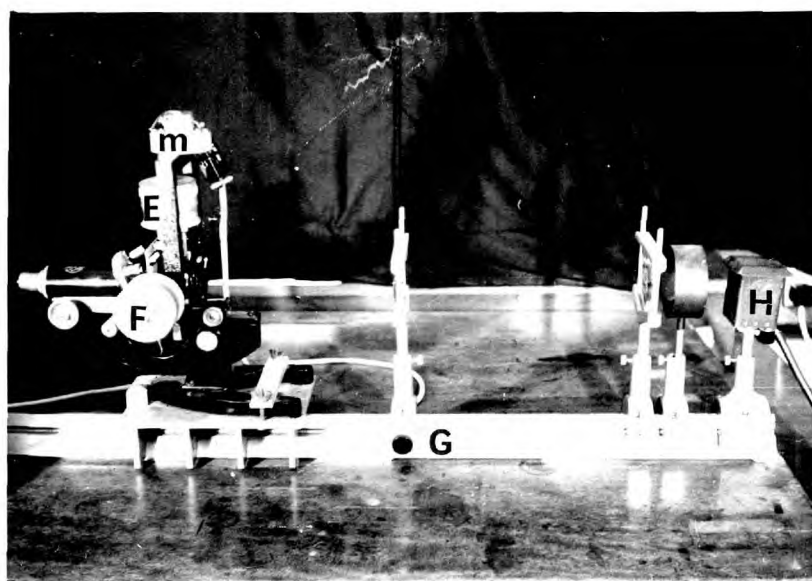


Fig. 16 Histoquantitation Equipment

- E. Vertical micrometer stage control with stepping electric motor (m).
- F. Horizontal micrometer stage control.
- G. Optical bench.
- H. Quartz iodine light source. (12 v. 100 w.)

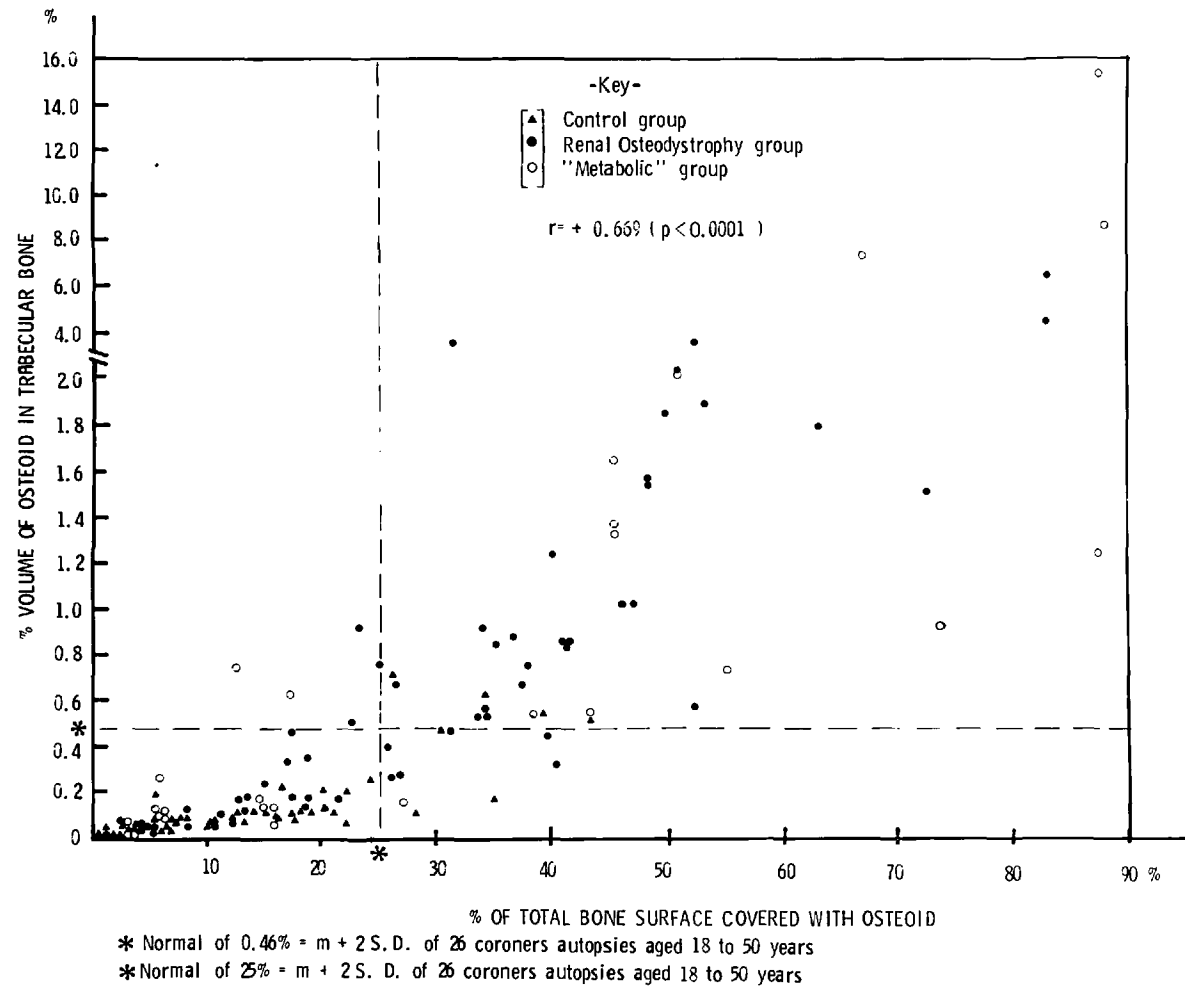
or osteoid, being softer than the neighbouring calcified bone, might spread if cut from celloidin embedded blocks. Border et al (1968) stated that the measurement of total bone surface covered with osteoid is sensitive indicator in detecting cases of osteomalacia. If the plastic embedded sections are cut properly and quantified accurately, both the percent surface covered by osteoid and the percent volume of osteoid can be reliably derived. It is theoretically possible to have considerable variation between the two variables; one can have a few large osteoid seams, another many very thin seams. A correlative study was therefore carried out between the percent volume of osteoid in trabecular bone and percent total bone surface covered with osteoid in all the cases which I have personally quantified. Indeed, there is a very good correlation between these two parameters as shown in Figure 17. ($r = + 0.67$, $p < 0.0001$). Inspection of the points show that the relationship is not a straight line but a curve; this, of course, would be expected in a situation where, to take an extreme case, the whole of the bone surface could be covered by osteoid but the percent volume osteoid could still increase. It is worthy of note that of the 156 points in Figure 17, only 4 are abnormal in terms of percent volume osteoid, but normal for percent surface osteoid; and 10 are abnormal in terms of percent surface osteoid but normal in percent volume osteoid. In this thesis the correlative studies with the biochemical and radiological findings have been carried out mainly with percent total bone surface covered with osteoid. The percent volume of osteoid in trabecular bone has been quantified in order to assess the amount of bone either calcified or non-calcified (osteoid) present, particularly in cases of renal osteodystrophy.

2.5. VALIDATION OF TECHNIQUE

In any quantitative study the results should clearly be reliable. To investigate this, three techniques have been employed :

Fig. 17

—COMPARISON OF THE AMOUNT OF OSTEOID AND THE PERCENTAGE OF BONE SURFACE COVERED WITH OSTEOID—



- (a) Requantitation of the same section by the same technique.
- (b) Requantitation of an immediately adjacent section by another technique.
- (c) Quantitation of a section from a different level of the same biopsy.

By using these three techniques, the degree of variation inherent in both the technique used and the biopsy itself can be estimated.

2.5.1. Reproducibility of Results

9 randomly selected sections were requantitated by the same technique. The results obtained from the two readings are shown in Table 1. In repeating the histoquantitation procedure, the line scans are set at regular intervals at 300-400 μ , but there was no overlapping with the previous line scans. It is interesting to note that there is no significant difference between the two readings.

2.5.2. Comparison of Results with those from an Automatic Technique

Histoquantitation results obtained by manual semi-automatic technique have been compared for 30 biopsies with those obtained by using an automatic multi-channel image analyser, the principle of which is discussed below. The results obtained by the two methods are shown in Table 2. The percent volume of calcified trabecular bone quantified by the two methods showed a very good correlation (Fig.18; $r = + 0.96$, $p < 0.0001$).

The working principle of the automatic multi-channel image analyser is as follows:-

An image from a microscope is transmitted by a black and white television camera to coder. Here the signals from the different components of the image are separated into seven channels depending on their light intensity. Each of these channels is arbitrarily assigned a colour code so that each colour shown on the television monitor corresponds to the light intensity radiated from the original image. The

information thus coded is also passed onto an electronic selector unit. The total length of the line of the television raster that is made up of each column^{our} is automatically measured and displayed on a counter.

In quantifying the amount of calcified bone present in the cancellous area, plastic embedded undecalcified sections of bone stained with von Kossa van Gieson are used. Histoquantitation of sections by the method of image analysis gives results within a short period of time as compared with the manual, semi automatic method which takes approximately two to two and a half hours from start to the final calculations. However, the manual method gives more information not only for the percent volume of calcified trabecular bone, but also for the other parameters, namely (a) percent volume of osteoid in the trabecular bone (b) percent of total bone surface covered with osteoid (c) percent of total bone surface showing resorption (d) percent of total bone surface showing resorption which is covered with osteoid. Moreover, artefacts which are inevitable in plastic embedded sections, could easily be ignored with the human eye, but the electronic eye is unable to differentiate between facts and artefacts.

2.5.3. Comparison of Results on Different Biopsy Levels

To observe the degree of variation in the amount of calcified trabecular bone present at different levels in the biopsy specimen; 10 randomly chosen blocks were re-embedded. Sections were prepared on the opposite side of the block (Fig.5, phase B) at a distance of 3-5 cms from the previous sections. The sections were quantified by the automatic image analyser. The results obtained are shown in Table 3. The minor variation thus seen from sample to sample is not statistically different.

These three studies validate the technique used and show that the degree of variation within the block is small.

TABLE 1REPRODUCIBILITY OF RESULTS

The following table shows the reproducibility of the results obtained from the same plastic embedded sections stained with Solochrome Cyanin R stain.

Case No.		Histological Measurements *				
		A	B	C	D	E
C.25 P.M. 12872	1st Reading	23.2	0	0	22.4	0
	2nd Reading	24.6	0.05	2.0	20.8	0
C.49	1st Reading	15.5	0.08	6.8	19.6	0
	2nd Reading	14.8	0.07	7.1	17.9	0
M.16	1st Reading	15.2	0.17	12.6	12.7	0
	2nd Reading	15.3	0.13	10.0	14.0	0
M.18	1st Reading	11.6	0.74	55.8	22.5	15.8
	2nd Reading	10.1	0.90	53.4	23.8	14.3
M.19	1st Reading	7.9	1.2	87.8	4.9	15.4
	2nd Reading	8.1	1.3	85.0	6.0	16.0
R.27	1st Reading	34.1	1.8	50.0	30.1	9.6
	2nd Reading	32.8	1.9	53.2	28.7	8.5
R.37	1st Reading	18.9	0.9	36.4	28.3	13.1
	2nd Reading	18.6	1.3	36.2	24.6	13.0
R.54	1st Reading	20.4	0.03	5.8	48.8	0.6
	2nd Reading	22.5	0.02	3.7	50.0	1.8
R.55	1st Reading	14.1	0.10	11.3	16.2	0.9
	2nd Reading	12.5	0.06	10.3	17.2	1.7

* A - % volume of calcified trabecular bone. B - % volume of osteoid in trabecular bone. C - % total bone surface covered with osteoid. D - % total bone surface showing resorption not covered with osteoid. (RS). E - % total bone surface showing resorption covered with osteoid (RS₀).

TABLE 3COMPARISON OF RESULTS ON DIFFERENT BIOPSY LEVELS

Comparison of results of the percent volume of calcified trabecular bone at different levels of the same biopsy obtained by the automatic image analysis

Case No.	1st Level	2nd Level
C.22	22.2	24.2
C.28	8.3	6.7
C.32	10.1	11.3
C.37	8.9	10.3
C.43	13.0	14.5
C.49	15.8	13.9
c*	7.3	9.6
M.14 (67/723)	12.7	13.9
M.19 (69/728)	7.5	8.1
R.39 (69/1262)	31.0	32.5

* aged female from coroner's case

TABLE 2

COMPARISON OF RESULTS WITH THOSE FROM AN AUTOMATIC TECHNIQUE

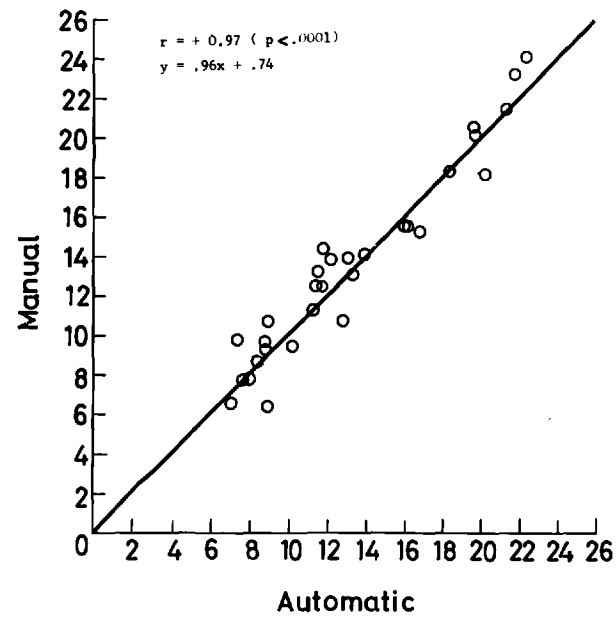
The following table shows results obtained by a manual method (A) as compared with the results obtained by means of an image analysis (B), on the same plastic embedded undecalcified sections from various cases; for the former method the sections are stained with solochrome cyanin R and for the latter the adjacent sections are stained with von Kossa-van Gieson stain. The figures represent the percent volume of calcified trabecular bone.

Case No.	Manual Method (A)	Image Analysis (B)
C.9.	24.1	22.5
C.22	20.2	22.2
C.25 (PM 12872)	23.2	20.9
C.28	8.8	8.3
C.32	9.5	10.1
C.33 (PM 12921)	8.1	7.9
C.34 (PM 12835)	15.6	16.0
C.35	16.4	13.5
C.36 (PM 12851)	12.5	10.9
C.37	10.7	8.9
C.41	9.4	8.7
C.43	10.8	13.0
C.45 (PM 12887)	9.7	8.4
C.47 (PM 12896)	20.8	20.8
C.49	15.5	15.8
C.*	9.8	7.3
M.14 (67/723)	13.3	12.7
M.16 (67/1200	15.2	16.9
M.19 (69/728)	7.8	7.5
M.23 (67/459)	18.2	19.7
M.31 (o/68/92)	13.9	13.7
M.33 (PM 12876)	6.6	7.0
M.36 (PM 12863)	11.4	11.1
R.18 (PM 12788)	18.3	18.4
R.19 (PM 12874)	13.9	12.2
R.21 (PM 12888)	20.6	19.7
R.22 (PM 12856)	13.1	13.3
R.25 (PM 12616)	14.1	13.9
R.42 (PM 12615)	6.4	8.6
R.56 (PM 12585)	12.5	11.4

* Aged female from coroner's case

Fig. 18

— COMPARISON OF % VOLUME OF CALCIFIED
TRABECULAR BONE BY TWO TECHNIQUES —



CHAPTER 3NORMAL BONE STRUCTURE3.1. INTRODUCTION

In any quantitative study of bone it is necessary to study the normal first, to determine its limits and thus define the abnormal. A number of studies have already been carried out on normals and the changes shown with age (Beck and Nordin, 1960, Lindahl and Lindgren, 1962, Garner and Ball, 1966, Sissons et al, 1967, Dunnill et al, 1967).

The relative frequency of bone disease in women suggests that sex may also be an important factor, and Dunnill et al (1967) did show a slightly lower proportion of vertebral trabecular bone in the female than the male. With this as a background it was obviously necessary to establish the normal range for different ages and sexes using the same techniques as would be used to study the abnormal group. It was not anticipated that the technique used here would give any different results from other workers' techniques, but there are certain subjective variables, in particular the definition of the border between trabecular and cortical bone, which may be interpreted differently by different workers.

The present chapter describes the results obtained from histoquantitative studies based on 8 μ undecalcified sections of plastic embedded iliac bone biopsies in a group of 50 normal cases representing 31 males and 19 females, age ranging from 18 to 89 years as shown in Table 4.

3.2. CASES STUDIED

Iliac bone samples were mainly obtained from coroners and routine autopsies performed at Finchley Mortuary and in Hammersmith Hospital. The cases represented those of sudden death (usually patients brought in dead, or dying within three days of admission), mainly due to road accidents, suicides, poisoning, cerebrovascular accidents, myocardial infarction, etc. Patients dying after three days of hospitalisation or with evidence of any chronic disease have been excluded from the control series.

TABLE 4 **The Control Group**
(From Coroner's autopsies of sudden death and from routine autopsies dying within 3 days of hospitalisation, excluding any hepatic, renal or alimentary disorders).

Case No	Sex & Age	HISTOLOGICAL MEASUREMENTS *				
		A	B	C	D	E
C.1	F.18	15.7	0.08	13.5	11.5	0
C.2	F.23	28.1	0.03	6.3	16.7	0
C.3	F.26	19.9	0	7.3	7.3	0
C.4	F.29	17.8	0.07	5.1	12.8	0
C.5	F.36	14.3	0.2	4.8	9.5	0
C.6	F.40	17.1	0	1.8	10.7	0
C.7	F.44	14.6	0.02	2.7	12.2	0
C.8	F.50	22.7	0.02	1.5	8.9	0
C.9	M.19	24.1	0.10	11.3	11.8	0
C.10	M.26	32.6	0	0	8.8	0
C.11	M.28	20.5	0.02	3.8	21.2	0
C.12	M.30	17.8	0.73	26.3	12.3	0
C.13	M.30	28.1	0.14	18.2	6.8	0
C.14	M.34	33.6	0.26	24.3	7.1	0
C.15	M.34	23.1	0.08	5.1	7.8	0
C.16	M.37	24.9	0.04	2.3	10.3	0
C.17	M.37	19.5	0.21	22.2	15.6	0
C.18	M.39	28.6	0.57	39.8	14.3	0
C.19	M.41	23.6	0.08	5.7	31.4	0
C.20	M.41	23.2	0.09	8.0	14.7	0
C.21	M.42	13.3	0.05	2.8	8.5	0
C.22	M.45	20.2	0.13	12.2	15.8	0
C.23	M.46	20.4	0.14	20.0	6.0	0
C.24	M.49	17.5	0.12	17.9	10.7	0
C.25(PM12872)	M.49	23.2	0	0	22.4	0
C.26	M.50	18.7	0.02	6.4	17.0	0
C.27(PM12877)	F.56	14.6	0.51	43.2	16.2	0
C.28	F.59	8.8	0.01	6.9	17.6	0
C.29(PM12838)	F.59	10.2	0.04	9.8	8.5	0
C.30	F.67	17.2	0.43	30.3	14.6	0
C.31	F.67	9.2	0.05	6.5	14.7	0
C.32	F.71	9.5	0.13	28.2	12.1	0
C.33(PM12921)	F.72	8.1	0.14	16.0	12.0	0
C.34(PM12835)	F.74	15.6	0.08	17.6	10.9	0
C.35	F.79	16.4	0.09	16.3	8.8	0
C.36(PM12851)	F.86	12.5	0.08	10.6	19.1	0
C.37	F.89	10.7	0.11	18.8	17.4	0
C.38	M.51	13.3	0.08	10.6	23.4	0
C.39	M.51	16.6	0.13	14.0	21.1	0
C.40	M.51	13.7	0	6.1	6.1	0
C.41	M.52	9.4	0.06	22.2	18.3	0
C.42	M.52	12.3	0	2.1	18.4	0
C.43	M.55	10.8	0.23	16.6	12.2	0
C.44(PM12871)	M.57	19.0	0.64	34.4	14.1	0
C.45(PM12887)	M.63	9.7	0.19	35.1	16.9	0
C.46(PM12880)	M.63	13.7	0.08	7.7	19.2	0
C.47(PM12896)	M.65	20.8	0.13	15.1	15.1	0
C.48(PM12886)	M.69	14.8	0.12	21.2	16.5	0
C.49	M.77	15.5	0.08	6.8	19.6	0
C.50	M.81	7.6	0.21	20.0	6.7	0

* A - % volume of calcified trabecular bone.

B - % volume of osteoid in trabecular bone.

C - % total bone surface covered with osteoid.

D - % total bone surface showing resorption not covered with osteoid (R.S.)

E - % total bone surface showing resorption covered with osteoid (R.S._o).

The iliac bone samples were taken at a point 2.5 cms below and behind the anterior superior iliac spine by a modified bone biopsy trephine needle as described by Sacker and Noëin (1954). Occasionally the relevant portion of the iliac crest was sawn off from the pelvis and later the tissue was trimmed down to a size comparable with the biopsies taken from the living subjects. The samples thus obtained were immediately preserved in buffered formol saline (pH 6.8-7.0) and then embedded in resin; the detailed procedure is given in the Appendix.

3.3. RESULTS

The overall results have been set out in the form of a tabulated chart at the end of this chapter for quick and easy reference.

The age changes have been studied not only decade by decade but also by comparing groups below and above 50 years of age. The results have been expressed in this way partly because a great majority of the renal failure patients are below 50 years of age and partly for comparison with the series of Garner and Ball (1966) who also used 50 as a dividing line.

Bone Volume

The amount of calcified bone in the cancellous area is expressed as a percentage of the area of cancellous bone measured. Bone volume is negatively correlated with age in both males and females where the correlation coefficient (r) equals -0.683 and -0.59 respectively (Figs. 19 and 20); if the male and female data are taken together, $r = -0.64$. The regression of bone volume in males and females on age were also studied by analysis of covariance (Moore and Edwards, 1965). The results of this are summarised in an Analysis of Variance Table. (Table 5).

TABLE 5

Analysis of Variance Table

Regression of Bone Volume in Males and Females on Age

Source	Sum of Squares of Observations (s.s.)	Degrees of Freedom (d.f.)	Mean Square M.S. = $\frac{s.s.}{d.f.}$	Mean Square Ratio (M.S.R.)
Overall slope for both sexes	797.45	1	797.45	36.60 ***
Comparison between male and female slopes	102.46	1	102.46	4.70 *
Average within group slope vs. slope of means	70.48	1	70.48	3.23 N.S.
Residual	1002.30	46	21.79	

*** Significant at 0.1% level

* Significant at 5% level

N.S. Not significant

Fig. 19

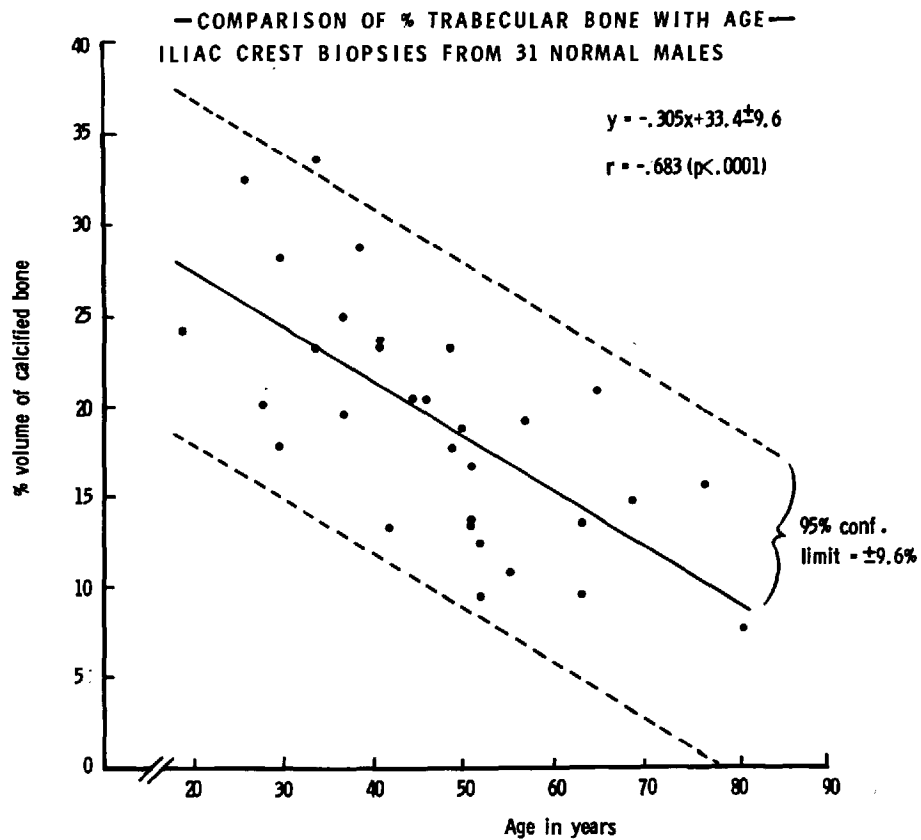
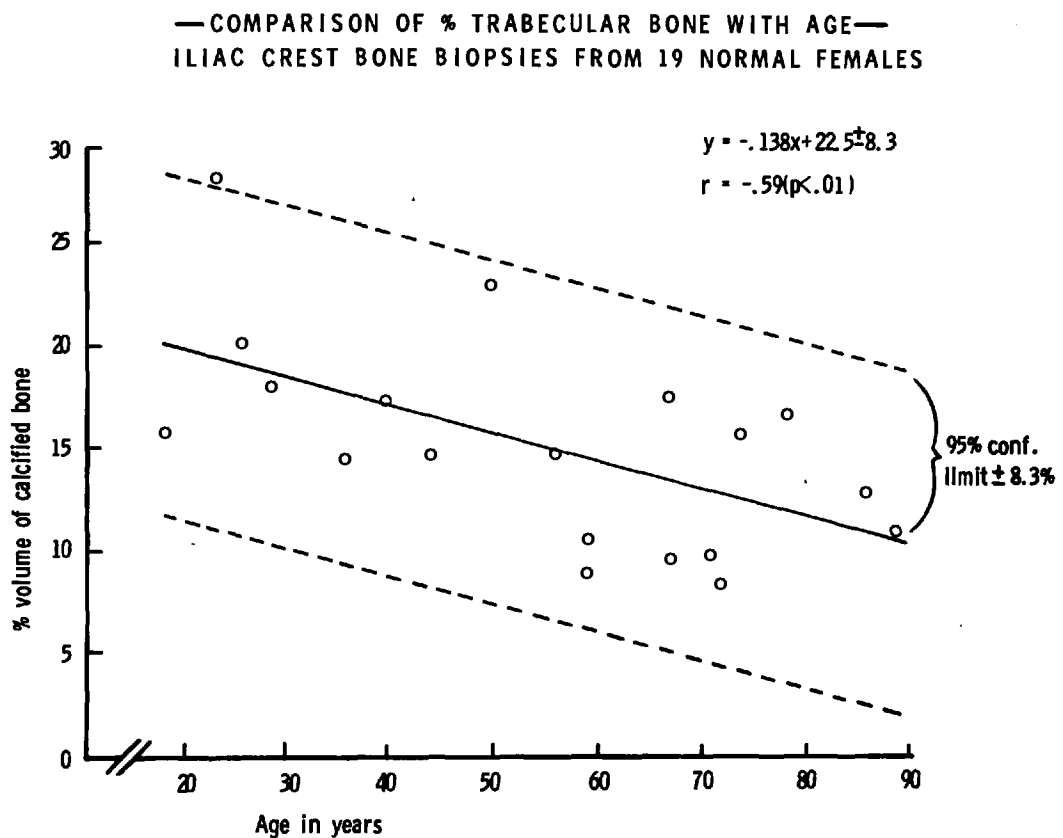


Fig. 20



It is shown that the overall slope of the regression is significantly different from zero ($p < 0.001$) and that there is a difference between the slopes for men and women ($p < 0.05$).

The variation in the amount of calcified trabecular bone with age and sex is also presented in Figure 21 by comparing the mean values for biopsies from patients aged 50 and below with the mean for those above 50. The mean calcified bone is significantly less in both men and women over the age of 50 than in men and women under 50. ($p < 0.01$ in women, $p < 0.001$ in men). Differences between men and women in both age groups are not significant. The results are as follows :-

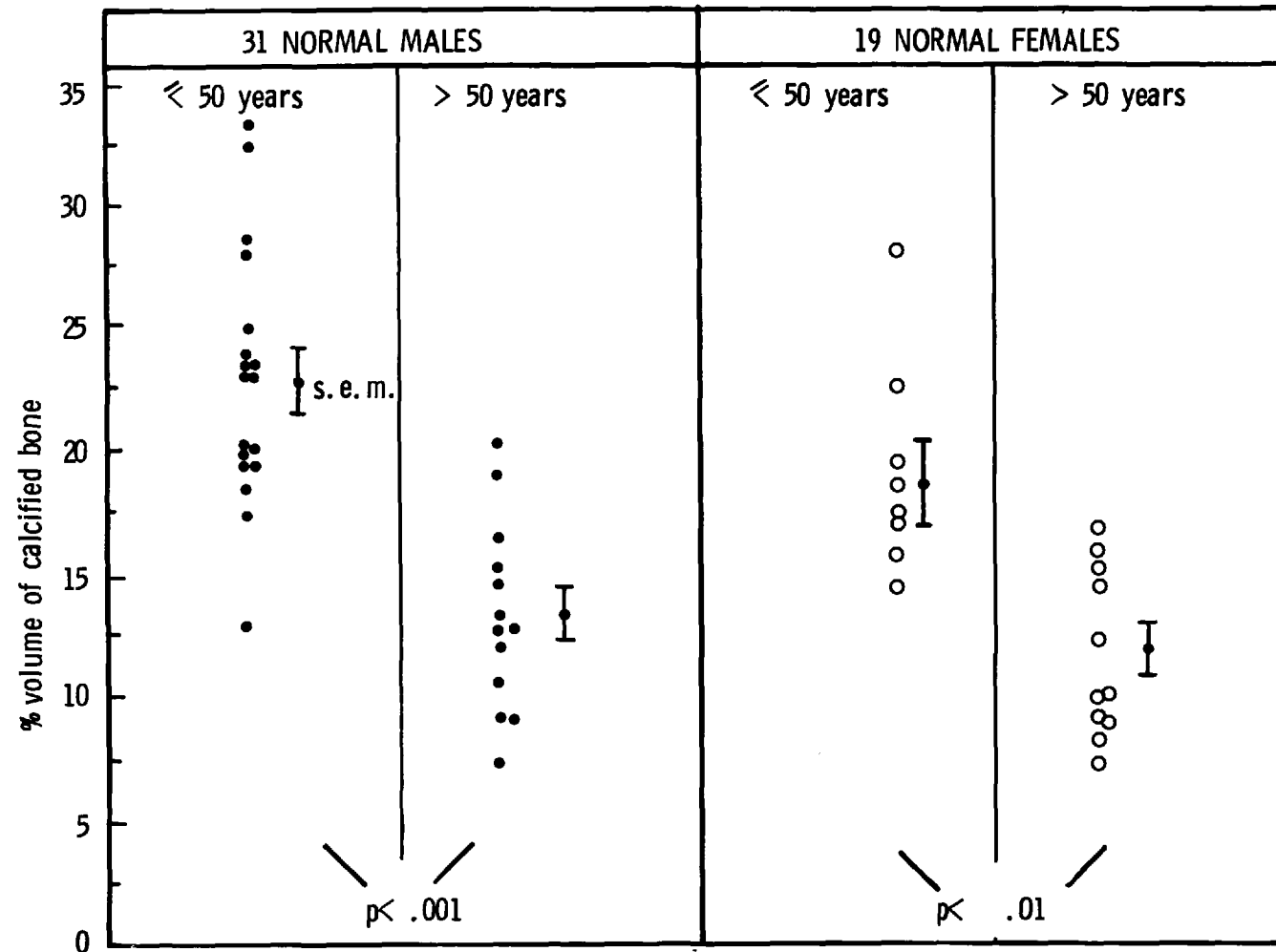
Age	<u>Males</u>		<u>Females</u>	
	≤ 50	> 50	≤ 50	> 50
Mean	22.94	13.63	18.77	12.07
S.D.	5.25	3.80	4.72	3.33
S.E.M.	1.24	1.05	1.67	1.00
n	18	13	8	11

When the sexes are combined the results are as follows :-

For males and females ≤ 50	$m \pm \text{s.e.m.} = 21.7 \pm 1.1$
	$m \pm 2 \text{ S.D.} = 21.7 \pm 10.7$
For males and females > 50	$m \pm \text{s.e.m.} = 12.9 \pm 0.7$
	$m \pm 2 \text{ S.D.} = 12.9 \pm 7.2$

Fig. 21

— COMPARISON OF % TRABECULAR BONE WITH AGE
IN ILIAC CREST BIOPSIES —



Osteoid Volume

The percentage amount of osteoid in trabecular bone has also been quantified. The mean for this parameter is not significantly different in men and women of either age group and is not significantly different in men and women aged 50 and less as compared with men and women over the age of 50. The values for the amount of osteoid in the trabecular bone are as follows :

	<u>Males</u>		<u>Females</u>	
Age	≤ 50	> 50	≤ 50	> 50
Mean	0.15	0.15	0.04	0.15
S.D.	0.19	0.16	0.04	0.16
S.E.M.	0.05	0.05	0.01	0.05
n	18	13	8	11

When the sexes are combined the results are as follows :

For males and females ≤ 50 $m \pm \text{S.E.M.} = 0.12 \pm 0.03$

$m \pm 2 \text{ S.D.} = 0.12 \pm 0.34$

For males and females > 50 $m \pm \text{S.E.M.} = 0.15 \pm 0.03$

$m \pm 2 \text{ S.D.} = 0.15 \pm 0.32$

Osteoid Surface

The percentage of total bone surface covered with osteoid has also been quantified. The results obtained for this parameter are shown in Figures 22 and 23. In the former the results are shown with the dividing line at the age of 50 years, whereas in the latter the data are compared directly with age. Four control cases (C18, PM12877, 12887 and 12871) were more than the three standard deviations above the means for the rest of the group and have not been used in calculating the normal range. These are considered to represent undiagnosed osteomalacia; and it is worth noting that three of the four cases are above 50 years of age. As shown in Fig.22, the difference between the mean in females ≤ 50 and > 50 is significant ($p < 0.01$). Difference between males ≤ 50 and > 50 and between males and females in both age groups are

Fig. 22

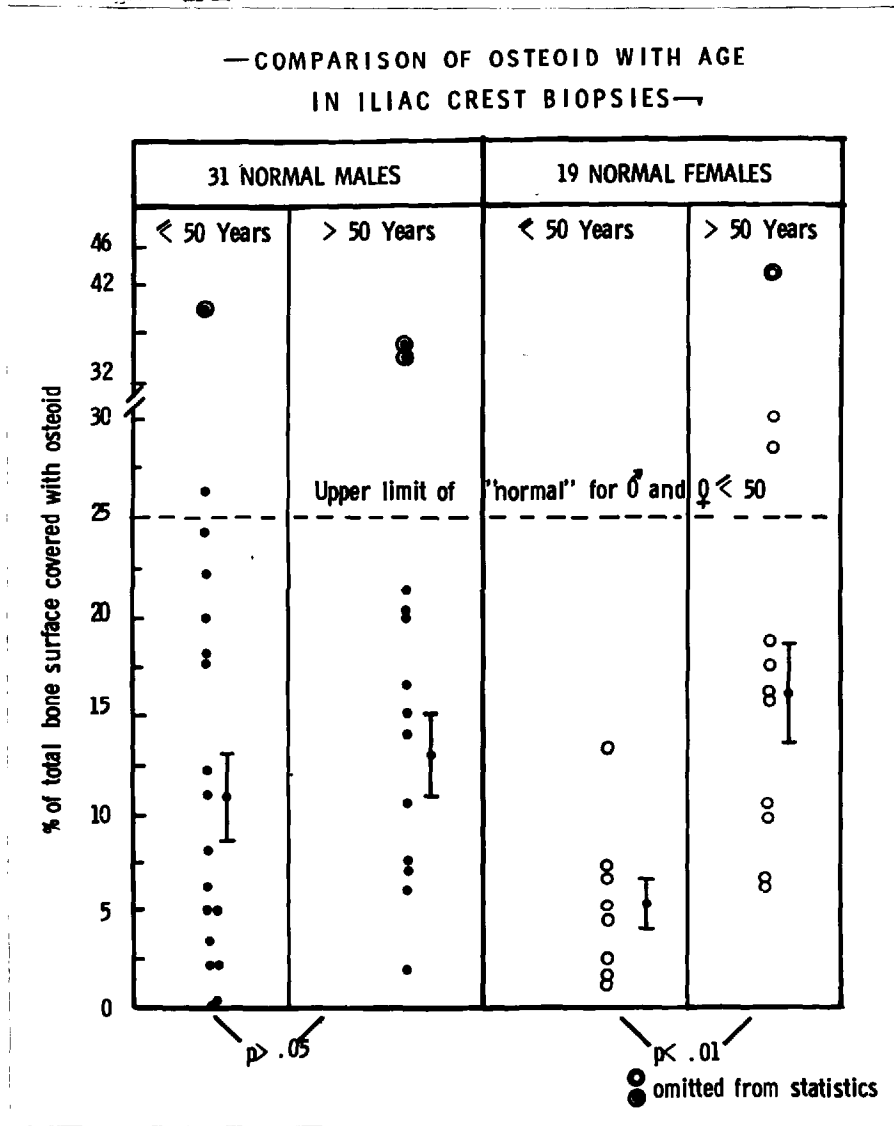
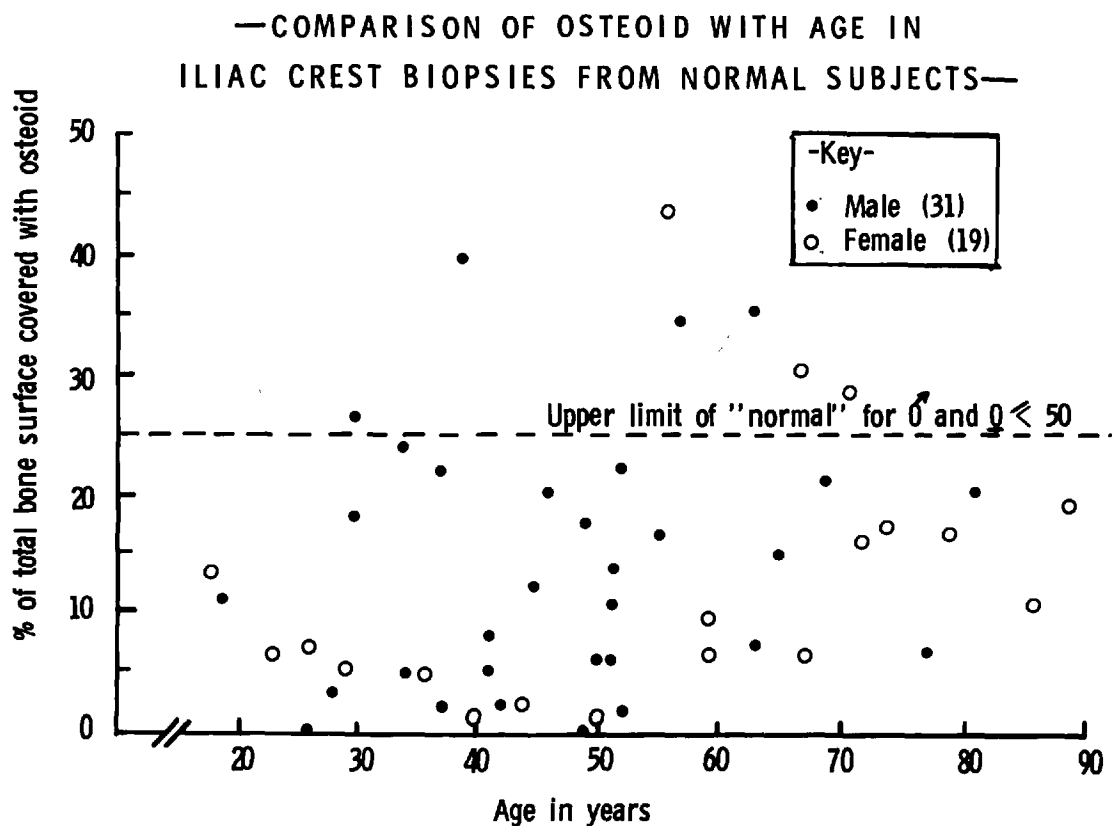


Fig. 23



Resorption Surface

Finally the percentage of bone surface showing evidence of resorption has also been quantified. The individual figures are plotted as shown in Figures 24 and 25. The measurement of resorption surface in on case (C19) is more than the three standard deviations above the mean for the group and has not been used to calculate the normal range. There is no significant difference between age groups of either sex or between men and women of either age group. While both sexes show a slight increase in resorption with age the difference between the means for the combined sexes is not significant.

Age	<u>Males</u>		<u>Females</u>	
	≤ 50	> 50	≤ 50	> 50
Mean	12.41	15.95	11.20	13.81
S.D.	4.91	5.17	2.85	3.61
S.E.M.	1.19	1.43	1.01	1.09
n	17	13	8	11

When the sexes are combined the results are as follows :-

For males and females ≤ 50 $m \pm \text{s.e.m.} = 12.0 \pm 0.9$

$m \pm 2 \text{ S.D.} = 12.0 \pm 8.7$

For males and females > 50 $m \pm \text{s.e.m.} = 14.9 \pm 0.9$

$m \pm 2 \text{ S.D.} = 14.9 \pm 9.1$

3.4. DISCUSSION

The results clearly show the necessity for age and sex matched controls in any study of bone disease. The most dramatic change is the loss of trabecular bone with age. This is in accord with previous workers and indeed there is good agreement between the actual figures, although separation between males and females is not usual in previous work.

Garner and Ball (1966) using undecalcified sections (6 u) of celloidin embedded iliac crest biopsies taken from above downwards found that the mean value for percent calcified bone below the age of 50 was 18.5%, and

Fig. 24

—COMPARISON OF BONE RESORPTION WITH AGE
IN ILIAC CREST BIOPSIES—

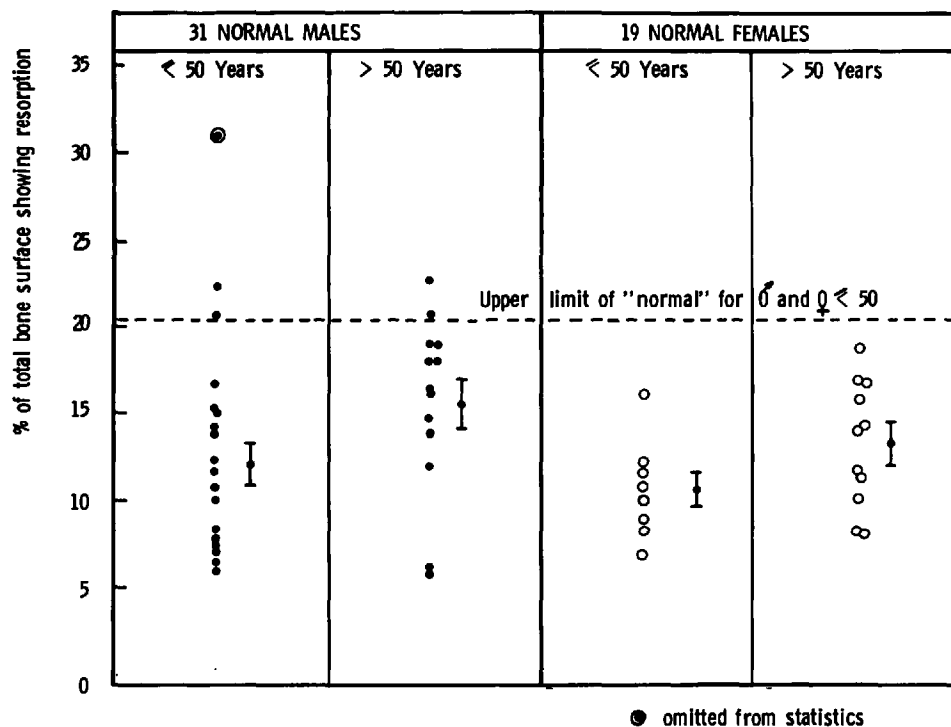
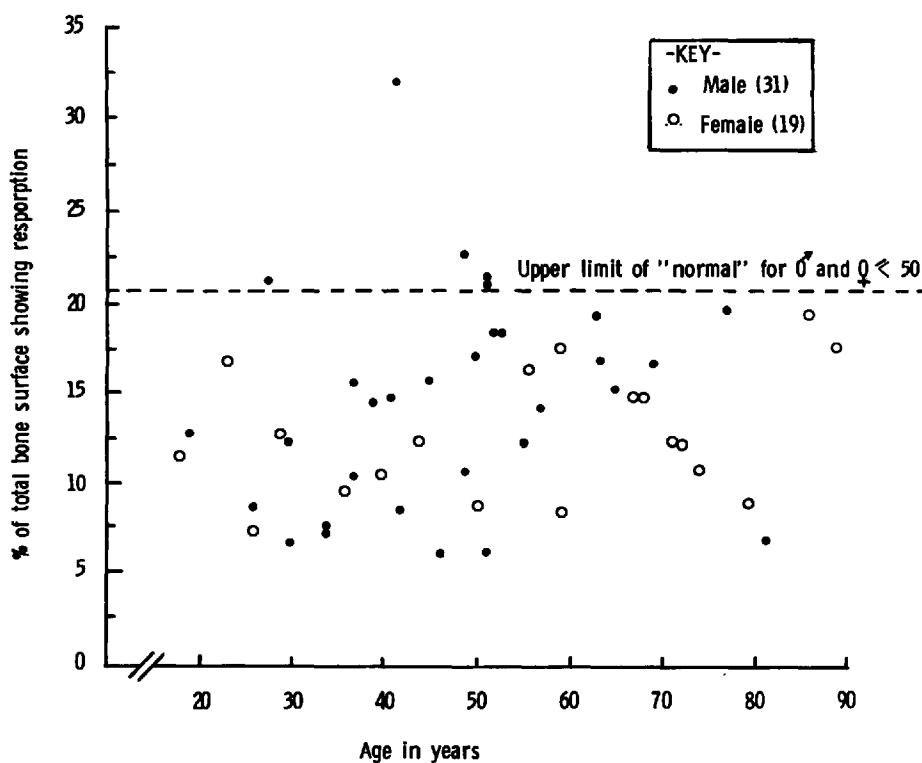


Fig. 25

—COMPARISON OF BONE RESORPTION WITH AGE
IN ILIAC CREST BIOPSIES FROM NORMAL SUBJECTS—



above 50 was 12.8%. The comparable figures for this control series are 21.7% and 12.9%. It is worth noting that the ratio of sexes differ in the two series, as do the scatter of ages; in addition, Garner and Ball included six children in their slightly smaller control series. They concluded that the amount of trabecular bone was fairly constant up to the age of 50 but declined progressively thereafter. The control series presented here appears to show a steady decline in the amount of calcified trabecular bone both for males and females throughout adult life. Sissons et al (1967) did not express their results in the same way, but it can be calculated that the mean value for bone area in iliac crest sections from control patients below 50 were about 21% and above 50 about 16% - correspond to 21.7% and 12.9% for this control series. Their figures for iliac crest can be interpreted as probably showing a steady decline with age; their figures for femoral cortices much more clearly show a steady decline in bone area with age.

The difference between the sexes in terms of the percent volume of trabecular bone was not significant in this study; comparable data are hard to find. Garner and Ball (1966) did not deal with their data separately, but it is possible to extract them from the tables given; there is no significant difference in the means for the different sexes. Arnold (1964) found that the ash content of the lumbar vertebra decreases progressively with increasing age in both sexes. Dunnill et al (1967) found a very slightly lower percent trabecular volume for vertebral bone in females than in males. Bordier et al (1967) and Sissons et al (1967) did not deal with the sexes separately. Although Sissons did plot the result for the sexes separately, he commented that no obvious sex difference was apparent.

The reasons for the drop in the amount of trabecular bone with age remain speculative; the fact that the drop is greater both absolutely and relatively in men than in women, and that no abrupt change at the menopause has been demonstrated suggests, that hormonal factors may not be as important as was once thought. The recent work of Doyle et al (1970) relating bone mass to muscle mass suggests an additional factor which may well prove to be important in age related bone loss.

The amount of bone surface covered with osteoid and the percent volume of osteoid have also been correlated with age and sex. One of the best studied comparable control series is again that of Garner and Ball, although they studied the volume of osteoid rather than the percent of bone surface covered with osteoid. They give the normal range for this measurement, based on a mixed group of 22 cases aged from 3 to 50, as 0.0 to 0.47 with a mean of 0.23%. This compares to a range of 0.0 to 0.46, with a mean of 0.12% for 26 cases aged from 18 to 50 for this control series. If the six youngest patients (aged 3 to 16) are excluded from the Garner and Ball series, the mean for the remaining 16 cases falls to 0.16%, much closer to the 0.12% for this control series. The mean figure for osteoid volume for 19 patients over the age of 50, calculated from the figures they give is 0.16%, compared to 0.15% for the 24 cases in my series. When the control figures for the percentage of bone surface covered with osteoid are studied, it is found that there is a statistically significant increase in the amount of osteoid in women over 50 as compared with younger women. This excluded one case, a woman of 56 with 4.3% of bone surfaces covered with osteoid, a figure more than three standard deviations above the mean. Two other women over 50 who gave figures for percent bone surface covered with osteoid that were more than two standard deviations above the mean for males and females were included. These figures could be interpreted as showing that the sub-clinical osteomalacia is more common in elderly females than in other age and sex groups; an observation in agreement with the findings of Chalmers

et al (1967). Indeed if the two cases who showed osteoid surfaces more than two standard deviations above the mean were excluded, the mean for females over 50 falls to 12.8% - extremely close to the figures for males of the same age group (12.9%). Sissons et al (1967) concluded that there was no great change in the amount of osteoid surface in iliac crest cancellous bone with age; the overall mean value being about 12%. They noted, as I have, the greater scatter of results in the elderly; but also commented on the presence of a considerable scatter of results in the younger age group. Bordier et al (1967) found a very similar mean figure (14.6%) for osteoid covered surfaces in iliac crest bone biopsies from 20 control subjects.

The amount of bone resorption surface has also been quantified in a manner similar to that for osteoid covered surfaces. It is of interest to document that the amount of bone resorption is relatively constant with increasing age. The results for this parameter shows a mean of 12.0 ± 0.9 (range 3.4-20.6). This finding is in agreement with that of Bordier et al (1967) who showed a figure of 13 ± 8 (mean ± 2 S.D.) for the percent of bone surface occupied by resorption in the cancellous area in 20 control patients. Jowsey (1960), and with her co-workers (1965) showed an increased bone resorption with age. Sissons et al (1961) also held the same view but their later studies suggested that increasing age has little effect on bone resorption, although values appeared to be somewhat increased above 60 years of age. They found an overall mean value of 11% (Sissons et al 1967).

The overall results of these control studies therefore show a drop in cancellous bone with age, the rate of fall being rather more steep in males than in females. The amount of osteoid covered surface rises slightly with age but this is significant only in females. The amount of resorption surface shows a very slight, but not significant, rise. The figures for these various parameters are in broad agreement with those quoted by other workers. The loss of trabecular bone with age despite a slight increase

in osteoid and a relatively constant resorption rate is paradoxical only if the osteoid covered surface is equated with formation surface. This is probably broadly true in the normal individual, Van der Sluis Veer et al (1964) found that 12% bone surfaces in iliac crest cancellous bone in 17 controls took up tetracycline; this indicating bone formation. This figure is remarkably close to the overall 13.7% from overall osteoid covered bone surfaces in my control series. Bordier et al (1967) used the 'calcification front' as a marker of bone formation and found that 75% of osteoid covered surfaces showed calcification fronts. However, as already described, the most likely interpretation of high osteoid figures in the elderly females is the presence of subclinical osteomalacia, in which the osteoid covered surfaces cannot be considered bone formation surfaces, as there is a block in the calcification of osteoid. These control studies then have provided the data necessary as a yard-stick against which the changes in metabolic bone disease and renal failure can be compared, and have also shown that while the amount of cancellous bone declines slowly with age, the amount of osteoid and the resorption surface remains broadly constant. The change in balance between formation of bone and bone resorption need only be slight to produce the observed fall in the percent of cancellous bone.

Summary of Results

The results are expressed $m \pm \text{s.e.m.}$, and the figures in brackets represent the normal range = $m \pm 2 \text{ S.D.}$

	<u>M & F ≤ 50</u>	<u>M & F > 50</u>
% volume of calcified trabecular bone.	21.7 ± 1.1 (11.1 - 32.2)	12.9 ± 0.7 (5.7 - 20.1)
% volume of osteoid in the trabecular bone.	0.12 ± 0.03 (0 - 0.46)	0.15 ± 0.03 (0 - 0.47)
% of total bone surface covered with osteoid.	9.2 ± 1.6 (0 - 25.0)	14.5 ± 1.6 (0 - 29.3)
% of total bone surface showing resorption.	12.0 ± 0.9 (3.4 - 20.6)	14.9 ± 0.9 (5.9 - 23.9)

The parameter for the amount of calcified trabecular bone has also been expressed on the basis of different age and sex groups and the results are shown below :-

% Volume of Calcified Trabecular Bone

In males ≤ 50	22.9 ± 1.2	(12.4 - 33.4)
In males > 50	13.6 ± 1.1	(6.4 - 21.2)
In females ≤ 50	18.8 ± 1.7	(9.3 - 28.2)
In females > 50	12.1 ± 1.0	(5.4 - 18.7)

3.5. SUMMARY

Histoquantitative studies were performed on 8 u undecalcified sections of plastic embedded iliac bone biopsies in a group of 50 normal cases representing 31 males and 19 females, ages ranging from 18 to 89 years. The overall results from this control group show a drop in the amount of calcified trabecular bone with advancing age, the rate of decline is steeper in males than in females. When the results from these patients of 50 or less are compared with those above 50, the amount of calcified trabecular bone is significantly less for both males and females in the older age group. The amount of bone surface that is covered with osteoid rises with age in both sexes, but the difference is significant only in females. The amount

of bone surface showing resorption is slightly higher in the older than in the younger age group of both sexes, but the difference is not significant.

CHAPTER 4.OSTEOPOROSIS

"The regular decay of nature which is called old age, is attended with changes which are easily detected in the dead body; one of the principal of these is found in the bones, for they become thin in their shell and spongy in their texture".

Sir Astley Cooper, 1824.

4.1. INTRODUCTION

The subject of osteoporosis has been of great interest for over a century. Formerly this disease process was thought to be that of old age only, but the disease although rare, has also been well recognised in young individuals. The disease process has different connotations to the histologist, the radiologist and the clinician. The general problem of osteoporosis is full of difficulties, mainly owing to the loose use of the term to denote any form of radiological bone rarefaction. It is more confused when the term has been used as a synonym for osteomalacia.

Osteoporosis is not a single disease entity, but it is a generic name for a group of disease. Since the pioneer work of Albright and co-workers (1940, 1948) the aetiologies of some types of osteoporosis have been fairly well known and many workers have also revealed the association of osteoporosis with various disease states, although the pathogeneses remain obscure and speculative. The pathogenesis of varied aetiologies of osteoporosis has been discussed repeatedly and extensively by Bartter (1957), Urist (1960), ^{Nordin et al} (1964), and Dent and Watson (1966).

Whatever the nature of speculations for the pathogenesis with respect to different aetiologies may be, the osteoporosis which is usually a clinical and radiological term is commonly classified into primary or idiopathic and secondary. In the former the cause is unknown, it is commonly seen not only in 'senile' or 'post-menopausal' states, but also in young adults (Jackson, 1958, Dent and Friedman, 1965). The cause of osteoporosis

in old age may be related to an imbalance of hormones, notably of oestrogens and androgens (Albright and Reifenshtein, 1948) or may occur as a part of general tissue atrophy (Fourman, 1955).

In the secondary group of osteoporosis most of the cases have been associated with different endocrine disorders, e.g., thyrotoxicosis, hyperadrenocorticism, acromegaly, and hypogonadism (Albright and Reifenshtein, 1948). Some are associated with nutritional disorders, primarily of protein malnutrition (Platt and Stewart, 1962), calcium deficiencies (Crawford et al, 1957, Nordin, 1960, Spencer et al, 1964). Dent and co-workers (1966) have also documented the association of osteoporosis with various diseases, e.g., chronic bronchitis, arachnodactyly, epilepsy and osteogenesis imperfecta tarda. A few workers have reported the finding of osteoporosis in heparin administration (Griffith et al, 1965) and in alcoholics (Saville, 1965).

In the past the diagnosis of osteoporosis has mainly been based on the clinical and radiological findings, but in recent years various workers have carried out correlative studies between the radiological findings and chemical estimations of bone in order to be able to evaluate general porosity of the skeletal system. For instance, Caldwell and Collins (1961) studied, by radiological, chemical and histological examination, lumbar vertebrae from necropsy specimens with a view to determine the range of variation of calcium content and radiographic density in normal and osteoporotic subjects. Lindahl and Lindgren (1962) also studied the apparent density of tibia and vertebrae in autopsy materials, to grade osteoporosis. Vost (1963) also stated that calcium expressed as weight per unit volume of bone is the best index for assessing osteoporosis in necropsy specimens. Some workers have also attempted to grade osteoporosis in the living subjects by means of radiological and bone density methods, e.g., the study of phalanges (Schraer and Neuman, 1958, Koch and Kaplan, 1961) and from ulna bones (Doyle 1961). Beck and Nordin (1960) also studied iliac crest samples obtained from necropsy examinations and prepared a visual 9 point scale for the estimation

of osteoporosis. They found fairly good agreement with spinal x-rays in patients with suspected osteoporosis.

Pommer (1885) recognised osteoporosis as 'simple atrophy' of bones. He distinguished it from osteomalacia with an excess of osteoid due to lack of vitamin D. The disease process affects the skeletal system in a generalised manner, and the vertebrae, sternum, sacrum, ribs and iliac crests are predominantly affected. Histologically osteoporosis has generally been defined as a reduction in the bone mass per unit volume of the skeleton, although the bone tissue is reduced in amount there is no considerable change in its chemical composition (Nordin, 1964).

While the bone overall may be of reduced strength, the individual trabeculum is not softened, unlike osteomalacia. The cortical bone becomes thin and well demarcated from the long, slender, widely separated trabeculae as shown in Figure 26. The diagnosis and assessment of severity of the disease process should, on the basis of the preceding comments, logically be based on histological measurements. However, any such measurement must, because of the known variations in the amount of bone with age and sex, use matched controls. Sampling presents another difficulty, the iliac crest biopsy material may not give a true picture of the state of the trabecular bone in the axial skeleton (Dunnill et al, 1967) although according to Jowsey and Gershon-Cohen (1964) and (Jowsey, 1965), the iliac crest bone biopsy is representative.

The biggest single difficulty is to perhaps define the disease under study. Clinically the term is used rather loosely as a diagnostic label for patients with radiologically 'thin bones' particularly if spontaneous vertebral fractures are present (Fig. 27) and no other cause can be found. 10 such cases in which a diagnosis of primary or idiopathic osteoporosis was made on clinical grounds have been studied and iliac crest bone biopsies quantified.

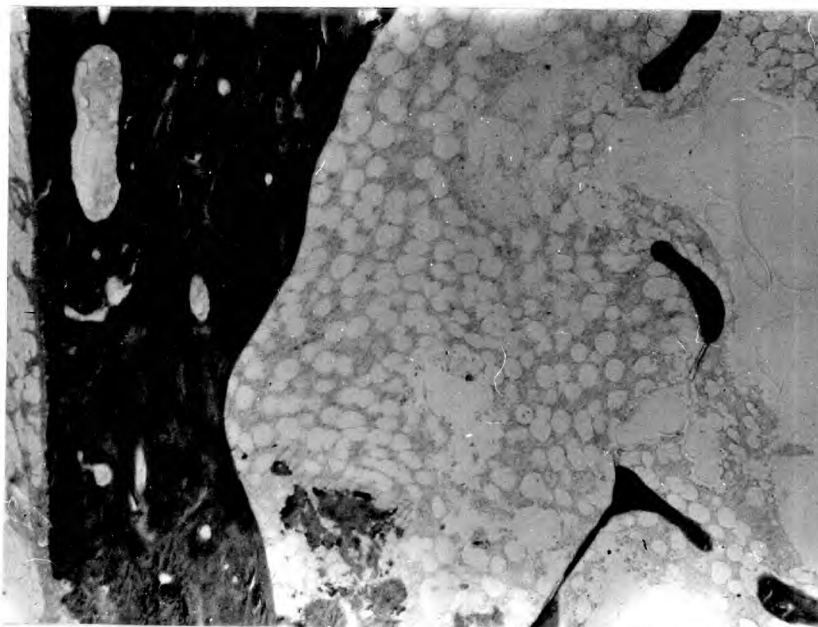


Fig. 26 An undecalcified section from a case of Osteoporosis showing a thin cortex and a trabecular bone.
(Pt. M.9. A.C., Solochrome cyanin x 30)



Fig. 27 Radiology of the thoracic spine from a patient with Osteoporosis, showing vertebral fractures.
(Pt. M.5. I.V.)

4.2. PATIENTS STUDIED

The patients studied in this group of selected osteoporotics shown in Table 6, were admitted to the Metabolic Ward of the Hammersmith Hospital for investigation and treatment of skeletal rarefaction with or without accompanying vertebral fractures. In all the patients except one, the serum biochemistry, including serum calcium, phosphorus, alkaline phosphatase and total proteins, was normal. The exception (M.5. I.V.) was diagnosed initially as possible hypophosphatasia on account of very low serum alkaline phosphatase; later this diagnosis was excluded because the patient had normal urinary excretion of phospho-ethanolamine. 24 hour strontium space was estimated in five patients and all were within the normal range.

4.3. RESULTS

The results obtained by histoquantitation of the iliac bone biopsies in 10 patients with clinical and suspect osteoporosis are set out in Figure 28 for the volume of calcified trabecular bone, Figure 29 for the percentage amount of bone surface covered with osteoid, and Figure 30 for the percentage amount of bone surface showing resorption. The results for the volume of calcified trabecular bone showed that 8 out of 10 patients studied lie in the lower half of the normal range (Mean ± 2 S.D.), one lying just above the mean value and one just below the lower limit of normal. The seven patients with vertebral fractures do not show significantly more loss of trabecular bone than the three without.

In 8 out of 10 of these patients the amount of osteoid present was within the normal range; in two, both women, the amount was slightly elevated.

In all these patients the amount of resorption present was within the normal range.

TABLE 6

CASES ADMITTED FOR INVESTIGATION OF 'CLINICAL AND SUSPECT' OSTEOPOROSIS

G E N E R A L				RADIOLOGY			B I O C H E M I S T R Y						HISTOLOGICAL MEASUREMENTS *				
Case No	Patient	Sex & Age	Clinical Diagnosis	Bone Biopsy Number	Thin Bones	Verterbral Fractures	Ca (mN)	PO ₄ (mN)	CaxP (mg%)	Alk. P'ase K.A.u	Tot. Pro. (G%)	Sr. Space (pl.u)	A	B	C	D	E
M.1.	A.F.	M 54	Idiopathic	68/1445	Yes	Yes	4.9	1.7	33.3	8	6.3	..	11.3	0.13	19.1	8.2	0
M.2	E.W.	M 54	Immobilisation	69/1057	Yes	Yes	4.9	2.1	41.2	12	6.5	..	7.86	0.05	23.3	10.0	0
M.3	F.A.	M 57	Senile	67/977	Yes	Yes	5.1	2.4	48.9	6	6.4	16.7	9.4	0.06	18.6	11.6	0
M.4	P.S.	M 65	Klinefelter's syndrome	68/1235	Yes	Yes	5.0	2.0	40.0	8	7.3	15.8	10.5	0.01	3.9	3.9	0
M.5	I.V.	F 39	Post pregnancy	67/3485	Yes	Yes	4.8	2.0	38.4	3	..	14.1	10.7	0.06	4.4	5.5	0
M.6	A.H.	F 48	Post menopause	67/1169	Yes	No	4.7	2.0	37.6	8	6.8	8.1	12.6	0.28	17.1	12.5	0
M.7	A.S.	F 50	Post menopause	67/1683	Yes	No	4.8	2.3	44.2	10	..	..	8.4	0.26	28.5	9.5	0
M.8	M.S.	F 55	Post menopause	68/437	Yes	No	4.8	2.1	40.2	12	..	18.6	9.8	0.08	7.8	10.4	0
M.9	A.C.	F 62	Post menopause	67/3246	Yes	Yes	4.9	2.2	43.1	9	..	..	10.7	0.10	9.6	5.5	0
M.10	L.B.	F 73	Post menopause	69/995	Yes	Yes	5.0	2.6	52.0	9	6.9	15.5	12.4	0.12	31.2	10.4	0
* A				% volume of calcified trabecular bone.			B			% volume of osteoid in trabecular bone.							
C				% total bone surface covered with osteoid.			D			% total bone surface showing resorption not covered with osteoid.							
E				% total bone surface showing resorption covered with osteoid (R.S.)													

4.4. DISCUSSION

The findings in this small group of patients highlight the difficulty in defining the concept of osteoporosis. The control studies have shown that loss of calcified trabecular bone is a normal part of ageing (Chapter 3). This has previously been shown by Sissons et al (1967) using ground sections embedded in methyl methacrylate both in the cancellous bone of iliac crest and the cortical bone from the mid-shaft of the femur. Dunnill et al (1967) also showed that with advancing age there is a decrease of bone mass based on the study of decalcified paraffin embedded sections in lumbar vertebrae. Beck and Nordin (1960) based on decalcified sections of iliac crest samples from necropsy specimens also have shown that there is a decrease of bone mass in old age, but were unable to give a proper assessment of age affect on bone mass because there were very few cases under the age of 40 years. The studies in this ill-defined group of 'clinical and suspect osteoporosis' showed that the majority fall into the lower part of the normal range for the percent volume of trabecular bone. If the group is more critically defined by the presence of vertebral fractures without known cause or known trauma the same remarks hold true. A comparison of these data with those of others is hindered by the lack of adequate control material in most studies. For instance, Bordier et al (1964) showed a reduced volume of trabecular bone in iliac crest bone biopsies from patients with clinical osteoporosis, but their group of osteoporotics was of mixed aetiology and the controls were very limited in number. The same remarks hold true for the selection of cases of 'osteoporosis' on radiological grounds, the bones are said to be 'thin' without reference to age and sex matched standard. When radiological studies are carefully quantified, it has also been shown that the majority of the cases labelled as osteoporosis with vertebral fractures usually fall into the lower half of the normal range for bone density (Doyle, F.H.,

Personal Communication). It is interesting to note that the single case of the group of miscellaneous endocrine bone disease (Chapter 6) with vertebral fractures showed the lowest percentage volume of trabecular bone of any biopsy personally studied.

In considering the cause of 'osteoporosis' a number of aetiologies have been suggested. Clearly a reduction in bone volume can be due to reduced formation or increased loss or both. For the group of idiopathic 'osteoporosis' the amount of bone breakdown was certainly not increased, most cases falling into the lower half of the normal range. Bordier et al (1964) claimed that osteoporotics showed an increase in the amount of resorption, but their group included two steroid induced osteoporotics and two cases of 'digestive' osteoporotics. If these are excluded the idiopathic group are not found to show increased resorption. The few personally studied cases of steroid osteoporosis also show evidence of increased resorption. It does not seem likely that idiopathic 'osteoporosis' is due to increased bone loss. The evidence of decreased bone formation in 'osteoporosis' is also rather poor. This study shows that the amount of bone surface covered with osteoid is within the normal range for all but two of the cases. Varying results have been reported from the use of tracer studies. Heaney and Whedon (1958) found a normal bone formation rate, as Fraser et al (1960). In contrast to Nordin (1959) who observed an increase in bone formation, Eisenberg and Gordan (1961) showed a decreased accretion rate !

Taking the findings together, it is apparent that a coherent picture can be presented. The amount of calcified bone decreases with age ; this change is more marked in men than in women, it may be related in part to loss of muscle mass (Doyle et al, 1970). If the loss of bone is accelerated, as for instance by steroid excess, spontaneous fractures may result. Spontaneous fractures may also occur in

a number of patients where trabecular bone mass is usually in the lower half of the normal range, matched for age and sex. If low trabecular bone mass alone was the cause of the fractures, these might be expected in about half of the population ! They have been looked for radiologically in a number of apparently normal people, and have been found in 4.6% of women over 47 years of age (Smith et al, 1960). It may be more common in an older age group (Gershon-Cohen et al, 1953). It would seem likely, therefore, that some other factor reducing the structural strength of bone is present in these patients; it is only speculation that this might be an abnormality in the collagen, or in the composition of laying down of the bone mineral. While this could be an acquired abnormality it could equally well be congenital and brought to light only by the slow wasting of trabecular bone that occurs with age.

It will be noticed that in the previous paragraph the term 'osteoporosis' has not been used. If it is to remain in use as a clinical term it must be clearly defined and the views of Urist (1960) and Doyle et al (1967) who placed great emphasis on the presence of vertebral fractures suggest one way of doing this. Osteoporosis is perhaps too firmly established in the literature to be disposed of altogether; it is suggested that the term 'clinical osteoporosis' should be used to denote the syndrome of relatively thin bones and spontaneous fractures. The disease can perhaps be renamed when the underlying factors leading to spontaneous fractures with a low normal trabecular bone mass are more clearly understood.

4.5. SUMMARY

Histoquantitative studies were performed in 10 cases of 'clinical and suspect' osteoporosis. All cases showed 'thin bones' on radiology, only 7 of which had any definite evidence of osteoporosis on the basis of vertebral fractures. Biochemical investigations revealed no marked abnormalities. The results of histological measurements for the value of calcified trabecular bone showed that 8 out of 10 patients studied lie in the lower half of the normal range ($\text{mean} \pm 2 \text{ S.D.}$), one being just above the mean value, and one just below the lower limit of normal. The 7 patients with vertebral fractures did not show significantly greater loss of trabecular bone than the 3 without vertebral fractures. In 8 patients, the amount of osteoid was within the normal range; in two, both women, the amount was slightly elevated. In all the patients, the amount of resorption was within the normal range. It is concluded that the ill defined syndrome of thin bones, commonly associated with spontaneous vertebral fractures, is not due solely to bone loss, but that some other cause for reduction in bone strength must exist.

Fig. 28

—AMOUNT OF CALCIFIED TRABECULAR BONE IN PATIENTS
WITH "METABOLIC BONE DISEASE"—

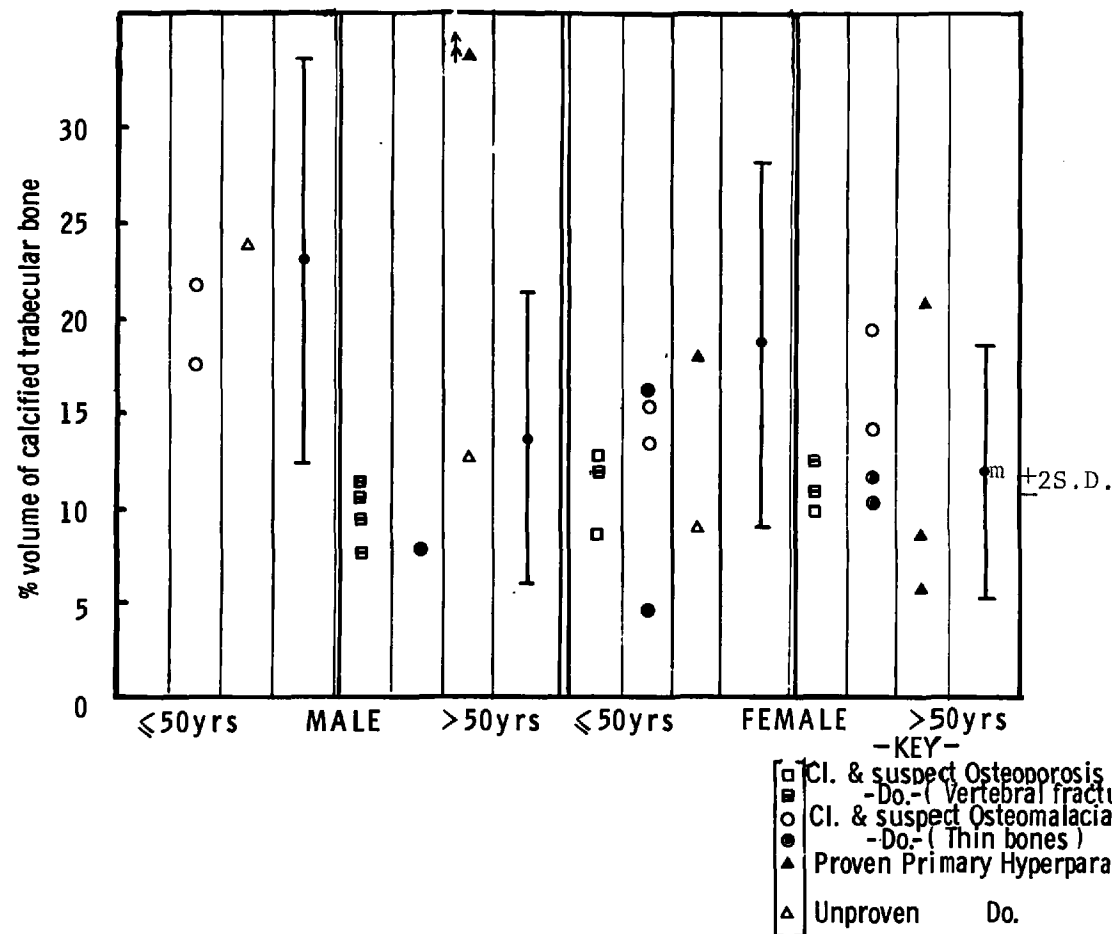


Fig. 29

—AMOUNT OF TOTAL BONE SURFACE COVERED WITH OSTEOID IN PATIENTS
WITH "METABOLIC BONE DISEASE"—

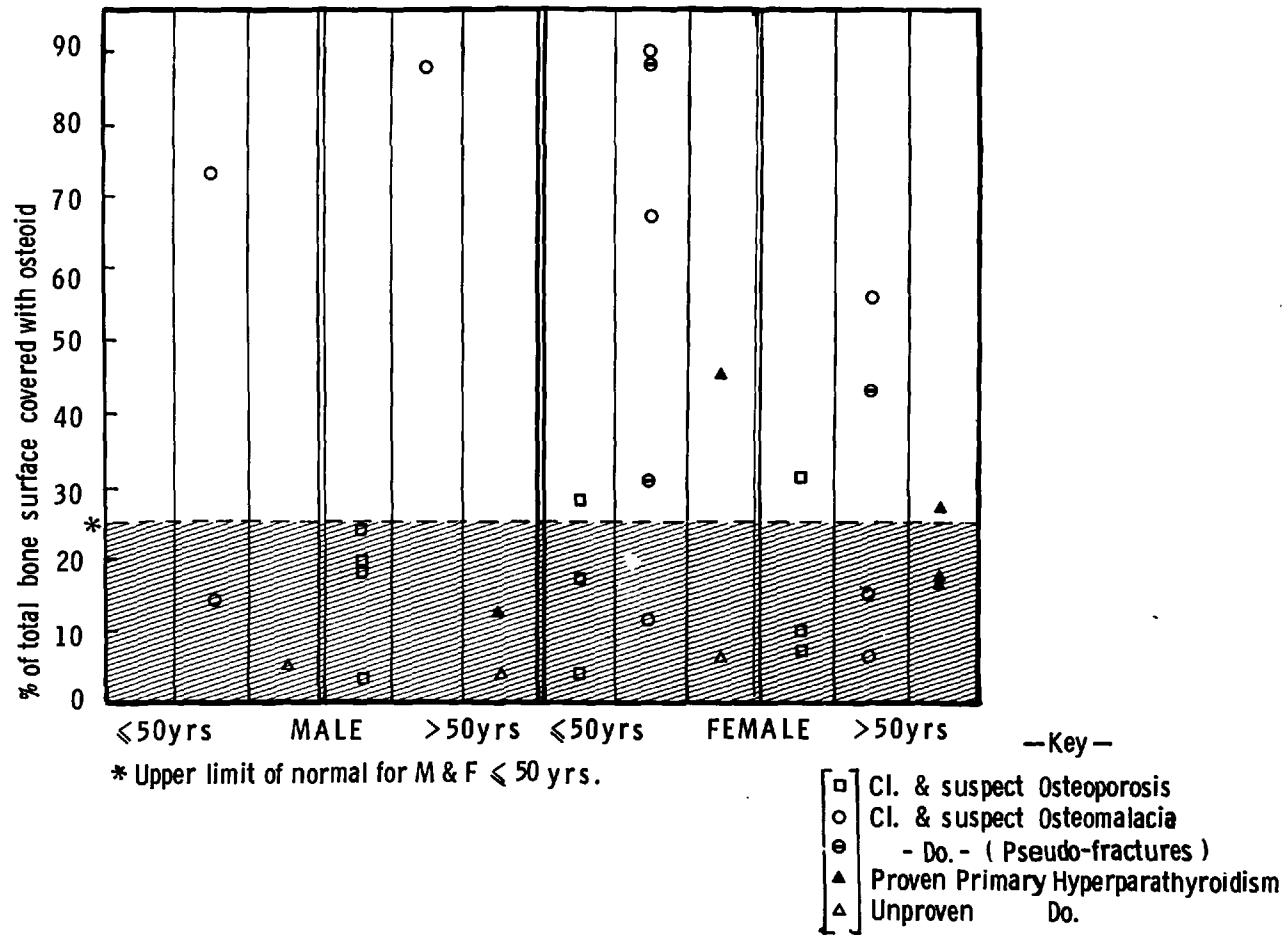
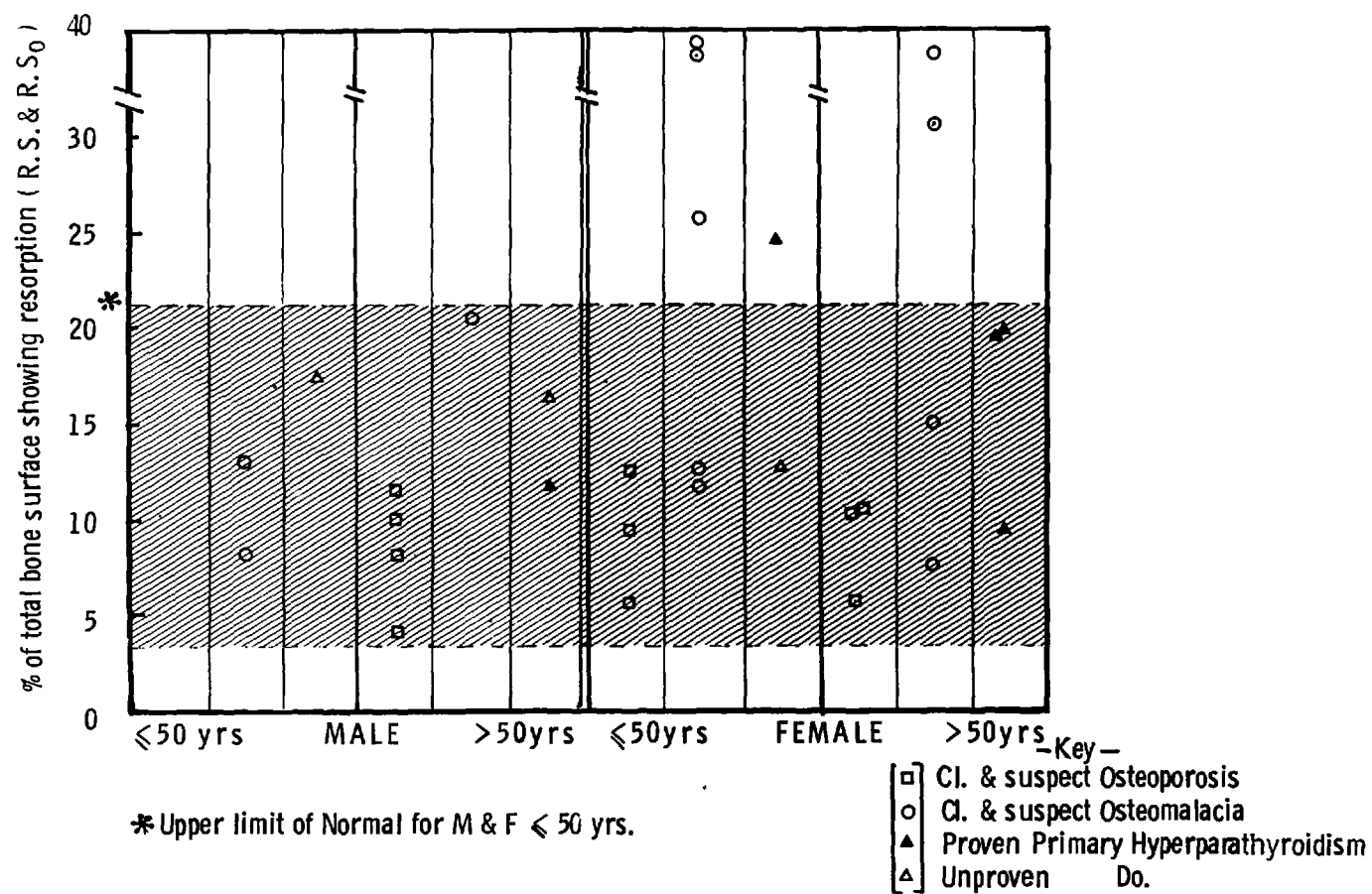


Fig. 30

— AMOUNT OF TOTAL BONE SURFACE SHOWING RESORPTION IN
PATIENTS WITH "METABOLIC BONE DISEASE" —



CHAPTER 5

OSTEOMALACIA

5.1. INTRODUCTION

The major problem in considering osteomalacia is the usage of the word itself. Some authors have used the term as a diagnosis implying a rather ill understood clinical entity, one of whose features is excess osteoid in the bones. Others have used osteomalacia to mean an increase in osteoid, which might be associated with a variety of different aetiologies of which some might not be explicable. Still others have used the term without any clear definition. Osteomalacia will be used here to imply an increase in the amount of osteoid above the normal range. The term clinical osteomalacia will be used to cover the rather vague clinical syndrome usually associated with the finding of excess osteoid on biopsy. Osteomalacia may thus occur with hyperparathyroidism, osteoporosis, or a variety of other bone diseases.

Clinically the patient may present with vague skeletal or muscle pains, muscle weakness, sometimes with bowing of the limbs, or occasionally with fractures. In a very severe case of osteomalacia the characteristic skeletal deformities, for example, collapsed pelvis ring, distorted thoracic cage, increased dorsal kyphosis, cora vara are evident as illustrated in Fig. 31. On radiology the bones may appear thin, and the characteristic features of severe osteomalacia is the presence of 'Looser's zones' or pseudofractures (Fig. 32). The biochemical features that usually accompany clinical osteomalacia are a normal or low serum calcium and phosphorus, and a high serum alkaline phosphatase. In most cases the ionic product of calcium and phosphorus is decreased (Albright and Reifstein, 1948). The same workers suggested that during the early stage of the disease, the ionic product may be reduced without significant increase in alkaline phosphatase. Some workers do not lay emphasis on the serum alkaline phosphatase and have observed



FIG. 31

A classical osteomalacic skeleton showing the characteristic deformation of the thoracic cage and pelvis. (Reproduced by permission of the author and of the editor from J.roy.Coll.Surg. Edinb. V.13, 1968).



FIG. 32

Radiology of the pelvis from a patient with Vitamin D resistant osteomalacia showing characteristic bilateral pseudo-fractures of severe osteomalacia. (Patient M.20. A.A.).

normal values of alkaline phosphatase in advanced cases of clinical osteomalacia (Pederson and McCarroll, 1951, Frame and Smith, 1958), Again these biochemical changes are not usually found in cases of chronic renal failure, with osteomalacia. The values of serum calcium and phosphorus or their ionic product may be variable in renal osteodystrophy (Dent et al, 1961).

Histologically the excess of osteoid tissue has been the characteristic feature of osteomalacia since its description by Pommer in 1885. The term osteomalacia is used in this thesis to imply an increase of osteoid. However, in the past, because an increased amount of osteoid was found in a variety of bone diseases, other than cases of clinical osteomalacia (Follis, 1957) biopsy is the only unequivocal evidence of osteomalacia (Chalmers, 1968). The definition of osteomalacia as a state of vitamin D deficiency is not satisfactory because there are conditions in which the term commonly used as a diagnosis, despite the normal intake of vitamin D.

Morgan et al (1965) suggested that the response to vitamin D might be used as a criterion in the diagnosis of clinical osteomalacia. A further possible diagnostic criterion, in this case a histological one, arises from the work of Bordier et al (1968) who noted that calcification fronts, present between osteoid and calcified bone normally, were very greatly reduced in some cases of osteomalacia, and became prominent after vitamin D administration. Bordier has suggested that reduction in the amount of calcification front might be used as the earliest diagnostic criterion of osteomalacia. However, it seems preferable to restrict the use of the term osteomalacia to patients with excess osteoid. Patients with reduced calcification fronts may indeed be deficient in vitamin D, and they may be going to develop the excess osteoid that is by definition osteomalacia.

The radiological diagnosis of metabolic bone disease has substantial limitations. Osteomalacia, frequently if not invariably

shows a reduction of the amount of mineral in the skeletal system, resulting in diminished x-ray density or rarefaction of the skeleton. In order to be detectable on routine x-ray, the loss of calcium from the skeleton needs to be in the region of at least 30-60% (Lachman, 1955). Radiological methods thus in general give a crude indication of the calcium content of the bones (Rose, 1964). In addition radiology is not able to distinguish between osteomalacia and osteoporosis unless the characteristic features of Looser's zones are present; at many times the two diseases can exist together (Hodgkinson et al, 1963). Osteomalacia may also often be associated with primary hyperparathyroidism without any demonstrable additional radiological changes.

Osteomalacia and rickets are part and parcel of the same disease process occurring in adults and growing children respectively, with varying aetiology, but all are ultimately linked with disturbances in the vitamin D metabolism (McLean, 1941, Lamm and Neuman, 1958, and Bohr, 1964).

5.2. PATIENTS STUDIED

The patients, as shown in Table 7, were admitted into the Metabolic Unit of the Hammersmith Hospital to undergo investigations for suspect osteomalacia, usually on the basis of vague and generalised muscle and skeletal pain, muscle weakness and stiffness, joint pains, skeletal deformities, or malabsorption syndrome. The 12 cases under study for histoquantitation comprised 3 cases of possible osteomalacia occurring 15-20 years after gastrectomy. Case M.11 (J.H.) had a polya partial gastrectomy in 1952, M.12 (I.T.) had a partial gastrectomy in 1948. The third case, M.13. (H.B.) had a polya partial gastrectomy in 1949. She was on intermittent high doses of calciferol until 1965 with

TABLE 7

CASES ADMITTED FOR 'CLINICAL AND SUSPECT' OSTEOMALACIA

G E N E R A L				RADIOLOGY			B I O C H E M I S T R Y						HISTOLOGICAL MEASUREMENTS *					
Case No.	Patient	Sex & Age	Clinical Diagnosis	Bone Biopsy Number	Thin Bones	Pseudo Fractures	Ca (mN) N=4.7-5.5	PO ₄ (mN) N=1.6-2.4	CaxP (mg%) 30-50	Alk. P'ase K.A.u 4-11	Tot. Sr. Pro. (G%) 6.5-7.5	Sr. Space (pl.u) N≤18	A	B	C	D	E	Remarks **
M.11	J.H.	M 44	Post gastrectomy osteomalacia	67/3016	No	No	4.1	1.8	29.5	16	..	25.5	17.8	0.93	73.8	5.5	2.7	Om.
M.12	I.T.	F 54	Post gastrectomy osteomalacia	68/2108	No	No	4.9	2.2	43.1	16	5.7	17.3	19.5	0.19	14.5	13.4	1.2	Normal
M.13	H.B.	F 77	Post gastrectomy osteomalacia	67/979	No	Yes	5.4	1.6	34.6	26	6.3	49.4	14.0	0.54	43.1	26.6	4.6	Om. & H
M.14	A.H.	F 47	Nutritional osteomalacia	67/723	No	No	4.0	1.5	24.0	23	6.2	23.0	13.3	8.77	88.6	3.8	35.6	Om. & H
M.15	E.I.	F 58	Adult coeliac disease	68/2489	Yes	No	4.8	2.6	49.9	17	..	19.3	10.3	0.08	5.5	7.7	0	Normal
M.16	M.E.	F 28	Adult coeliac disease	67/1200	No	No	4.7	2.2	41.4	4	..	12.4	15.2	0.17	12.6	12.7	0	Normal
M.17	M.D.	M 32	Adult coeliac disease	67/980	No	No	4.6	2.5	46.0	8	7.8	..	21.6	0.11	14.5	12.9	0	Normal
M.18	H.H.	F 55	Adult coeliac disease, probable idiopathic hypoparathyroidism	68/513	Yes	No	3.9	2.7	42.1	19	..	27.0	11.6	0.74	55.8	22.5	15.8	Om. & H

/continued over

19	A.H.	M 58	Uretero-colic anastamosis for Ca. bladder	69/728	Yes	No	4.6	1.1	20.2	18	6.3	..	7.9	1.22	87.8	4.9	15.4	Om. & H.
M.20	A.A.	F 41	Vit. D resistant osteomalacia	67/2237	Yes	Yes	4.8	1.0	19.2	21	7.3	13.3	4.4	15.45	88.3	6.7	5.0	Om.
M.21	M.E.	F 40	Vit. D resistant osteomalacia	68/1619	Yes	Yes	4.7	1.1	20.7	20	7.6	14.6	..	..	39.7	12.8	12.8	Om. & H.
M.22	A.L.	F 32	Autonomous hyperparathyroidism with pseudo-hypoparathyroidism	69/644	Yes	No	5.1	1.3	26.5	173	6.7	55.5	16.2	7.21	66.9	14.5	23.5	Om. & H.

- * A % volume of calcified trabecular bone
 B % volume of osteoid in trabecular bone
 C % total bone surface covered with osteoid
 D % total bone surface showing resorption not covered with osteoid (R.S.)
 E % total bone surface showing resorption covered with osteoid (R.S._o)
- ** Om. Histological osteomalacia (upper limit of normal for per cent total bone surface covered with osteoid for males and females ≤ 50 is 25%).
 H. Histological hyperparathyroidism (upper limit of normal for per cent total bone surface showing resorption (RS & RS_o) for males and females ≤ 50 is 20.6%).

fluctuating levels of serum calcium. At the time of bone biopsy in April, 1967, she was normocalcemic but had a relapse of her bone pain and weakness and vitamin D in a dose of 9,000 units/day was re-instituted and after two days her serum calcium rose to 6.7mEq/L. She had a raised serum alkaline phosphatase and strontium space and also increased phosphaturia as shown by a high phosphate excretion index (Nordin and Fraser, 1960) of $+ 0.32$ ($N = \pm 0.09$). In view of these biochemical and radiological abnormalities she was diagnosed as a case of post gastrectomy osteomalacia with tertiary hyperparathyroidism and the findings are in accord with the histological measurements which also showed a state of severe osteomalacia and increased parathyroid activity. Four months later subtotal parathyroidectomy was performed; two enlarged glands were found, one showed a mixed cell adenoma and the other mainly chief cell hyperplasia. There was one case of nutritional osteomalacia. 4 cases (M.15-M.18) of adult coeliac disease or idiopathic steatorrhea all showed 'sub-total villous atrophy' in the jejunum, (M.15 also had a complete deficiency of immunoglobulin (IgA) in her serum.) One patient (M.18) on the basis of low serum calcium and high serum phosphorus and a decrease of phosphaturia as shown by low phosphate excretion index of -0.14 was also thought to have idiopathic hypoparathyroidism; 2 cases were diagnosed as vitamin D resistant osteomalacia, 1 case had had ureterocolic anastomosis for carcinoma of the bladder 12 years previously, and finally in one, rare complex case, the diagnosis of autonomous hyperparathyroidism with ^{/pseudo}hypoparathyroidism, renal tubular acidosis and osteitis fibrosa cystica was made. (A detailed record of this case has been reported at the end of this chapter).

In none of the patients studied was there any evidence of chronic renal failure except for the patient with ureterocolic anastomosis (M.19. A.H.) who showed a blood urea of 80 mg% for several years.

5.3. RESULTS

The various parameters obtained from histological measurements together with the clinical status of the patient and the radiological and bio-chemical findings are set out in Table 7. Out of the 12 patients studied, 5 showed a low serum calcium, 3 of these also showed a low CaxP product. There were three other patients (M.20, 21, and 22) who showed a low CaxP product, but their serum calcium values were normal. 10 out of 12 patients had an elevated level of serum alkaline phosphatase. Strontium space was estimated in 10 patients, in 6 there was a definite increase but the other 4 cases were within the normal limits, including two cases of vitamin D resistant osteomalacia. Serum total proteins were estimated in 8 cases and 4 showed low values. In 6 patients there was radiological evidence of 'thin bones'; 3 showed pseudo-fractures; 2 patients (M.13 H.B. and M.22 A.L.) showed the presence of subperiosteal cortical resorption in the phalanges, one of these patients also showed pseudo-fractures.

The histological measurements are shown in Table 7 and also in Figures 28, 29 and 30. The percent volume of calcified trabecular bone for all but two of the patients was within the normal range. All five cases reported as having 'thin bones' were below the mean value for calcified bone for the comparable control group and one was well beyond the normal range. They did not show any evidence of vertebral fractures.

Regarding the percent of total bone surface covered with osteoid, 8 out of 12 patients showed a definite increase. All 3 patients with evidence of pseudofractures showed an increase of osteoid, two were cases of vitamin D resistant osteomalacia (Figs. 33 and 34).

With regard to the percent of bone surface showing resorption, 5 out of 12 patients showed a marked increase; 2 of those also showed radiological evidence of subperiosteal cortical resorption in the

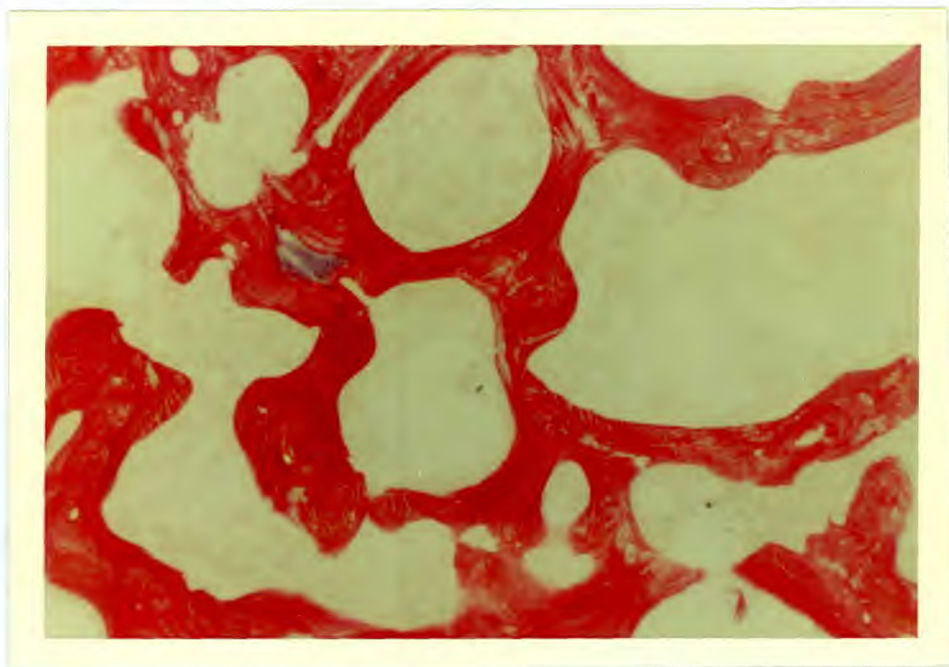


Fig. 33

An undecalcified section from a case of Vitamin D resistant osteomalacia showing trabeculae composed almost entirely of osteoid tissue. (Patient M.20. A.A. Solochrome cyanin x 30).

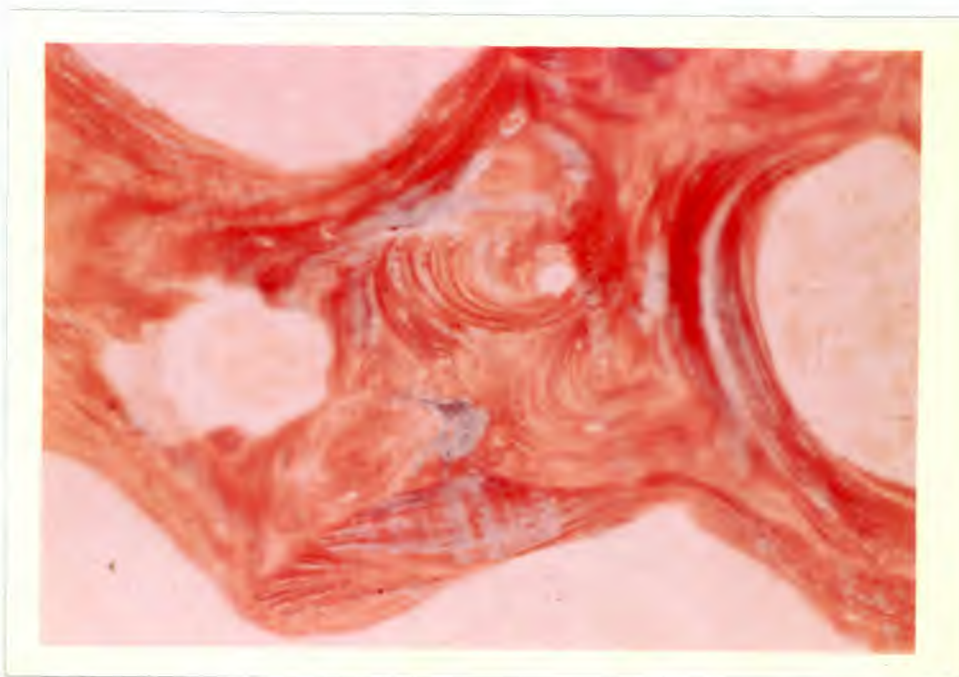


Fig. 34

An undecalcified section from the previous patient showing partially calcified osteoid tissue with resorption cavity. (Solochrome cyanin x 120).

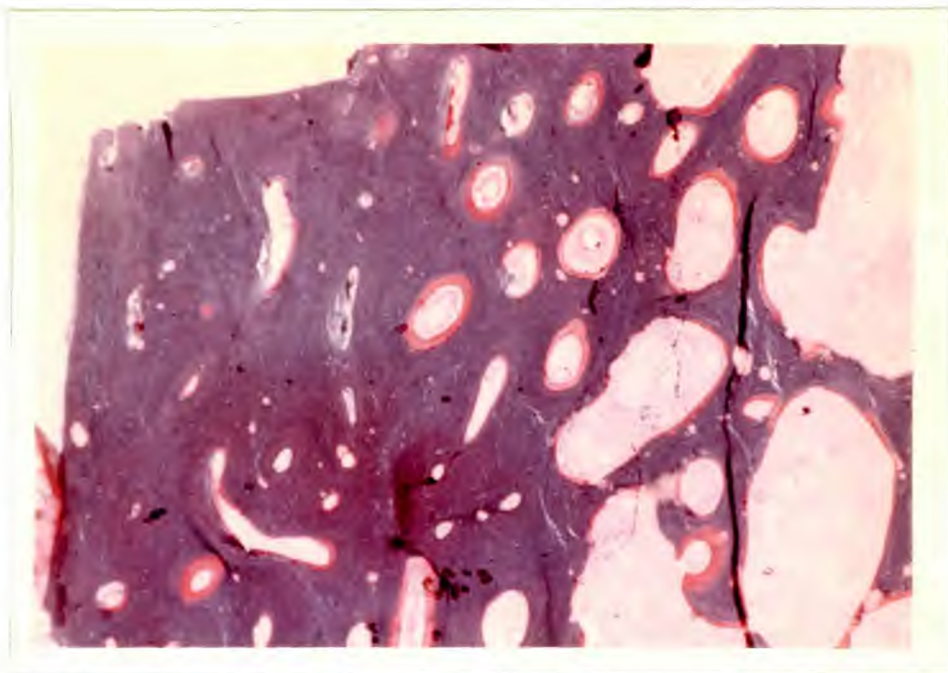


Fig. 35

An undecalcified section from a case of dietary osteomalacia with secondary hyperparathyroidism showing an increase of osteoid tissue. (Patient M.14, A.H. Solochrome cyanin x 30).

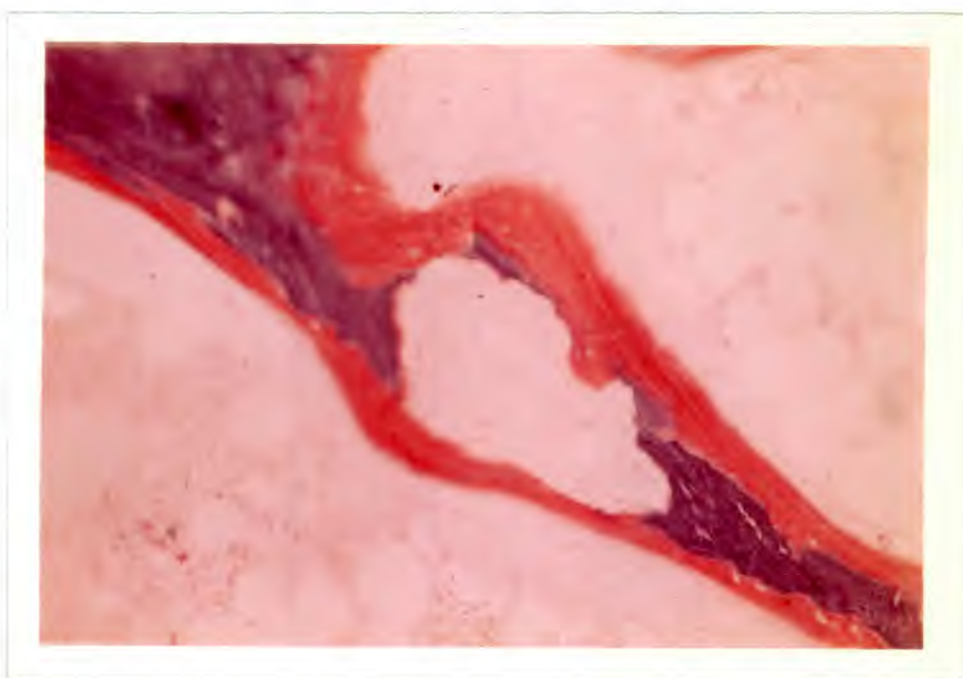


Fig. 36

An undecalcified section from the previous patient showing 'Tunnelling resorption' with the overlying osteoid untouched. The adjacent trabecular bone shows osteoid covering an irregular scalloped surface. (R.S._o). (Solochrome cyanin x 120).

phalanges. In case M.14(A.H.), although there is a marked increase in resorptive activity, and an excess of osteoid (Figs. 35 & 36), there was no evidence of radiological bone disease.

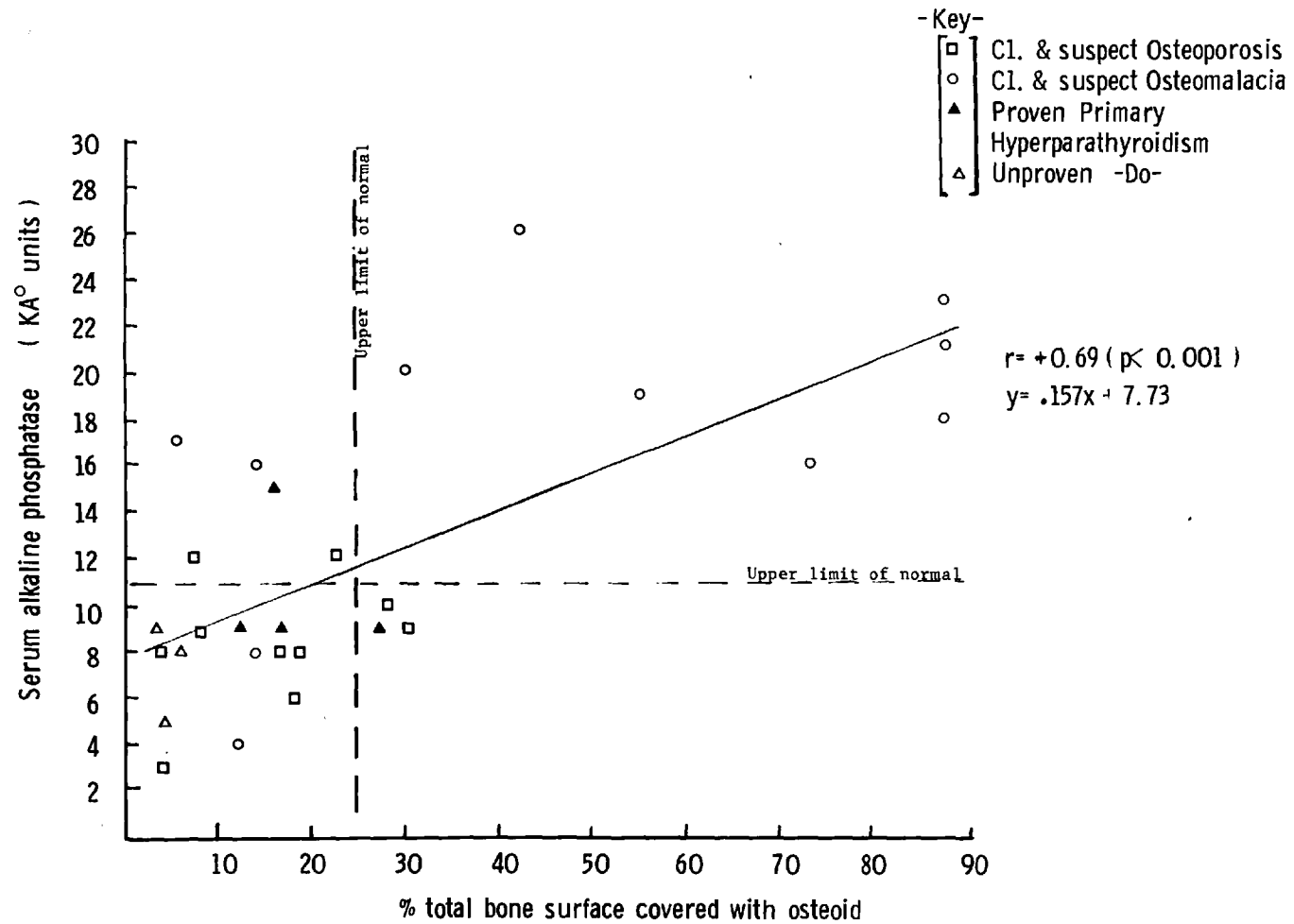
5.4. DISCUSSION

The patients whose studies are reported in this chapter illustrate the wide range of aetiologies associated with osteomalacia. However, in this series of 12 patients in whom the diagnosis of osteomalacia was made on suspected or clinical grounds, only 8 showed the histological features of osteomalacia on quantitative study. This was expected as ever since Looser described these decalcified bands in 1920, their presence have been usually accepted as pathognomonic of osteomalacia. Similar radiological appearances have been described in patients with severe Paget's disease or osteogenesis imperfecta who were not thought to have osteomalacia in addition. Although all cases in this study with Looser's zones had osteomalacia, the reverse is not true, only one of the six patients in whom over half the available trabecular bone surface was covered with osteoid showed Looser's zones.

In the absence of Looser's zones one of the commonly accepted important abnormalities to be looked for in the diagnosis of osteomalacia is a raised alkaline phosphatase. It can be seen from Table 7 that all but two of the 12 patients suspected of osteomalacia showed a raised serum alkaline phosphatase and that in both of them the osteoid surface was found on quantitation to be normal. The other two patients with a normal amount of osteoid showed only small elevations of alkaline phosphatase. These findings suggest a direct relationship between the two variables and they are compared for a variety of patients with the clinical diagnosis of osteomalacia, osteoporosis and hyperparathyroidism in Figure 37. It can be seen that there is good correlation ($r = +0.69$, $p < 0.001$). The upper limits of normal of the two investigations are marked as dotted lines and in 5 cases the serum alkaline phosphatase was raised with a normal osteoid, and in three the

Fig. 37

—RELATIONSHIP BETWEEN SERUM ALKALINE PHOSPHATASE AND
THE AMOUNT OF OSTEOID—



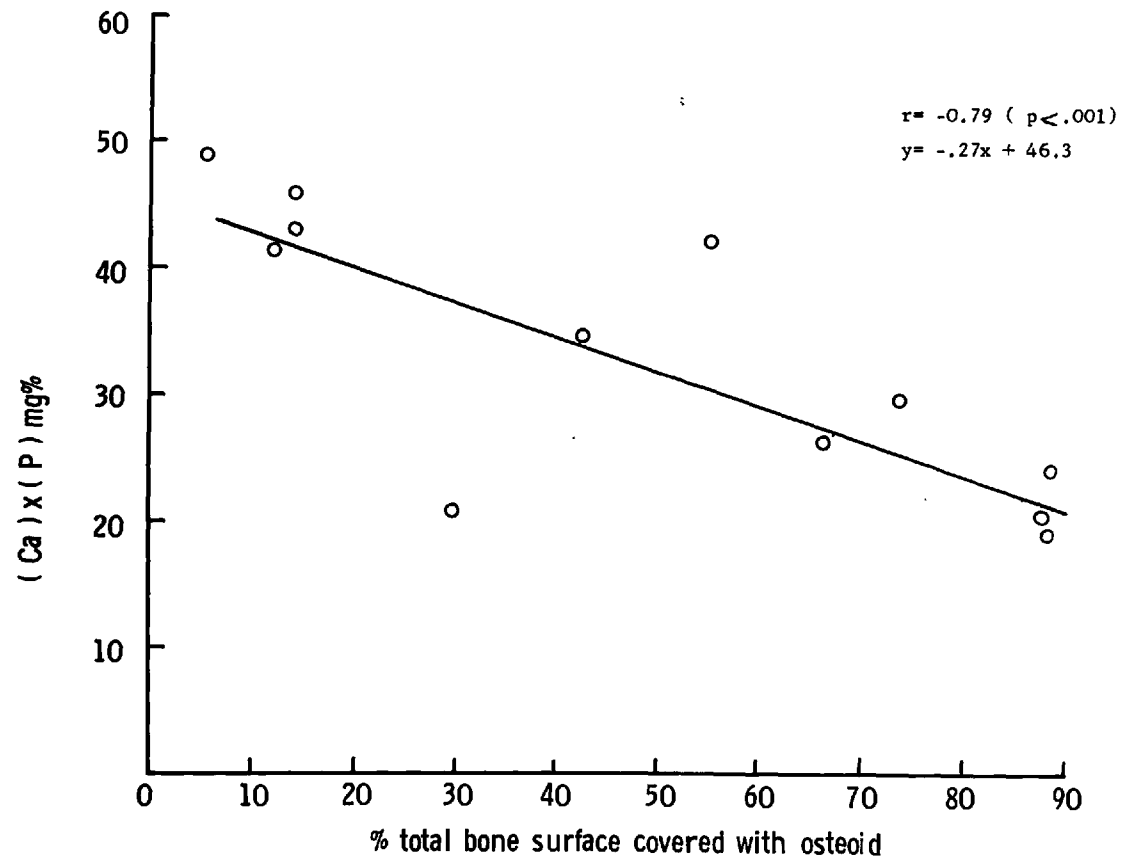
osteoid was elevated with the normal alkaline phosphatase. However, the elevation in all these instances was only slight. It can be concluded from these observations that unless some other cause for raised alkaline phosphatase exists, there is likely to be a good correlation between an elevated alkaline phosphatase and the amount of osteoid covered trabecular bone surfaces in an iliac crest biopsy.

The serum calcium levels were abnormally low in 5 of the 12 cases and in 4 severe osteomalacia was present. In the fifth case (M.17 M.D.) the osteoid surface was normal in amount, and it is interesting to note that the alkaline phosphatase was also normal. A low CaxP product has been recognised as a feature of osteomalacia, whether vitamin D sensitive or vitamin D resistant for many years (Howland and Kramer, 1922, Nordin and Smith, 1967); although cases with chronic renal failure must be excluded (Stanbury, 1967). In this study all 6 patients with a low CaxP product (less than 30 expressed in terms of mg%) showed osteomalacia, mostly severe. In this selected group of 12 patients there was a surprisingly good correlation between the CaxP product and the amount of osteoid as shown in Figure 38. ($r = -0.79$, $p < 0.001$). The only two points remote from the regression line were a case of vitamin D resistant osteomalacia with a rather inadequate biopsy and a case of adult coeliac disease with possible idiopathic hypoparathyroidism.

The strontium space is a relatively simple test which is an index of the exchangeable calcium in the bone. It has been found to be high in osteomalacia by Joplin et al (1967) who claim that it was a simple useful screening test for osteomalacia. Of the 10 patients from this group on whom the test was performed, it was normal in 4, 2 of them were patients with vitamin D resistant osteomalacia, and high values for osteoid; 2 were patients in whom the clinical suspicion of osteomalacia was not confirmed histologically. Of the 6

Fig. 38

—RELATIONSHIP OF (Ca)x (P) WITH AMOUNT OF OSTEOID IN PATIENTS
WITH "CLINICAL & SUSPECT" OSTEOMALACIA—

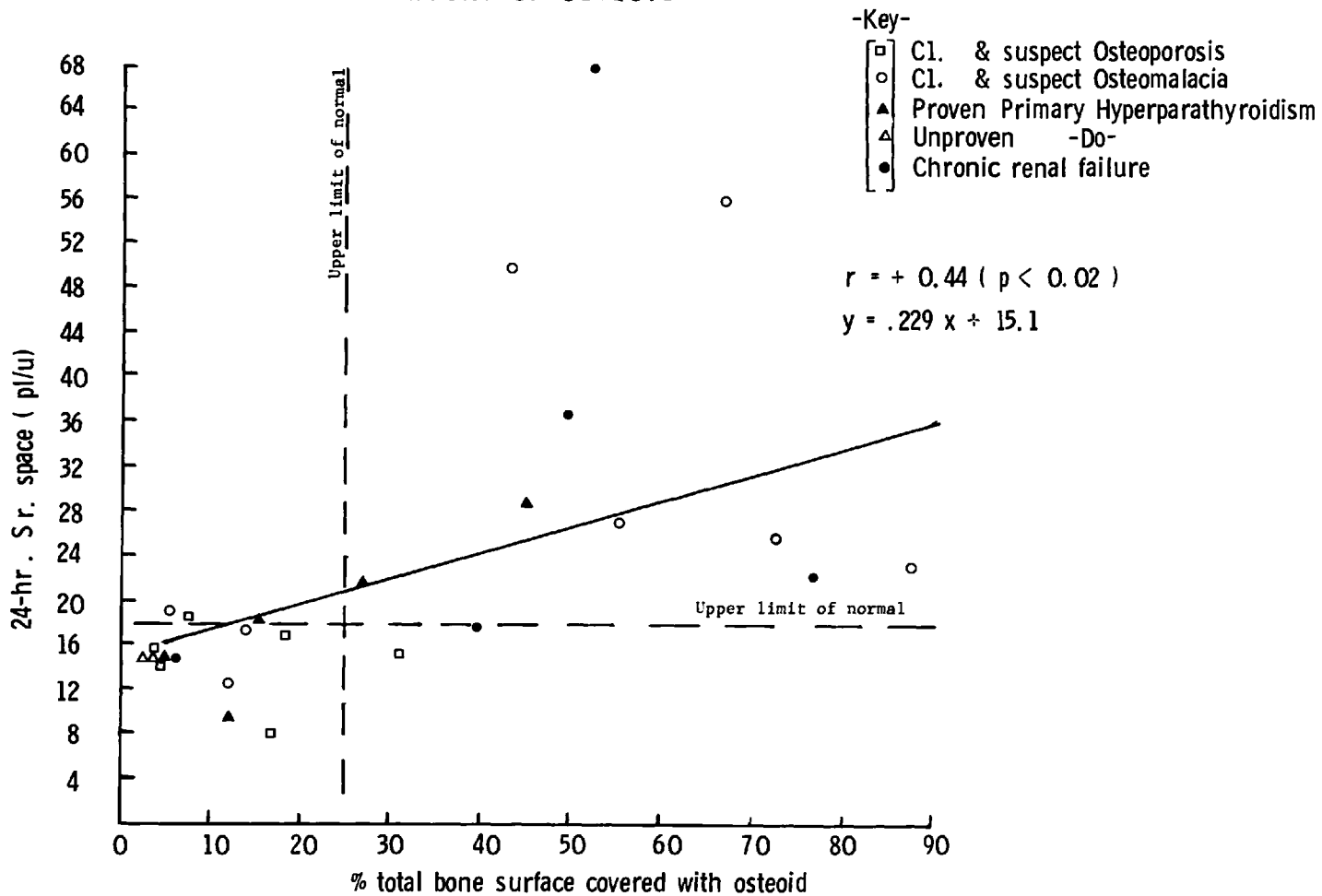


patients with elevated strontium space, 5 showed osteomalacia, one did not. If the patients with vitamin D resistant osteomalacia are excluded and the study is broadened to include all patients with bone biopsies which I have quantified on whom the strontium space has been performed, a modest correlation between this and the amount of osteoid is found as shown in Figure 39. ($r = + 0.44$, $p < .02$). The upper limit of this test might be more usefully set at 20 plasma units; all the patients whose biopsies showed normal amounts of osteoid will thus show a normal strontium space, while of the 12 biopsies with excess osteoid the strontium space was abnormal in 10. The contrast between the findings for the strontium space in the two patients with vitamin D resistant osteomalacia and the rest is marked. Both showed a normal strontium space despite the presence in one of moderate and in one of extensively severe osteomalacia. This observation is open to a number of interpretations but underlines the difference in aetiology between vitamin D resistant osteomalacia and osteomalacia of other causes.

The finding that in histological osteomalacia a low serum calcium, a low CaxP product, a high alkaline phosphatase and a high strontium space occur, is not unexpected. This work has, however, shown that a correlation exists between the amount of osteoid and the level of the CaxP product, the serum alkaline phosphatase and the strontium space. The correlation in each case is moderate but all the patients with osteomalacia studied show an abnormal value for at least two of these three investigations. If the upper limit of normal for the strontium space is taken as 20 plasma units, none of the normals show an abnormal value for more than one of these three tests. These studies suggest that a combination of these three tests may prove useful in predicting the presence and the severity of osteomalacia in patients suspected on clinical grounds of this diagnosis.

Fig. 39

—RELATIONSHIP BETWEEN THE 24-hour STRONTIUM SPACE AND THE
AMOUNT OF OSTEOID—



5.5. SUMMARY

In the series of 12 patients in whom the diagnosis of osteomalacia was made on clinical grounds, only 8 showed histological features of osteomalacia. All patients who showed radiological evidence of Looser's zones showed an excess of osteoid on quantifying, but the reverse is not true. 10 out of 12 patients who showed an elevated level of serum alkaline phosphatase also have evidence of histological osteomalacia and a good correlation has been shown between an elevated serum alkaline phosphatase and the amount of bone surface covered with osteoid in iliac bone biopsies, in particular, clinical and suspect osteomalacia and primary hyperparathyroidism (proven or unproven). This correlation does not exist in patients with chronic renal failure and has been discussed in Chapter 7.

In this study all 6 patients with a low CaxP product (less than 30 mg%) showed severe osteomalacia. In this selected group of 12 patients, a good negative correlation exists between the ionic product of calcium and phosphorus and the bone surface covered with osteoid. The estimation of 24 hour strontium space is also a useful test in predicting histological osteomalacia. A positive correlation exists between 24 hour strontium space and the amount of osteoid. All the patients under study for this group of 'clinical and suspect' osteomalacia showed an abnormal value for at least two of the three investigations, in particular serum calcium, serum alkaline phosphatase and 24 hour strontium space. The correlative studies thus suggest that a combination of these tests may prove useful in predicting the severity of osteomalacia in patients suspected on clinical grounds.

CASE RECORD (M.22 A.L.)

A female patient aged 32 was a dwarf and mentally defective. She was normal at birth and during infancy. She started walking at the age of $2\frac{1}{2}$ years and went to school at the age of 5 years, but due to lack of response to teaching, was kept at home and did little other than help her mother in doing household work. Two years ago she fell and fractured her clavicle. In June, 1968, after an injury to her left elbow, radiology showed marked changes of osteitis fibrosa cystica. She was admitted to the Hammersmith Hospital for further investigations. These showed :-

(a) Biochemistry

Serum calcium	5.1 mEq/L	(N = 4.7-5.5)
Serum phosphorus	1.3 mEq/L	(N = 1.6-2.4)
Serum alkaline phosphatase	173 K.A. ⁰ units	(N = 4-11)
Serum total proteins	6.7 g%	(N = 6.5 -7.9)
Serum potassium	2.6-3.5 mEq/L	(N = 3.8-5.2)
Serum chlorides	105 mEq/L	(N = 100-107)
Blood urea	16 mg%	(N = 20-40)
Creatinine clearance	94 ml/min	(N = 120-130)
Blood Bicarbonate (HCO_3)	25 mEq/L	(N = 24-30)

Urinary acidification test showed the urine pH to be 6.1, 6.2, 6.1 after 4, 6, and 8 hours of injection of ammonium chloride (0.1 G/Kg) (N = $\text{pH} < 5.3$).

Urine showed a slight generalised aminoaciduria but glycosuria was not present.

Parathyroid hormone assay	2-12 ng/ml	(N = 0.25 mg/ml)
Urinary hydroxyproline	678-970 mg/G (Of creatinine)	(N = 45 mg/G (of creatinine)
Phosphate excretion index	+ 0.30	(N = $\pm$ 0.09)

(b) Radiology showed gross widespread changes of hyperparathyroid activity in the form of subperiosteal cortical resorption and

cystic changes in the hands.

In view of the above mentioned abnormalities in the biochemical and radiological findings the provisional diagnosis was autonomous hyperparathyroidism with pseudo hypoparathyroidism, renal tubular acidosis and osteitis fibrosa cystica.

(c) Histology

Biopsy from the iliac bone, showed an outer cortex of reduced thickness, containing many wide Haversian systems. The trabeculae showed a marked increase in resorption. Many of the trabecular surfaces including the resorptive ones were covered by thick osteoid seams, the largest measuring 36 u in thickness. A large area of the trabecular bone was occupied by many small trabeculae composed largely of woven osteoid with a small amount of woven bone included in it (Figs 40 and 41). Periosteocytic osteolysis was also a remarkable feature.

The histological measurements showed abnormal results in most of the parameters as shown in Table 7.

The picture was thus suggestive of severe hyperparathyroid activity with severe osteomalacia.

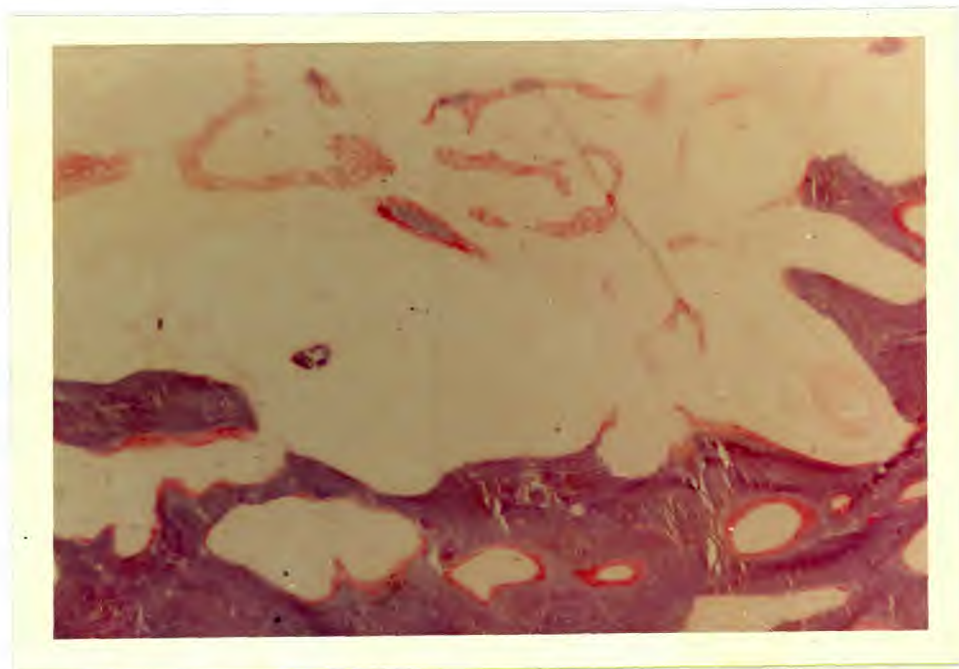


Fig. 40

An undecalcified section from a case of autonomous hyperparathyroidism with pseudo-hypoparathyroidism showing a marked increase in resorptive activity and osteoid tissue. (Patient M.22. A.L. Solochrome cyanin x 30).

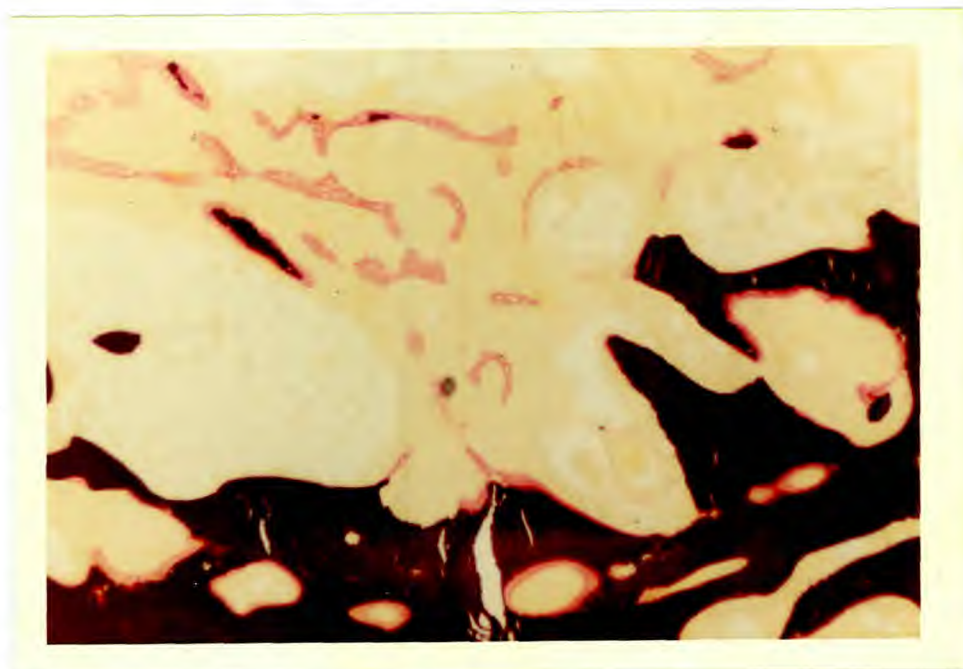


Fig. 41

An adjacent section from the previous patient. (Von Kossa Van Gieson x 30).

CHAPTER 6

ENDOCRINE BONE DISEASE

6.1. INTRODUCTION

Bone disease is common in a wide variety of endocrine disorders. In hyperparathyroidism, bone disease may dominate the clinical presentation, in Cushing's syndrome and thyrotoxicosis osteoporosis may be an important feature. The field is too broad for a detailed review of previous work on endocrine bone disease to be relevant. The key papers will be referred to in discussion. Bone biopsies from 15 patients with known or suspected endocrine bone disease have been quantified and the results of these studies are reported in this chapter. The patients have been divided into two groups, one with known or suspected hyperparathyroidism and the other a non-parathyroid group.

6.2. PATIENTS STUDIED

Patients with definite or suspected hyperparathyroidism are shown in Table 8. They were admitted into the Metabolic Unit of Hammersmith Hospital for investigation of renal calculi, joint or bone pains, or neuro-psychiatric symptoms such as apathy and depression. These patients were investigated for primary hyperparathyroidism because of renal calculi, or because of persistently or intermittently raised serum calcium.

The group of non-parathyroid endocrine bone disease (Table 9) represents a mixed collection of diagnoses, all with a cause for possible bone disease.

6.3. RESULTS

Out of 8 patients admitted for investigation of probable primary hyperparathyroidism, 5 had a definite increase of serum calcium representing the mean of at least 3 separate estimations. Two cases of

TABLE 8

CASES ADMITTED FOR INVESTIGATION OF PROBABLE PRIMARY HYPERPARATHYROIDISM

G E N E R A L					RADIOLOGY	BIOCHEMISTRY				HISTOLOGICAL MEASUREMENTS *					
Case No	Patient	Sex & Age	Bone Biopsy Number	Presenting Complaint	Phalangeal Erosions	Ca (mN)	PO ₄ (mN)	Alk. P'ase K.A.u	Sr. Space (pl.u)	A	B	C	D	E	Parathyroids (explored)
M.23	C.M.	F 41	67/459	Painful cystic lesion in tibia	No	6.7	2.1	..	28.6	18.2	1.4	45.1	15.9	8.5	Yes, (adenoma)
M.24	E.W.	F 57	68/2829	Depression	No	5.7	1.9	9	..	20.7	0.63	17.2	9.2	0	Yes, 69/629, Adenoma in R. superior gland. Weighs 340 mg. Mainly of transitional & water-clear cells.
M.25	D.F.	F 68	68/800	Osteoarthritis	No	5.6	2.0	15	18.1	8.7	0.07	16.1	19.4	0	Yes, 68/2429, Adenoma in R. lower gland. Weighs 150 mg. Mainly of oxyphil cells.
M.26	A.G.	F 58	66/3563	Bone pain	No	5.8	1.8	9	21.6	6.7	0.17	27.6	19.7	0	Yes, 67/248, Adenoma found. Weighs 350 mg. Mainly of chief cells.
M.27	A.C.	M 53	68/2881	Renal calculi	No	5.8	1.7	9	9.5	46.9	0.73	12.7	11.9	0	Yes, 69/1130, Adenoma in L. inferior gland. Weighs 3.29 gms. Mainly of chief cells.
M.28	D.B.	F 38	67/3345	Renal calculi	No	5.1	2.2	8	..	8.7	0.07	6.3	12.9	0	Not explored.

/continued over

M.29	C.L.	M 36	66/3599 Renal calculi	No	5.4	2.3	5	14.5	23.4	0.27	4.9	17.3	0	Not explored.
M.30	G.W.	M 65	69/709 Hypertension, mild diabestes mellitus.(Once hypercalcaemic)	No	4.9	2.1	9	14.6	12.5	0.05	3.5	16.5	0	Not explored

- * A % volume of calcified trabecular bone
 B % volume of osteoid in trabecular bone
 C % total bone surface covered with osteoid
 D % total bone surface showing resorption not covered with osteoid (R.S.)
 E % total bone surface showing resorption covered with osteoid (R.S_o)

TABLE 9

CASES OF NON-PARATHYROID ENDOCRINE BONE DISEASE

Case No	Patient	Sex & Age	Bone Biopsy Number	Clinical Diagnosis	HISTOLOGICAL MEASUREMENTS*					MISCELLANEOUS ** (Radiology, Biochemistry, etc.)
					A	B	C	D	E	
.31	M.M.	F 19	0/68/92	Medullary carcinoma of thyroid (Hypercalcitonin-aemia)	13.9	0.11	15.8	14.1	0	Serum Ca 5.1mN, Serum PO ₄ 2.4mN, Serum Alk. P'ase 9 K.A.u, Plasma calcitonin 2.8mU/ml. (N=0.16-0.32)
.32	J.A.	F 47	68/3305	Medullary carcinoma of thyroid (Hypercalcitonin-aemia)	18.3	0.14	6.6	8.3	0	Serum Ca 5.1mN, Serum PO ₄ 2.2mN, Serum Alk. P'ase 10 K.A.u, Plasma calcitonin 2.0-3.0mU/ml.
.33	R.B.	M 58	PM. 12876	Cushing's syndrome, Adrenal hyperplasia	6.6	0.14	5.7	67.8	0	Thin bones and compressed vertebral fractures.
.34	H.W.	M 50	PM 12828	Hypophysectomy in 1965 for acromegaly. On prednisone and thyroxine treatment.	7.6	0.55	38.5	37.6	0	
.35	K.R.	F 40	67/1342	Pituitary implants in 1959 1960 for acromegaly. On prednisone and thyroxine treatment.	18.6	0.08	12.2	31.7	0	Minimal anterior lipping in the thoracic spine.
.36	D.D.	M 60	PM 12863	Ca lung with ? ectopic ACTH secretion and hypercalcaemia.	11.4	0.02	3.8	25.3	0	Serum Ca 6.2mN, Serum PO ₄ 0.9mN, Serum Alk. P'ase 8 K.A.u, Serum K 1.2mN, Serum Na 138mN, Serum Cl 70mN, Serum HCO ₃ 55mN, Blood urea 30mg%, Active large adrenals weighing 16.5gms, Parathyroids weigh 190 mgms.

/continued over

M.37	M.W.	F 59	68/3061	Thyrototoxic nodular goitre	22.7	1.61	45.6	21.1	0	Serum Ca 4.5mN, Serum PO ₄ 1.8mN, Serum Alk. P'ase 16 K.A.u, "Thin bones" present.
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* A % volume of calcified trabecular bone
 B % volume of osteoid in trabecular bone
 C % total bone surface covered with osteoid
 D % total bone surface showing resorption not covered with osteoid (R.S.)
 E % total bone surface showing resorption covered with osteoid (R.S.)_o

** Serum Ca N = 4.7-5.5 mN
 Serum PO₄ N = 1.6-2.4 mN
 Serum Alk.P'ase N = 4-11 K.A.u.
 Serum K N = 3.8-5.2 mN
 Serum Na N = 136-149 mN
 Serum Cl N = 100-107 mN
 Serum HCO₃ N = 24-30 mN
 Blood urea N = 14-38 mg%
 Plasma
 calcitonin N = 0.16-0.32 mU/ml

renal calculi showed values of serum calcium consistently within the normal range, although in one case rather high normal. One case of mild diabetes mellitus was hypercalcaemic (5.6 mEq/L) on one occasion two years prior to bone biopsy. Serum phosphorus was normal in all the patients. Serum alkaline phosphatase too was normal in all the patients except in one case which was slightly elevated.

Strontium space was estimated in 6 patients; two patients showed a definite increase, in one patient it was marginally elevated and in three it was normal.

Out of the 5 hypercalcaemic patients, 4 also showed a definite increase in bone turnover rates studied by kinetic tracer studies; as a result of which the patients underwent parathyroidectomy. All 5 proved to have primary hyperparathyroidism with a single adenoma.

The results of histological measurements are shown in Table 8, and also in Figures 28, 29 and 30. The percent amount of calcified trabecular bone is variable; one case (M.23) lies slightly below the mean, two cases (M.24 and M.27) lie beyond the upper limit of the normal range and two cases (M.25 and M.26) lie in the lower half of the normal range, for their comparable age and sex group. One case (M.27) shows a very unusual histological picture (Figure 43). The amount of calcified trabecular bone is 46.9%; the bony trabeculae are thick and crowded. Resorption is normal and few of the surfaces are covered by osteoid seams, the largest measuring 31 μ in thickness with three collagen lamellae, shows some patchy calcification. The broad interlocking thick trabeculae show some mosaic pattern.

For the three cases of unproven primary hyperparathyroidism, the amount of calcified trabecular bone is normal in two and greatly reduced in one.

The amount of bone resorption is towards the upper limit

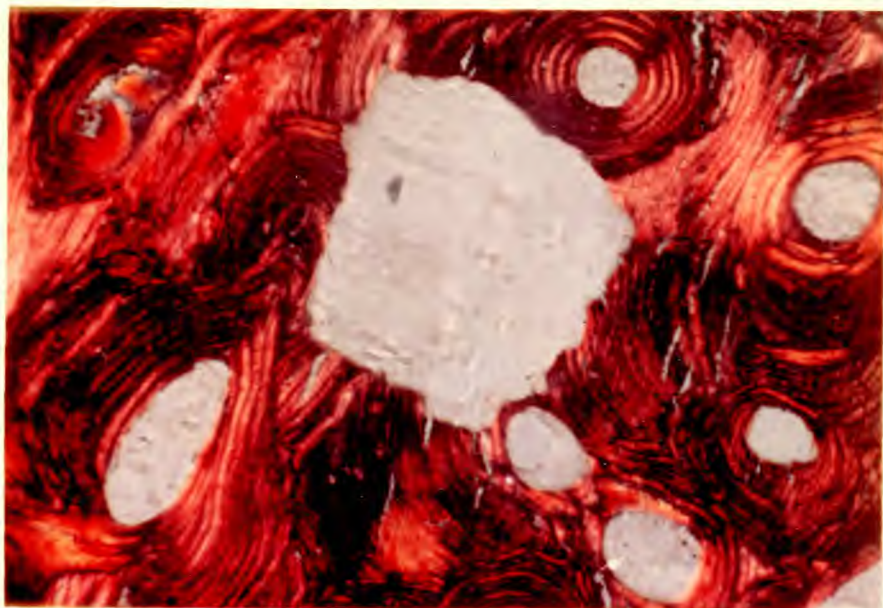


Fig. 42

An undecalcified section from a patient with primary hyperparathyroidism showing the birefringence of the cortical lamellar pattern. A large cortical resorption cavity with resorption cutting across the fibre pattern is seen. (Patient M.26. A.G. Solochrome cyanin x 120. Polarized light).

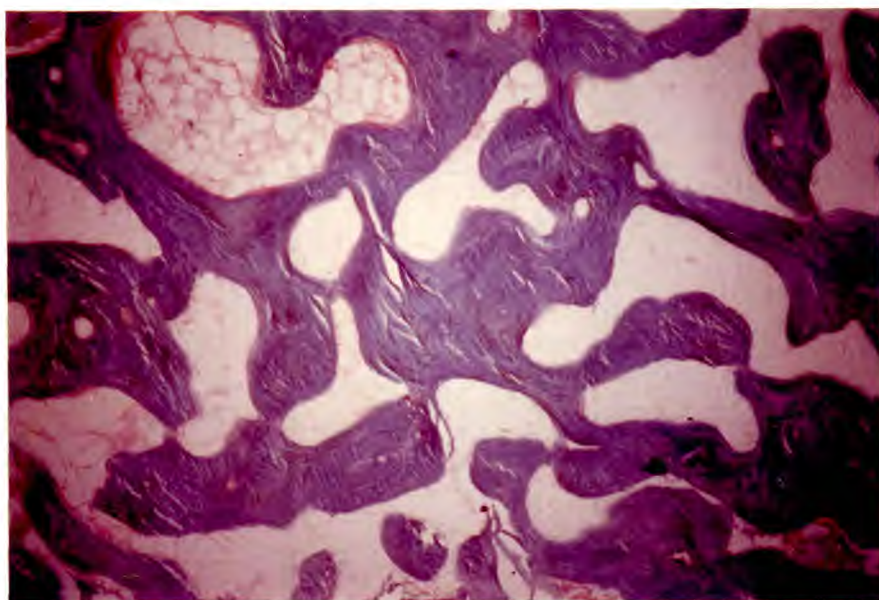


Fig. 43

An undecalcified section from a case of primary hyperparathyroidism showing a very unusual broad, thick, trabecular pattern, with no definite demarcation from the cortical bone. Neither resorption surface nor osteoid seams are prominent. (Patient M.27. A.C. Solochrome cyanin x 30).

of the normal range in the biopsies of two of the patients with adenoma, one of which shows a striking feature ^{case 17 B} in resorptive activity (Figure 42); and well above in one patient (M.23) if osteoid covered resorption is also included. This patient also showed a great excess of osteoid. A second patient (M.26) showed a moderate degree of osteomalacia.

The bone changes encountered in non-parathyroid endocrine disease are presented in Table 9. These cases consisted of two cases of hypercalcitoninaemia due to medullary carcinoma of thyroid, one case of Cushing's syndrome and two cases treated with prednisone and thyroxine for hypopituitarism as a result of hypophysectomy, one case of carcinoma lung with probable ectopic ACTH secretion and finally one case of thyrotoxic nodular goitre. The rather heterogenous nature of this group makes it easier to draw attention to the abnormal results during the discussion.

6.4. DISCUSSION

The results from this small series of patients with a variety of endocrine bone disease do not suggest that bone biopsy should be used as a means of establishing the primary diagnosis. It is of value for confirming or excluding the presence of osteomalacia and for contributing to an understanding of mechanisms involved in the production of bone disease.

Of the five proven cases of primary hyperparathyroidism, three showed figures for resorption near or beyond the upper limit of normal; two were entirely normal. It is interesting to note that the three with the highest figures for resorption were those who presented with symptoms referable to bone, the other two presented with depression attributed to hypercalcaemia and with renal calculi. The proportion of cases with hyperparathyroidism presenting with bone lesions has been decreasing due in part to the emphasis laid on the occurrence of primary

hyperparathyroidism in patients with urinary calculi, 5% or 15% of patients with recurrent calculi (Lee et al 1955). It has been suggested by various authors that in primary hyperparathyroidism two hormones could be produced, one primarily influencing bone metabolism, the other kidney, and that this could account for the dramatic difference in presentation between 'bones' and 'stones' in hyperparathyroidism (Dent et al, 1961). While the normal figures for resorption of one patient (M.27 A.C.) with proven hyperparathyroidism and renal stones studied, could be used to support this, it is worth stressing that this patient was most unusual in several ways. The massive increase in trabecular bone in the biopsy led to the suspicion that it had been taken very close to the iliac crest and also with the partial mosaic histological pattern, that the patient might have had Paget's disease. As far as could be ascertained the biopsy was correctly taken and sectioned. No radiological evidence of Paget's disease was present, but a diffuse increase in bone density was noted by the radiologist. Microdensitometry of radiographs of the ulna bone confirmed an increased density; this was very marked at one centimetre, where the bone is largely trabecular (Doyle F.H., Personal Communication, 1970). The amount of resorption in relation to controls would be slightly greater in this case if it were expressed as an absolute amount rather than a percent figure. However, the reason for the increased amount of trabecular bone in this patient must remain speculative, although it might be related to the slight increase in blood urea (52 mg%). It is possible that the second parathyroid hormone is concerned; it is also possible that an excess secretion of calcitonin as well as parathyroid hormone was present and the combination of hormones led to osteosclerosis. While further studies of bone in patients with hyperparathyroidism and renal stones would be of interest, too much weight should not be placed on one case.

The amount of osteoid in patients with hyperparathyroidism has been known to be occasionally increased since attention was drawn to it by Albright and Reifstein (1948), and recently the quantitative studies of Boyce and Jowsey (1966) showed that patients with hyperparathyroidism may show an increase in the width of osteoid seams. Of the five patients studied here, two showed an increase in osteoid, while three showed normal amounts of osteoid. However, as was the case with Boyce and Jowsey's patients, all were above the mean figures for controls. It is possible that the increased amount of osteoid represent an increase in bone formation rate, and this interpretation has been placed on the increased amount of osteoid found in thyrotoxicosis (Bordier et al, 1967). An alternative explanation is that the hyperparathyroidism may not be primary, but may be 'tertiary hyperparathyroidism'; that is autonomous hyperparathyroidism arising in glands stimulated as a result of some other disease process, in this instance dietary deficiency or malabsorption (Davies et al, 1968). I have no evidence that the patient (M.23 C.M.) with 45% of bone surface showing osteoid, suffered from any of these diseases; but tertiary hyperparathyroidism did develop in two patients in this series, one following prolonged renal failure (R.14) and one with post gastrectomy osteomalacia (M.13).

One interesting correlation that it is possible to study as a result of this work is the relationship of the phosphate excretion index (PEI) to the amount of resorption present in the iliac bone biopsy. The PEI can be regarded as an indirect measurement of parathyroid hormone level, as it reflects the action of parathyroid hormone on the renal handling of phosphate (Brush et al, 1955, Nordin and Fraser, 1960), and the amount of resorption would, of course, be expected to be related to the level of parathyroid hormone. Thalassinios et al (1970) have also shown that there is a close relationship between serum calcium and the PEI.

If the resorption surface (R.S. and R.S._o) is plotted against the PEI for all cases where this measurement is available (Table 10 and Figure 44), most of the patients lie on a line showing a correlation between the two variables, with a few points showing a discrepancy. A study of the table shows that most of the discrepant cases had a large proportion of bone surface covered with osteoid. The surface available for osteoclasts to act would therefore be reduced and a case can be made out for expressing the resorption surface as a percentage of the non-osteoid covered surface. The correlation between these figures and the PEI is shown in Figure 45, the only widely discrepant point is that for a most unusual patient with adult coeliac disease and suspected idiopathic hypoparathyroidism. It is possible that in this patient the response of the kidney to parathyroid hormone was impaired more than the response of the bone. Excluding the patient the coefficient correlation between PEI and the amount of resorption was $+0.84$ ($p < .0001$), confirming the value of the PEI in the prediction of parathyroid function.

While the main effect of parathyroid hormone on bone is generally accepted as stimulation of osteoclasts (Barnicot, 1948, Chang, 1951); the main effect of calcitonin on bone is thought to be an inhibition of osteoclastic activity (MacIntyre, 1967). There are some difficulties in accepting this for normal man as patients with medullary carcinoma of thyroid show considerable increase in serum calcitonin levels without any hypocalcaemia (Cunliffe et al, 1968). Also while calcitonin is important in lowering elevated serum calcium, its effect in normal men may be quite small (MacIntyre, 1967). It is of considerable interest therefore to see that the two patients with medullary carcinoma and hypercalcitoninaemia included in this group both showed normal amounts of resorption. Parathyroid hormone assay results are available for one

TABLE 10

ESTIMATION OF PHOSPHATE EXCRETION IN PATIENTS WITH 'METABOLIC BONE DISEASE'

Case No	Patient	Sex & Age	Clinical Diagnosis	P.E.I. ($N=+0.09$)	HISTOLOGICAL MEASUREMENTS *				
					C	D	E	D + E	$\frac{D}{100-C} \times 100$
M.14	A.H.	F 47	Nutritional osteomalacia	+ .26	88.6	3.8	35.6	39.4	33.3
M.13	H.B.	F 77	Post gastrectomy osteomalacia, tertiary hyperparathyroidism	+ .22	43.1	26.6	4.6	31.2	46.7
M.23	C.M.	F 41	Primary hyperparathyroidism	+ .17	45.1	15.9	8.5	24.4	28.9
M.26	A.G.	F 58	Primary hyperparathyroidism	+ .16	27.6	19.7	0	19.7	27.2
M.24	E.W.	F 57	Primary hyperparathyroidism	+ .04	17.2	9.2	0	9.2	11.1
M.29	C.L.	M 36	Idiopathic renal calculi	+ .024	4.9	17.3	0	17.3	18.2
M.27	A.C.	M 53	Primary hyperparathyroidism	- .003	12.7	11.9	0	11.9	13.6
M.30	G.W.	M 65	Mild diabetes mellitus, probable primary hyperparathyroidism	- .013	3.5	16.5	0	16.5	17.1
M.28	D.B.	F 38	Idiopathic renal calculi	- .016	6.3	12.9	0	12.9	13.8
M.11	J.H.	M 44	Post gastrectomy osteomalacia	- .02	73.8	5.5	2.7	8.2	20.9
M.15	E.I.	F 58	Adult coeliac disease, IgA deficiency	- .04	5.5	7.7	0	7.7	8.2
M.16	M.E.	F 28	Adult coeliac disease	- .07	12.6	12.7	0	12.7	14.5
M.20	A.A.	F 41	Vit.D resistant osteomalacia	+ .36	88.3	6.7	5.0	11.7	57.3
M.21	M.E.	F 40	Vit.D resistant osteomalacia	+ .07	30.7	12.8	12.8	25.6	18.5
M.22	A.L.	F 36	Autonomous hyperparathyroidism with pseudo-hypoparathyroidism	+ .06	66.9	14.5	23.5	38.0	43.8
M.18	H.H.	F 55	Adult coeliac disease, probable idiopathic hypoparathyroidism	- .13	55.8	22.5	15.8	38.3	50.9

* C % total bone surface covered with osteoid. D % total bone surface showing resorption not covered with osteoid (R.S.)
 E % total bone surface showing resorption covered with osteoid (R.S_o). D+E % total bone surface showing resorption (R.S.+R.S_o)
 $\frac{D}{100-C} \times 100$ % non-osteoid covered surface showing resorption.

Fig. 44

— RELATIONSHIP BETWEEN PHOSPHATE EXCRETION INDEX AND
BONE RESORPTION SURFACE —

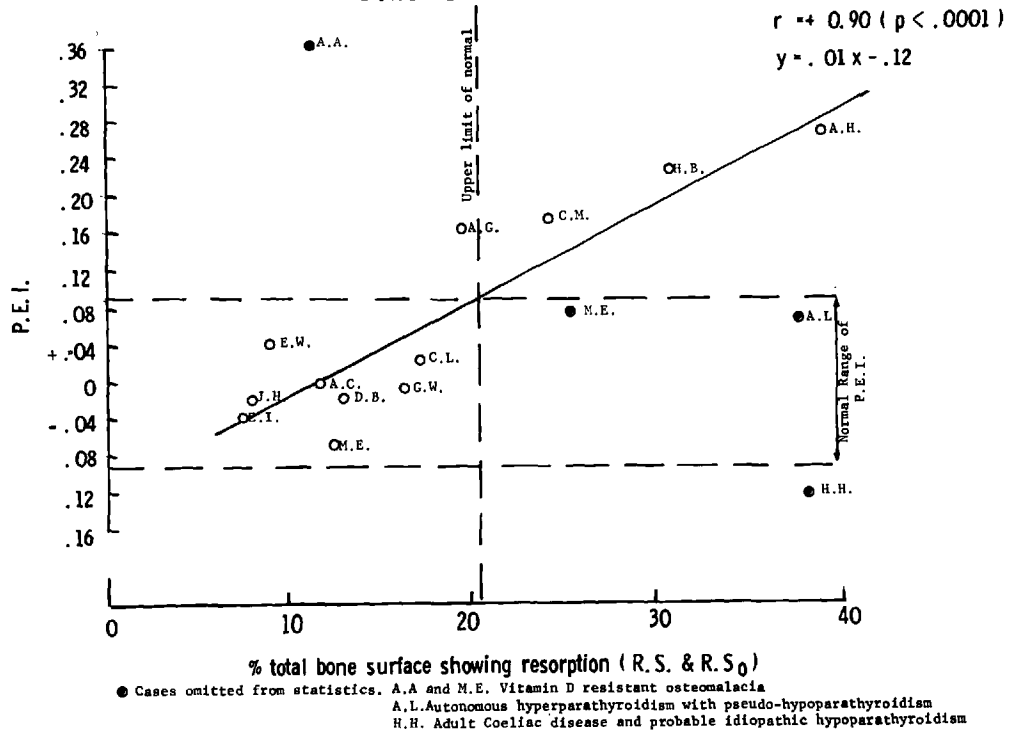
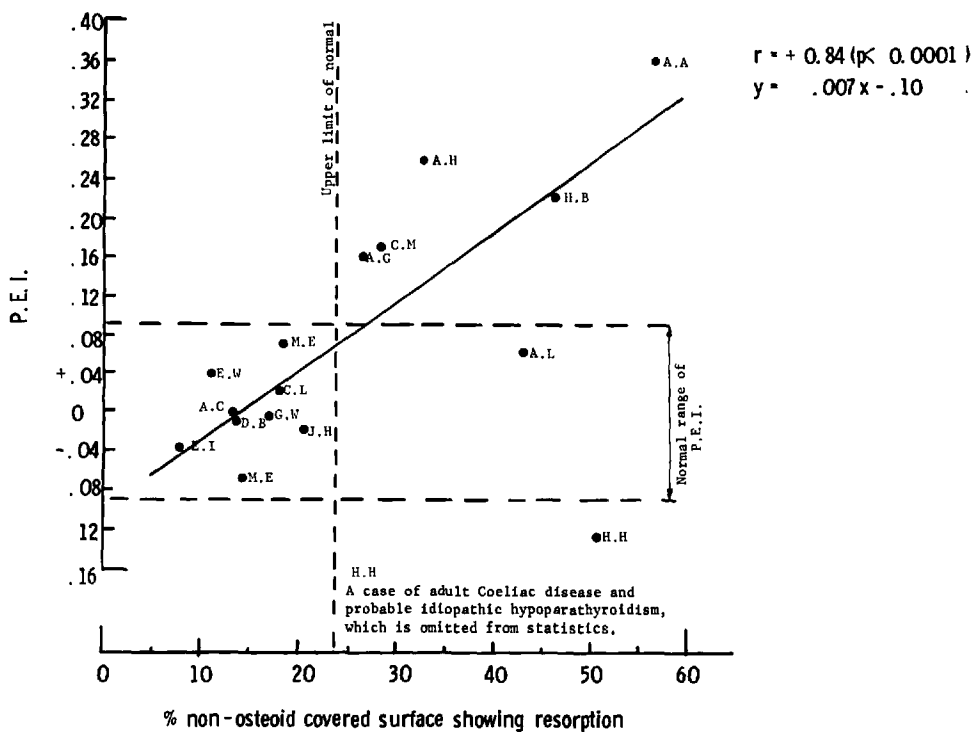


Fig. 45

— RELATIONSHIP BETWEEN PHOSPHATE EXCRETION INDEX AND
BONE RESORPTION SURFACE —



of these patients (M.32 J.A.) and the mean value for 5 estimations lies just above the upper limit of normal, (i.e. 0.34 ng/ml. Normal ≤ 0.3 ng/ml.). It is possible therefore that a gross excess of calcitonin secretion is balanced by a small increase in parathyroid hormone production. It may well be that the role of calcitonin is much more important in children, with their greater bone turnover, than in adults.

The other endocrine patients all showed abnormal bone biopsies. Excess resorption was present in the patient with thyrotoxicosis (M.37 M.W.), and in both the patients with Cushing's syndrome and in the two receiving steroid therapy. Bordier et al (1967) also found excess resorption in two patients with thyrotoxicosis. Earlier work has suggested that the osteoporosis of Cushing's syndrome, well illustrated by low figure of 6.6% for trabecular bone in one patient (M.33) was due to impaired bone formation rather than increased destruction (Mooser, 1921, Follis, 1951, Sissons, 1956,). While these do not represent quantitative measurement of resorption, there is a surprising unanimity of results in view of my findings. However, close study of the article by Bordier et al (1964) shows that two steroid treated patients were included with a variety of other causes in a study of osteoporosis. Although this was not commented on, both patients with steroid osteoporosis showed a marked increase in resorption, enough in fact to bring the mean for the group to slightly above that for their controls. Bordier's figures, together with mine, suggest that excess osteoplastic activity is an important factor in steroid osteoporosis, as do the results of Birkenhager et al (1967) who found that in clinically severe cases of hypercortisonism a high osteoclastic activity was always found. Excess osteoid was found in one of the steroid treated patients and in the thyrotoxic patient. Whether this was due to a direct hormonal effect or to some other factor is uncertain.

These findings stress the importance of regarding 'endocrine osteoporosis' as a different disease entity from 'idiopathic osteoporosis', and suggest that the mechanisms involved may be quite different.

6.5. SUMMARY

The cases under study in this group were divided into two, parathyroid (8 cases) and non-parathyroid endocrine bone disease (7 cases).

Of the 5 proven cases of primary hyperparathyroidism, 3 showed figures for resorption near or beyond the upper limit of normal, 2 were entirely normal. The former three also presented with symptoms referable to bone, the other two presented with renal calculi and with depression attributed to hypercalcaemia. Of the 5 proven cases of primary hyperparathyroidism, two showed a definite increase in osteoid while 3 showed normal amounts of osteoid, though all were above the mean figures for controls.

One interesting finding as a result of this study is a good correlation between phosphate excretion index and the amount of bone resorption surface present in iliac bone biopsies.

The other endocrine patients (Non-parathyroid group) all showed abnormal bone biopsies. An increase in resorptive activity was present in the patient with thyrotoxicosis, in both patients with Cushing's syndrome and in the two receiving steroid therapy. An excess amount of osteoid was present in one of the steroid treated patients and in the thyrotoxic patient. The amount of calcified trabecular bone was within the normal range for all the patients in this group. In one case of Cushing's syndrome where a radiological diagnosis of thin bone was made, and vertebral fractures were present, the amount of calcified trabecular bone was right at the lower limit of the normal range. This patient was also the one who showed excessive resorptive activity. In two cases

of hypercalcitoninaemia due to medullary carcinoma of thyroid, the histology of bone showed no abnormalities, in particular the amount of resorption surface was well within the normal range.

CHAPTER 7

RENAL OSTEODYSTROPHY

7.1. INTRODUCTION

Since the observations of Lucas in 1883, it has been well known that patients with chronic renal failure commonly show skeletal abnormalities. Rickets, osteomalacia, osteoporosis, osteosclerosis and osteitis fibrosa have all been described. (Follis and Jackson, 1943, Snapper, 1949, Crawford et al, 1954, Stanbury, 1957, Kaye et al, 1960). The term renal osteodystrophy was, however, introduced by Liu and Chu in 1943 to embrace the various skeletal complications of chronic renal disease. Bone disease is a common complication of chronic renal failure whether this results from glomerular failure, both glomerular and tubular insufficiency, or from tubular insufficiency only. The pathogenesis of renal osteodystrophy is still a matter of dispute (Relman, 1968, Stanbury, 1968). However, the generally accepted view is that in chronic renal failure an acquired resistance develops to the effects of vitamin D on both intestine and bone, resulting in hypocalcaemia, skeletal abnormalities and secondary hyperplasia of the parathyroid glands (Liu and Chu, 1943, Stanbury, 1957, Stanbury and Lumb, 1966, Kleeman et al, 1967). Fairbank, 1951, stated that bone disease is a relatively infrequent complication of renal disease in the adult. This statement might hold true to a certain extent if the incidence of bone disease is mainly judged on clinical and radiological findings, however, a more accurate index of bone changes will be revealed only by histopathological studies. Follis and Jackson (1943) studied the vertebrae of patients dying with renal failure and found the histological changes of osteomalacia and osteitis fibrosa either occurring singly or in combination. About half their cases showed osteomalacia and one third osteitis fibrosa and the remainder showed features of both conditions. Berner (1944) also found similar changes in a series of 138 cases.

In children the clinical features of renal osteodystrophy are more pronounced when compared with those of adults. Presumably in the former the rapid turnover and remodelling of bone makes it likely that abnormalities will develop more rapidly and skeletal deformities similar to those of late nutritional rickets (Barber, 1926) may develop.

While it is generally agreed that the bone is abnormal in renal failure, quantitation of the changes has rarely been carried out. The most significant paper is that of Garner and Ball, who studied undecalcified sections and measured the amount of calcified bone and the amount of osteoid and compared them with the control series. They did not quantify the amount of resorption and their osteoid measurements were based on volume only, not on the surface area of bone covered. Their main conclusion was that the trabecular bone mass increased in renal failure.

The occurrence of bone disease in renal failure has become of much greater practical importance since the introduction of haemodialysis and transplantation. With haemodialysis particularly patients are now being kept alive in a prolonged state of mild renal failure, and in some centres bone disease has become an important clinical problem.

The purpose of this chapter is five-fold - first to quantify the bone changes prevalent in patients with chronic renal disease, second to study the effects of haemodialysis and kidney transplantation and parathyroidectomy on renal osteodystrophy, third to study the relationship of biochemical investigations (serum calcium, serum inorganic phosphorus, the ionic product of calcium and phosphorus, serum alkaline phosphatase, total proteins, and blood urea) to the state of renal osteodystrophy, fourth to study the relationship of roentgenographic observations and the predominant type of bone disease, (the radiological and

histological assessment of hyperparathyroidism and a correlative study between the ulnar mineral thickness and the percent volume of calcified trabecular bone in an iliac bone biopsy) and finally to study the association of parathyroid hyperplasia and the type of bone disease in patients with chronic renal failure.

7.2. PATIENTS STUDIED

The majority of the patients were admitted into the Artificial Kidney Unit to undergo haemodialysis for chronic renal azotaemia of considerable duration. The patients were also selected as suitable for kidney transplantation and were maintained on haemodialysis until this could be carried out. The patients under study consisted of 34 males and 26 females. Patients were usually in their third and fourth decade and the average age was 34 years. The underlying renal disease was established either by renal biopsy or from the kidney removed in preparation for transplantation or at autopsy in the majority of the cases; but in a few instances the diagnosis was made on clinical grounds only. Iliac bone biopsies were obtained in 28 patients who had not had haemodialysis, in 22 patients during haemodialysis, in 19 patients after kidney transplantation and in 3 patients before and after parathyroidectomy for persistent hypercalcaemia. The renal failure was due to chronic glomerulonephritis (48%), chronic pyelonephritis (32%), congenital polycystic kidneys (10%), and miscellaneous nephropathies (Hydronephrosis, renal amyloidosis, disseminated lupus erythematosus, and congenital obstructive uropathy, 10%).

7.3. RESULTS

The cases under study in this chapter are set out in Tables 14, 15, 17, 18 and 19 depicting the general clinical status of the patients, together with the biochemical findings and histological measurements. The figures quoted for the biochemical investigations represent the mean of at least two estimations which were performed on fresh blood samples taken shortly before the commencement of haemodialysis, and within one week of iliac bone biopsy.

7.3.1. General Studies

The group of patients with renal failure was looked at as a whole for the purpose of comparing the figures with certain biochemical radiological and histological data. The serum calcium, phosphorus, ionic product of calcium and phosphorus, and serum alkaline phosphatase showed no consistent pattern. The total proteins were usually low, with a few exceptions, the blood urea was of course raised in all. Some of these findings will be dealt with in greater detail later. Radiological studies of the hands, pelvis and spine were performed in nearly all patients, and particular attention was paid to the presence or absence of subperiosteal cortical erosions of the phalanges, pseudo fractures and the so-called 'rugger jersey' appearance of the spine. None of these patients showed pseudo fractures nor any radiological evidence of osteosclerosis. Cortical erosions were seen in a number of patients and an attempt was made to grade these into minimal, moderate and florid. The comparison between the radiological assessment of cortical erosions and the histological assessment of bone resorption is shown in Table 11, Figure 46. It can be seen that the two cases classified as moderate or florid on radiological grounds show a high value for resorption surface. Six out of the seven cases described as minimal on radiology showed a high value for resorption surface. However, about half (12 out of 23) of those normal on radiological grounds showed an abnormally high value for resorption in iliac bone biopsies. It is concluded that radiological assessment of resorption is, even with very careful study of the films, only of value if abnormal.

The amount of trabecular bone present in these biopsies, was when possible, compared with the ulnar bone density measurements. These are based on the radiographic density of the ulna, the x-ray being taken under standard conditions and the density compared with that of a

TABLE 11

The relationship between the radiological assessment of hyperparathyroidism based on the subperiosteal resorption in the phalanges of fingers (A) and the percentage of total bone surfaces showing resorption (R.S. & R.S.O) in iliac crest biopsy (B)

Case No	Patient	Sex & Age	Bone Biopsy Number	Nature of Therapy	A*	B
R.7.	J.T.	M 28	68/415	Not dialysed	1	51.6
R.10.	A.U.	M 33	69/95	Post Transplant. 6 mts.	0	14.4
R.11.	J.B.	F 23	68/1566	Pre. Transplant	0	41.4
R.11.	J.B.	F 23	69/482	Post Transplant. 6 mts.	0	14.9
R.14.	L.G.	F 50	67/1341	Pre.Parathyroidectomy	3	64.7
R.14.	L.G.	F 50	68/1893	Post Parathyroidectomy.1yr.	2	5.3
R.12.	D.O.	F 31	68/3354	Post Transplant. 6 mts.	0	9.5
R.13.	M.B.	F 38	69/143	Post Transplant. 6 mts.	0	23.6
R.26.	G.B.	F 40	69/240	Post Parathyroidectomy. 6 mts	0	21.4
R.27.	K.B.	M 23	69/1077	Haemodialysis. 5 wks.	1	39.7
R.30.	A.M.	M 33	69/1081	Haemodialysis. 8 wks.	0	18.3
R.31.	J.K.	F 47	68/316	Haemodialysis. 10 wks.	0	33.1
R.33.	T.R.	M 56	69/1084	Haemodialysis. 8 wks.	0	15.7
R.34.	R.L.	F 34	69/239	Haemodialysis. 6 wks.	0	55.9
R.35.	R.P.	F 47	68/1569	Haemodialysis. 12 wks.	0	41.4
R.36.	A.B.	F 43	PM12745	Haemodialysis 20 wks.	0	16.9
R.37.	J.D.	M 32	69/176	Haemodialysis 40 wks.	0	17.8
R.38.	D.P.	F 45	69/269	Haemodialysis 28 wks.	0	39.7
R.39.	M.C.	F 37	68/460	Pre Transplant	0	57.8
R.39.	M.C.	F 37	69/1262	Post Transplant.1 yr.	0	34.6
R.40.	B.B.	F 20	69/1261	Post Transplant. 1 yr.	1	16.5
R.43.	A.W.	F 28	69/479	Post Parathyroidectomy.1yr.	1	31.1
R.44.	A.B.	M 38	68/682	Haemodialysis > 3 yrs.	0	29.2
R.46.	D.C.	M 27	68/1997	Haemodialysis 4 wks.	0	41.4
R.47.	A.R.	M 24	69/645	Haemodialysis 44 wks.	2	43.8
R.48.	W.M.	M 34	68/2758	Haemodialysis 36 wks.	1	27.1
R.45.	J.S.	F 37	69/154	Pre.Transplant	1	23.8
R.50.	A.F.	F 23	69/175	Post Transplant. 2½yrs.	0	12.2
R.52.	D.F.	M 19	69/54	Post Transplant 1 yr.	1	21.1
R.53.	R.S.	F 21	69/708	Post transplant 2 yrs.	0	9.7
R.54.	M.K.	M 22	69/304	Post Transplant 2 yrs.	0	49.4
R.57.	B.S.	M 35	69/1082	Post Transplant 1 yr.	0	10.7

* Grading of subperiosteal cortical bone resorption in phalanges of fingers.

Grade 0 - No subperiosteal resorption. Grade 1- Minimal but definite.

Grade 2 - Moderate. Grade 3 - Florid

TABLE 12

The relationship between the mineral thickness at a point one centimetre from the distal end of the ulna bone (A) and the per cent volume of calcified trabecular bone in an iliac crest biopsy (B)

Case No	Patient	Sex & Age	Bone Biopsy Number	Nature of Therapy *	A	B
R.11	J.B.	F 23	68/1566	Pre.Transplant	256	24.9
R.11	J.B.	F 23	69/482	Post Transplant. $\frac{1}{2}$ yr.	228	19.5
R.12	D.O.	F 31	68/3354	Post Transplant. $\frac{1}{2}$ yr.	318	28.5
R.13	M.B.	F 38	69/143	Post Transplant. $\frac{1}{2}$ yr.	302	25.2
R.14	L.G.	F 50	67/1341	Pre.P.	28	17.8
R.14	L.G.	F 50	68/1893	Post P. 1 yr.	77	20.5
R.26	G.B.	F 40	69/240	Post P. $\frac{1}{2}$ yr.	251	19.9
R.27	K.B.	M 23	69/1077	Haemodialysis. 5 wks.	561	34.1
R.29	A.K.	M 27	69/1078	Haemodialysis. 5 wks.	237	14.9
R.30	A.M.	M 33	69/1081	Haemodialysis. 8 wks.	384	12.7
R.31	J.K.	F 47	68/316	Pre.Transplant	60	20.1
R.33	T.R.	M 56	69/1084	Haemodialysis. 8 wks.	116	22.7
R.34	R.L.	F 34	69/239	Haemodialysis. 6 wks.	212	12.3
R.35	R.P.	F 47	68/1569	Haemodialysis. 12 wks.	125	17.9
R.37	D.C.	M 27	68/1997	Haemodialysis. 4 wks.	507	18.9
R.38	D.P.	F 45	69/269	Haemodialysis. 28 wks.	175	11.8
R.39	M.C.	F 37	68/460	Pre.Transplant	408	31.7
R.40	B.B.	F 20	69/1261	Post Transplant. 1 yr.	309	20.3
R.43	A.W.	F 28	68/418	Pre. P.	307	35.9
R.43	A.W.	F 28	69/479	Post P. 1 yr.	273	31.2
R.44	A.B.	M 38	68/682	Haemodialysis > 3 yrs.	228	20.3
R.45	J.S.	F 37	69/154	Haemodialysis. 32 wks.	233	22.8
R.46	J.D.	M 32	69/176	Haemodialysis. 40 wks.	268	30.2
R.47	A.R.	M 24	69/645	Haemodialysis. 44 wks.	322	26.3
R.48	W.M.	M 34	68/2758	Haemodialysis. 36 wks.	384	17.3
R.50	A.F.	F 23	69/175	Post. Transplant. $2\frac{1}{2}$ yrs.	123	15.1
R.52	D.F.	M 19	69/54	Post Transplant. 1 yr.	257	16.8
R.53	I.S.	F 21	69/708	Post Transplant. 2 yrs.	232	27.5
R.54	M.K.	M 22	69/304	Post Transplant. 2 yrs.	277	20.4
R.57	B.S.	M 35	69/1082	Post Transplant. 1 yr.	293	14.2

* Pre.P. Pre Parathyroidectomy.
 Post P. Post Parathyroidectomy.

Fig. 46

— COMPARISON BETWEEN THE RADIOLOGICAL & HISTOLOGICAL ASSESSMENT OF HYPERPARATHYROIDISM IN PATIENTS WITH CHRONIC RENAL DISEASE—

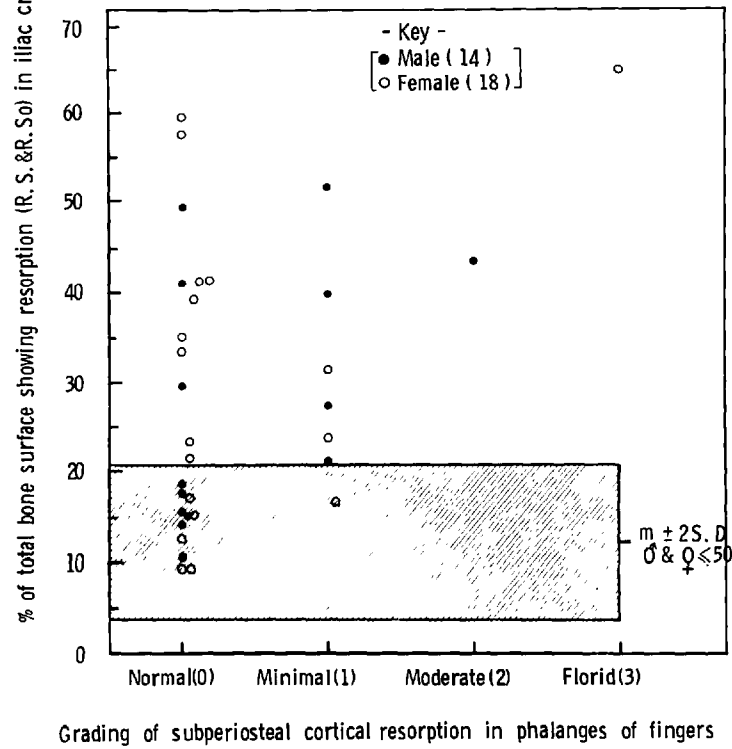
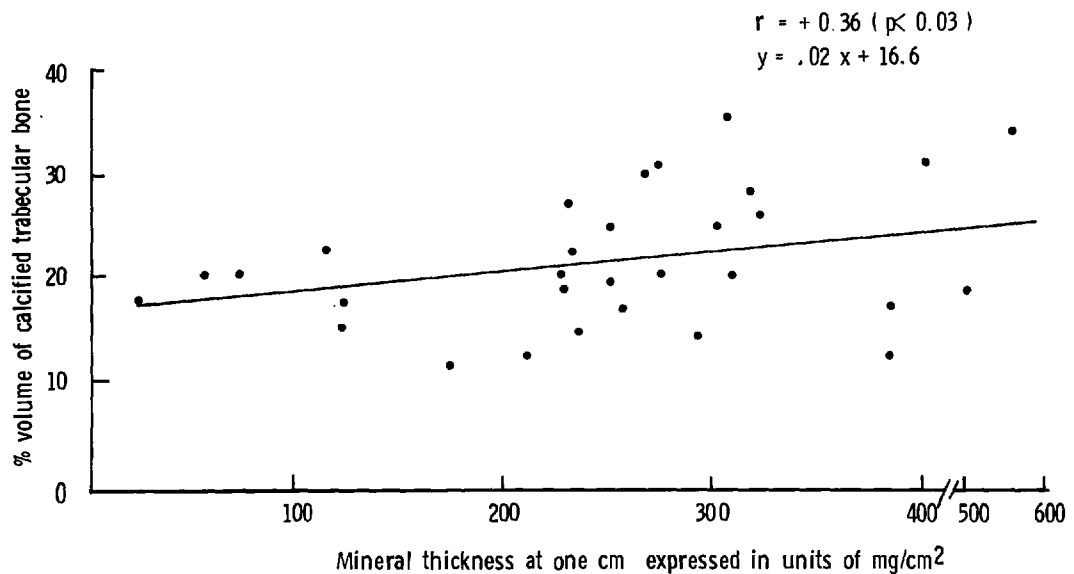


Fig. 47

—RELATIONSHIP BETWEEN THE MINERAL THICKNESS AT A POINT ONE CM. FROM THE DISTAL END OF ULNA BONE AND THE AMOUNT OF CALCIFIED TRABECULAR BONE IN AN ILIAC CREST BIOPSY IN PATIENTS WITH CHRONIC RENAL DISEASE—



standard aluminium step wedge. As the cortical thickness is very low at its distal end, increasing proximally, (Doyle, 1969) the trabecular bone volume of the iliac bone biopsies was compared with the ulnar mineral thickness at one centimetre, where the ulna is very largely composed of trabecular bone. The results are set out in Table 12, Figure 47, and show a modest correlation between the two variables.

In addition to the histological studies on the iliac bone biopsies whenever possible the parathyroids were taken for study. This proved possible in 15 cases, all with elevated blood urea (Table 13). In 13 cases the glands were obtained at autopsy and in 2 at surgery. These glands were weighed carefully in 9 cases and as in some cases fat was present within the gland, a 'glandular' weight was calculated. This was obtained by calculating in a representative section for each gland, the area occupied by fat and by glandular tissue. A simple point counting grid technique was used for this (See Appendix). The glandular weight of the parathyroids was calculated by multiplying the gross weight by the figure for the proportion of the gland which was occupied by glandular tissue. These figures show a clear correlation with the percentage of bone surface showing resorption (Figure 49).

Apart from these general points, the results are best considered under the various sub-groups of patients studied.

7.3.2. Chronic Renal Failure Patients not on Haemodialysis

The results are set out in Table 14. There is no consistent pattern in serum calcium, phosphorus or ionic product of calcium and phosphorus; although the serum calcium tends to be low and the phosphorus high, as expected. In Figures 50 and 51, these values are set out graphically, with the dominant histological finding of increased osteoid or resorption shown as 'osteomalacia' and 'hyperparathyroidism' respectively. Serum alkaline phosphatase was estimated in 23 patients, of this group only 10 patients lie within the normal range, (Figs. 52 and 53)

TABLE 13

PARATHYROID STATUS IN CHRONIC RENAL DISEASE

G E N E R A L					HISTOLOGICAL MEASUREMENTS ϕ					P A R A T H Y R O I D S				MICROSCOPIC FEATURES
Case No	Patient	Sex & Age	Haemo-dialysis	Blood Urea (mg%)	A	B	C	D	E	Section Number	Size *	Gross Weight (mg)	Net Weight (mg)**	
R.14	L.G.	F 50	No	160	17.8	1.9	53.1	42.2	22.5	67/1630	+	14000	11200	Diffuse and nodular chief cell hyperplasia some transition to water clear cell hyperplasia.
R.16	P.M.	F 34	No	198	15.1	0.04	5.2	43.2	1.0	PM/12631	+	330	283	Diffuse and nodular chief cell hyperplasia and areas of oxyntic cell hyperplasia.
R.17	P.C.	F 34	No	146	12.9	0.07	6.8	24.7	0	PM/12669	+	320	250	Diffuse and nodular chief cell hyperplasia some transition to water clear cell hyperplasia.
R.18	E.S.	F 43	No	102	18.3	0.06	8.0	40.2	1.2	PM/12788	+	350	259	Diffuse and nodular chief cell hyperplasia.
R.19	D.B.	F 36	No	212	13.9	0.05	4.0	34.7	0	PM/12874	+	250	210	Diffuse chief cell hyperplasia.
R.20	E.F.	M 30	No	159	14.6	0.47	31.5	24.2	6.3	PM/12627	+	..	..	Diffuse chief cell hyperplasia.
R.21	M.D.	F 29	No	..	20.6	0.03	3.5	15.2	0	PM/12888	-	120	86	Normal.
R.24	R.N.	M 68	No	..	9.5	4.30	82.9	9.4	24.8	PM/12795	+	..	..	Diffuse chief cell hyperplasia.
R.25	R.H.	M 59	No	117	14.1	1.51	48.3	17.5	10.8	PM/12616	+	140	120	Diffuse chief cell hyperplasia.
R.28	K.K.	M 24	Yes. 6 mts.	293	24.9	0.18	21.9	23.4	3.1	PM/13112	+	..	..	Diffuse chief cell hyperplasia.
R.36	A.B.	F 43	Yes 5 mts.	125	28.7	0	1.2	16.9	0	PM/12745	+	..	..	Diffuse chief cell hyperplasia.

continued over/

R.41	P.A.	F 32	Yes 1½ yrs.	..	17.2	0.28	26.9	46.9	6.9	PM/ 12175	+	..	..	Diffuse and nodular chief cell hyperplasia some transition to water clear cell and oxyntic cell hyperplasia.
R.42	T.M.	M 53	Yes 1½ yrs.	136	6.4	0.04	10.8	23.9	2.2	PM/ 12615	+	..	..	Diffuse chief cell hyperplasia.
R.43	A.W.	F 28	Yes 2½ yrs.	92	35.9	3.46	52.6	23.7	16.5	68/444	+	1220	976	Diffuse and nodular chief cell hyperplasia some transition to water clear cell hyperplasia.
R.44	A.B.	M 38	Yes >3 yrs.	175	20.3	1.78	63.4	14.8	14.4	PM/ 12996	+	615	461	Diffuse chief cell hyperplasia some transition to water clear cell hyperplasia.

Ø A % volume of calcified trabecular bone. B % volume of osteoid in trabecular bone.
 C % total bone surface covered with osteoid. D % total bone surface showing resorption not covered with osteoid (RS)
 E % total bone surface showing resorption covered with osteoid (R.S.₀)

* + Parathyroids enlarged. - Parathyroids not enlarged.

** Net weight is the gross parathyroid weight multiplied by the per cent of the gland occupied by glandular tissue (based on a point count measurement, separating glandular from fatty and connective tissue).

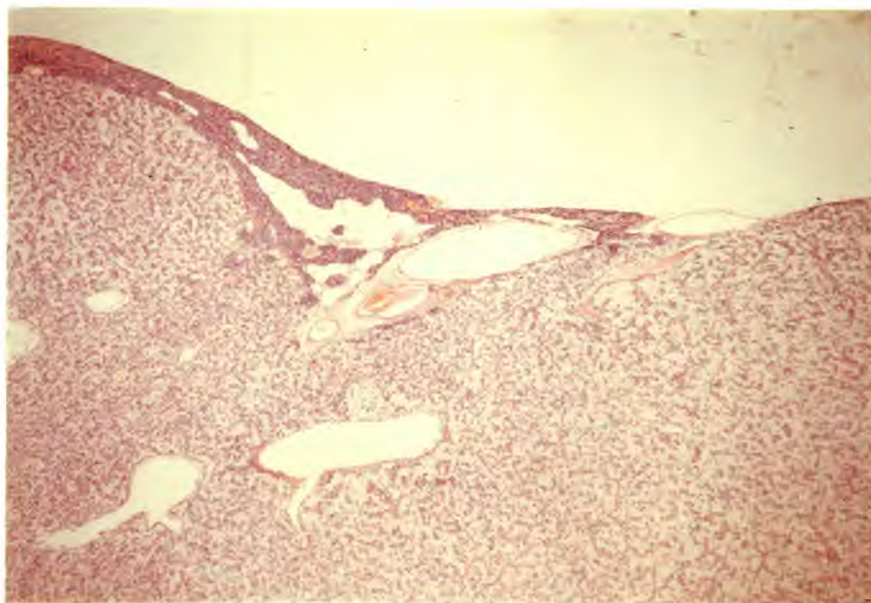


Fig. 48

Section of one of two parathyroid adenomas found at total parathyroidectomy in a patient with long standing renal failure. Note the diffuse water clear cell pattern in the adenoma and the fat in the rim of compressed chief cells. (Patient R.14. L.G. Haematoxylineosin x 30).

Fig. 49

— RELATIONSHIP BETWEEN MEAN PARATHYROID GLANDULAR WEIGHT
AND THE AMOUNT OF BONE RESORPTION SURFACE IN PATIENTS
WITH CHRONIC RENAL DISEASE—

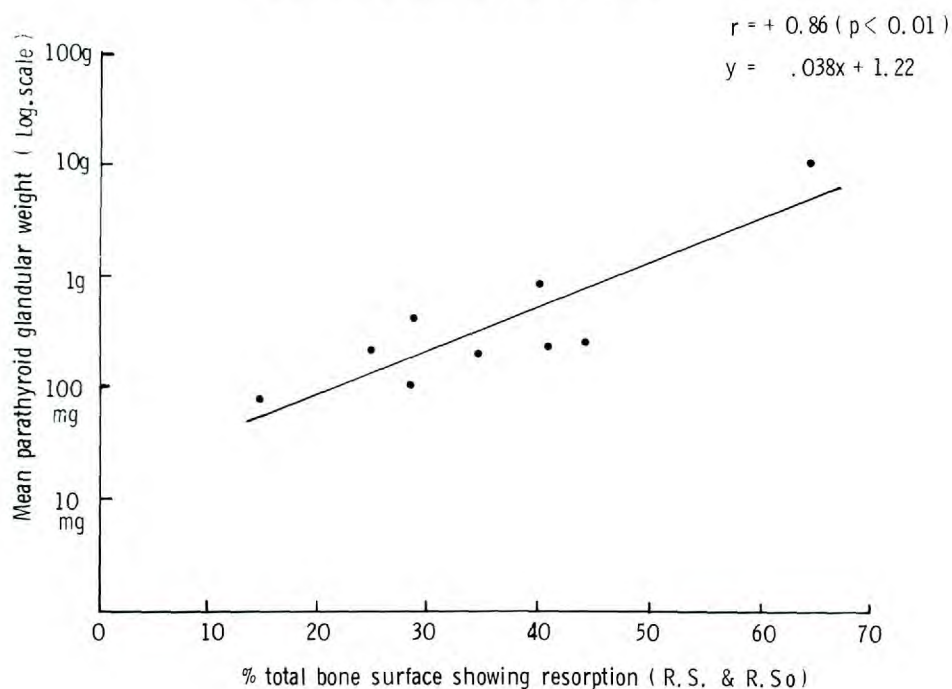


Fig. 50

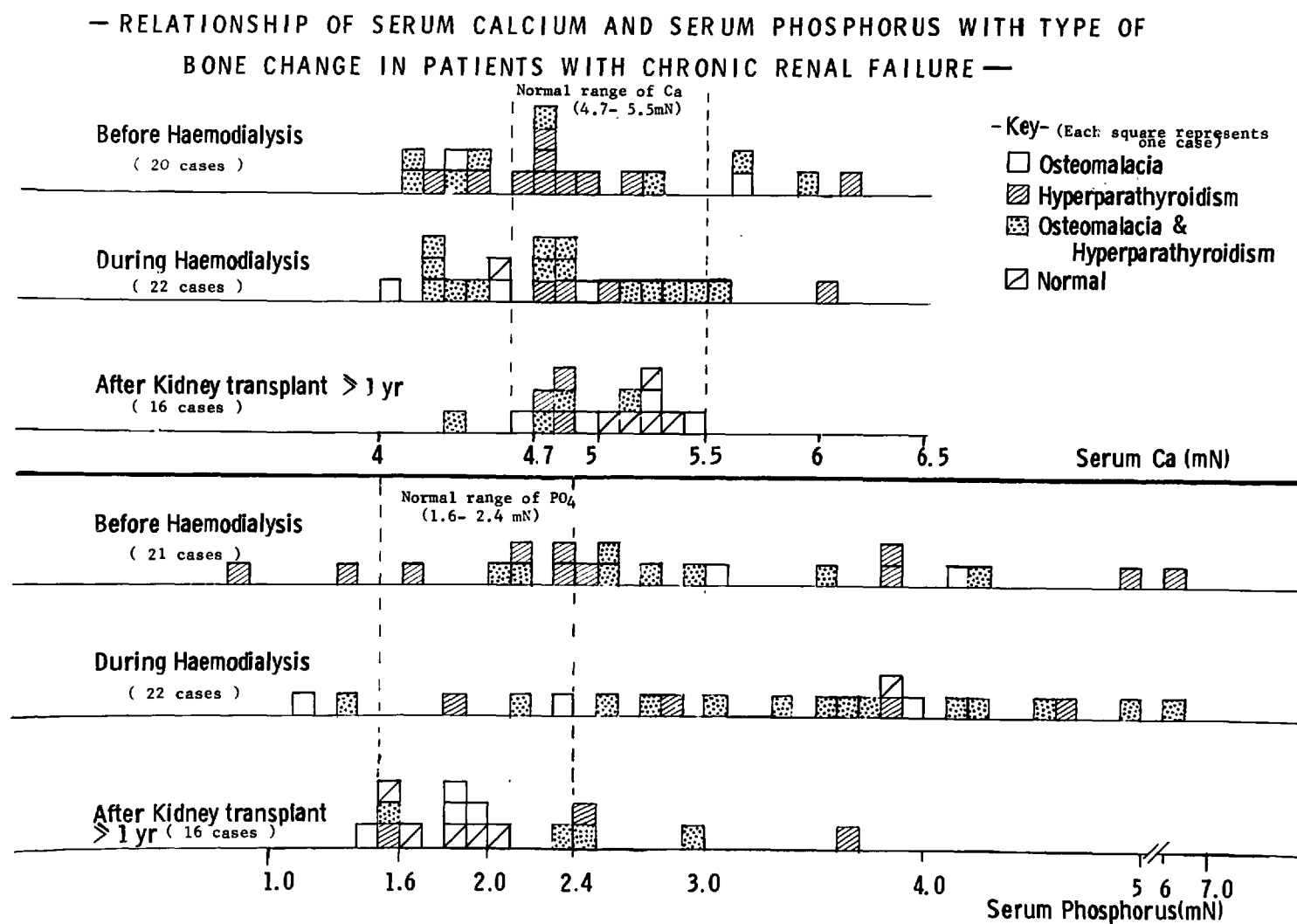


Fig. 51

— RELATIONSHIP OF $(Ca) \times (P)$ WITH TYPE OF BONE CHANGE IN
PATIENTS WITH CHRONIC RENAL FAILURE —

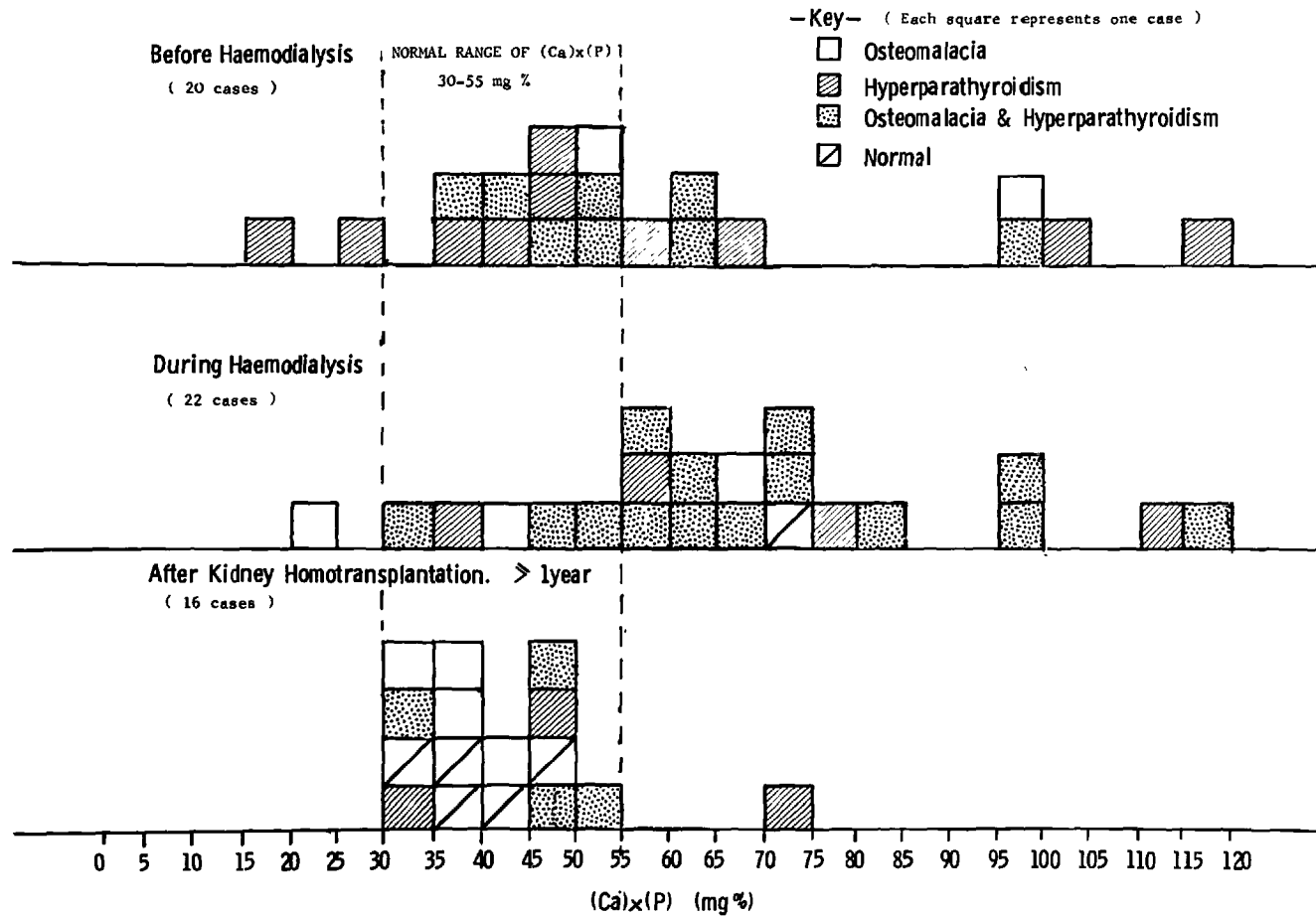


Fig. 52

— RELATIONSHIP BETWEEN SERUM ALKALINE PHOSPHATASE AND THE AMOUNT
OF OSTEOID IN PATIENTS WITH CHRONIC RENAL FAILURE —

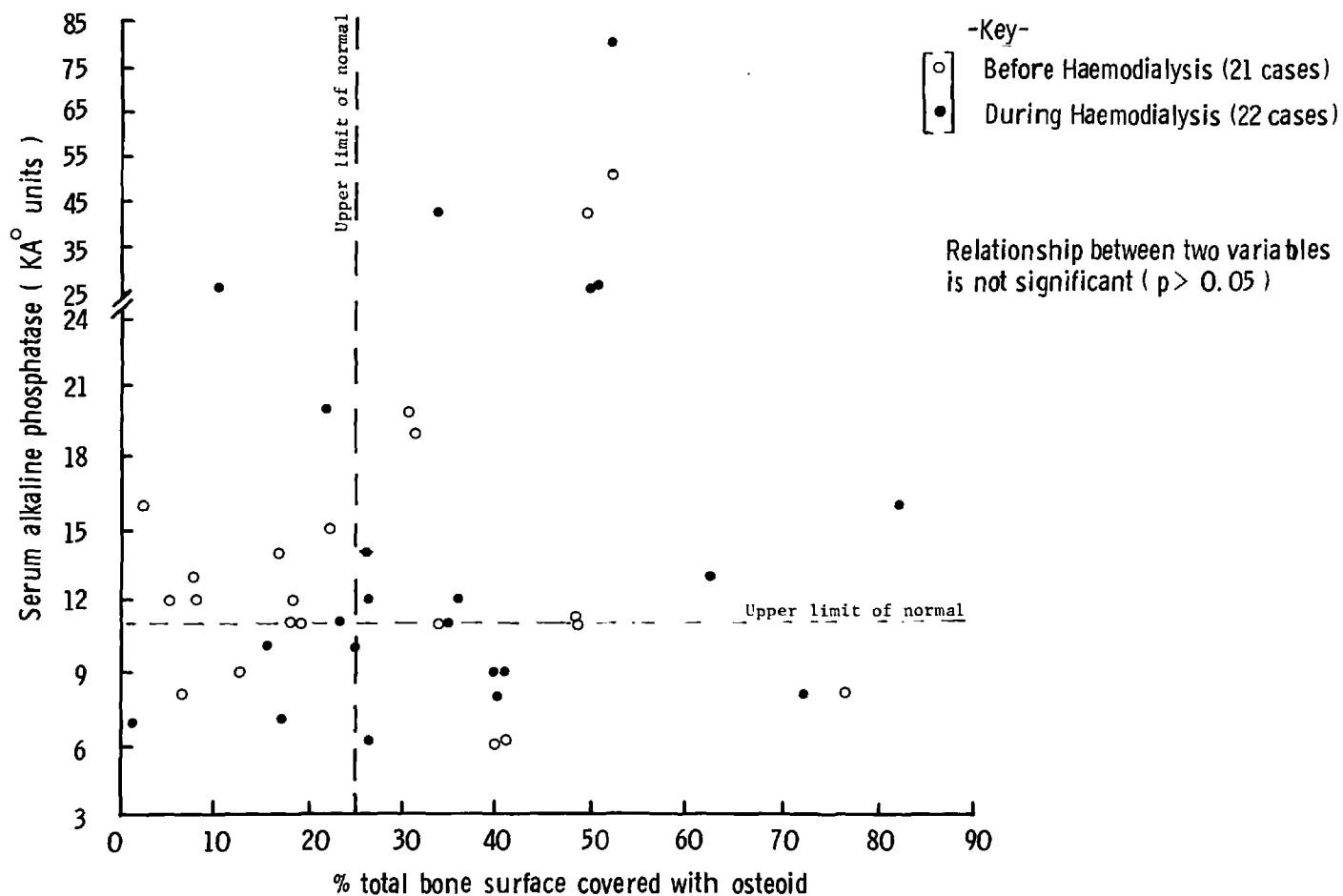


Fig. 53

— RELATIONSHIP OF SERUM ALKALINE PHOSPHATASE WITH TYPE OF BONE
CHANGE IN PATIENTS WITH CHRONIC RENAL FAILURE —

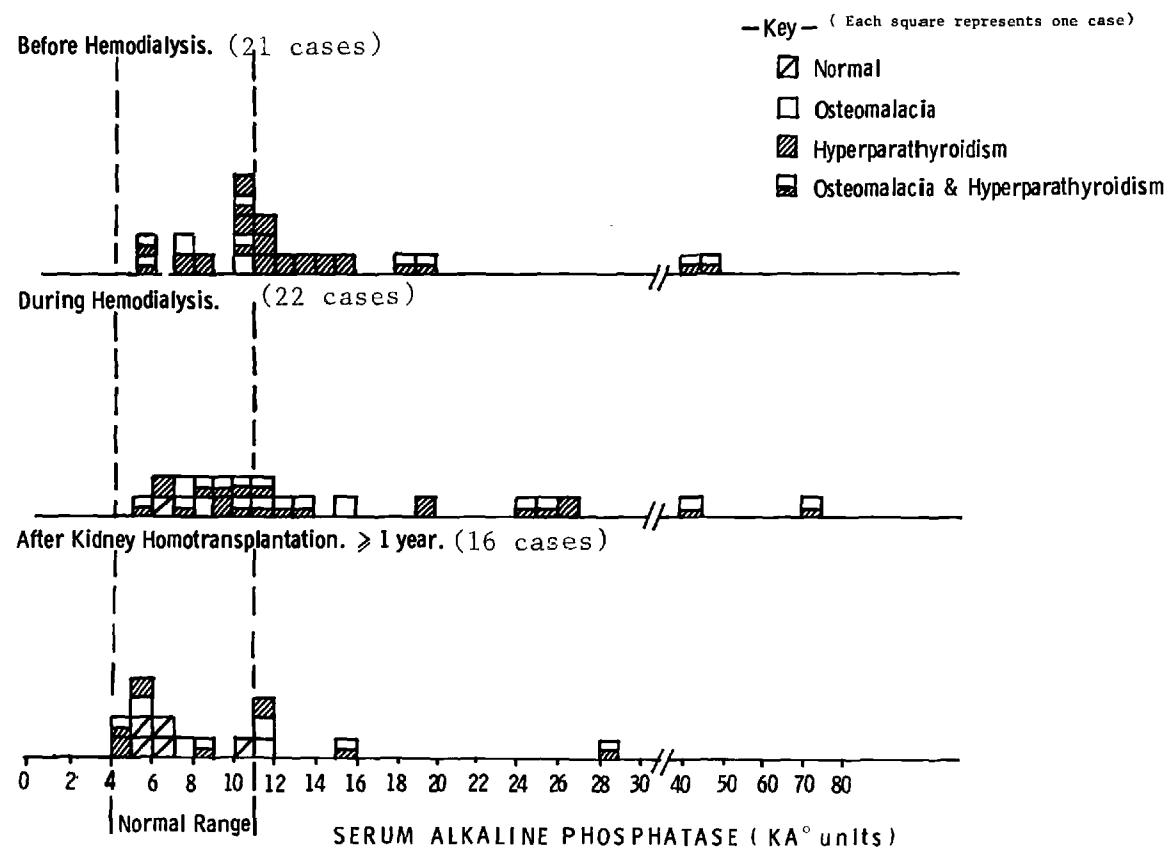


Fig. 54

—AMOUNT OF CALCIFIED TRABECULAR BONE IN
PATIENTS WITH CHRONIC RENAL FAILURE—

-Key-

- Before Haemodialysis (28 cases)
- △ During Haemodialysis < 6mths (11 cases)
- ▲ During Haemodialysis > 6mths (11 cases)
- After kidney transplant > 1 yr. (16 cases)

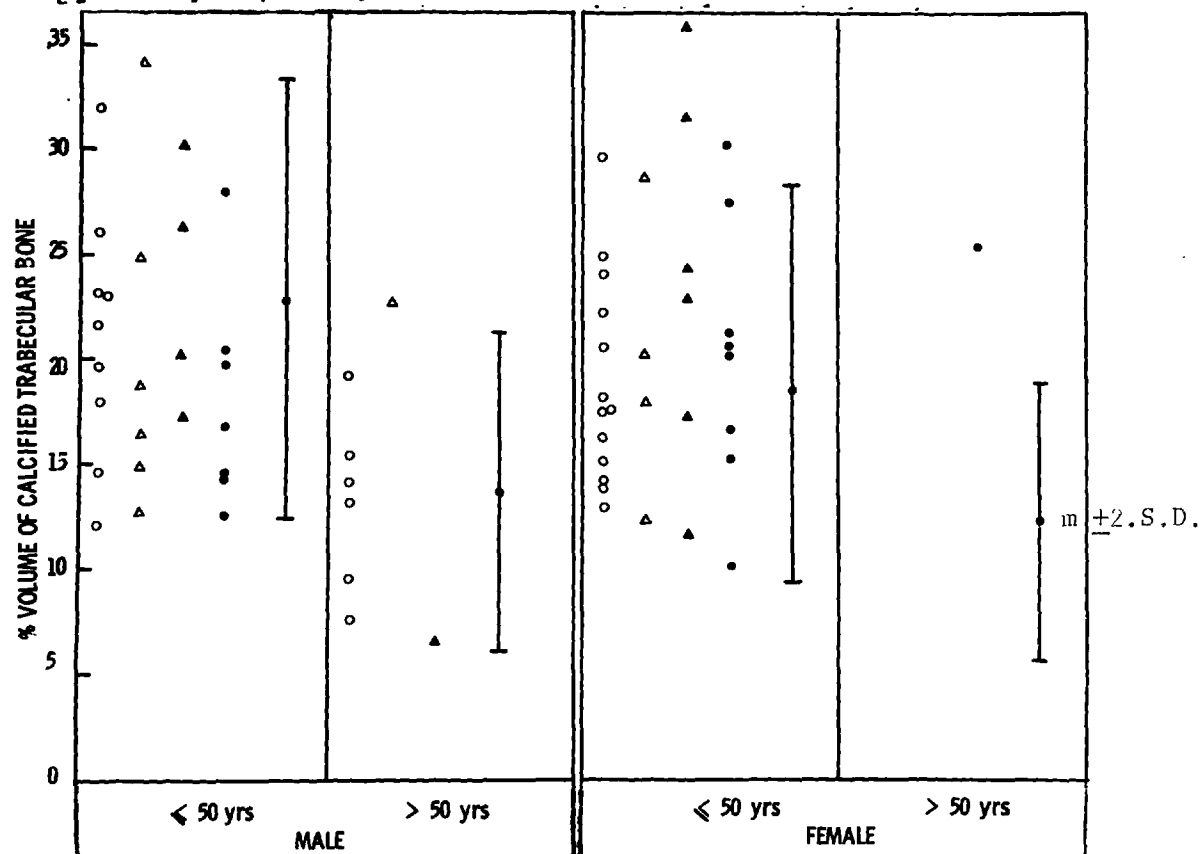


Fig. 55

— AMOUNT OF TOTAL BONE SURFACE COVERED WITH OSTEOID
IN PATIENTS WITH CHRONIC RENAL FAILURE —

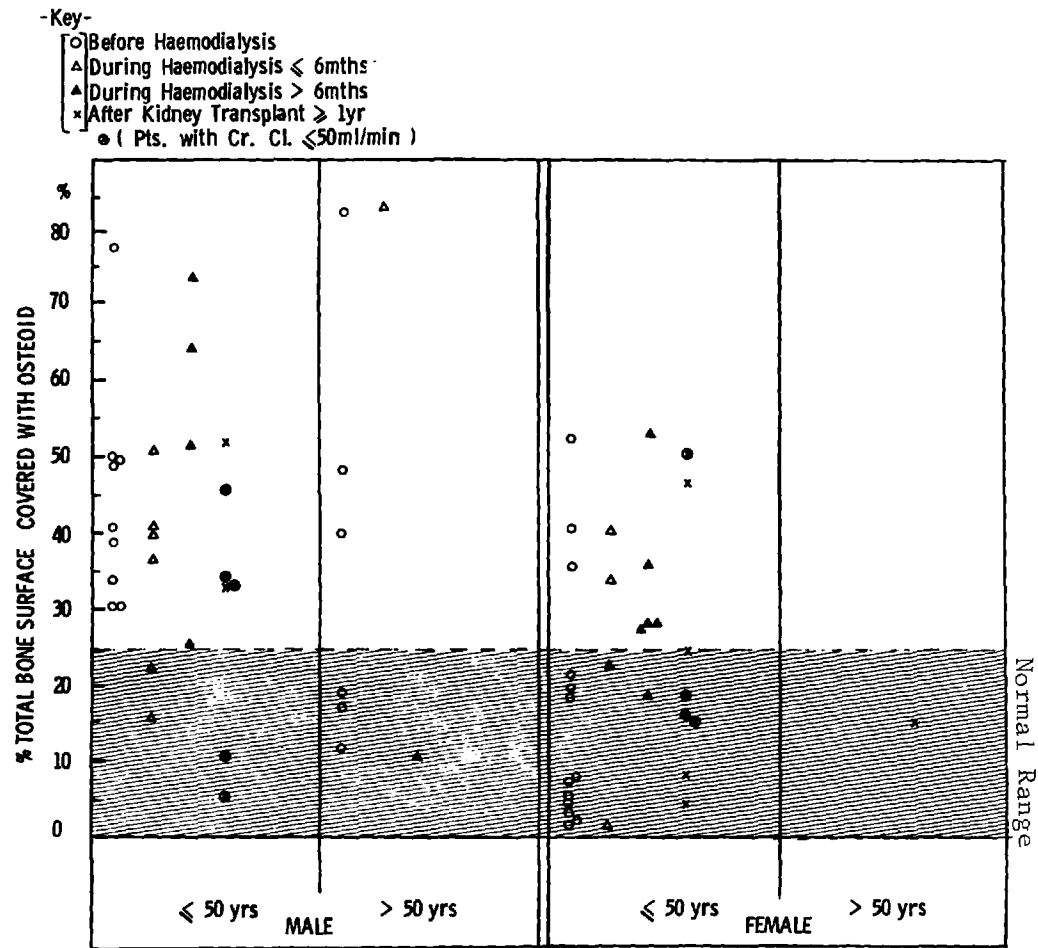
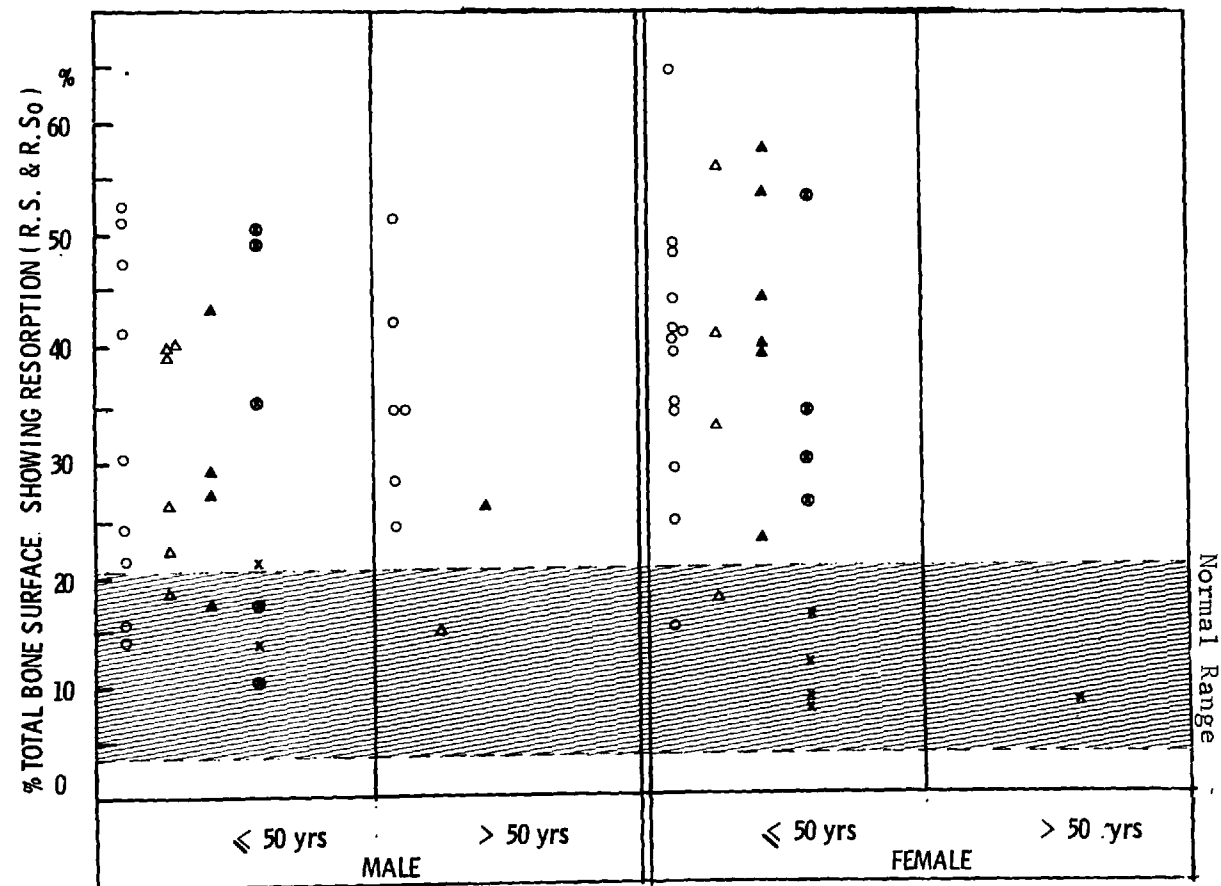


Fig. 56

— AMOUNT OF TOTAL BONE SURFACE SHOWING RESORPTION
(R.S. & R.S₀) IN PATIENTS WITH CHRONIC RENAL FAILURE —

-Key-
○ Before Haemodialysis
△ During Haemodialysis ≤ 6mths
▲ During Haemodialysis > 6mths
× After Kidney Transplant ≥ 1yr
(● Pts. with Cr. Cl. ≤ 50ml/min)



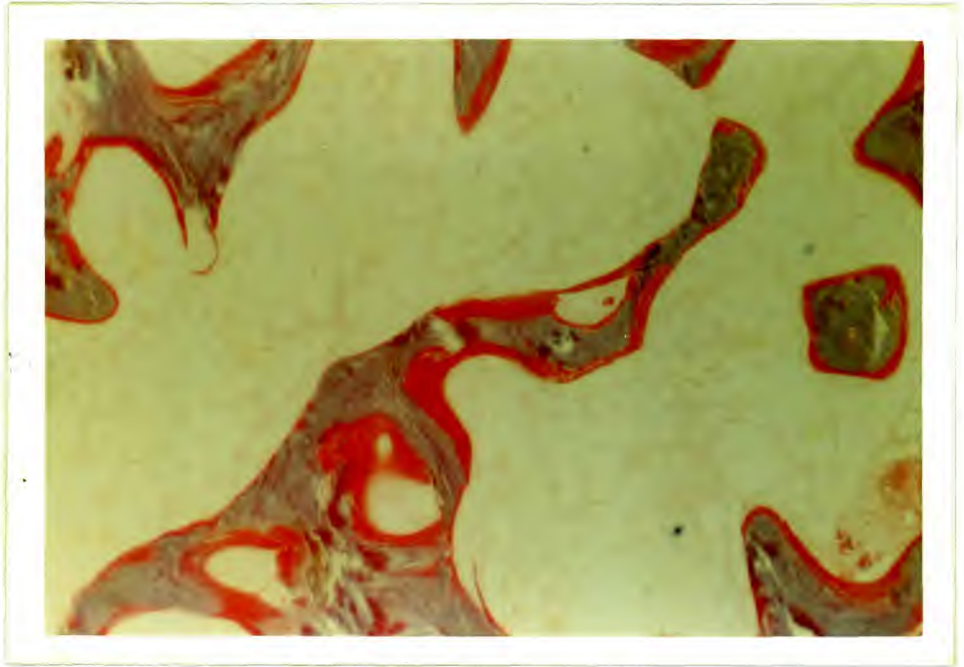


Fig.57

An undecalcified section from a case of renal osteodystrophy showing an excess of osteoid tissue. (Patient R.33 T.R. Solochrome cyanin $\times 30$).

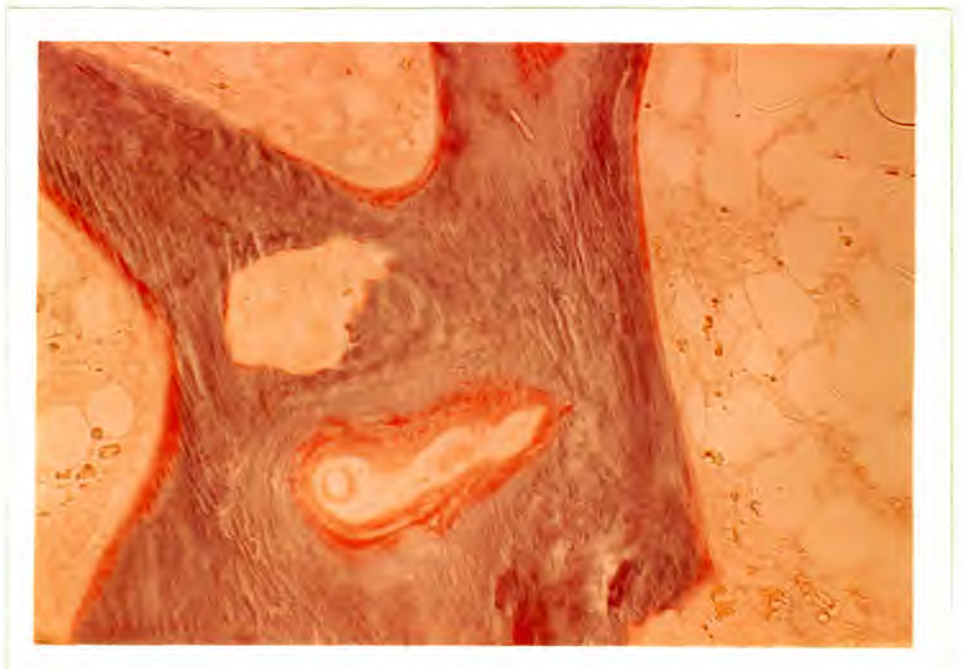
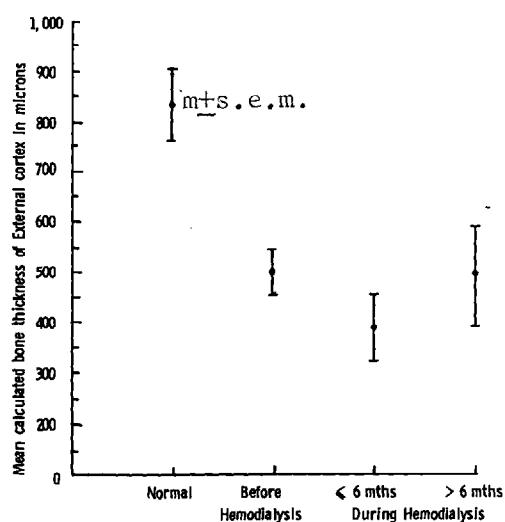


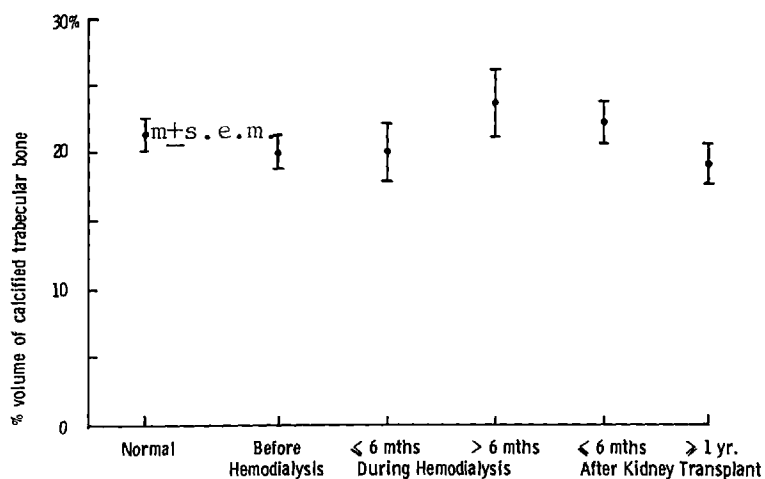
Fig.58

An undecalcified section from a case of renal osteodystrophy during haemodialysis showing an increase in resorption as well as in osteoid tissue. (Patient R.47. A.R. Solochrome cyanin $\times 120$).

—EFFECT OF HAEMODIALYSIS ON THE EXTERNAL CORTEX—

Fig.
59

—EFFECT OF HEMODIALYSIS & KIDNEY TRANSPLANT ON THE AMOUNT OF CALCIFIED TRABECULAR BONE—

Fig.
60

—EFFECT OF HEMODIALYSIS & KIDNEY TRANSPLANT ON THE AMOUNT OF TOTAL BONE SURFACE COVERED WITH OSTEOID—

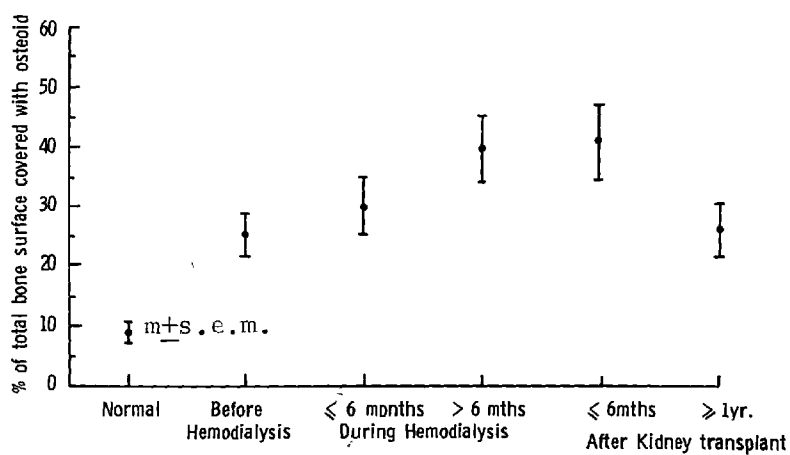
Fig.
61

TABLE 14

CHRONIC RENAL FAILURE PATIENTS (NOT DIALYSED)

GENERAL				BIOCHEMISTRY							HISTOLOGICAL MEASUREMENTS **						Remarks	***
Case No	Patient	Sex & Age	Diagnosis $\emptyset$	Bone Biopsy Number	Ca (mN)	PO ₄ (mN)	Ca _x P (mg%)	Alk. P'ase K.A.u	Tot. Pro. (G%)	Blood Urea (mg%)	A	B	C	D	E	F		
R.1	H.F.	M 42	*C.G.N.	68/1892	5.7	4.2	95.8	11	8.1	115	12.8	0.93	34.0	11.0	3.0	356	Om.	
R.2	J.B.	M 29	*C.G.N.	68/2113	..	..	..	..	..	..	23.7	0.76	37.9	27.3	14.3	550	Om. & H.	
R.3	C.D.	F 25	*C.P.N.	69/242	5.2	1.7	35.4	16	7.4	84	17.5	0.05	2.5	28.7	0	484	H.	
R.4	N.S.	M 31	*C.G.N.	68/2998	..	..	..	..	..	..	32.1	0.83	41.2	38.3	14.2	264	Om. & H.	
R.5	C.P.	F 12	*C.G.N.	68/1825	..	..	..	..	..	..	29.8	0.68	36.8	36.8	11.8	616	Om. & H.	
R.6	A.D.	M 14	*C.G.N.	68/2324	..	..	..	..	..	..	21.7	1.02	47.9	33.5	14.3	292	Om. & H.	
R.7	J.T	M 28	C.P.N.	68/415	6.0	2.1	50.4	11	6.0	143	26.1	1.53	48.5	32.9	18.7	476	Om. & H.	
R.8	E.C.	F 36	C.P.N.	68/2114	4.5	0.9	16.2	12	5.5	187	16.3	0.11	7.9	34.8	5.8	475	H.	
R.9	J.B.	M 56	Solitary hydronephrosis	68/2852	4.3	3.9	67.1	14	6.3	188	19.2	0.48	17.2	39.1	2.9	685	H.	
R.10	A.U.	M 33	C.P.N.	68/799	4.2	2.6	43.7	20	6.3	105	22.9	3.51	30.6	12.5	9.0	1018	Om. & H.	
R.11	J.B.	F 23	C.G.N.	68/1566	4.9	2.4	47.0	12	6.2	235	24.9	0.38	18.9	34.2	7.2	539	H.	
R.12	D.O.	F 31	C.G.N.	68/525	4.4	2.2	38.7	6	5.9	106	22.2	0.85	41.3	21.8	13.3	534	Om. & H.	
R.13	M.B.	F 38	C.P.N.	68/683	4.8	1.4	26.8	15	6.4	128	24.1	0.51	22.5	40.6	8.7	451	H.	
R.14	L.G.	F 50	Polycystic kidneys	67/1341	5.7	4.3	98.0	50	..	160	17.8	1.88	53.1	42.2	22.5	285	Om. & H.	
R.15	J.C.	M 59	Amyloidosis kidneys	68/436	4.5	2.6	46.8	6	4.5	180	7.6	0.34	40.3	17.5	7.01	..	Om. & H.	

continued over/

R.16	P.M.	F 34	Focal G.N.	PM 12631	4.8	6.1	117.1	12	..	198	15.1	0.04	5.2	43.2	1.0	..	H.
R.17	P.C	F 34	Mem. G.N.	67/748	4.8	2.2	42.2	8	5.3	146	12.9	0.07	6.8	24.7	0	..	H.
R.18	E.S.	F 43	C.P.N. (analgesic)	PM 12788	5.0	5.0	100.0	13	6.4	102	18.3	0.06	8.0	40.2	1.2	..	H.
R.19	D.B.	F 36	Mem. G.N.	PM 12874	4.4	3.0	52.8	78	4.6	212	13.9	0.05	4.0	34.7	0	..	H.
R.20	E.F.	M 30	C G.N.	PM 12627	4.2	3.6	60.5	19	7.9	159	14.6	0.47	31.5	24.2	6.3	..	Om.& H.
R.21	M.D.	F 29	D.L.E.	PM 12888	4.3	2.0	34.4	59	6.4	..	20.6	0.03	3.5	15.2	0	..	Normal
R.22	G.S.	M 67	C.G.N.	PM 12856	4.7	2.5	47.0	9	5.7	172	13.1	0.10	13.0	47.0	4.0	..	H.
R.23	R.H.	M 62	C.P.N.	PM 12640	..	3.9	..	11	6.1	74	15.5	0.18	19.5	27.8	6.8	..	H.
R.24	R.N.	M 68	C.P.N.	PM 12795	..	..	..	..	..	..	9.5	4.30	82.9	9.4	24.8	..	Om.& H.
R.25	R H.	M 59	C.P.N.	PM 12616	4.8	2.8	53.8	11	6.7	117	14.1	1.51	48.3	17.5	10.8	..	Om. & H.
R.26	G.B.	F 40	C.G.N.	68/1502	6.2	2.4	59.5	11	6.3	180	14.2	0.14	18.9	32.4	5.4	473	H.
R...	Z.L.	M 48	*C.P.N.	69/3501	5.3	3.0	63.6	41	6.0	178	19.9	2.0	50.0	19.0	5.6	..	Om.& H.
R...	F.S.	M 49	Focal G.N.	69/2374	4.4	3.1	54.6	8	5.3	184	17.6	1.3	77.2	12.3	3.5	..	Om.

Ø C.G.N. Chronic glomerulonephritis; C.P.N. Chronic pyelonephritis; Focal G.N. Focal glomerulonephritis;
 Mem. G.N. Membranous glomerulonephritis; * Diagnosis on clinical impression.

** A % volume of calcified trabecular bone. B % volume of osteoid in trabecular bone.
 C % total bone surface covered with osteoid. D % total bone surface showing resorption not covered with osteoid (RS).
 E % total bone surface showing resorption covered with osteoid (R.S₀).
 F Mean calculated bone thickness of external cortex in microns.

*** Om. Histological osteomalacia (upper limit of normal for per cent total bone surface covered with osteoid for males and females ≤ 50 is 25%).
 H. Histological hyperparathyroidism (upper limit of normal for per cent total bone surface showing resorption(RS & RS₀). for males and females ≤ 50 is 20.6%).

In 2 patients, R.19 and R.21, the values were markedly increased, the former case also showed hepatosplenomegaly and macroglobulinaemia with 'M' band; and the latter was associated with systemic lupus erythematosus, who also showed a high 5'-nucleotidase of 132 i.u., (N = 2-17 i.u./Litre). These two patients were excluded from correlative studies, as the elevated alkaline phosphatase may well have been due to non-bony causes. Plasma total protein was estimated in 21 patients and in 17 the amount was less than 6.5 G%. Blood urea was estimated in 22 patients and found to be raised in all of them. The main quantitative biopsy measurements are set out graphically in Figures 54, 55 and 56. In each case the normal range (mean $\pm$ 2 S.D.) is shown for males and females below 50, separately for trabecular bone, jointly for osteoid and resorption. The results show that the amounts of trabecular bone lie within the normal range except for one case (R.5.) Slightly over half (15 out of 28) of these patients show an increase in osteoid, more noticeable in the young males than in the other groups. Nearly all (25 out of 28) show an increase in resorption, often marked.

7.3.3. Chronic Renal Failure Patients on Haemodialysis

These patients had been on haemodialysis from a minimum period of time of two weeks to a maximum of more than three years. They are divided into two groups those who have been on dialysis for less than six months and those who have been on regular dialysis for six months or more. The main results are set out in Table 15.

In these two sub-groups also, the serum calcium, serum phosphorus, ionic product of calcium and phosphorus, serum alkaline phosphatase, were not consistent (Figures 50 - 53). Serum alkaline phosphatase was normal in 11 out of 22 patients. Plasma total protein was estimated in 17 patients, in 10 it was found to be abnormal (less than 6.5 G%). Blood urea was estimated in 20 patients and was markedly elevated in all of them.

TABLE 15

CHRONIC RENAL FAILURE PATIENTS (ON HAEMODIALYSIS)

G E N E R A L			B I O C H E M I S T R Y								HISTOLOGICAL MEASUREMENTS **							Remarks***
Case No	Patient	Sex & Age	Diagnosis Ø	Bone Biopsy Number	Duration Of Haemo- dialysis	Ca (mN)	PO ₄ (mN)	CaxP (mg%)	Alk. P'ase K.A.u	Tot. Pro. (G%)	Blood Urea (mg%)	A	B	C	D	E	F	
R.27	K.B.	M 23	C.P.N.	69/1077	5 wks.	4.4	4.2	73.9	25	6.1	190	34.1	1.84	50.0	30.1	9.6	274	Om. & H.
R.28	K.K.	M 24	C.G.N.	69/1074	8 wks.	5.1	1.9	38.7	20	6.5	293	24.9	0.18	21.9	23.4	3.1	237	H.
R.29	A.K.	M 27	C.G.N.	69/1078	5 wks.	5.3	2.6	55.1	8	6.2	136	14.9	1.23	40.0	33.3	6.7	474	Om. & H.
R.30	A.M.	M 33	*C.G.N.	69/1081	8 wks.	4.6	2.4	44.2	9	6.8	147	12.7	0.47	39.8	15.1	3.2	93	Om.
R.31	J.K.	F 47	C.G.N.	68/316	10 wks.	4.8	5.0	96.0	11	6.3	152	20.1	0.94	23.5	27.7	5.4	600	Om. & H.
R.32	S.K.	M 18	C.P.N.	68/1169	2 wks.	4.9	3.9	76.4	10	..	170	16.3	0.25	15.6	18.0	4.1	474	H.
R.33	T.R.	M 56	Polycystic kidneys	69/1084	8 wks.	4.1	4.0	65.6	16	5.7	..	22.7	6.53	82.9	3.9	11.8	246	Om.
R.34	R.L.	F 34	*C.P.N.	69/239	6 wks.	5.6	4.3	96.3	11	6.3	236	12.3	0.58	34.5	32.1	23.8	380	Om. & H.
R.35	R.P.	F 47	C.P.N.	68/1569	12 wks.	5.5	1.4	30.8	9	6.9	141	17.9	0.84	41.2	23.5	17.6	345	Om. & H.
R.36	A.B.	F 43	C.G.N.	PM/12745	20 wks.	4.6	3.9	71.8	7	5.8	125	28.7	0	1.2	16.9	0	..	Normal
R.37	D.C.	M 27	C.P.N.	68/1997	4 wks.	4.8	3.1	59.5	12	..	202	18.9	0.89	36.4	28.3	13.1	772	Om. & H.
R.38	D.P.	F 45	C.P.N.	69/269	28 wks.	6.1	4.7	114.6	7	6.6	212	11.8	0.19	17.8	32.9	6.8	280	H.
R.39	M.C.	F 37	C.P.N.	68/460	32 wks.	4.5	4.6	82.8	6	5.9	284	31.7	0.67	26.8	46.5	11.3	455	Om. & H.
R.40	B.B.	F 20	Solitary hydro- nephrosis	68/527	36 wks.	4.3	3.6	61.9	42	5.6	164	24.2	0.85	34.9	27.6	16.5	397	Om. & H.
R.41	P.A.	F 32	C.P.N.	PM/12175	1½ yrs.	4.9	6.1	119.6	12	..	..	17.2	0.28	26.9	46.9	6.9	..	Om. & H.

continued over/

R.42 T.M.	M 53	C.P.N.	PM/12615	1½ yrs.	4.8	2.9	55.7	27	6.7	136	6.4	0.04	10.8	23.9	2.2	..	H.
R.43 A.W.	F 28	C.G.N.	68/418	2½ yrs.	5.4	2.2	47.5	77	7.2	92	35.9	3.46	52.6	23.7	16.5	246	Om. & H.
R.44 A.B.	M 38	Polycystic kidneys	68/682	>3 yrs.	4.3	3.8	65.3	13	..	175	20.3	1.78	63.4	14.8	14.4	314	Om. & H.
R.45 J.S.	F 37	C.G.N.	69/154	32 wks.	4.9	2.8	54.8	14	6.9	220	22.8	0.26	26.3	21.3	2.5	1226	Om. & H.
R.46 J.D.	M 32	Familial nephritis	69/176	40 wks.	5.0	1.2	24.0	8	6.4	219	30.2	1.50	72.6	6.5	11.3	479	Om.
R.47 A.R.	M 24	Congenital obstructive uropathy	69/645	44 wks.	4.3	3.7	63.6	26	6.0	193	26.3	2.02	51.3	33.8	10.0	433	Om. & H.
R.48 W.M.	M 34	C.G.N.	68/2758	36 wks.	5.2	3.4	70.7	10	..	188	17.3	0.76	25.0	25.0	2.1	587	Om. & H.

Ø	C.G.N.	Chronic glomerulonephritis;	C.P.N.	Chronic pyelonephritis;	*Diagnosis on clinical impression.	
**	A	% volume of calcified trabecular bone.			B	% volume of osteoid in trabecular bone.
	C	% total bone surface covered with osteoid.			D	% total bone surface showing resorption not covered with osteoid (RS)
	E	% total bone surface showing resorption covered with osteoid (R.S ₀).				
	F	Mean calculated bone thickness of external cortex in microns.				
***	Om.	Histological osteomalacia (upper limit of normal for per cent total bone surface covered with osteoid for males and females ≤ 50 is 25%).				
	H.	Histological hyperparathyroidism (upper limit of normal for per cent total bone surface showing resorption (RS & RS ₀) for males and females ≤ 50 is 20.6%).				

Regarding the histological measurements the amounts of calcified trabecular bone lie within the normal range, except for 5 cases showing an increase (R.27, 33, 36, 43 and 39).

No significant change in the amount of trabecular bone occurred with haemodialysis. Quantification of the cortical thickness was attempted, this was possible in the external cortex only because in some biopsies the internal cortex was missing, in others the muscle which is frequently attached to the external cortex and not to the internal cortex was absent (Table 16). The total thickness was not satisfactory because of the variation in the percentage of bone present, the product of these two factors was therefore taken as the mean cortical thickness. The precautions taken to align the biopsy, and avoid cutting the cortex at an angle have been considered. The results of the measurements are shown in Figure 59, it can be seen that renal failure alone led to a considerable decrease in cortical thickness with a slight but not significant further drop in the short term dialysis group.

The majority of the patients in this group, 16 out of 22, showed an increase in osteoid, without any obvious sex difference. Nearly all (18 out of 22) also showed an increase in resorption surface. The effect of long continued haemodialysis on the variables measured was not pronounced. There was a slight rise in the amount of osteoid in the group dialysed for more than 6 months (Fig. 61), and also a slight rise in the amount of calcified trabecular bone (Fig. 60). Neither rise was significant.

7.3.4. Chronic Renal Failure Patients Before and After Kidney

Transplantation

The results for patients in this group are shown in Tables 17 and 18. 9 patients shown in Table 17 had pre and post transplant biopsies. The pre-transplant biopsies also have been included in one of the previously discussed groups. 10 patients shown in Table 18 had only post-transplant biopsies. The post transplant biopsies were studied at

TABLE 16

The following table shows the mean calculated bone thickness of the external cortex in microns (M.C.B.T.) which is derived from the percentage of the amount of calcified bone present in the cortex and its mean thickness. The iliac crest bone samples are obtained from routine and coroner's autopsies where external cortex can be definitely identified. The external cortex was chosen because it is consistently present in the bone biopsy material.

Case No.	Sex & Age	External M.C.B.T.
.. P.M. 12859	M 13	739
C9	M 19	990
..P.M. 12767	F 31	752
.. P.M. 12573	F 33	1159
C18	M 39	448
C22	M 45	870
.. P.M. 12883	M 49	594
.. P.M. 12672	M 49	1049
.. P.M. 12642	M 54	897
.. P.M. 12847	F 54	1589
.. P.M. 12842	F 56	954
C27 P.M. 12877	F 56	1179
C29 P.M. 12838	F 59	376
C46 P.M. 12880	M 63	785
.. P.M. 12846	F 63	580
C45 P.M. 12887	M 63	585
.. P.M. 12735	M 66	827
C48 P.M. 12886	M 69	668

6 months (8 cases) and at one year or more (16 cases). All patients in this group were, at the time of biopsy, living after renal transplant *that* was successful in the sense that the patient was not being treated with haemodialysis. The renal function had in all cases, compared to the pre-transplant condition (in which case the creatinine clearance was always less than 5 ml/min), improved. However, the post transplant patients show a scatter of renal function. In 8 of the 19 patients the creatinine clearance was more than 50 ml/min. The majority of the patients in this group showed serum calcium, phosphorus and alkaline phosphatase within the normal range; the occasional exception being in general the patients with poor renal function. The blood urea was variable, and the plasma proteins were frequently low, here too the quality of the residual renal function was obviously important.

In relation to the histological measurements, the amount of calcified trabecular bone was normal in 14 cases and increased in 2 (R.39 and 49). Figure 54. Out of 16 patients biopsied at one year or more after transplant (Figure 55), 7 showed an increasing amount of osteoid, 4 of these showed poor renal function (creatinine clearance less than 50 ml/min). On the other hand 9 patients showed a normal amount of osteoid, in 5 poor renal function was present.

In Figure 56, 8 out of the 16 patients biopsied one year or more after transplantation showed an increased amount of resorption surface, 7 of these 8 had poor renal function. On the other hand 8 patients showed normal amounts of resorption, only 2 were associated with poor renal function. In other words, the state of renal function in these post-transplant cases seems to have a profound effect on the amount of bone resorption surface but not a very striking effect on the amount of osteoid.

The effects of renal transplantation on bone changes in 9 individual patients who had both pre and post transplant biopsies are

TABLE 17

BONE BIOPSIES STUDY IN CHRONIC RENAL FAILURE PATIENTS BEFORE AND AFTER KIDNEY TRANSPLANTATION

G E N E R A L						B I O C H E M I S T R Y						HISTOLOGICAL MEASUREMENTS **						Remarks***
Case No	Patient	Sex & Age	Diagnosis	Bone Biopsy Number	Nature of Therapy *	Cr. Cl. (ml/min)	Ca. (mN)	PO ₄ (mN)	CaxP (mg%)	Alk. P'ase K.A.u	Tot. Pro. (G%)	Blood Urea (mg%)	A	B	C	D	E	
R.10	A.U.	M 33	C.P.N.	68/799	No.H.D.													
					Pre.T.	..	4.2	2.6	43.7	20	6.3	105	22.9	3.5	30.6	12.5	9.0	Om. & H.
				69/95	Post.T.													
					6mts.(C)	22	4.7	2.5	47.0	29	6.9	77	22.9	2.01	29.5	9.1	5.3	Om.
				69/1864	Post.T.1yr.	21	4.8	2.5	48.0	29	7.4	64	28.0	3.4	34.6	38.5	11.5	Om. & H.
R.11	J.B.	F 23	C.G.N.	68/1566	No.H.D.													
					Pre.T.	..	4.9	2.4	47.0	12	6.2	235	24.9	0.38	18.9	34.2	7.2	H.
				69/482	Post.T.													
					6mts.(C)	16	5.0	1.3	26.0	7	6.8	113	19.5	0.63	25.5	13.8	1.1	Om.
				69/2591	Post.T.1yr.	16	5.4	2.1	45.4	7	6.5	101	21.1	0.34	17.2	9.4	0	Normal
R.12	D.O.	F 31	C.G.N.	68/525	No.H.D.													
					Pre.T.	..	4.4	2.2	38.7	6	5.9	106	22.2	0.85	41.3	21.8	13.3	Om. & H.
				68/3354	Post.T.													
					6mts.(C)	8	4.9	2.6	50.9	9	7.0	163	28.5	0.76	40.9	5.7	3.8	Om.
				69/1865	Post.T.1yr.	9	4.4	3.0	52.8	5	5.9	107	20.1	0.97	51.6	31.3	21.9	Om. & H.
R.13	M.B.	F 38	C.P.N.	68/683	No.H.D.													
					Pre.T.	..	4.8	1.4	26.8	15	6.4	128	24.1	0.51	22.5	40.6	8.7	H.
				69/143	Post.T.													
					6mts.(L)	38	5.2	2.0	41.6	11	7.4	32	25.2	0.83	27.3	15.0	8.6	Om. & H.

continued over/

R.31	J.K.	F 47	C.G.N.	68/316	H.D.10wks. Pre.T.	..	4.8	5.0	96.0	11	6.3	152	20.1	0.94	23.5	27.7	5.4	Om. & H.
				68/3196	Post.T. 6mts.(C)	77	5.3	1.7	36.0	14	7.2	47	22.1	1.51	64.6	3.1	10.9	Om.
				69/1260	Post.T.1yr.	77	5.3	2.0	42.4	12	6.3	37	16.5	0.50	39.9	6.9	1.5	Om.
R.32	S.K.	M 18	C.P.N.	68/1169	H.D.2wks. Pre.T.	..	4.9	3.9	76.4	10	..	170	16.3	0.25	15.6	18.0	4.1	H.
				68/3021	Post.T. 6mts.(L)	58	5.6	1.7	38.0	14	..	..	14.4	0.64	35.2	21.8	1.2	Om. & H.
R.39	M.C.	F 37	C.P.N.	68/460	H.D.32wks. Pre.T.	..	4.5	4.6	82.8	6	5.9	284	31.7	0.67	26.8	46.5	11.3	Om. & H.
				69/1262	Post.T.1yr.	19	4.9	1.6	31.4	5	6.2	120	31.0	0.46	19.2	34.6	0	H.
R.40	B.B.	F 20	Hydro- nephrosis	68/527	H.D.36 wks. Pre.T.	..	4.3	3.6	61.9	42	5.6	164	24.2	0.85	34.9	27.6	16.5	Om. & H.
				68/3079	Post.T. 6 mts.(C)	70	5.9	1.8	42.5	11	7.0	35	25.3	0.82	42.9	22.8	13.0	Om. & H.
				69/1261	Post.T.1yr.	72	5.3	2.0	42.4	11	6.3	29	20.1	0.20	9.55	14.6	1.9	Normal
R.45	J.S.	F 37	C.G.N.	69/154	H.D.32wks. Pre.T.	..	4.9	2.8	54.8	14	6.9	220	22.8	0.26	26.3	21.3	2.5	Om. & H.
				69/2221	Post.T. 6mts.(C)	19	5.4	1.5	32.4	10	7.6	123	20.5	2.35	68.4	15.2	16.5	Om. & H.

* H.D. Haemodialysis. Pre.T. Pre kidney transplant. (C) Cadaver. (L) Live donor. Post T. Post kidney Trasnplant.

** A % volume of calcified trabecular bone. B % volume of osteoid in trabecular bone.
C % total bone surface covered with osteoid. D % total bone surface showing resorption not covered with osteoid (R.S.)
E % total bone surface showinf resorption covered with osteoid(R.S._o).

*** Om. Histological osteomalacia (upper limit of normal for per cent total bone surface covered with osteoid for males and females ≤ 50 is 25%).
H. Histological hyperparathyroidism (upper limit of normal for per cent total bone surface showing resorption (R.S. & R.S._o) for males and females ≤ 50 is 20.6%).

TABLE 18

BONE BIOPSIES STUDY IN CHRONIC RENAL FAILURE ONLY AFTER KIDNEY HOMOTRANSPLANTATION

Case No	Patient	Sex & Age	Diagnosis	Bone Biopsy Number	Nature of Therapy *	Cr. Cl. (ml/min)	Ca (mN)	PO ₄ (mN)	CaxP (mg%)	Alk. P'ase K.A.u	Tot. Pro. (G%)	Blood Urea (mg%)	A	B	C	D	E	Remarks ***
													HISTOLOGICAL MEASUREMENTS**					
R.49	V.N.	F 58	Polycystic kidneys	69/481	Post.T. 1½yrs.(C)	88	5.2	1.9	39.5	6	6.8	47	25.3	0.19	13.7	8.9	0	Normal
R.50	A.F.	F 23	C.G.N.	69/175	Post.T. 2½yrs.(L)	65	5.3	1.7	36.0	6	6.7	68	15.1	0.02	5.4	11.5	0.7	Normal
R.51	I.T.	M 21	C.G.N.	69/194	Post.T. 3yrs.(L)	170	5.5	1.5	33.0	8	7.1	..	19.7	0.54	33.6	11.7	2.3	Om.
R.52	D.F.	M 19	C.G.N.	69/54	Post.T. 1yr.(L)	75	5.2	1.6	33.3	16	6.7	33	16.8	0.58	52.2	13.7	7.4	Om. & H.
R.53	I.S.	F 21	C.P.N.	69/708	Post.T. 2yrs.(C)	82	4.7	1.9	35.7	6	7.3	43	27.5	0.41	25.6	8.5	1.2	Om.
R.54	M.K.	M 22	C.G.N.	69/304	Post.T. 2yrs.(C)	34	4.8	2.5	48.0	6	5.7	134	20.4	0.03	5.8	48.8	0.6	H.
R.55	M.S.	M 28	C.G.N.	69/53	Post.T. 1½yrs.(C)	17	5.1	1.6	32.6	7	6.1	44	14.1	0.10	11.3	16.2	0.9	Normal
R.56	C.T.	M 49	Polycystic kidneys	PM/12585	Post.T. 1yr.(C)	20	4.9	2.4	47.0	9	5.5	58	12.5	1.01	45.9	24.8	10.1	Om. & H.
R.57	B.S.	M 35	C.G.N.	69/1082	Post. T. 1yr.(C)	37	5.0	1.9	38.0	12	6.8	57	14.2	0.52	33.9	6.8	3.9	Om.
R.58	M.W.	F 46	Polycystic kidneys	69/1259	Post.T. 1yr.(C)	13	4.9	3.7	72.5	12	6.4	215	20.2	0.37	16.9	28.3	1.9	H.

* Post.T. Post kidney transplantation. (C) Cadaver. (L) Live donor.

shown in Table 17 and Figures 62, 63 and 64.

The amount of calcified trabecular bone largely lay within the normal range for both the pre and post transplant biopsies. There was little variation between these, with a single exception; the maximum difference was 8.4%. The patient with the highest figure for calcified trabecular bone on the first biopsy (R.39) showed no appreciable change in the second biopsy (31.7-31%). In Figure 62, 5 out of 9 cases showed an excess of osteoid before transplant; at 6 months and at one year, three cases still showed an increased amount. All 3 (R.45, 12 and 10) had poor renal function (creatinine clearance less than 50 ml/min). The other two cases with excess osteoid (R.39 and 40) returned to the normal range, although one still showed poor renal function.

In the 4 cases (R.31, 13, 11 and 32) with a normal amount ~~for~~ osteoid in the pre-transplant biopsies, the post-transplant biopsies at six months and at one year showed an excess amount of osteoid although two cases had good renal function. One case (R.11) did not show any appreciable change in the amount of osteoid despite poor renal function.

In other words, the amount of osteoid does not seem to be much affected by the functional status of the kidneys. This feature could well be seen when the post-transplant biopsies at six months and at one year were taken as a group, and compared with the normal control group (Figure 61). It is also apparent that the amount of osteoid in the post-transplant biopsies taken at six months or less and at one year or more does not differ significantly from the rest of the chronic renal failure patients who had or had not haemodialysis.

The effects of renal transplantation on the amount of bone resorption surface, i.e., resorption surface not covered with osteoid (R.S.) and resorption surface covered with osteoid (R.S._o) were also studied and are shown in Figures 63 and 64.

Fig. 62

- EFFECT OF RENAL TRANSPLANTATION ON
THE AMOUNT OF OSTEOID -

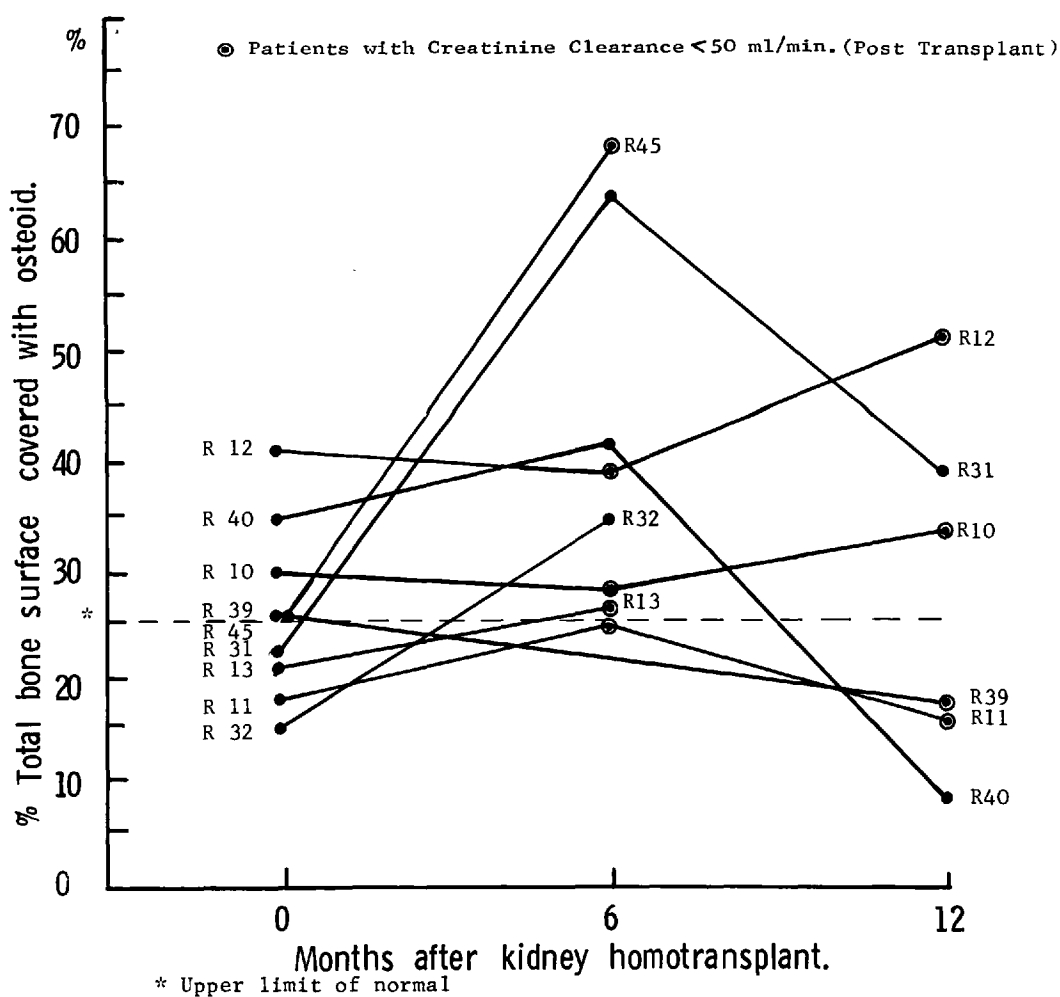


Fig. 63

— EFFECT OF RENAL TRANSPLANTATION ON BONE RESORPTION
SURFACES NOT COVERED WITH OSTEOID (R.S) —

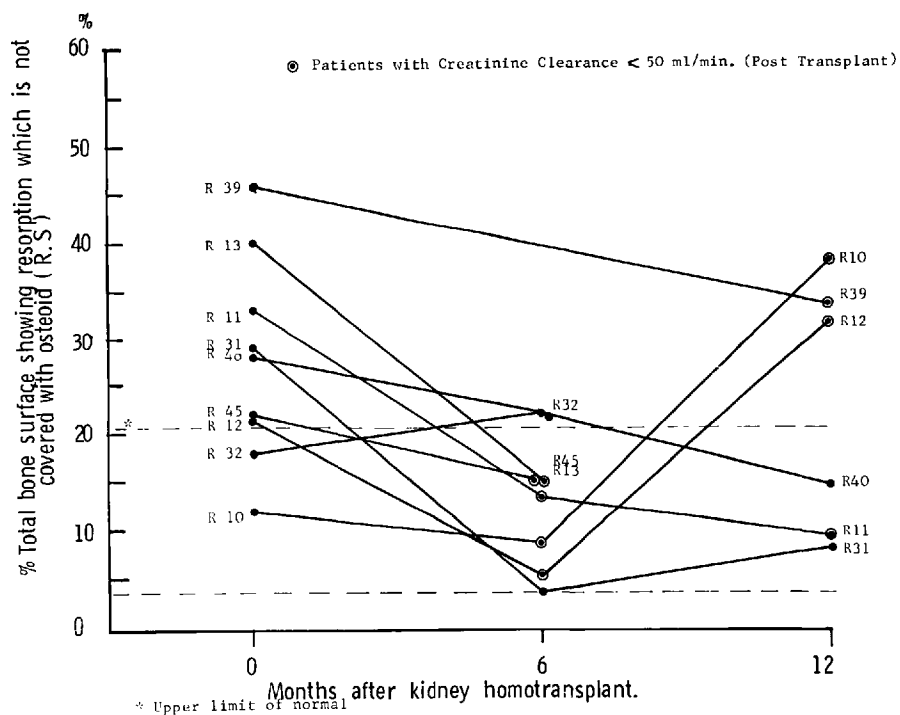


Fig. 64

— EFFECT OF RENAL TRANSPLANTATION ON BONE RESORPTION
SURFACES COVERED WITH OSTEOID (R.S₀) —

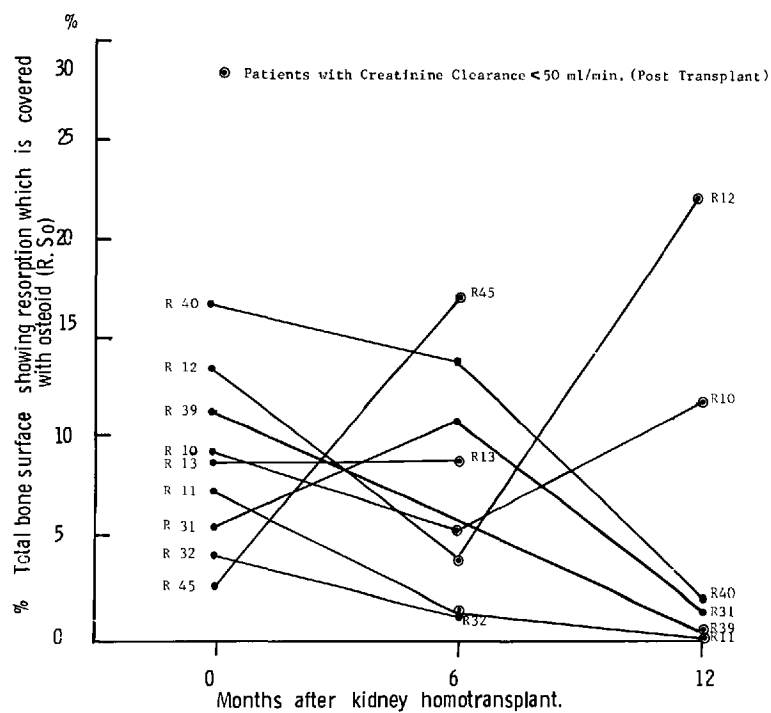
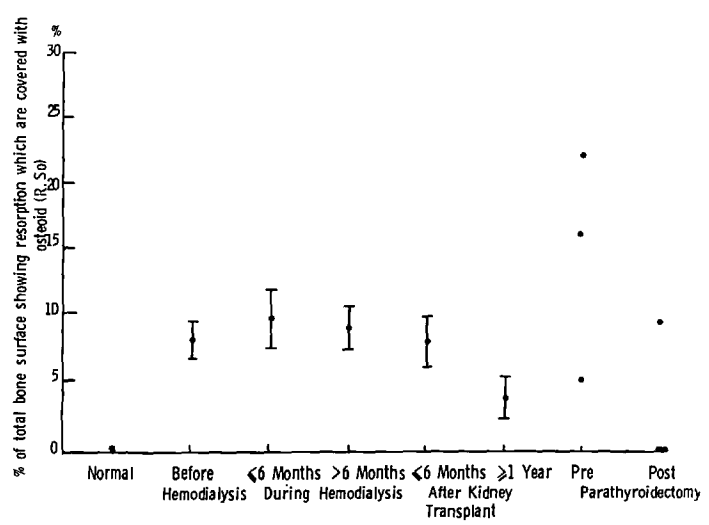


Fig. 65

- EFFECT OF RENAL TRANSPLANTATION ON BONE RESORPTION SURFACE WHICH IS COVERED WITH OSTEIOD -



In relation to the former parameter, 7 cases showed a definite increase in resorption in the pre-transplant biopsies, only 2 were abnormal at one year post transplant, both of these showed poor renal function; the other 5 were in the normal range although three of them still had poor renal function. On the other hand there were two cases (R.10 and 32) who showed a normal amount of resorption before kidney transplant. The former showed a marked increase in resorption at one year post-transplant and was associated with poor renal function; whereas the latter case with good renal function showed a moderate increase in resorption at six months post-transplant.

All the pre-transplant biopsies from these cases showed the presence of resorption surfaces covered with osteoid (R.S._o) Figure 64, a feature which was not seen in any of the control biopsies. Six months after transplant all biopsies again showed this, one year after transplant two of the six biopsies showed no osteoid covered resorption, and two others a very low figure. All these cases which showed a considerable increase in R.S._o, two at six months, two at one year, were patients with poor renal function. The mean value for R.S._o at one year was below that for any of the renal failure groups (Figure 65). If the 7 patients with good renal function after transplant were chosen the mean figure would fall to 2.1%.

7.3.5. Chronic Renal Failure Patients Before and After Parathyroidectomy

Parathyroidectomy was carried out because of persistent hypercalcaemia in these patients, whose results are shown in Table 19. In two patients (R.14 L.G., and R.26 G.B.) the parathyroidectomy was total, in one (R.43 A.W.) three glands were totally removed, one gland was partially removed. In Patient R.43, although the serum calcium at the time of biopsy was 5.4 mN, a month previously there had been a definite hypercalcaemia. Even 5.4 mN is probably abnormal in view of the low serum proteins. In all three cases, there was a dramatic reduction of serum calcium after parathyroidectomy, but serum phosphorus remained

TABLE 19

BONE BIOPSIES STUDY IN CHRONIC RENAL FAILURE PATIENTS BEFORE AND AFTER PARATHYROIDECTOMY

G E N E R A L						B I O C H E M I S T R Y						HIS TOLOGICAL MEASUREMENTS**					
Case No	Patient	Sex & Age	Diagnosis	Bone Biopsy Number	Nature of Therapy*	Ca (mN)	PO ₄ (mN)	CaxP (mg%)	Alk. P'ase K.A.u	Tot. Pro. (G%)	Blood Urea (mg%)	A	B	C	D	E	Remarks ***
R.14	L.G.	F 50	Polycystic Kidneys	67/1341	No H.D.	5.7	4.3	98.0	50	..	160	17.8	1.88	53.1	42.2	22.5	Om. & H.
				68/1893	Pre. P. Post P.1yr.	4.0	3.7	59.2	7	5.2	113	20.5	0.45	15.0	5.3	0	Normal
R.26	G.B.	F 40	C.G.N.	68/1502	No H.D.	6.2	2.4	59.5	11	6.3	180	14.2	0.14	18.9	32.4	5.4	H.
				69/240	Pre. P. Post P.½yr.	5.0	5.7	114.0	11	6.4	174	19.9	0.04	8.5	21.4	0	Normal
R.43	A.W.	F 28	C.G.N.	68/418	H.D. 2½yrs.	5.4	2.2	47.5	77	6.2	92	35.9	3.5	52.6	23.7	16.5	Om. & H.
				69/479	Pre. P. Post P.1yr.	4.6	3.4	62.5	12	6.3	196	31.2	0.62	24.5	21.3	9.8	Om. & H.

* H.D. Haemodialysis. Pre.P. Pre parathyroidectomy. Post.P. Post parathyroidectomy.

** A % volume of calcified trabecular bone. B % volume of osteoid in trabecular bone.
C % total bone surface covered with osteoid. D % total bone surface showing resorption not covered with osteoid(RS).
E % total bone surface showing resorption covered with osteoid (R.S_O).

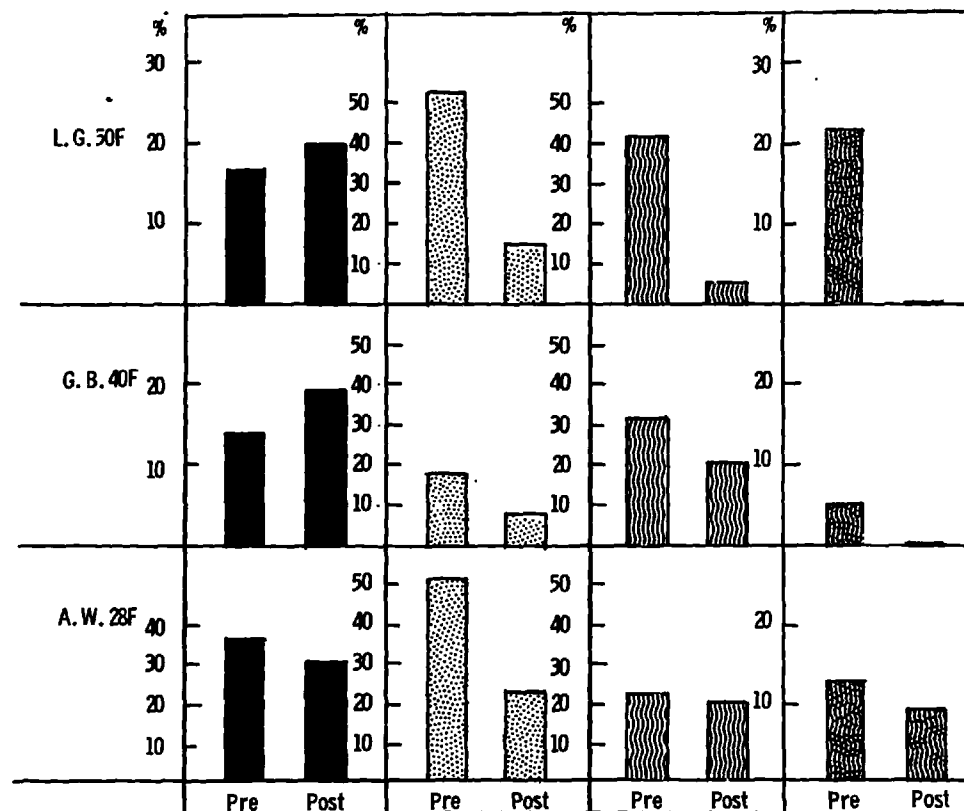
*** Om. Histological osteomalacia (upper limit of normal for per cent total bone surface covered with osteoid for males and females 50 is 25%).
H. Histological hyperparathyroidism (upper limit of normal for per cent total bone surface showing resorption (RS & RS_O) for males and females 50 is 20.6%).

Fig. 66

—EFFECTS OF PARATHYROIDECTOMY ON RENAL OSTEODYSTROPHY—

—Key—

- % volume of calcified trabecular bone
- ▨ % of total bone surface covered with osteoid
- ▤ % of total bone surface showing resorption not covered with osteoid (R.S.)
- ▥ % of total bone surface showing resorption covered with osteoid (R.So)



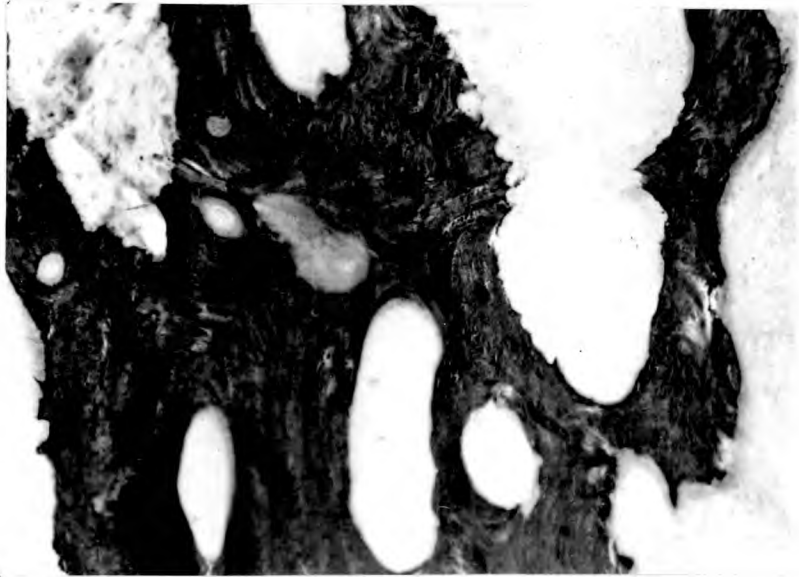


Fig. 67

An undecalcified section from a case of renal osteodystrophy with tertiary hyperparathyroidism showing a marked scalloping of the trabecular surfaces. (Patient R.14. L.G. Solochrome cyanin x 120).



Fig. 68

Radiograph of the hand from a case of renal osteodystrophy with tertiary hyperparathyroidism to show the classical subperiosteal cortical resorption in the phalanges. Cystic changes are also prominent. (Patient R.14. L.G.).

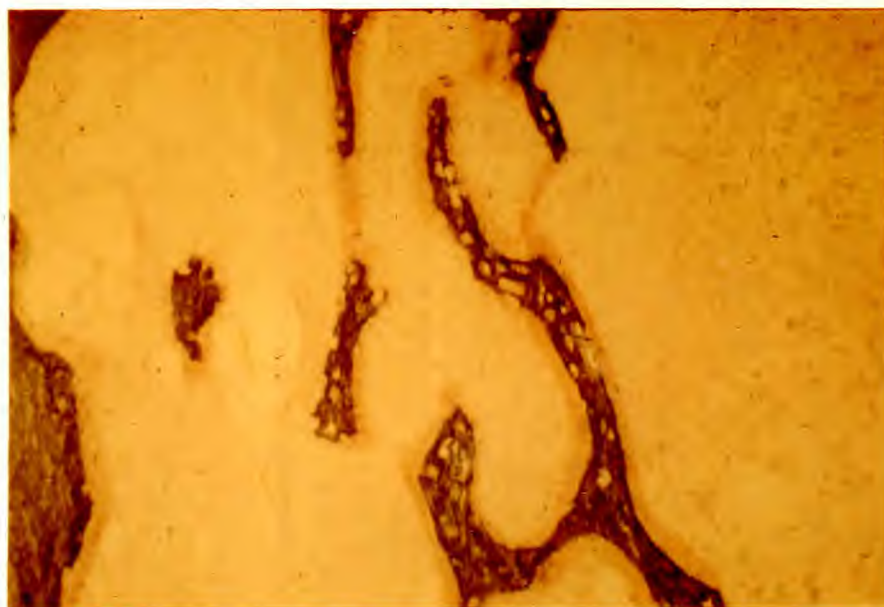


Fig. 69

An undecalcified section from a case of renal osteodystrophy with severe tertiary hyperparathyroidism. There is gross bone resorption much of which is covered with osteoid. The prominent osteocyte cavities lie in trabeculae made up of woven bone. (Patient R.14. L.G. Solochrome cyanin x 30).

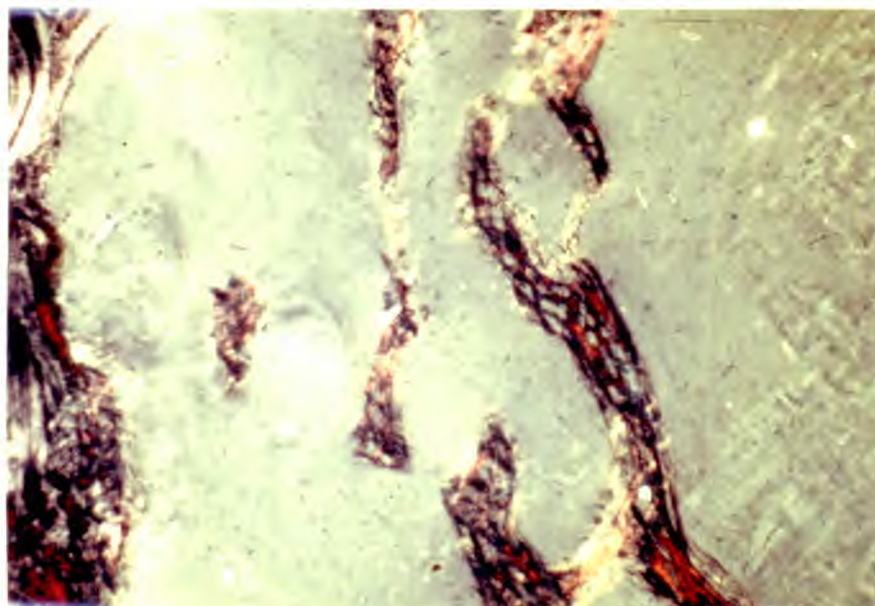


Fig. 70

An undecalcified section from the previous patient showing the criss-cross birefringent fibre pattern of woven bone. (Solochrome cyanin x 30. Polarized light).

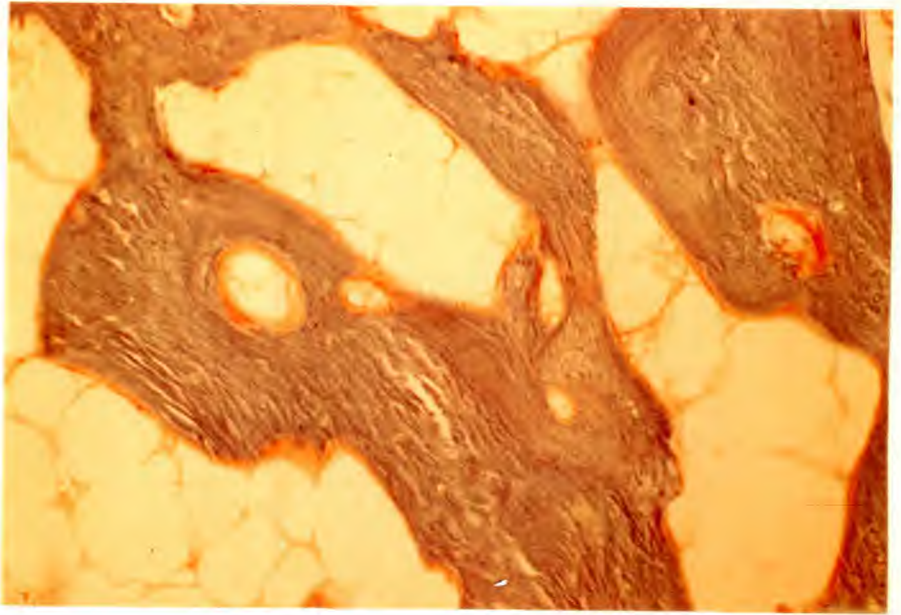


Fig. 71

An undecalcified section from the previous patient a year after total parathyroidectomy. Note a marked decrease in osteoclastic resorption. (Solochrome cyanin x 120).

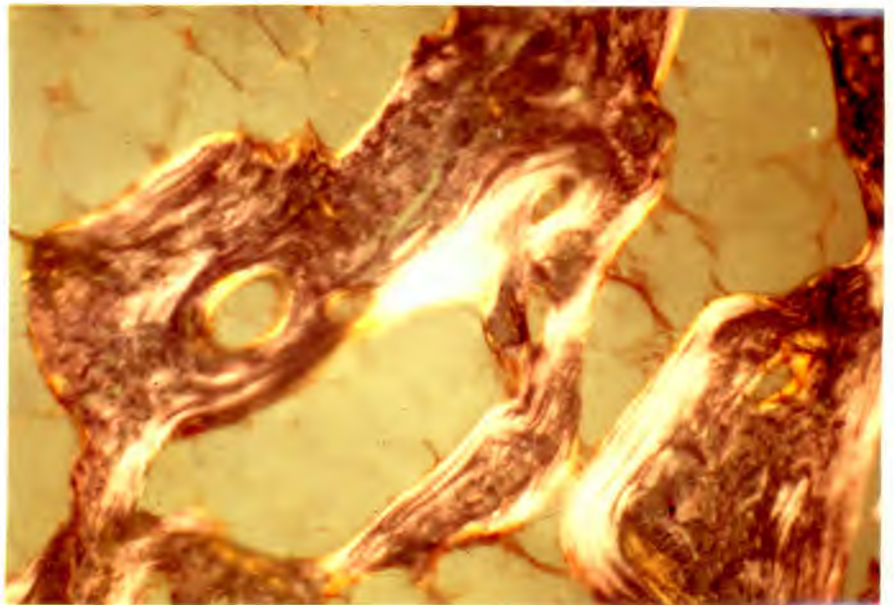


Fig. 72

An undecalcified section from the patient one year after total parathyroidectomy. Note the appearance of the birefringent lamellar fibre pattern. (Solochrome cyanin x 120. Polarized light).

elevated. Serum alkaline phosphatase was markedly elevated in two patients before parathyroidectomy, returned to normal in one patient and was marginally elevated in the other. Plasma total protein was reduced in all three cases and was not appreciably altered by parathyroidectomy. Blood urea remained elevated in all the three cases.

The results of histological measurements are shown in Figure 66. In patient R.14, L.G., there was a dramatic reduction in the amount of osteoid and resorption one year after total parathyroidectomy. Prior to operation this patient showed severe osteomalacia with a very marked increase in resorptive activity (Figures 67-70); after the operation the histological structure of the bone showed a marked change, becoming indistinguishable from normal (Figures 71 and 72).

In patient R.26, G.B., prior to total parathyroidectomy the bone histology showed a marked increase in parathyroid activity on the basis of an increase in resorption surfaces, but these features reverted to near normal after the operation. In both these two patients the amount of calcified trabecular bone was normal showing a slight rise after total parathyroidectomy.

Finally in patient R.43, A.W., features of severe osteomalacia and hyperparathyroidism were present prior to subtotal ($3\frac{1}{2}$ gland) parathyroidectomy. One year after operation the amount of osteoid surface was greatly reduced, but was still abnormal and little change had occurred in resorption surfaces. One point of interest in relation to this patient was a high figure for calcified trabecular bone. This though slightly reduced, remained high after the operation.

7.4. DISCUSSION

The results from this study illustrate the variable and interesting features of bone lesions prevalent in patients with chronic renal failure. In the past many workers have laid emphasis on the value of biochemical findings, in particular the serum calcium, serum phosphorus, ionic product of calcium and phosphorus and serum alkaline phosphatase and on radiological observations, in predicting the type of bone lesions prevalent in chronic renal disease. The patients studied before and during haemodialysis and after kidney transplantation as shown in Figures 50-53, have variable levels of serum calcium, phosphorus, ionic product of calcium and phosphorus and a variable pattern of bone disease ranging from normal to severe osteomalacia or marked hyperparathyroidism or at times exhibiting both features.

Shelling and Remsen (1935), Dreskin and Fox (1950) claimed that in azotaemic hyperparathyroidism based on radiological observations, the serum phosphorus is almost always increased. Stanbury and Lumb (1962), Wilson et al (1965) and Stanbury (1967) stated that in patients with renal failure, a high CaxP product (more than 86.5mg%) and roentgenographic abnormalities were found when azotaemic hyperparathyroidism was the dominant bone lesion; they suggested that subtotal parathyroidectomy would be beneficial to the patient. These two claims are clearly linked, as elevated levels of serum phosphorus will contribute to an elevated ionic product.

In my series there were only four patients with chronic renal failure that were not dialysed and with a CaxP product above 86.5 (Figure 51). Two of these showed hyperparathyroid features, one hyperparathyroidism and osteomalacia and one osteomalacia with a figure for resorption within the normal range. Both the patients with abnormally low CaxP product showed dominant hyperparathyroid features in their iliac

bone biopsies and Figure 51 also showed that there was no clear association of hyperparathyroidism with any particular Ca²⁺P product range.

The situation in relation to radiology is a little clearer. The characteristic radiological findings of hyperparathyroidism is the presence of multiple, small, scalloped surfaces on the periosteal margins of phalanges (Pugh, 1951). They do not seem to be consistently present in patients of the present series with increased parathyroid activity based on histological assessment.

Johnson et al (1969) noted that the absence of significant hyperparathyroidism on the basis of subperiosteal resorption in patients with long term haemodialysis. They made a speculation that the relative absence of severe bone disease in these patients may be related to the absence of significant hyperparathyroidism. The results of my studies show that histological evidence of hyperparathyroidism is common in patients with renal failure, and that radiological evidence of hyperparathyroidism is a relatively crude way of detecting this (Fig. 46). The relevance of the histological evidence of increased resorption to the problem of hyperparathyroidism is suggested by the study of the relationship of resorption surface to parathyroid weight. In relation to this, attempts have been made in the past to correlate quantitatively the bone changes and the degree of parathyroid hyperplasia; and the well known one is that of Gilmour (1947) who found a close correlation between parathyroid hypertrophy and the degree of osteitis fibrosa in 20 cases with renal osteodystrophy. Although this could be studied in only a few cases in this series, there is a good correlation between the histological measurements and the glandular mass (Figure 49). Katz et al (1969) have shown, as might be expected, a close correlation between parathyroid gland weight at surgery and plasma levels of parathyroid hormone.

The degree of resorptive activity in the undialysed and

dialysed patients had no correlation with the level of serum calcium. Reiss and Canterbury (1969) also found lack of correlation between parathyroid hormone and serum calcium levels in patients with chronic renal failure. The assessment of the parathyroid status in patients with renal failure is clearly more reliable in iliac bone biopsy than on radiology or on indirect biochemical assessment; and increased parathyroid activity is clearly a major component of renal osteodystrophy. Dialysis did not induce any major change in the amount of resorption but successful transplant did so. At one year after transplant, 2 of the 6 cases with creatinine clearance more than 50 ml/min showed amounts of resorption surface within the normal range. Renal transplant, if successful therefore leads to a considerable drop in resorption surface. Total parathyroidectomy leads to a dramatic drop in resorption, particularly if the osteoid covered surfaces are included. One of the three cases subjected to parathyroidectomy showed adenoma on histological examination of the glands (Fig. 48). This case is presumed to be an example of so-called tertiary hyperparathyroidism. The finding that resorption is increased in the great majority of cases of renal failure suggests that the continued stimulation of the glands present during dialysis as well as in undialysed patients, is likely to lead to an increase in the incidence of this complication. The evidence from this investigation suggests that patients with excess parathyroid activity cannot be identified with certainty by radiological means or by using the ionic product of calcium and phosphorus. Bone biopsy with quantitation assessment of the amount of bone resorption will give a reliable indication of parathyroid activity, and could be used as a guide to treatment. One patient (R.54) who shows a very high figure for resorption two years after a partially successful transplant is currently under consideration for parathyroidectomy. Autonomous hyperparathyroidism after renal transplant has been described (McIntosh et al,

1966, Alfrey et al, 1968).

One interesting observation as a result of this study is the finding of scalloped irregular bone surfaces covered with varying amounts of osteoid. This feature is present in most of the patients with chronic renal failure (Fig.65), however, it is also occasionally seen in cases of dietary osteomalacia with evidence of secondary hyperparathyroidism (Fig.36). Strictly speaking these surfaces cannot be regarded as active resorption surfaces. However, scalloped surfaces without obvious osteoclastic activity may also not represent active resorption, and it is reasonable to consider all scalloped surfaces whether or not they have osteoclasts, and whether or not they are covered by osteoid, as evidence of current or recent active resorption. Resorption surfaces covered by osteoid (R.S._o) certainly are found most often in high turnover situations. Moreover, Jowsey et al (1969) have also considered these resorption surfaces covered by osteoid to be resorption surfaces on the basis of micro-radiographic studies in patients with chronic renal failure. Their main conclusion was resorption exceeded formation in chronic renal failure patients.

The other main component of renal osteodystrophy is osteomalacia. The majority of patients studied showed a marked increase in the amount of osteoid, both in terms of the absolute volume and percent of total bone surface covered with osteoid. According to Albright and Reifenstein (1948) the osteoid seams are narrow in azotaemic /and Royer osteodystrophy and Fourman (1968) also stated that the width of osteoid seams is less than that of rickets. However, Follis et al (1952) and Ball (1960) postulated that the seams may be thin at first and later show an increase in width. As shown in Figure 61, azotaemic osteomalacia seems to progress despite haemodialysis and is not dramatically arrested by kidney transplant. After a year of kidney transplant, although the amount of osteoid seems to decline, it is still increased

when compared with the control group. The amount of osteoid does not depend appreciably on the state of renal functions. The osteomalacia that is shown by the majority of the patients with chronic renal failure is obviously a significant part of renal osteodystrophy, and it too may become more prominent with the increase in dialysis. The absence of improvement of the increase in osteoid after transplantation is surprising. It may be due to the continued remodelling that takes place as the bones return to normal. This possibility is supported by the observed changes in trabecular morphology illustrated in Figures 73 and 74, when the broad osteoid covered trabeculae of chronic renal failure are converted to a near normal pattern one year after transplant. The marked drop in osteoid in the patient treated by parathyroidectomy is probably related to the vitamin D therapy these patients were given.

The in vivo diagnosis of osteomalacia is again best achieved by iliac bone biopsy. In the patients with dietary osteomalacia there was a significant correlation between the serum alkaline phosphatase and the amount of osteoid (Figure 37, Chapter 5), by contrast, in renal failure patients no significant correlation was shown (Fig.52). However, the finding of a raised serum alkaline phosphatase in renal osteodystrophy is not an unusual feature. It may be due to the bone disease or to other causes, for example, clinically occult hepatitis, as a result of administration of immunosuppressive drugs. The finding of normal serum alkaline phosphatase even in the presence of severe osteomalacia or with increased parathyroid activity, though not surprising, has never been well documented in the literature. In the patients thus studied any type of bone disease could be seen with either normal or high serum alkaline phosphatase (Fig.53). Katz et al (1969) stated that there is a close relationship between serum alkaline phosphatase with the extent of bone involvement in renal osteodystrophy, based on radiological findings. The scatter diagram in Figure 52 shows that there is no relationship

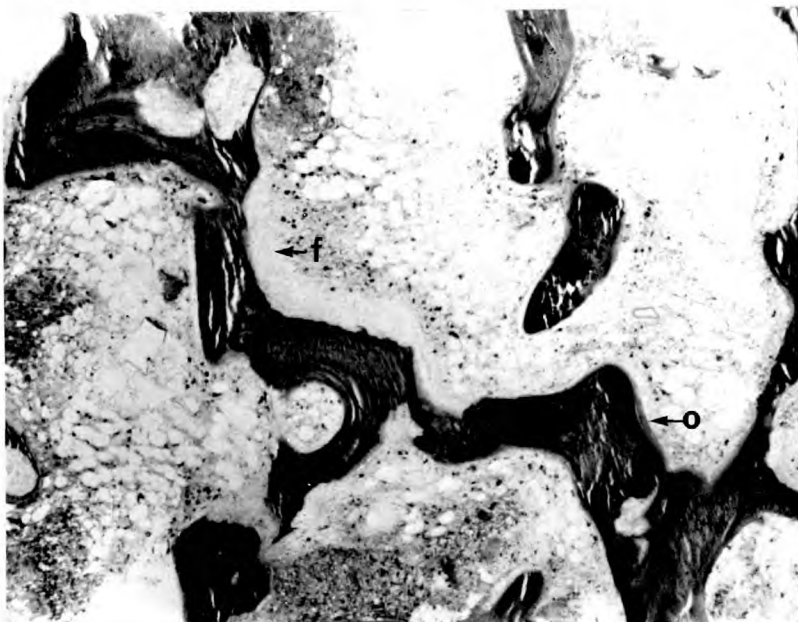


FIG. 73

An undecalcified section from a case of renal osteodystrophy showing an increase in resorption and osteoid tissue which is pale stained (o). Note also prominent fibrous tissue bands in the marrow spaces (f) and deposition of iron pigment. (Patient R.40. B.B. Solochrome cyanin x 30).

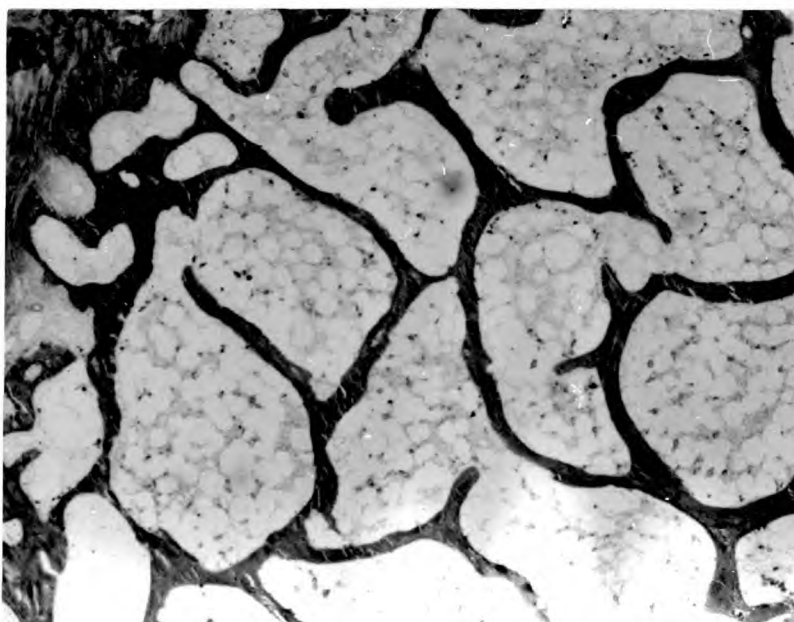


FIG. 74

An undecalcified section from the same case as the previous illustration one year after successful kidney transplant. Note the near normal cortical and trabecular pattern, the iron pigment remains. (Solochrome cyanin x 30).

between serum alkaline phosphatase and the amount of osteoid ($p > .05$). There are 8 cases who showed an excess of osteoid, with normal serum alkaline phosphatase and 9 patients who showed normal amounts of osteoid with a raised serum alkaline phosphatase. Thus it was quite evident that serum alkaline phosphatase is not a good indicator of the presence or absence of osteomalacia in renal failure. Serum alkaline phosphatase can easily be detected in urine, but this does not represent the normal major route of excretion in man (Roberts (1933.) However, it is possible that in patients with chronic renal failure, with proteinuria, serum alkaline phosphatase could be excreted through the damaged nephrons. Butterworth et al (1965) studied the characteristics of alkaline phosphatase in human urine in acute renal failure cases and stated that the urine contains mostly fast migrating alkaline phosphatase derived mainly from the kidney. However, they also observed a serum-like alkaline phosphatase band in urine in a single case of homotransplant recipient. One speculative explanation for the lack of correlation between the serum alkaline phosphatase and the amount of osteoid in the bone in patients with renal failure is that the rate of loss of alkaline phosphatase via the kidney is variable, and related to the type of renal damage.

In relation to the amount of calcified trabecular bone in patients with chronic renal failure only 7 cases (12%) showed increased calcified trabecular bone mass. ^(Fig 54) Although osteosclerosis is far less common than osteomalacia, in a recent review the occurrence is given as about 20% in patients with chronic renal failure (Valvassori and Pierce, 1964). Kaye (1964) also found an increase in the calcium, phosphorus and nitrogen content of whole lumbar vertebral bodies in patients with chronic renal failure. Garner and Ball (1966) stressed that the amount of total bone mass, including mineralised and non-mineralised bone is usually increased in cases of chronic renal azotaemia. However, the former is usually within the normal limits so that the majority of the increase was due to an increase in osteoid. The degree of osteomalacia

their patients showed was far higher than in my series. They also documented a few cases with an increased calcified trabecular bone mass in renal failure.

The histological evidence shows that in renal failure there is usually excessive bone formation and destruction occurring simultaneously, kinetic studies, for example those of Heaney (1969) support this. A minor imbalance could obviously lead to considerable changes in total bone mass. While it is obviously possible to implicate calcitonin in this situation, this would be expected to inhibit resorption, and it does not seem likely to play a major role in the face of the high resorption values I have found.

The majority of chronic renal patients either undialysed or during haemodialysis showed an increased resorption, more so than cases of primary hyperparathyroidism. This observation is in agreement with those of Jowsey et al (1969) on the basis of microradiographic studies and also of Berson and Yalow (1966) who stated that the concentration of parathyroid hormone in the plasma of patients with severe chronic uraemia is frequently much higher than it is in the majority of cases having adenomatous hyperparathyroidism.

While the calcified trabecular bone mass in chronic renal failure is normal or increased, and does not change significantly with therapy (Fig.60), the cortical bone does alter. There is a considerable drop in cortical bone thickness in patients with undialysed chronic renal failure, and a further loss may well occur in the first 6 months of dialysis. Pellegrino and Biltz (1965) found a significant decrease in the density of the cortex of the tibia, in patients with chronic renal failure, based on microradiographic studies and concluded that increased porosity was a feature of uraemia. Zech et al (1969) in an abstract of a meeting, also stated that thinning of the cortex and reduction in bone mass occurs with prolonged haemodialysis. Variations

from centre to centre, with different techniques of dialysis are to be expected, and further studies of biopsies from different centres may lead to a better understanding of the control of bone growth in renal failure patients.

7.5. SUMMARY

Iliac bone biopsies from 60 patients with chronic renal failure were studied. All but two biopsies from 50 patients with chronic renal failure untreated or on haemodialysis were histologically abnormal, irrespective of biochemical or radiological changes. In the majority of the cases the radiological changes which were noted in the hands, in form of subperiosteal resorption were mild and the biochemical abnormalities were variable.

The changes observed were an increase in the percentage of bone surfaces covered with osteoid, or the percentage of bone surfaces showing resorption, or both. An additional striking feature in these cases was a definite increase in the percentage of irregular scalloped surfaces covered with osteoid.

Biopsies from a group of patients who had been on regular dialysis for at least 6 months showed on average a significant increase in the percentage of bone surfaces covered with osteoid as compared to a group of patients with renal failure who had not had dialysis. There was also a definite decrease of mineralised cortical bone but in the mineralised cancellous bone there was no change.

Many of the parameters obtained from histoquantitation were correlated with the biochemical and radiological findings.

The percentage of osteoid covering bone surfaces did not correlate with the amount of serum alkaline phosphatase either before or during dialysis. Serum alkaline phosphatase was not a true indicator of the degree of osteomalacia in cases of renal osteodystrophy; the possibility that this was due to the loss of the enzyme from a renal

leak is discussed.

The percentage bone resorption surfaces did not correlate with serum calcium, but showed a close relationship with the glandular weight of the parathyroids, and is therefore a true reflection of parathyroid activity.

The calcium and phosphorus product is of no value in the prediction of the type of bone disease found in iliac bone biopsy.

Three patients with chronic renal failure have been studied before and after parathyroidectomy. The first patient (L.G.) showed a marked reduction in osteoid and a very marked decrease in resorption surface a year after total parathyroidectomy. The second patient (G.B.) showed a marked reduction in osteoid and a moderate reduction in osteoclastic activity 7 months after total parathyroidectomy. The third patient (A.W.) showed a marked decrease in osteoid, but only a slight decrease in resorption a year after subtotal parathyroidectomy.

19 patients have been studied by bone biopsy after transplantation. Biopsies from 9 patients have been quantitated both before and after transplantation. These showed on average an increase in the amount of osteoid and a reduction in the amount of resorption surface at intervals ranging from 6 months to 1 year after transplant.

Biopsies from a further group of 10 patients who had not had a pre-transplant bone biopsy have also been studied. The mean figures of all the parameters for this group were very similar to that of the post-transplant group. One patient (R.54, M.K.) showed an extremely high amount of resorption surface 2 years after transplant, and was suspected of having tertiary hyperparathyroidism.

It is concluded that quantitative bone histology is of value in revealing the true frequency and nature of the bone disease in patients with chronic renal failure.

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APPENDIX

1. PREPARATION OF UNDECALCIFIED SECTIONS OF BONE

1.1. Fixative (10% buffered formol saline)

3.5 grams of anhydrous sodium dihydrogen phosphate (NaH_2PO_4) and 6.5 grams of anhydrous disodium hydrogen phosphate (Na_2HPO_4) was dissolved in 900 ml of water. 100 ml of concentrated formalin was added. The pH of this medium is between 6.8 and 7.0.

1.2. Technique of Resin Embedding

(a) Fixation : Through and through iliac bone biopsy (Fig.4) usually about 10 mm in length and 4 mm in diameter is fixed in phosphate buffered formol saline (pH 6.8-7.0) for a period of 48 to 72 hours.

(b) Dehydration : 70% alcohol for 24 hours
90% alcohol for 24 hours
100% alcohol for 48-72 hours
Tertiary butyl alcohol for 8-10 hours
All at room temperatures.

(c) Embedding : The biopsy is then impregnated in varying proportions of styrene and resin in universal glass containers.

Pure styrene for 24 hours
75% styrene, 25% resin for 24 hours
50% styrene, 50% resin for 24 hours
25% styrene, 75% resin for 24 hours
Pure resin for 48 hours

All these stages are carried out at room temperature, the final stage in pure resin is carried out at a negative pressure of about 200 mm of mercury (Fig.7). The tissue is then embedded in resin with 2% catalyst for 48-72 hours at 37°C . This is carried out in 2 oz polythene specimen containers (Messrs. Raymond Lamb). Polymerisation is usually complete in 72 hours but if the block does not feel solid, can be continued for a further 24 hours at $40-50^\circ\text{C}$. When fully polymerised the blocks can be removed by squeezing the thin plastic container see Fig.5 (3). The sides are then trimmed as close to the bone as possible with an electric saw.

(d) Re-embedding : The block is then re-embedded in resin with 2% catalyst and 0.2% accelerator at 37° for a period of 2-3 days to pro-

duce a hard outer supporting framework. The block, which should now be rock-hard, is then trimmed with an electric saw to a size of 2.5 x 1.5 x 1.0 cm. The surface to be cut as shown in Figure 5 (4), phase A, is polished with sandpaper. It should be smooth and even; if rough the block should be re-embedded and cut at phase B.

N.B. The required temperature in the embedding procedure can be obtained either by using an incubator or by a direct heat from a 60 watt bulb. The latter is preferred.

1.3. Cutting

The trimmed block is mounted between the two jaws of the microtome. The block is then adjusted to be in line with the knife by means of a spirit level and the screws are then tightened. The block is then trimmed until the surface of the bone is exposed by a 'Trimmer'.

Sections are then cut at 8 μ thickness on a Jung K microtome by a very sharp knife 'Cutter'. The surface of the block is kept moist with a 70% alcohol and a camel hair brush is used to pick up the sections. Sellotape or cigarette paper is not needed if the bone is well impregnated, although these have been suggested by some workers to prevent curling and fragmentation.

1.4. Staining Procedure

(a) Solochrome cyanin R stain

- (I) Transfer sections to water
- (II) Stain for 10 mins in the following mixture :
 - Solochrome cyanin R - 1 G
 - Glacial acetic acid - 2 ml
 - Distilled Water - 98 ml
- (III) Wash in warm water (37°) until the sections become bluish and the water is no longer coloured red, for about 10 seconds.
- (IV) Dehydrate in ascending alcohols, clear in xylol and mount in D.P.X. (Distrene-Plasticizer-Xylene). The differentiation period is very critical sometimes microscope aid is necessary. Osteoid tissue stains a reddish colour, in contrast to calcified bone which appears bluish, and the calcification front appears at the junction of osteoid and calcified bone as a dark purplish granular layer.

(b) Von Kossa Van Gieson Stain

- (1) Transfer sections to distilled water
- (11) Place in 1.5% aqueous silver nitrate (Freshly prepared from 10% stock solution) and leave in natural or artificial light for $\frac{1}{2}$ -1 hour.
- (111) Wash in distilled water
- (1V) Remove excess silver solution with hypo (5% sodium thiosulphate) for 3-5 minutes
- (V) Rinse in distilled water
- (VI) Counterstain in van Gieson's solution for 3-5 minutes.
- (VII) Rinse in tap water
- (VIII) Dehydrate, clean and mount in D.P.X.

(c) Perls' Prussian Blue (see page 172)

1.5. Materials

The plastic materials were obtained through Raymond Lamb Laboratory Equipment Supplier of Alperton, Middlesex. They are manufactured by Bondaglass Ltd., Beckenham, Kent.

- (i) Resin : This is a polyester resin, is a synthetic resinous compound formed by the reaction between a polyhydric alcohol and a polybasic acid. It is also a clear casting resin, water white, highly reactive and cures in the presence of small amounts of cobalt naphenate.
- (ii) Styrene : This is a monomer used for thinning polyester resin.
- (iii) Catalyst : This is a methyl ethyl ketone peroxide.
- (iv) Accelerator : This is a cobalt naphenate. When the catalyst is added to resin which has had a requisite amount of accelerator an exothermal chemical reaction commences which eventually solidifies the resin.

N.B. Catalyst and accelerator should not be mixed together in order to avoid an explosion. Styrene and resin mixtures should be handled in a fume cupboard.

2. TECHNIQUE OF HISTOQUANTITATION

The section to be quantified is first studied with a microscope to determine its adequacy. If satisfactory the edge of the section, excluding areas with gross artefact, is outlined with a grease pencil. The dimensions of this area are measured with a micrometer eyepiece. The section is placed on the stage of the microscope which

is part of the histoquantitation equipment (Fig.16); it is then projected onto the white screen, placed at a distance of 4 feet away from the microscope by means of a powerful quartz iodine lamp. The magnification used is 240 x., i.e., using an eyepiece x .6, and an objective x 40. The section is then adjusted so that the projected image of the cortex would lie parallel to an arbitrary vertical line drawn on the screen and the point selected for the start of the line scan overlies a reference spot on the screen. The section is then kept firmly on the stage by means of two clamps, the exact location of the slide is read on the horizontal scale (F) and on the vertical scale (E).

The ^{slide}slight component quantitator (B) as shown in Figure 15 is switched on and all the electro-magnetic counters are set to zero by pressing the 'counter reset' button.

The section is quantitated with relation to a reference point on the screen. The number of line scans vary from section to section, but on an average there are about 14 to 18 scans between two cortices, with a distance interval of 200-400 u between each scan, which can be adjusted with the horizontal scale (F). The scans are spaced more closely together over cortical bone than on trabecular bone (Fig.75).

In exceptional cases the line scan passes through an osteoid seam without hitting the calcified bone (Scan No.6) and the line scan passes through an osteoid seam laid down between two bone surfaces (Scan No.10). In such circumstances the total bone surfaces covered with osteoid should be carefully calculated, i.e., by subtracting or adding one to H_o .

The abbreviated terms used in Table 20 and Figure 75 are as follows:

L_b , L_o and L_m	The summated length for each component in microns, namely L_b for calcified bone, L_o for osteoid and L_m for marrow space.
L_t	Total length of line scanned = $L_b + L_o + L_m$
H_b , H_o and H_m	Total number of hits for each component
H_r and H_{ro}	are eventmarkers for the total number of resorption surfaces not covered and covered with osteoid respectively.
eH	Denotes the number of hits (edge hits) for the calcified bone lying at the edge of the area to be measured.
pH	Denotes the total number of hits for the rare trabecular surface which is so shattered that it is impossible to decide whether resorption or osteoid had been present (Pseudo hits).

Calculations

$$\% \text{ volume of calcified trabecular bone} = L_b / L_t \times 100 = \frac{13183}{52309} \times 100 = 25.2\%$$

$$\% \text{ volume of osteoid in trabecular bone} = L_o / L_t \times 100 = \frac{442}{52309} \times 100 = 0.84\%$$

$$\begin{aligned} \% \text{ volume of bone marrow in trabecular bone} &= L_m / L_t \times 100 = \frac{38684}{52309} \times 100 \\ &= 73.95\% \end{aligned}$$

For the purpose of a histogram the percent volume of different components for each line scan also be calculated.

Total number of bone surfaces (S) is calculated as thus:

$$(H_b \times 2) - (eH + pH) = (119 \times 2) - 18 = 220$$

Therefore % total bone surfaces covered with osteoid =

$$\frac{H_o}{S} \times 100 = \frac{60}{220} \times 100 = 27.3\%$$

TABLE 20

HISTOQUANTITATION PROCEDURE

Case No. R.13 M.B.	Distance in u between each scan starting from cortical edge	Channel Number *								Total Length in u **	Stage Scale Reading in u ***	eH and pH Ø		
		1		2		3		4					5	
		Calcified Bone		Osteoid		Bone Marrow		Resorption Surface not covered with osteoid 'R.S.'					Resorption Surface covered with osteoid 'R.S. _o '	
		Lb	Hb	Lo	Ho	Lm	Hm	Hr	Hro	Lt				
Cortical Bone	150	3253	2	..	..	22	1	..	..	3275	3277	2		
	150	2774	4	5	3	519	3	..	..	3298	..	2		
	150	2871	5	21	3	427	3	..	2	3319	3317	2		
Transition Zone	150	2165	8	14	4	1137	8	3	..	3316	..	1		
	150	1701	7	194	5	1487	7	1	2	3382	3382	1		
Trabecular Bone	150	881	5	57	4	2445	4	..	2	3383	..	2		
	300	876	6	109	7	2329	6	4	3	3314	3316	1		
	300	545	3	4	1	2719	4	2	1	3268	..	..		
	300	1335	6	15	3	1961	7	3	..	3311	3313	..		
	300	150	2	8	1	3062	2	..	..	3220	..	1		
	300	810	2	..	..	2522	3	..	..	3332	3330	1		
	300	1335	5	39	3	1674	6	2	1	3048	..	..		
	300	618	6	24	3	2664	6	1	1	3306	3308	1		
	300	528	3	33	1	2699	4	4	1	3260	..	..		
	300	731	3	6	1	2613	3	..	..	3350	3352	..		
	300	925	5	68	5	2326	6	1	2	3319	..	..		
	300	874	5	11	1	2294	5	2	1	3179	3177	1		
	300	921	3	11	1	2301	4	..	..	3233	..	..		
	300	809	4	34	2	2442	5	..	1	3285	3287	..		
	300	939	5	14	1	2279	5	1	..	3232	..	..		
	300	915	2	9	1	2354	3	..	..	3278	3278	..		

/continued over

		Lb	Hb	Lo	Ho	Lm	Hm	Hr	Hro	Lt		
Transition Zone	300	1799	3	13	1	1405	3	..	..	3217	3215	1
	200	2139	4	23	1	1302	5	5	1	3464	..	..
	200	1421	5	37	3	1981	5	2	..	3439	3437	1
	200	843	3	6	1	2614	4	1	..	3463	..	..
	200	1752	5	..	..	1708	5	1	..	3460	3460	..
Cortical Bone	200	2670	5	21	3	833	6	..	1	3524	..	..
	200	3223	3	7	1	237	3	..	..	3467	3469	1

* L_b , L_o and L_m - The summated length for each component in microns, namely L_b for calcified bone, L_o for osteoid, and L_m for marrow space.

H_b , H_o and H_m - Total number of hits for each component; H_r and H_{ro} are event markers for the total number of resorption surfaces not covered and covered with osteoid respectively.

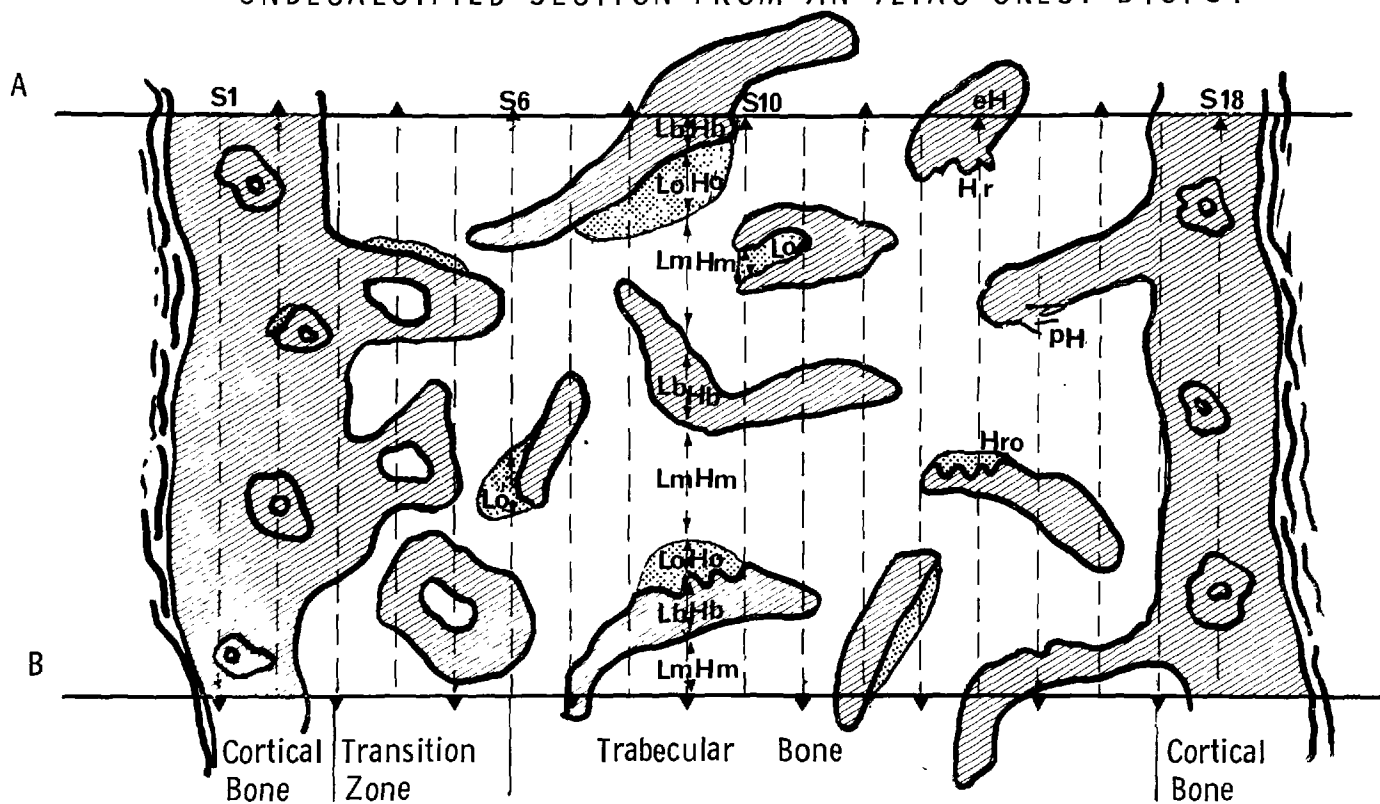
** L_t - The total length of line scanned = $L_b + L_o + L_m$.

*** - The stage scale consists of a vertical scale ; 1 division = 500 u and a vernier circular scale; 1 division = 2 u. The readings taken at the beginning and the end of histoquantitation provide a check on the total length of line scan as recorded on the counters(i.e. $L_b + L_o + L_m$).

Ø eH and pH - The former denotes the number of hits "edge hits" for the calcified bone lying at the edge of the area to be measured and the latter denotes the total number of hits for the rare trabecular surface which is so shattered that it is impossible to decide whether resorption or osteoid had been present "pseudo hits".

Fig. 75

— DIAGRAMMATIC REPRESENTATION OF HISTOQUANTITATION ON AN UNDECALCIFIED SECTION FROM AN ILIAC CREST BIOPSY—



N.B. A and B are two lines drawn to form the edge of the area to be measured.

S1 to S18 are line scans with direction.

L_b, L_o and L_m are lengths of calcified bone, osteoid and marrow traversed.

H_b, H_o and H_m are hits at end of traverse across calcified bone, osteoid and marrow.

H_r and H_{ro} are hits at surfaces of calcified bone showing resorption only, or resorption covered with osteoid.

% total bone surface showing resorption not covered with osteoid =

$$\frac{H_r}{S} \times 100 = \frac{33}{220} \times 100 = 15.0\%$$

% total bone surface showing resorption covered with osteoid =

$$\frac{H_{ro}}{S} \times 100 = \frac{19}{220} \times 100 = 8.6\%$$

The overall histoquantitation results are thus recorded in the form of a tabulated chart as shown in Table 20 and also presented in the form of the histogram (Figure 76) which enables the results to be taken in at a glance and also enables the separation of cortical bone, transitional zone, and trabecular bone to be made.

3. RADIOLOGICAL INVESTIGATIONS

The majority of the patients studied had x-ray films of the hands and other parts of the skeletal system usually spine and pelvis in order to ascertain the presence or absence of 'thin bones', pseudo-fractures and phalangeal erosions. Vertebral body fractures or collapse were sought as radiological evidence of osteoporosis.

In the patients with chronic renal failure the grading of subperiosteal cortical erosions was carried out by Dr. F.H. Doyle.

The average mineral thickness of the distal 1 cm of the ulnar bone which mainly reflects the amount of trabecular bone is obtained from the comparison of the density of the radiograph with that of step-wedge using a microdensitometer (Doyle, 1961, 1969).

4. BIOCHEMICAL INVESTIGATIONS

4.1. Serum calcium, phosphorus and serum alkaline phosphatase, total proteins and blood urea were all estimated in the Department of Chemical Pathology by the standard Technicon Autoanalyser Method (Wootton, 1964).

(i) Before August 1968 the serum calcium was estimated by Zeiss flame photometer and after August 1968 was estimated by the standard technicon autoanalyser method using o-cresolphthalein complexione and calibrated

Fig. 76

— HISTOGRAM SHOWING PERCENTAGE COMPOSITION OF CALCIFIED BONE,
OSTEOID AND MARROW IN AN ILIAC CREST BIOPSY—

(EACH COLUMN REPRESENTS A LINE SCAN PARALLEL TO THE EDGE OF CORTEX. THE
DISTANCE OF THE SCAN FROM CORTICAL AREA IS SHOWN ALONG THE ABSCISSA)

— KEY —

Name, Sex & Age M.B., F 38

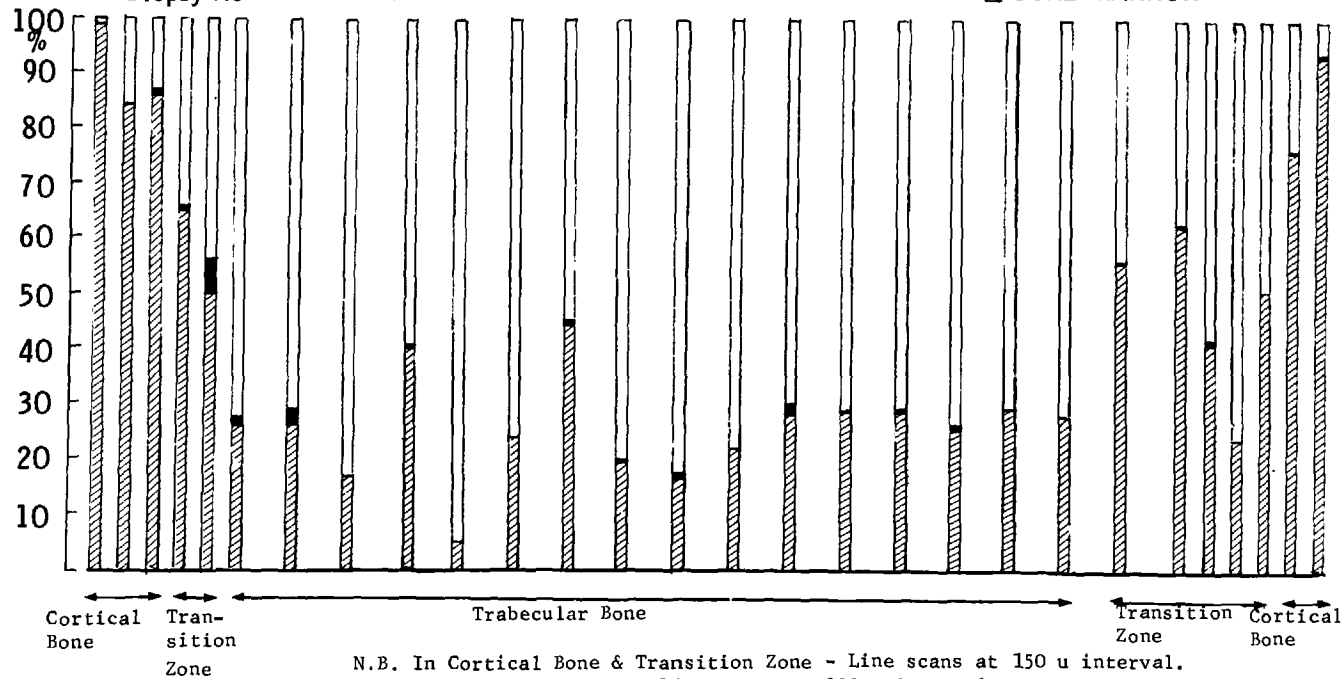
Diagnosis Chronic renal disease
(6 mts. after renal transplant)

Biopsy No S 69/143 (R.13)

▨ CALCIFIED BONE

■ OSTEOID

□ BONE MARROW



by the Zeiss flame photometer (Normal serum calcium = 4.7-5.5 mN).

(ii) The serum inorganic phosphorus was also estimated by the standard Autoanalyser method using amino-naphthol sulphonic acid as a reducing agent (Normal = 1.6 -2.4 mN).

(iii) Serum alkaline phosphatase was also estimated by an Auto-analyser method (King-Armstrong) with dialysis using disodium phenyl phosphate as substrate (Normal = 4 - 11 KA^o units).

(iv) Serum total protein was estimated by Autoanalyser Biuret method. (Normal = 6.5-7.9 G%).

(v) Bloodurea was estimated by an Autoanalyser method using diacetyl monoxime thiosemicarbazide (Normal = 20-40 mg%).

(vi) Creatinine clearance - the serum and urine creatinine estimated by an Autoanalyser method using a Jaffe reaction in which a red colour is produced with an alkaline picrate. Clearance value determined according to the formula UV/P . U = urine concentration of creatinine in mg per 100 ml. V = urine production at a rate of V ml per minute. P = plasma concentration of creatinine in mg per 100 ml. (Normal = 120-130 ml/min).

4.2. Phosphate Excretion Index (PEI)

This was measured in all cases of 'clinical and suspect' osteomalacia and 'probable' primary hyperparathyroidism. This test was performed as described by Nordin and Fraser (1960). After an overnight fast, the patients were kept on a low calcium diet containing about 750 mg of phosphorus. For each patient at least two estimations were carried out and the mean values were considered. This index takes into account the level of serum phosphate so that the phosphate/creatinine clearance ratio is expressed as the departure from the value which would be expected in the normal subject for the prevailing serum phosphate concentration (Normal = ± 0.09).

4.3. 24-Hour Strontium Space

This test was performed as described by Joplin et al (1967). Strontium space was estimated in most of the cases in the 'Metabolic Group' including a few chronic renal failure patients (R 14 L.G., R.15 J.C., R.17 P.C., R.. Z.L., R.. F.S.).

It is an index of the exchangeable calcium. 9.4 mEq of stable strontium is given intravenously (as strontium gluconate), and the strontium is measured in the subsequent 24 hour urine and in the serum taken at 24 hours. The 24 hour space corrected for body weight is $\pm$

$$\frac{24\text{-Urinary Strontium (mEq)}}{\text{Serum strontium (mEq/L)}} \times \frac{20}{\text{Body Weight (Kg)}} = \text{Strontium Space (Plasma units)}$$

(Normal $\leq$ 18 plasma units).

N.B. In all the above mentioned biochemical estimations the tests were performed within one week of iliac bone biopsy.

In patients receiving haemodialysis the blood samples were taken shortly before the commencement of haemodialysis.

5. CALCULATION OF MEAN PARATHYROID GLANDULAR WEIGHT

The Section of parathyroid glands is projected onto the screen by means of a Leitz slide projector (using an eyepiece x 6 and an objective x 10). The former is kept at a distance of 6 feet away from the latter. On the screen, points are mapped out to produce a point-counting grid system. The points are arranged at the apices of equilateral triangles. The number of points falling on each component is proportional to the area of that component and thus to the volume of that component (Delesse, 1848, Hennig, 1959).

The number of points falling on different components of the glands, in particular, the glandular tissue, fibrovascular connective tissue and fatty tissue are recorded in the '3 figure tabulator' used in haematological practice. In this way all the glandular elements in all the four glands can easily be separated out from the fibrovascular and

fatty connective tissue, and by multiplying the gross weight of the four parathyroid glands by the percent of the glandular tissue, the mean parathyroid glandular weight or 'net weight' could be derived accurately.

1.4. Staining Procedure (Cont. from page 162)

(c) Perls' Prussian Blue.

The principle of this stain is that ferric iron combines with ferrocyanide to form an insoluble complex, Prussian Blue.

(I) Transfer sections to water.

(II) Place for 5 mins. in the following mixture (Perls' reagent) which is slightly warmed.

Potassium ferrocyanide	1 G.
Distilled Water	50 ml.
Hydrochloric acid(conc)	1 ml.

(III) Rinse in distilled water.

(IV) Counterstain in 1% aqueous Neutral red or Carmalum.

(V) Wash rapidly in tap water.

(VI) Dehydrate, clean and mount in D.P.X.

N.B. Iron-free analytical reagents and clean glass ware must be used to avoid false reactions.

6.1

TBS+U VS 20ST VUL 6.3 0.03, 5.1 0.07, 13.5 0.03, 7.3 0, 1.2 0,
 4.5 0.2, 1.5 0.02, 2.7 0.02, 11.3 0.1, 26.3 0.73, 3.3 0.02,
 18.2 0.14, 0 0, 39.3 0.57, 2.3 0.04, 24.3 0.26, 22.2 0.21, 5.1 0.03,
 12.2 0.13, 17.9 0.12, 2.3 0.05, 6.4 0.02, 5.7 0.03, 20.0 0.14,
 3.0 0.09, 0 0, 6.9 0.01, 9.3 0.04, 43.2 0.51, 23.2 0.13, 16.3 0.09,
 13.3 0.11, 30.3 0.43, 6.5 0.05, 17.6 0.03, 10.6 0.03, 16.0 0.14,
 20.0 0.21, 6.3 0.03, 16.6 0.23, 2.1 0, 10.6 0.03, 14.0 0.13,
 6.1 0, 22.2 0.06, 34.4 0.64, 35.1 0.19, 15.1 0.13, 21.2 0.12,
 7.7 0.03, 3.9 0.01, 4.4 0.06, 9.6 0.10, 19.1 0.13, 13.6 0.06,
 31.2 0.12, 23.3 0.05, 7.8 0.03, 17.1 0.23, 23.5 0.26, 45.1 1.36,
 17.2 0.63, 16.1 0.07, 27.6 0.17, 12.7 0.73, 6.3 0.07, 4.9 0.27,
 3.5 0.05, 73.3 0.93, 14.5 0.19, 43.1 0.54, 53.6 3.8, 5.5 0.03,
 12.6 0.17, 14.5 0.11, 55.3 0.74, 37.3 1.2, 33.3 15.5, 66.9 7.2,
 33.5 0.55, 5.7 0.14, 12.2 0.03, 3.3 0.02, 45.6 1.65, 6.6 0.14,
 15.3 0.11, 34.0 0.93, 37.9 0.76, 2.5 0.05, 41.2 0.33, 36.3 0.63,
 47.9 1.02, 43.5 1.53, 7.9 0.11, 17.2 0.43, 30.6 3.51, 13.9 0.33,
 41.3 0.35, 22.5 0.51, 53.1 1.88, 40.3 0.34, 5.2 0.04, 6.8 0.07,
 6.0 0.06, 4.0 0.05, 31.5 0.47, 3.43 0.03, 13.0 0.10, 19.5 0.13,
 32.9 4.3, 43.3 1.5, 18.9 0.14, 50.0 1.34, 21.9 0.13, 40.0 1.22,
 39.3 0.47, 23.5 0.94, 15.6 0.25, 32.9 6.53, 34.5 0.53, 41.2 0.34,
 1.2 0, 72.0, 72.6 1.50, 17.3 0.19, 26.8 0.67, 34.9 0.35, 26.9 0.23,
 10.3 0.04, 52.6 3.46, 63.4 1.73, 26.3 0.26, 36.4 0.39, 51.3 2.02,
 25.0 0.76, 9.55 0.20, 34.6 3.4, 39.9 0.5, 19.2 0.46, 17.2 0.34,
 51.6 0.97, 27.3 0.33, 35.2 0.64, 63.4 2.4, 13.7 0.19, 5.4 0.02,
 33.6 0.54, 52.2 0.53, 25.6 0.41, 5.3 0.03, 11.3 0.10, 45.9 1.01,
 33.9 0.52, 16.9 0.37, 15.0 0.45, 3.5 0.04, 24.5 0.62; CALC4:
 TBS+U VS 20ST VUL
 MEAN X 24.20 S.D. 20.14 S.E.M. 1.613
 MEAN Y .7030 S.D. 1.693 S.E.M. .1355
 156 POINTS. R= .6690
 P= .0000000144 FOR 154 D.F.F
 REGRESSION LINE IS

$$Y = .056X + .652$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.2

AUTO VS MANUAL 15.6 15.5 8.3 8.7 22.5 22.1 8.9 10.7 22.2
 13.0 10.3 7.3 9.3 13.5 14.4 10.1 9.5 8.7 9.3 16.0 15.6
 10.9 12.5 20.9 23.2 8.4 9.7 20.5 21.3 7.7 7.6 18.4 13.3
 12.2 13.9 19.7 20.6 13.3 13.1 13.9 14.1 11.1 11.4 6.6 6.4
 11.4 12.5 12.7 13.3 16.7 15.2 7.5 7.6 12.7 13.2 7.0 6.3
 13.7 13.9; CALC4:

AUTO VS MANUAL

MEAN X 13.38 S.D. 4.874 S.E.M. .5393
 MEAN Y 13.60 S.D. 4.350 S.E.M. .3355
 30 PLINTS. R= .9660
 P= .0000000143 FOR 28 D OF F
 REGRESSION LINE IS

$$Y = .961X + .738$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

COMPARE DIFFERENT LEVEL %VO;L CB 22.2 8.3 10.1 8.9 13.0 15.6
 7.3 12.7 7.5 31.0,
 24.2 6.7 11.3 10.3 14.5 13.9 9.6 13.9 8.1 32.5; CALC3:

COMPARE DIFFERENT LEVEL %VO;L CB

NO IN EACH SAMPLE= 10

SAMPLE 1 MEAN 13.68 S.D. 7.616 S.E.M. 2.408

SAMPLE 2 MEAN 14.50 S.D. 7.976 S.E.M. 2.522

MEAN DIFFERENCE-.8200

T= 1.81 P= .10 FOR 9 D OF F

*** NB. TWO-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.2 (Contd.)

'COMPARE SAME LEVEL %VOL CB' 23.2 15.5 15.2 11.6 7.9 34.1
18.9 20.4 14.1,
24.6 14.8 15.3 10.1 8.1 32.8 18.6 22.5 12.5; CALC3:

COMPARE SAME LEVEL %VOL CB
NO IN EACH SAMPLE= 9
SAMPLE 1 MEAN 17.85 S.D. 7.624 S.E.M. 2.541
SAMPLE 2 MEAN 17.70 S.D. 7.831 S.E.M. 2.610
MEAN DIFFERENCE .1778
T= .415 P= .69 FOR 8 D OF F
*** NB. TWO-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

'COMPARE SAME LEVEL %VOL OST' 0 .08 .17 .74 1.2 1.8 .9 .03
.10,
.05 .07 .13 .90 1.3 1.9 1.3 .02 .06; CALC3:

COMPARE SAME LEVEL %VOL OST
NO IN EACH SAMPLE= 9
SAMPLE 1 MEAN .5578 S.D. .6407 S.E.M. .2136
SAMPLE 2 MEAN .6367 S.D. .7229 S.E.M. .2410
MEAN DIFFERENCE-.0789
T= 1.70 P= .13 FOR 8 D OF F
*** NB. TWO-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

'COMPARE SAME LEVEL TBS+R.S' 22.4 19.6 12.7 22.5 4.9 30.1 28.3
48.8 16.2,
20.8 17.9 14.0 23.8 6.0 28.7 24.6 50.0 17.2; CALC3:

COMPARE SAME LEVEL TBS+R.S
NO IN EACH SAMPLE= 9
SAMPLE 1 MEAN 22.83 S.D. 12.43 S.E.M. 4.143
SAMPLE 2 MEAN 22.56 S.D. 12.23 S.E.M. 4.078
MEAN DIFFERENCE .2778
T= .450 P= .66 FOR 8 D OF F
*** NB. TWO-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

'COMPARE SAME LEVEL TBS+R.SO' 0 0 0 15.8 15.4 9.6 13.1 0.6 0.9,
0 0 0 14.3 16.0 8.5 13.0 1.8 1.7; CALC3:

COMPARE SAME LEVEL TBS+R.SO
NO IN EACH SAMPLE= 9
SAMPLE 1 MEAN 6.156 S.D. 7.165 S.E.M. 2.388
SAMPLE 2 MEAN 6.144 S.D. 6.783 S.E.M. 2.261
MEAN DIFFERENCE .0111
T= .039 P= .97 FOR 8 D OF F
*** NB. TWO-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.3

'AGE CB MALE' 19 24.1, 30 17.8, 28 20.5, 30 23.1, 26 32.6,
 39 23.6, 37 24.9, 34 33.6, 37 19.5, 34 23.1, 45 20.2, 49 17.5,
 42 13.3, 50 18.7, 41 23.6, 46 20.4, 41 23.2, 49 23.2, 31 7.6,
 77 15.5, 55 10.8, 52 12.3, 51 13.3, 51 16.6, 51 13.7, 52 9.4,
 57 19.0, 63 9.7, 65 20.8, 69 14.8, 63 13.7; CALC4:

AGE CB MALE

MEAN X 47.23	S.D. 14.74	S.E.M. 2.648
MEAN Y 19.04	S.D. 6.577	S.E.M. 1.181
31 POINTS.	R=-.6827	

P= .0000117 FOR 29 D OF F
 REGRESSION LINE IS

$$Y = -.305X + 33.4$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

'AGE CB FEMALE' 23 28.1, 29 17.8, 18 15.7, 26 19.9, 40 17.1,
 36 14.3, 50 22.7, 44 14.6, 59 8.8, 59 10.2, 56 14.6, 71 9.5,
 79 16.4, 89 10.7, 67 17.2, 67 9.2, 74 15.6, 86 12.5, 72 8.1; CALC4:

AGE CB FEMALE

MEAN X 55.00	S.D. 21.81	S.E.M. 5.004
MEAN Y 14.89	S.D. 5.127	S.E.M. 1.176
19 POINTS.	R=-.5332	

P= .00404 FOR 17 D OF F
 REGRESSION LINE IS

$$Y = -.138X + 22.5$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.3(Contd.)

177

'CB MF<50' 28.1 17.3 15.7 19.9 17.1 14.3 22.7 14.6 24.1 17.3
 20.5 28.1 32.6 28.6 24.9 33.6 19.5 23.1 20.2 17.5 13.3 13.7
 23.6 20.4 23.2 23.2; CALC1:

CB MF<50
 NO IN SAMPLE 26 MEAN 21.66
 S.D. 5.368 S.E.M. 1.053

'CB MF>50' 8.8 10.2 14.6 9.5 16.4 10.7 17.2 9.2 15.6 12.5
 8.1 7.6 15.5 10.8 12.3 13.3 16.6 13.7 9.4 19.0 9.7
 20.8 14.8 13.7; CALC1:

CB MF>50
 NO IN SAMPLE 24 MEAN 12.92
 S.D. 3.604 S.E.M. .7357

'CB MALE 50 BELOW & ABOVE' 24.1 17.8 20.5 28.1 32.6 28.6 24.9
 33.6 19.5 23.1 20.2 17.5 13.3 18.7 23.6 20.4 23.2 23.2
 7.6 15.5 10.8 12.3 13.3 16.6 13.7 9.4 19.0 9.7 20.8
 14.8 13.7; CALC2:

CB MALE 50 BELOW & ABOVE
 SAMPLE 1 N= 18 MEAN= 22.94 S.D. 5.258 S.E.M. 1.239
 SAMPLE 2 N= 13 MEAN= 13.63 S.D. 3.803 S.E.M. 1.055
 DIFFERENCE BETWEEN MEANS= 9.308
 T= 5.43 P= .000 FOR 29 D OF F
 *** NB. TWO-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

'CB FEMALE 50 BELOW&ABOVE' 23.1 17.8 15.7 19.9 17.1 14.3
 22.7 14.6
 8.8 10.2 14.6 9.5 16.4 10.7 17.2 9.2 15.6 12.5
 8.1; CALC2:

CB FEMALE 50 BELOW&ABOVE
 SAMPLE 1 N= 8 MEAN= 18.77 S.D. 4.695 S.E.M. 1.660
 SAMPLE 2 N= 11 MEAN= 12.07 S.D. 3.328 S.E.M. 1.004
 DIFFERENCE BETWEEN MEANS= 6.702
 T= 3.65 P= .002 FOR 17 D OF F
 *** NB. TWO-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

JUST MALE 50 BELOW & ABOVE 11.3 26.3 3.8 18.2 0 2.3 24.3
 22.2 5.1 12.2 17.9 2.8 6.4 5.7 20.0 8.0 0,
 20.0 6.8 16.6 2.1 10.6 14.0 6.1 22.2 15.1 21.2
 7.7; CALC2:

JUST MALE 50 BELOW & ABOVE

SAMPLE 1	N= 17	MEAN= 10.97	S.D. 6.833	S.E.M. 2.142
SAMPLE 2	N= 11	MEAN= 12.95	S.D. 6.767	S.E.M. 2.040

DIFFERENCE BETWEEN MEANS= 1.975

T= .630

P= .534 FOR 26 D OF F

*** NB. TWO-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

JUST FEMALE 50 BELOW & ABOVE 6.3 5.1 13.5 7.3 1.8 4.8 1.5 2.7,
 6.9 9.8 28.2 16.3 18.8 30.3 6.5 17.6 10.6 16.0; CALC2:

JUST FEMALE 50 BELOW & ABOVE

SAMPLE 1	N= 8	MEAN= 5.375	S.D. 3.690	S.E.M. 1.375
SAMPLE 2	N= 10	MEAN= 16.10	S.D. 8.195	S.E.M. 2.591

DIFFERENCE BETWEEN MEANS= 10.73

T= 3.39

P= .004 FOR 16 D OF F

*** NB. TWO-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

TBS JUST M F 6.3 5.1 13.5 7.3 1.8 4.8 1.5 2.7 11.3 26.3 3.8
 13.2 0 2.3 24.3 22.2 5.1 12.2 17.9 2.8 6.4 5.7 20.0
 8.0 0; CALC1:

TBS JUST M F

NO IN SAMPLE 25
 S.D. 7.970

MEAN 9.180
 S.E.M. 1.594

TBS JUST M F 50 6.9 9.8 28.2 16.3 18.8 30.3 6.5 17.6 10.6
 16.0 20.0 6.8 16.6 2.1 10.6 14.0 6.1 22.2 15.1 21.2 7.7; CALC1:

TBS JUST M F 50

NO IN SAMPLE 21
 S.D. 7.465

MEAN 14.45
 S.E.M. 1.629

6.5

TBS RS MF 16.7 12.3 11.5 7.3 10.7 9.5 8.9 12.2 11.8 12.3
 21.2 6.8 8.8 14.3 10.3 7.1 15.6 7.3 15.3 10.7 3.5 17.0 6.0
 14.7 22.4; CALC1:

TBS RS MF
 NO IN SAMPLE 25 MEAN 12.03
 S.D. 4.341 S.E.M. .8682

TBS RS M F 50 17.6 8.5 16.2 12.1 8.8 17.4 14.6 14.7 10.9 19.1
 12.0 6.7 19.6 12.2 18.4 23.4 21.1 6.1 18.3 14.1 16.9 15.1
 16.5 19.2; CALC1:

TBS RS M F 50
 NO IN SAMPLE 24 MEAN 14.98
 S.D. 4.557 S.E.M. .9301

6.6

TBS+U VSCXP 73.8 29.5, 14.5 43.1, 43.1 34.6, 88.6 24.0,
 5.5 49.9, 12.6 41.4, 14.5 46.0, 87.8 20.2, 88.3 19.2,
 30.7 20.7, 55.8 42.1, 66.9 26.5; CALC4:

TBS+U VSCXP
 MEAN X 48.51 S.D. 32.42 S.E.M. 9.360
 MEAN Y 33.10 S.D. 11.09 S.E.M. 3.202
 12 POINTS. R=-.7983
 P= .000927 FOR 10 D OF F
 REGRESSION LINE IS

$$Y = -.273X + 46.3$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.7

'TBS + U VS ALK PHQS' 3.9 8, 4.4 3, 9.6 9, 19.1 8, 18.6 6,
 31.2 9, 23.3 12, 7.8 12, 17.1 8, 28.5 10, 73.8 16, 14.5 16,
 43.1 26, 88.6 23, 5.5 17, 12.6 4, 14.5 8, 55.8 19, 87.8 18,
 88.3 21, 30.7 20, 17.2 9, 16.1 15, 27.6 9, 12.7 9, 6.3 8,
 4.9 5, 3.5 9; CALC4:

TBS + U VS ALK PHQS

MEAN X 27.39

S.D. 26.80

S.E.M. 5.064

MEAN Y 12.04

S.D. 6.028

S.E.M. 1.139

28 POINTS.

R= .6982

P= .0000181 FOR 26 D OF F

REGRESSION LINE IS

$$Y = .157X + 7.73$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.8

'TBS+U VS SR SPACE' 3.9 15.8, 4.4 14.1, 18.6 16.7, 31.2 15.5,
 7.8 18.6, 17.1 8.1, 73.8 25.5, 14.5 17.3, 43.1 49.4, 88.6 23.0,
 5.5 19.3, 12.6 12.4, 55.8 27.0, 88.3 13.3, 30.7 14.6,
 66.9 55.5, 45.1 28.6, 16.1 18.1, 27.6 21.6, 12.7 9.5, 4.9 14.5,
 53.1 67.4, 40.3 17.7, 50.0 36.7, 77.2 22.0, 6.8 14.7; CALC4:

T

TBS+U VS SR SPACE

MEAN X 34.48

S.D. 27.47

S.E.M. 5.387

MEAN Y 22.96

S.D. 14.33

S.E.M. 2.811

26 POINTS.

R= .4386

P= .0125 FOR 24 D OF F

REGRESSION LINE IS

$$Y = .229X + 15.1$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.9

D/100-C X 100. %NON-OSTEIOD SUR COVERED BY RES IN NORMAL 17.8
 13.5 13.3 7.9 10.8 9.9 9.0 12.5 13.3 16.7 22.0 8.3 8.3 23.7 10.5
 9.4 20.1 8.2 17.9 13.0 8.7 18.2 7.5 15.9 22.4; CALC1:

D/100-C X 100. %NON-OSTEIOD SUR COVERED BY RES IN NORMAL
 NO IN SAMPLE 25 MEAN 13.57
 S.D. 5.042 S.E.M. 1.008

RS (D/100-CX 100) VS PEI 33.3 .26, 46.7 .22, 28.9 .17,
 27.2 .16, 11.1 .04, 18.2 .024, 13.6 -.003, 17.1 -.013,
 13.8 -.016, 20.9 -.02, 8.2 -.04, 14.5 -.07, 57.3 .36,
 18.5 .07, 43.8 .06; CALC4:

RS (D/100-CX 100) VS PEI
 MEAN X 24.87 S.D. 14.55 S.E.M. 3.758
 MEAN Y .0801 S.D. .1259 S.E.M. .0325
 15 POINTS. R= .8378
 P= .0000482 FOR 13 D OF F
 REGRESSION LINE IS

$$Y = .007X + -.100$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

RS&RSU VS PEI 11.9 -.003, 7.7 -.04, 19.7 .16, 31.2 .22,
 9.2 .04, 8.2 -.02, 24.4 .17, 16.5 -.013, 12.9 -.016,
 17.3 .024, 39.4 .262, 12.7 -.07; CALC4:

RS&RSU VS PEI
 MEAN X 17.59 S.D. 9.759 S.E.M. 2.817
 MEAN Y .0595 S.D. .1122 S.E.M. .0324
 12 POINTS. R= .9040
 P= .0000273 FOR 10 D OF F
 REGRESSION LINE IS

$$Y = .010X + -.123$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.10

MT VS CB 256 24.7, 225 19.5, 313 23.5, 302 25.2, 23 17.3,
 77 20.5, 251 19.9, 561 34.1, 237 14.9, 334 12.7, 60 20.1, 116 22.7,
 212 12.3, 125 17.9, 263 30.2, 175 11.3, 403 31.7, 309 20.3,
 307 35.9, 273 31.2, 228 20.3, 233 22.3, 507 13.9, 334 17.3,
 277 20.4, 123 15.1, 257 16.5, 293 14.2, 322 26.3, 232 27.5; CALC4:

MT VS CB

MEAN X 258.4 S.D. 120.1 S.E.M. 21.92
 MEAN Y 21.72 S.D. 6.560 S.E.M. 1.201
 30 POINTS. R= .3629
 P= .0244 FOR 28 D OF F
 REGRESSION LINE IS

$$Y = .020X + 16.6$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.11

RS VS PARATH WT 44.2 283, 24.7 250, 41.3 259, 34.7 210,
 15.2 86, 28.3 120, 40.2 976, 29.2 461, 64.7 11200; CALC11:

RS VS PARATH WT

MEAN X 35.83 S.D. 14.16 S.E.M. 4.720
 MEAN Y 2.589 S.D. .6269 S.E.M. .2090
 9 POINTS. R= .8600
 P= .00147 FOR 7 D OF F
 REGRESSION LINE IS

$$Y = .038X + 1.22$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

6.12

Mean Calculated bone thickness M.C.B.T. (External) Control group

'E8' 585 668 897 739 594 752 1049 954 827 1159 376 1179 785

580 1539 870 448 990; CALC1:

E8

 NJ IN SAMPLE 18
 S.D. 294.4

 MEAN 835.6
 S.E.M. 69.39

M.C.B.T (External) Before Hemodialysis

'E9' 356 550 434 264 616 292 476 475 685 1018 539 534 451 285

473; CALC 1:

E9

 NJ IN SAMPLE 15
 S.D. 187.4

 MEAN 499.9
 S.E.M. 43.39
M.C.B.T. During Hemodialysis $\leq$ 6 months

'E10' 274 237 474 93 600 474 246 330 345 772; CALC1:

E10

 NJ IN SAMPLE 10
 S.D. 197.8

 MEAN 339.5
 S.E.M. 62.54
M.C.B.T. During Hemodialysis $>$ 6 months

'E11' 479 280 455 397 246 314 1226 433 537; CALC1:

E11

 NJ IN SAMPLE 9
 S.D. 295.6

 MEAN 490.8
 S.E.M. 98.53

6.13

184

'CB NJ HD MF<50' 12.2 23.7 17.5 32.1 29.8 21.7 26.1 17.3

22.9 24.9 22.2 24.1 17.3 15.1 12.9 18.3 13.9 14.6 20.6

14.2; CALC1:

'CB NJ HD MF<50'

NJ IN SAMPLE 20

S.D. 5.683

MEAN 20.05

S.E.M. 1.271

'CB HD MF<50 <6' 34.1 24.9 14.9 12.7 20.1 16.3 12.3 17.9

28.7 18.9; CALC1:

CB HD MF<50 <6

NJ IN SAMPLE 10

S.D. 7.121

MEAN 20.83

S.E.M. 2.252

CB HD MF <50 >6' 30.2 11.8 31.7 24.2 17.2 35.9 20.3 22.3

6.3 17.3; CALC1:

CB HD MF <50 >6

NJ IN SAMPLE 10

S.D. 7.443

MEAN 23.77

S.E.M. 2.355

'CB POST T MF <50 <6' 25.3 22.9 22.1 19.5 28.5 25.2 14.4

20.5; CALC1:

CB POST T MF <50 <6

NJ IN SAMPLE 8

S.D. 4.305

MEAN 22.30

S.E.M. 1.522

P

'ABOVE RPT' 20.1 28.0 16.5 31.0 21.1 10.1 15.1 19.7

16.8 27.5 20.4 14.1 12.5 14.2 20.2; CALC1:

ABOVE RPT

NJ IN SAMPLE 15

S.D. 5.933

MEAN 19.15

S.E.M. 1.545

OST NO HD MF<50 34.0 37.9 2.5 41.2 36.8 47.9 48.5 7.9

30.6 18.9 41.3 22.5 53.1 5.2 6.8 8.0 4.0 31.5 3.5 18.9; CALC1:

%total bone surface covered with osteoid (T.B.S.+0) Before hemodialysis

OST NO HD MF<50

NO IN SAMPLE 20 MEAN 25.05
S.D. 17.24 S.E.M. 3.856

OST HD MF <50 <6 50.0 21.9 40.0 39.8 23.5 15.6 34.5

41.2 1.2 36.4; CALC1:

%TBS+0 ≤ 6months During Hemodialysis

OST HD MF <50 <6

NO IN SAMPLE 10 MEAN 30.41
S.D. 14.63 S.E.M. 4.626

OST HD MF <50 >6 72.6 17.8 26.8 34.9 26.9 52.6 63.4 26.3

51.3 25.0; CALC1:

% TBS+0 > 6months During Hemodialysis

OST HD MF <50 >6

NO IN SAMPLE 10 MEAN 39.76
S.D. 18.73 S.E.M. 5.933

OST POST T MF<50 <6 42.9 29.5 64.6 25.5 40.9 27.3 35.2 68.4; CALC1:

% TBS+0 ≤ 6months After kidney transplant

OST POST T MF<50 <6

NO IN SAMPLE 8 MEAN 41.79
S.D. 16.48 S.E.M. 5.825

OST POST T MF <50 1 YR 9.6 34.6 39.9 19.2 17.2 51.6 5.4

33.6 52.2 25.6 5.8 11.3 45.9 33.9 16.9; CALC1:

%TBS+0 > 1yr. After kidney transplant

OST POST T MF <50 1 YR

NO IN SAMPLE 15 MEAN 26.85
S.D. 16.12 S.E.M. 4.162

6.15

(R.S.₀) Before Hemodialysis

'E15' 3.0 14.3 0 14.2 11.3 14.3 18.7 5.8 2.9 9.0 7.2 13.3 8.7
22.5 7.0 1.0 0 1.2 0 6.3 0 4.0 6.8 24.8 10.3 5.4; CALC1:

E15

NO IN SAMPLE 26 MEAN 8.192
S.D. 6.961 S.E.M. 1.365

(R.S.₀) During Hemodialysis ≤ 6 months

'E17' 9.6 3.1 6.7 3.2 5.4 4.1 11.8 23.8 17.6 113.1; CALC1:

E17

NO IN SAMPLE 10 MEAN 9.840
S.D. 6.845 S.E.M. 2.165

(R.S.₀) During Hemodialysis > 6 months

'E18' 11.3 6.8 11.3 16.5 6.9 2.2 16.5 14.4 2.5 10.0 2.1; CALC1:

E18

NO IN SAMPLE 11 MEAN 9.136
S.D. 5.462 S.E.M. 1.647

(R.S.₀) Post kidney transplant ≤ 6 months

'E19' 13.0 5.3 10.9 13.4 1.1 3.3 3.6 1.2 16.5; CALC1:

E19

NO IN SAMPLE 9 MEAN 8.200
S.D. 5.630 S.E.M. 1.877

(R.S.₀) Post kidney transplant ≥ 1 year

'E20' 1.9 11.5 1.5 0 0 21.9 0.7 0 2.3 7.4 1.2 0.6 0.9 10.1 3.9
1.9; CALC1:

E20

NO IN SAMPLE 16 MEAN 4.112
S.D. 5.950 S.E.M. 1.488

6.16

E40\ 11 34.0, 16 2.5, 11 48.5, 12 7.9, 14 17.2, 20 30.6, 12 18.9,
 6 41.3, 15 22.5, 50 53.1, 6 40.3, 12 5.2, 8 6.8, 13 8.0, 19 31.5,
 9 13.0, 11 19.5, 11 48.3, 11 18.9; CALC4:

E40

MEAN X 14.05 S.D. 9.454 S.E.M. 2.169
 MEAN Y 24.63 S.D. 16.10 S.E.M. 3.693
 19 POINTS. R= .3349
 P= .0305 FOR 17 D OF F
 REGRESSION LINE IS

$$Y = .570X + 16.6$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

E41\ 25 50.0, 20 21.9, 8 40.0, 9 39.8, 11 23.5, 10 15.6, 16 82.9,
 11 34.5, 9 41.2, 7 1.2, 8 72.6, 7 17.8, 6 26.8, 42 34.9, 12 26.9,
 27 10.8, 77 52.6, 13 63.4, 14 26.3, 12 36.4, 26 51.3, 10 25.0; CALC4:

E41

MEAN X 17.27 S.D. 15.94 S.E.M. 3.398
 MEAN Y 36.15 S.D. 19.93 S.E.M. 4.261
 22 POINTS. R= .1998
 P= .19 FOR 20 D OF F
 REGRESSION LINE IS

$$Y = .251X + 31.3$$

*** NB. ONE-TAIL TEST OF SIGNIFICANCE HAS BEEN USED.

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