AGE CHANGES IN CANCELLOUS BONE

by

Kenneth John Holley

A thesis submitted for the degree
of Doctor of Medicine in the
University of London.

Institute of Orthopaedics
December 1968.

ABSTRACT

This thesis records a quantitative morphological study of cancellous bone. Changes in amount of bone, extent of surface, and extent of bone formation and resorption with aging were studied, one object being to clarify the understanding and facilitate the recognition of osteoporosis (reduction in the amount of bone present).

The literature relating to these problems is reviewed.

Iliac crest bone was studied in 93 normal subjects, aged up to 93 years, using perspex embedded, undecalcified, ground sections. Measurements were made by point counting and line sampling techniques.

The reproducibility of these methods and the biological variation in parameters measured was studied within a section, a block, and the length of the iliac crest.

After a rise from childhood, the main study shows a progressive reduction with age in the amount of bone present, starting earlier than is generally realised. No differences are apparent between males and females.

This process is universal; there is no evidence of a pathological group of individuals with less bone.

The results do not support commonly accepted theories of postmenopausal and senile osteoporosis. It is suggested that postmenopausal and senile osteoporosis

is a physiological manifestation of aging, rather than a pathological process seen in old age.

Bone surface area also falls with age; if related to the amount of bone present, lowest values are seen in early adult life.

The percentage of surface occupied by formation and resorption is higher than is obvious from routine decalcified sections, and varies with age, highest values being seen in childhood and old age. Additional information is derived from these figures by relating them to the available surface area.

It is concluded that these changes from childhood to adulthood represent a reduced bone turnover rate, and in old age probably compensate for a reduced rate at which bone is laid down at any site.

CONTENTS

FIGURES

TABLES

SECTION ONE

INTRODUCTION

INTRODUCTION

Bone is a living and active tissue, and undergoes many changes throughout life. It has been known for many years that bone mass is diminished in old age, and that bone formation and resorption are continuous processes, bone tissue being continually turned over and remodelled throughout life. Sir Astley Cooper wrote in 1823 (Cooper 1823) of the bone becoming thin and spongy in old age, while towards the end of the eighteenth century John Hunter (Hunter 1837) postulated the concept of a continuous turnover and remodelling of bone by the removal of bone substance, and the laying down of new bone.

Until quite recently, however, our knowledge of the extent, magnitude, and timing of these processes was little more advanced than when they were first described, the technical limitations of the available methods of study of calcified tissues having retarded progress. Recent technical progress has meant that more methods are available for the study of bone aging, and a number of published results are now available.

However, not all the published results are in agreement with each other, while in particular much of the histological study of bone aging has not been detailed, and few results have been expressed in quantitative terms. Urist (1959) stressed that more information is needed about the normal progress of skeletal aging.

The lack of results is particularly apparent in relation to cancellous bone. Again largely because of difficulties inherent in processing and studying a complex tissue like cancellous bone (Bauer 1962, 1964), previous workers have almost entirely confined their studies to cortical bone, or cortical and cancellous bone together, and the study of cancellous bone has been neglected (Hall 1965).

This neglect is unfortunate since cancellous bone may well show greater metabolic activity than cortical bone. Cancellous bone has long been regarded as possessing a more rapid rate of turnover than cortical bone (Bauer, Aub & Albright, 1929). Cancellous bone presents a proportionately much greater surface area than cortical bone, and it is thus probable that cancellous bone is more likely to take part in metabolic changes than cortical bone. Amprino & Engström (1952) wrote "the trabecular bone should be more sensitive than the compacta to physiological stimuli which control liberation and fixation of minerals". Cancellous bone might therefore be expected to be more labile than cortical bone, and might show greater changes, and at an earlier stage, in response to physiological or pathological stimuli than cortical bone. Thus the study of cancellous bone might be more profitable from a morphological aspect than cortical bone.

The aim of this investigation has been to study the

morphology and bone forming and resorbing activity of normal human cancellous bone (as exemplified by iliac crest cancellous bone) at various ages throughout life, results being expressed in quantitative terms.

Iliac crest was chosen for this study because it provides large areas of cancellous bone. It is, in addition, a standard bone biopsy site (Ball 1963) and results obtained from such a study will have practical importance in relation to the interpretation of diagnostic bone biopsies.

The features studied were:-

1. The amount of bone present.
2. The total amount of surface area available for bone formation and resorption.
3. The extent of bone formation and resorption.
4. The degree of variation of these quantities within a small area of bone, in order to assess the reliability of a single diagnostic biopsy.
5. The variations in the parameters with age.

Such a study, it was hoped, would prove of value not only in connection with normal bone morphology and the changes of aging, but would also, by establishing the limits of normality, contribute to the understanding of metabolic bone disease, in particular senile osteoporosis. This is already a very common bone disease (Smith, Tyler & Hellinger 1960); its incidence and importance will increase in the future as the average

age of the population increases. Moon & Urist (1962) calculated that by 1980 all the hospital beds now in existence in the United States could be occupied by cases of senile osteoporosis.

From a practical standpoint it was hoped that the studies might facilitate the histological recognition of such metabolic bone diseases, and that the limits of normality might be established to act as a base line for diagnostic biopsy studies.

SECTION TWO

REVIEW OF LITERATURE

REVIEW OF LITERATURE

This section is in several parts.

An introductory review of bone as a tissue is followed by a review of previous work on the problems of quantitative changes in bone with age, and quantitation of bone formation and resorption. In this section emphasis is laid upon the approach of other workers to the problems, and the methods employed.

Full consideration of the results obtained will be dealt with later.

BONE AS A TISSUE

Bone is a specialised form of connective tissue. It consists of branching cells, termed osteocytes, forming a syncytium lying in lacunae within an organic intercellular matrix, composed of bundles of collagen fibres, bound together by an amorphous cementing ground substance, mucopolysaccharide in nature, thought to be predominantly chondroitin sulphates A and C (Meyer, Davidson, Linker & Hoffman, 1956). The intercellular matrix is calcified, giving bone its rigidity. The major calcium salt of bone is generally agreed to be in the form of hydroxyapatite, a crystal lattice structure with the general formula of 3 $Ca(PO_4)_2$. $Ca(OH)_2$ (Neuman & Neuman, 1953).

Early work with the electron microscope suggested that the bone salt was deposited in the ground substance, orientated along the collagen fibres (Robinson, 1952), but from more recent studies it is believed that some, if not most, of the mineral is deposited within the collagen fibres (Glimcher, 1959) which are composed of a three stranded helix, with a cylindrical hollow in the centre of the helix (Glimcher, 1960).

Bone differs from most connective tissues by its calcification, and from other calcified tissues (e.g., calcified cartilage) by the organised nature of the formed intercellular elements, and by the manner in

which the mineral element is related to them.

The calcification and consequent rigidity of bone confer its distinctive functions. The mechanical function of bone, that of providing support, protection of organs, weight bearing, and transmission of the forces of muscular contraction is obvious, and contributed to the development of the view that bone is a stable and immutable tissue and of little physiological interest.

Only more recently was it realised that bone, containing as it does 99% of the calcium of the body (Cooke, 1955) acts as a storehouse or reservoir for this and other minerals, and is an important part of the homeostatic mechanism for controlling the level of ionised calcium in blood and tissue fluids where calcium is of importance in the maintenance of neuromuscular activity and blood coagulation.

Urist (1962) defined the physiological function of the skeleton, as distinguished from the mechanical function, as that of storage and turnover, and it is now realised that bone has a high metabolic turnover, and is a most active and plastic tissue. Indeed it has been remarked (Le Gros Clark, 1945) that, next to blood, bone is the most plastic tissue in the body.

BONE STRUCTURE

a) Fibre Pattern. In the foetus and infant and in the adult under pathological conditions, such as

fracture healing, bone may be laid down, the collagen fibres lacking orientation, being arranged in a feltwork or woven fashion. In the adult under normal conditions bone is laid down in lamellae or sheets. In each lamella the collagen fibres are parallel to each other, but their orientation differs from that in adjacent lamellae. This study is concerned almost entirely with lamellar bone.

b) Spatial Arrangement. At the gross anatomical level bone may be arranged in two forms - compact or cancellous (Pritchard, 1956a). Compact (or cortical) bone consists mainly of lamellae of bone arranged in cylindrical systems known as Haversian systems or osteons. Each osteon consists of concentric lamellae arranged around a vascular space, known as a Haversian canal. These are small and unrecognisable macroscopically; indeed compact bone appears solid to the naked eye, and only under the microscope can its slightly porous nature be recognised.

Cancellous (or spongy bone) is on the other hand a far more porous structure. It consists of thin plates, struts and bars of bone, which interconnect to form a light spongy honeycomb or lattice like structure. This arrangement is not apparent however in thin sections, and here the pattern seen is the more familiar one of a network of thin trabeculae or rods of bone (Figures 8 & 9).

Compact and cancellous bone are not two fundamentally different types of bone : they do however represent two different spatial arrangements of lamellar bone, and by virtue of their different arrangements presumably subserve different functions.

Compact bone is thought to play the major part in weight bearing. Hirsch & Brodetti (1956) showed that in the femoral neck 70% of the total weight bearing capacity lay in the cortical layers of compact bone. Cancellous bone does play some part in weight bearing, the remaining 30% of the total weight bearing capacity being due to cancellous bone.

Cancellous bone, on the other hand, due to its more porous nature presents a relatively greater area of surface than cortical bone, and is thus likely to play the major role in metabolic activity of bone, and particularly in the storage and turnover of calcium and other minerals.

SITES OF CANCELLOUS AND COMPACT BONE

Compact bone forms the shafts of long bones, and at the ends of long bones forms a peripheral shell, filled with a lattice of cancellous bone. In iliac crest, cancellous bone represents the bulk of bony tissue, and is bounded by a relatively thin shell of compact bone. As Pritchard (1956a) pointed out, morphologically there is no absolute distinction between compact and

cancellous bone, and any separation of the two must be to some extent arbitrary. However in transverse sections of iliac crest a clear distinction may be drawn between the central fine network of spongy bone, and the peripheral limiting shell of compact bone.

FUNCTIONS OF ILIAC CREST CANCELLOUS BONE

Using "stress-coat" studies developed by de Forest and Ellis (1940) in which a bone is coated with a strain sensitive lacquer, and then subjected to a deforming force, Evans & Lissner (1955) showed that the iliac crest is concerned with weight bearing, being subjected to tensile strain. Evans & King (1961) suggested that the cancellous bone may function as an energy absorbing material, while probably the most important mechanical function of iliac crest cancellous bone lies in resisting forces developed by contraction of those muscles attached to the iliac crest - the external and internal oblique and transverse muscles of the abdomen, the iliacus and glutei. The cancellous bone will be concerned in calcium homeostasis, and metabolic exchanges.

Iliac crest cancellous bone is subjected to many influences, and is likely to respond to many physiological and pathological changes. Any change in the bone may not easily be assigned to one particular cause.

BONE FORMATION AND RESORPTION

Bone Formation

Bone formation is usually described as being either endochondral or intramembranous; as Ham (1965) points out, this distinction refers only to the particular environment in which the bone forms. The actual process of bone tissue formation is identical in either case. As bone is a rigid substance, bone formation can occur only at bone surfaces, where it is the result of cellular activity.

Three morphologically distinct types of bone cell were recognised and described by the 19th century German histologists Gegenbauer (1864, 1867) and Kölliker (1873, 1889). Each cell type is confined to specific sites in bone; this difference in localization suggests that each type has a special function. The branching osteocytes, situated within bone substance and thought to be concerned in its maintenance have been described above. In areas of active bone formation, such as fracture callus and epiphyses, the bone surfaces are consistently lined by a single layer of plump mononuclear cells known as osteoblasts (Figures 1 & 2), which because of their site are thought to be responsible for bone formation.

These cells were first noted by Goodsir (1845) who described "on the surface of young and vigorous bone... numerous cells... more or less turgid" and

Figure 1. Bone formation. The upper surface of the lower trabecula is covered by a single layer of osteoblasts. At the upper end of the trabecula tangential sectioning of the layer of osteoblasts gives an impression of multilayering. Decalcified section. Haematoxylin and eosin (× 160).

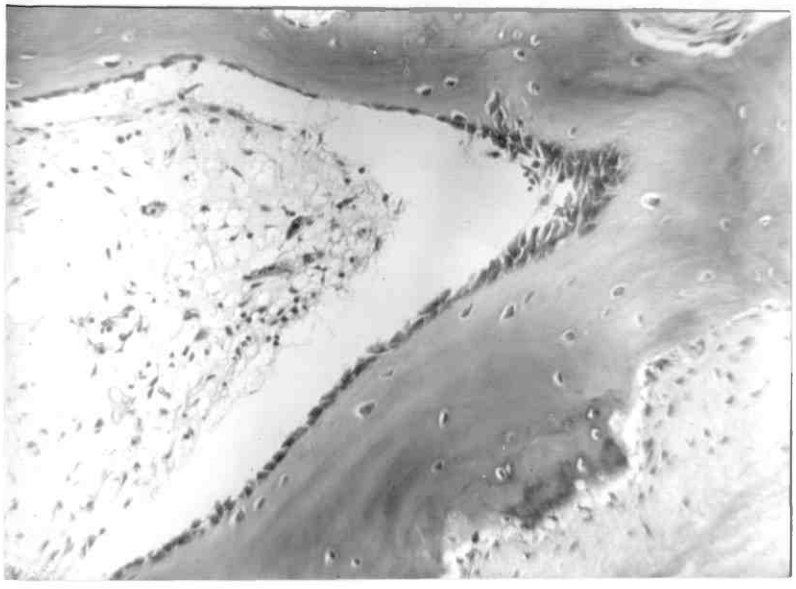
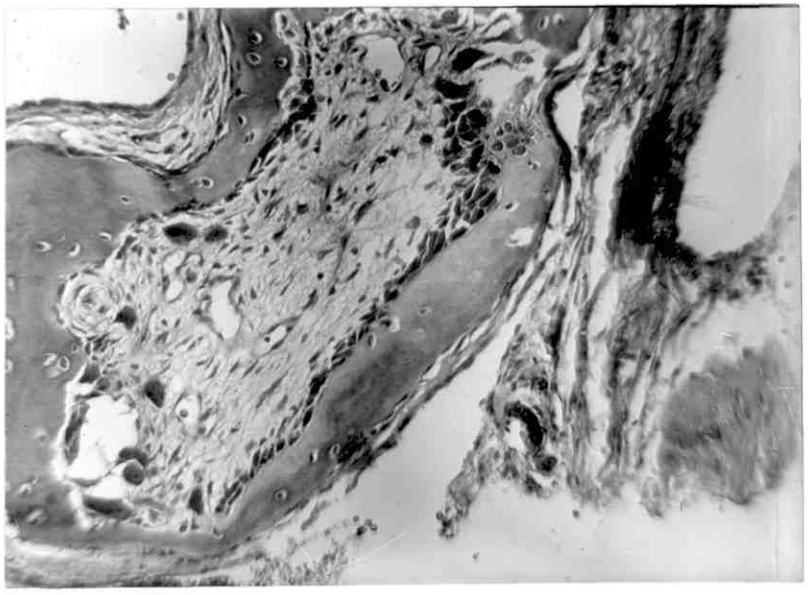


Figure 2. Bone formation. Plump mononuclear osteoblasts line the upper surface of the central trabecula. The larger cells at either end of the trabecula are osteoclasts. Decalcified section. Haematoxylin and eosin (x 160).



wrote "that the cellular layer plays an important part in the economy of bone appears probable from the prominent position it holds in its development". In 1853 Tomes and De Morgan noted similar cells on the surface of developing bones, but, not realizing their significance, merely termed them "osteal cells". Gegenbauer studied the cells in detail, and considering that they were responsible for bone formation named them osteoblasts. Their morphology was recently reviewed by Pritchard (1956b).

In areas where bone formation is not occurring, the surfaces may be lined by a thin inconspicuous layer of flattened cells which may be regarded as resting or inactive osteoblasts (Pritchard 1956b). In areas of bone formation, however, the cells are larger, rounded or cuboidal, their nuclei become plumper and more crowded together, and the cytoplasm becomes more abundant (Baker 1959).

Most workers accepted without reserve the concept that, because of their location, osteoblasts were actively concerned in bone formation. Keith (1919) wrote "osteoblasts seem to conduct the work of bone building as if they had been given the training of expert and unerring engineers". Others, however, believed that bone formation was a humoral process controlled by chemical changes in body fluids, and that the association of osteoblasts with growing bone was fortuitous and

unconnected. Leriche and Policard (1926) in reviewing this concept expressed the view that osteoblasts represented no more than reactionary forms of the ordinary connective tissue cells, that they played no part whatsoever in the formation of bone matrix, but instead tended to oppose the deposition of matrix.

Suggestive evidence in favour of the view that the osteoblasts are concerned in the elaboration of the protein matrix of bone is given by the observation that the cytoplasm of osteoblasts is strongly basophilic (Pritchard 1956b). Such basophilia is seen in other cells, such as plasma cells, which are engaged in the elaboration of proteins. The degree of basophilia varies with the activity of the osteoblast. Pritchard (1952) showed that in developing bone of rat embryo the basophilia reaches a maximum as deposition of bone begins, and then decreases as the osteoblasts become quiescent.

Follis (1951) by demonstrating that the cytoplasm of osteoblasts stained red with methyl green - pyronin (the reaction being abolished by preliminary treatment with ribonuclease) proved that osteoblasts contained ribonucleic acid, while Pritchard (1952) showed that the ribonucleic acid content was responsible for the basophilia of the osteoblasts by demonstrating that the basophilia disappeared after ribonuclease digestion.

In other cells a high ribonucleic acid content of the cytoplasm is associated with a high rate of new protein formation (Caspersson 1947). It is thus likely that osteoblasts have a high rate of new protein formation, and this strongly suggests that they are concerned with elaboration of bone matrix.

The problem was settled by the work of Carneiro and Leblond (1959) following that of Neuberger and Slack (1953), who showed that radioactive labelled glycine is incorporated into the collagen of bone matrix. Carneiro and Leblond administered tritium-labelled glycine, and showed by radioautographs that the glycine was taken up by osteoblasts and appeared later in the bone matrix deep to the osteoblasts. It was absent from the rest of the bone tissue.

It is thus clear that osteoblasts are directly responsible for the elaboration of bone matrix. The actual mechanism, however, by which the matrix is elaborated has not been definitely determined. Cameron (1963) in reviewing recent experimental work on this problem felt that the evidence pointed to a soluble collagen precursor being formed by osteoblasts, transported across the cell membrane, and once outside the cell being aggregated into collagen fibrils.

Although osteoblasts are directly responsible for the elaboration of bone matrix they appear to play no

direct role in calcification; although an indirect role cannot be excluded. Electron microscopy has not shown any evidence of crystals of hydroxyapatite within osteoblasts (Cameron, 1963). Radioautographic studies after Ca^{45} injection into young rats showed the entry of this ion into the superficial layers of bone, dentine and enamel during growth, but not into the matrix forming cells themselves (Kumamoto and Leblond 1956, Carneiro and Leblond 1959).

Bone Formation - Osteoid Tissue

During the process of bone deposition, the organic matrix is laid down before the mineral crystallites, and thus in areas of bone formation a layer of as yet uncalcified matrix is present between the osteoblasts and the calcified bone. This layer of matrix prior to calcification is known as osteoid or osteoid tissue, and a discrete layer of osteoid is termed an osteoid seam (Frost 1963).

In suitably processed specimens of bone, stained with haematoxylin and eosin, osteoid may be recognised as a narrow border of pale staining eosinophilic material on the surface of the haematoxyphilic blue staining calcified bone (Baker 1959). In specimens of bone processed by routine methods, however, all distinction between osteoid and calcified bone is frequently lost, particularly if acid decalcification

is prolonged (Ball 1963).

The fact that bone matrix is deposited before bone mineral has been known for many years. Tomes and De Morgan in 1853 noted that bone was not calcified when deposited, while at the same time Virchow (1851, 1853, 1860), as a result of experiments on crustacean shells and human bone, suggested that bone formation was a two stage process, the deposition of a fibrous matrix being followed by calcification : Virchow applied the name "osteoid" to this matrix tissue. Pommer (1885) studied the process of bone formation thoroughly, using partly decalcified sections. His findings confirmed those of the earlier workers, and demonstrated that osteoid tissue was seen in adults, and was not present only in growing individuals.

Osteoid Tissue - Physiological or Pathological ?

The presence of osteoid tissue implies that calcification follows at an interval after matrix formation. If calcification follows very shortly after matrix formation, or if the two processes are synchronous, osteoid will not be seen. It is natural, therefore, that certain workers should question whether osteoid was always seen in the physiological state or whether the presence of osteoid denoted a pathological state, the more so because much of the earliest work on osteoid was performed using rachitic bone.

Wieland (1909) studied undecalcified celloidin sections of normal and abnormal bone from human embryos and children. He concluded that osteoid was consistently present and coined the term "physiologische osteoid" to imply its normality. Erdheim (1914) studied growing and adult rats, both normal and rachitic, concluding that osteoid was present in normal animals (during growth and in adult life) as well as in rachitic animals.

Other workers, however, did not agree with these findings. Weidenreich (1923a and b) denied that osteoid was normally present during osteogenesis. He ascribed most of the osteoid tissue seen by previous workers to artefacts caused by faulty techniques. Studying bone development in human embryos Watt (1928) concluded that osteoblasts laid down both organic matrix and calcium salts simultaneously. He therefore agreed with Weidenreich that osteoid was absent from normal bone formation.

McLean, Bloom and Bloom carried out a series of experiments on calcification and ossification in bones from embryonic and young rats, young kittens and puppies (McLean and Bloom 1940; Bloom and Bloom 1940), and pigeons (Bloom, Bloom and McLean 1941), using undecalcified bone sections, stained for calcium salts by a modification of the von Kossa technique (von Kossa 1901). They concluded that, in these species at least,

bone matrix was calcifiable when laid down and that under optimal conditions of supply and transport of bone minerals, the matrix was usually calcified simultaneously with its deposition, or so soon after that no intermediate stage of osteoid tissue formation could be demonstrated. They were unable to find osteoid seams on primary spongiosa (cancellous bone formed initially, by endochondral ossification of the epiphyseal plate). The incidence of osteoid seams increased with increasing distance from the epiphyseal plate. The secondary spongiosa (cancellous bone formed by remodelling of the primary spongiosa) was normally free from osteoid, but this was commonly seen in the shafts. From these observations they stated that osteoid was not a necessary stage in bone formation, and that when osteoid was seen under physiological conditions this was due to a lag in calcification attributable to a local deficiency of bone mineral.

Subsequent investigators have disagreed with these findings. It has recently become possible to study the mineral distribution in undecalcified sections of bone by the process of microradiography (Engström 1949), in which the mineral content of a thin bone section is determined by the projection of soft X-rays on to a photographic emulsion, which is subsequently studied microscopically. Several workers have compared the

appearances of microradiographs from undecalcified sections with the microscopical appearances of the same section (Vincent 1955; Meyer 1956; Lacroix 1956). Their results make it clear that at sites of bone formation the tissue most recently laid down does not appear in the microradiograph, and is, therefore, uncalcified. A border of matrix (the osteoid seam) is completely free from mineral. Deeper layers show an abrupt onset of almost complete calcification. These findings confirm that osteoid is consistently found as a stage in osteogenesis.

Using undecalcified sections of human and animal bones, stained by von Kossa's method to demonstrate bone mineral, Meyer (1956) and Löe (1959) confirmed that recently formed bone matrix is uncalcified and forms a well defined morphological layer. Further proof of the physiological nature of osteoid was provided by Robinson and Cameron (1958), investigating bone formation in the femoral primary spongiosa in human infants with the electron microscope. They observed a zone of uncalcified tissue lying between the osteoblasts and calcified bone. This was a consistent feature at sites of bone formation. Dudley and Spiro (1961) also using the electron microscope demonstrated that that part of the bone surface which was lined by cells of inactive appearance, and which was therefore

regarded as inert, was free from osteoid.

Together these observations provide a convincing body of evidence leaving no doubt that in bone formation osteoid is consistently present, and is the normal precursor of mineralized bone.

If osteoid may be identified with accuracy, then its presence may be used as an index or indicator of bone formation.

Bone Resorption

The concept of cellular resorption of bone, as opposed to humoral dissolution, was advanced by Tomes and De Morgan (1853) who noted that the surface of bone undergoing absorption was pitted, and "hollowed with numerous minute cavities". The cavities were occupied by masses of granular nucleated cells, which lay in immediate contact with the bone and were thought to be responsible for its erosion.

Their techical methods did not allow them to characterise the masses of cells further. Later workers described the presence of multinucleated giant cells on bone surfaces, Rindfleisch (1873) noting the occurrence of such multinucleated cells in the erosion pits. (Figures 3 and 4). At the same time, Kölliker (1873) made a thorough study of these giant cells and the process of bone resorption, and concluded that they were the cellular agents of bone resorption, corresponding to the masses of granular cells described by Tomes and

Figure 3. Bone resorption. Much of the bone surface is irregular, showing a number of crenated Howship's lacunae in which large multinucleated giant cells - osteoclasts - are present. Decalcified section. Haematoxylin and eosin (x160).

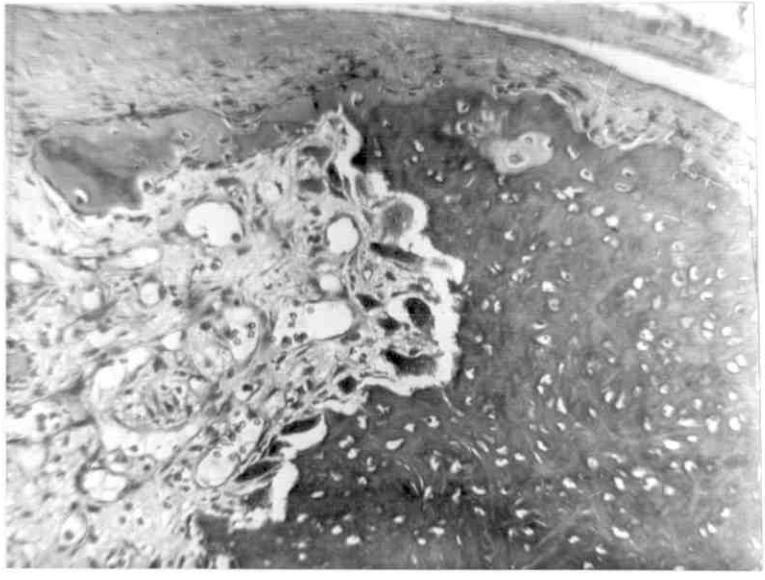
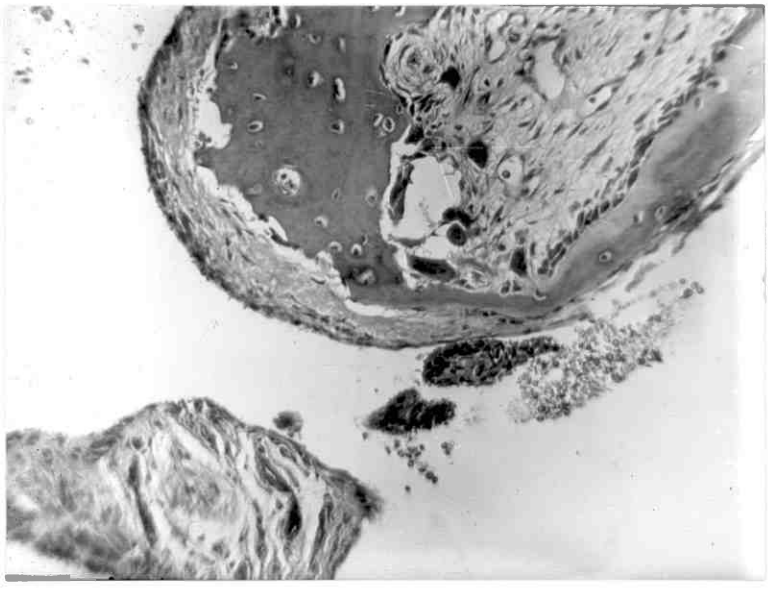


Figure 4. Bone formation and resorption. In the centre of the figure large multinucleated osteoclasts are present in crenated Howship's lacunae; to the right the trabecular surface is covered by mononuclear osteoblasts. Decalcified section. Haematoxylin and eosin (x160).



De Morgan. Kölliker termed the cells "ostoklast" i.e., bone-breaker, later changed to "osteoclast".

The hollowed out pits were thought to be eroded by the action of osteoclasts, and are therefore, like the presence of osteoclasts, an indication of bone resorption. They are generally known as "Howship's lacunae" (after Howship (1819), who first described them but failed to realise their significance) or "resorption cavities".

Kölliker's views were not accepted without debate. An alternative school of thought was initiated by Ribbert (1880) who suggested that a preliminary process of decalcification, termed halisteresis, occurs in all bone resorption. In his view, osteoid borders were considered to be a stage of bone resorption, representing bone matrix from which the mineral had been removed by halisteresis. Later Retterer (1906) and Leriche and Policard (1926) expanded this theory, describing a process which the latter workers termed "osteolysis". They postulated that in bone resorption, after a preliminary process of decalcification, the bone matrix simply reverts or "dedifferentiates" to fibrous tissue.

There is little or no evidence to support such a view. It has been abundantly demonstrated that the uncalcified borders of bone represent a stage in bone formation and not resorption. At the level of the light

microscope, in areas of bone resorption, bone matrix and bone salt always disappear together with no evidence of preliminary decalcification (Baker 1959; McLean and Bloom 1941).

As Baker (1959) points out, it is clear that Retterer and Lériche and Policard, the proponents of the theory of "osteolysis", mistook bone formation for bone resorption and misconstrued the appearance of uncalcified borders of matrix at bone formation sites as representing decalcification before resorption.

It has been postulated that osteoclasts may play a minor role in bone resorption, being attracted to bone that is disintegrating under the action of some other agent (Cameron 1963). Jaffe (1930, 1933) believed that the function of the osteoclast was to remove previously demineralized bone debris. Other workers believed that the osteoclast is the result of, rather than the cause of, bone resorption, the most recent proponents of this theory being Heller, McLean and Bloom (1950) who suggested that osteoclasts might simply represent masses of fused osteocytes freed from the bone resorption of the surrounding matrix.

This theory is rendered untenable by the observation of Ham (1952) that grafts of dead (thrice frozen and thawed) bone after four weeks show considerable resorption and a large number of osteoclasts.

In these circumstances clearly osteoclasts cannot represent fused osteocytes.

The histological evidence that osteoclasts are actively engaged in bone resorption stems from two findings. The histological picture is striking. The irregular surface of bone undergoing erosion suggests a cellular process, and osteoclasts are seen to occupy lacunae and to surround spicules of bone in a fashion suggesting cause and effect (Hancox 1949). The localization of osteoclasts is important. They are concentrated in sites where bone erosion is known to be taking place, for example about the bony walls of the dental alveoli of human and pig embryos (Arey 1919) where bony resorption is occurring to accommodate the rapidly growing teeth. This histological evidence is suggestive but circumstantial, and it must be conceded that on histological grounds alone one cannot disprove that osteoclasts are merely incidental to bone resorption.

Dynamic Studies

Recent in vivo studies have provided further evidence to support the classical views of Kölliker. Sandison (1928) and Kirby-Smith (1933) studied living bone fragments transplanted to, and maintained in , transparent chambers in the rabbits ear. Sandison gives a series of camera lucida drawings. In one a trabecula of bone is shown. After 48 hours a cell with the appearances of an

osteoclast is present in a deep Howship's lacuna which has formed during the same time. Forty eight hours later the trabecula is bisected and the osteoclast has disappeared.

Kirby-Smith studied the process in greater detail, and illustrated the disappearance of bone in contact with large granular cells considered morphologically to be osteoclasts. The eroded bone surface presents an irregular surface. He was able to show that absorption never occurred in the absence of osteoclasts, that bone was absorbed from beneath the cell body of the osteoclasts, and only from those parts of the bone beneath the cell body, He was able to observe the presence of an osteoclast before resorption, which later occurred in contact with the osteoclast. This rules out the possibility that the osteoclast is the result of absorption of bone.

Gaillard (1955, 1957) and Goldhaber (1958, 1960, 1963) studied the behaviour of living bone in tissue culture, recording the changes in the bone and cells by time lapse microcinematography. Gaillard observed bone in contact with osteoclasts disappearing. He calculated the rate of disappearance of matrix, and noted that when this was greatest, the number of osteoclasts was at a maximum, but also claimed that resorption was seen initially before typical osteoclasts were visible, and that resorption might continue if the osteoclast moved

away from the bone.

Initially Goldhaber too thought that early bone resorption took place without osteoclastic resorption, the earliest sign of resorption in tissue culture being the appearance of small crenations or "holes" which enlarged and coalesced to form typical Howship's lacunae. He was at first unable to demonstrate osteoclasts in relation to this resorbing bone surface, but in later experiments, using higher powers of magnification, he was able to observe giant cells "participating actively against the bone surface". The bone appeared to melt away in response to a "bubbling and boiling" activity at the surface and within the cell cytoplasm. It is thus probable that the earlier failures to demonstrate osteoclasts at the site of all bone resorption were due to technical reasons. This is favoured by the experiments of Irving and Handelman (1963). They studied autogenous devitalized bone implants in rats and noted that loss of weight of the implants was first evident at the time by which giant cells, which they believed to be osteoclasts, began to appear in significant numbers.

These experiments further support the concept that osteoclasts play an active role in bone resorption, and that the appearance of Howship's lacunae may be taken to indicate an area of bone resorption.

Resorption without Osteoclasts

In some bone sections, it is not uncommon to find that few Howship's lacunae appear to contain osteoclasts (Baker 1959), this having been put forward as evidence that bone resorption can occur without osteoclasts. It is more logical to suppose with Baker (1959) and Hancox (1956) that the osteoclast has a short functional life at any one site and having brought about resorption it disappears, leaving an empty Howship's lacunae. In vivo studies support this view. In Sandison's experiments (1928) an osteoclast arose, bone was resorbed and the cell disappeared within 48 hours. The longest time that Goldhaber (1963) was able to follow an osteoclast in tissue culture was 48 hours. Furthermore Bhaskar, Mohammed and Weinmann (1956) showed that the presence or absence of osteoclasts from areas of resorption cannot be satisfactorily determined without serial sections. Studying serial sections they found that osteoclasts were much larger, and extended more widely than hitherto supposed, large areas of cytoplasm being free from nuclei. Such non nucleated cytoplasm, lying in Howship's lacunae, is easily overlooked in a single section. A section may also pass through an osteoclast process containing one of the many nuclei. This is easily mistaken for a mononuclear cell (e.g., osteoblast) lying on the bone surface. The workers found that occasionally an osteoclast

appeared to be at some distance from a trabecula, but serial sections showed that the bulk of the cytoplasm was in contact with the bone surface.

Electron Microscope Studies

Detailed studies support the view that osteoclasts actively erode bone. The subject has been recently reviewed by Cameron (1963) and Hancox and Boothroyd (1964).

All workers are agreed that the osteoclast is applied to the bone surface. At the site of contact, the cell border (in other areas smooth) is ruffled into folds and finger-like processes, which bound fine channels running inwards to vacuoles in the interior of the osteoclast. At the site of contact, the bone surface is irregular and disorganised, and shows detached bone salt crystals and mineralised collagen fibrils. All workers have observed bone salt crystals in the channels leading into the interior of the osteoclasts and in the vacuoles, this evidence pointing to the osteoclasts being responsible for the dissolution of bone mineral. Detached collagen fibrils can be seen between the processes of the cell border. These observations show that the osteoclast is intimately associated with bone resorption, and strongly suggest that it has a causal relationship to resorption, although the actual mechanism whereby the matrix and mineral are

freed is not yet known.

Conclusion

Taken together, the observations reviewed in these sections leave little doubt that osteoclasts actively resorb bone, forming the irregular eroded Howship's lacunae, which are therefore an indicator of bone resorption.

Bone Resorption by Other Cells

It has been suggested at various times that cells other than osteoclasts are capable of bone resorption.

Macrophages

Goldhaber (1963) reported that in tissue culture studies mononuclear cells, described as macrophages, as well as osteoclasts could resorb bone. The work of Bhaskar et al. (1956) described above, suggests that these mononuclear cells may in fact be processes of osteoclasts.

Endothelial cells

a) Pathological. Jaffe (1930) described the occurrence of "vascular resorption" (disappearance of bone through the agency of blood vessels or granulation tissue) in inflammatory states of bone, and Starr (1947) reviewed the subject. Both gave illustrations showing thinned bone trabeculae, and surrounding dilated thin walled blood vessels or granulation tissue. Resorption of bone had presumably occurred but there was no evidence

that it was actually in process at the time. Many typical Howship's lacunae were present and it is likely that the resorption had been mediated by osteoclasts which had subsequently disappeared.

b) In Endochondreal Ossification. In electron photomicrographs of rat tibial epiphysis, Cameron (1961) described an irregular appearance of the bone surface, deep to a capillary, which he thought indicative of bone resorption, but there was no evidence of removal of bone material by the capillary endothelium.

There is no evidence to suggest that bone resorption by these possible mechanisms is ever seen under physiological conditions in cancellous bone.

Resorption by Osteocytes

Recently Bélanger, Robichon, Migicovsky, Copp and Vincent (1963) described enlarged osteocytes, the lacunae of which are surrounded by bone showing alterations in organic matrix and a lowered mineral density. The changes were interpreted as implying resorption of bone mineral by the osteocytes. The authors termed the process "osteolysis". The process is not related to the "osteolysis" postulated by Léricheand Policard (v. Bone Resorption). The significance of these observations is debatable. The phenomenon was observed mainly in pathological states (e.g., bone treated in vivo with parathyroid hormone or EDTA - a

chelating agent used in the laboratory in the decalcification of bone) in animals. It does not appear to have been seen in normal human material, and would in any case only remove mineral, not matrix. The work has yet to be confirmed; Young (1963), using similar materials to Bélanger et al., found no significant alterations in osteocytes or lacunae.

There is no evidence that this process, if confirmed, is concerned in physiological resorption of bone in the human.

Conclusion. The observations reviewed above provide a body of evidence leaving no doubt that resorption of bone is an active cellular process, which is mediated exclusively, or almost entirely, by osteoclasts, the Howship's lacunae being a sign of bone resorption.

AGE CHANGES IN BONE TISSUE

Sir Astley Cooper wrote in 1823 that in old age "the bones become thin in their shell, and spongy in their texture... The process of absorption and deposition varies at different periods of life... This is well seen in the natural changes of the bones, their increase in youth, their bulk, weight and little comparative change during the adult period, and the lightness and softness they acquire in the more advanced stages of life".

Quantitatively this reduction in amount of bone is termed osteoporosis.

OSTEOPOROSIS

Osteoporosis refers to a reduction in the amount of bone present, either with regard to the skeleton as a whole or to any particular part of it, the bone remaining appearing to be of normal composition (Sissons 1964). Nordin (1963) defined the condition as a reduction in bone mass per unit volume. Minor chemical changes in bone composition have on occasion been reported (Nordin 1964a), but no significant or consistent changes have been reported. Although the total amount of calcium in the skeleton may be greatly reduced, the degree of calcification of the bone which remains is normal, unless there is coexistent osteomalacia. Osteoporosis has recently been reviewed and classified by Cooke (1955), Urist (1962) and Nordin (1964a).

Osteoporosis may be local or general. Local osteoporosis is frequently seen as a result of immobilisation (e.g., of a joint). Generalised osteoporosis may also result from immobilisation (e.g., prolonged recumbency) and is a concomitant of many endocrine disorders, such as Cushing's syndrome, acromegaly and hyperthyroidism. In the great majority of examples of generalised osteoporosis the cause is unknown, and the term idiopathic osteoporosis is used.

This osteoporosis of unknown cause occurs most commonly after middle age, and the terms involutional, postmenopausal and senile osteoporosis are used to describe this, the loss of bone from the skeleton with aging. The terminology is somewhat confused, Albright and Reifenstein (1948) termed osteoporosis seen in females aged from 45 to 65 years "postmenopausal" and that seen in individuals aged over 65 years "senile". Ball (1960) stated that in the absence of other known aetiological factors osteoporosis in females over 45 years and males over 55 years is somewhat arbitrarily termed "postmenopausal" or "senile" osteoporosis, while in younger individuals such osteoporosis is termed "idiopathic". The somewhat arbitrary nature of the terminology suggests that there is no clear distinction between these entities.

The possible distinction of senile osteoporosis from normal loss of bone in old age is complicated by

the limited amount of information as to the extent of the latter. Senile osteoporosis has been regarded as a pathological condition, but it is not clear whether any distinction can, or indeed should, be drawn between this and normal aging changes.

Idiopathic osteoporosis was distinguished histologically by Pommer (1885) and a clear account of the morbid anatomical changes was provided by the studies of Schmorl of a series of 7,000 spines (Beadle 1931), but the entity was not clearly defined clinically until the studies of Albright and his colleagues, published from 1940 onwards (Albright, Bloomberg and Smith 1940, Albright and Reifenstein 1948). Albright put forward a theory of aetiology, basing this on the age and sex distribution of the cases studied. Of 42 cases initially studied, 40 were female and 2 male. No cases were seen in females before the menopause. Aware of the clear relationship in birds between ovarian activity and the amount of bone tissue, increased amounts of bone tissue being seen during ovulation, and of the hyperossification produced in male birds and some animals by oestrogen administration (Gardner and Pfeiffer 1943), Albright suggested that in women the menopause and development of osteoporosis were causally related. It was postulated that postmenopausal osteoporosis was caused by impaired osteoblastic activity as a result of

diminished oestrogen secretion following the menopause. Androgens too were known to affect bone (Gardner and Pfeiffer 1943) and Albright (1947) postulated that idiopathic osteoporosis in men, which he considered to be less common, and of later onset, than in women, was also due to impaired osteoblastic activity from gradually diminishing androgenic and adrenal cortical anabolic hormone production occurring after the age of sixty years.

Clinically, oestrogens and androgens were found to promote a positive nitrogen and calcium balance in cases of postmenopausal and senile osteoporosis, and to bring about rapid relief of symptoms. In consequence Albright's theory gained general acceptance. Reifenstein (1957) suggested that imbalances between the anabolic and antianabolic hormones of gonads and adrenals was of more importance in the genesis of osteoporosis than absolute deficiency of anabolic hormones (i.e., androgens and oestrogens).

Despite its acceptance, however, no direct evidence in favour of Albright and Reifenstein's theory has ever been adduced. No conclusive evidence of recovery from senile or postmenopausal osteoporosis (as measured by a return of radiological density of osteoporotic bone towards normal) has ever been brought forward, despite oestrogen therapy for periods up to twenty years (Henneman and Wallach 1957). Nor does it seem that any

positive calcium balance achieved by oestrogen therapy can be maintained for a long period (Rose 1964). Although anabolic hormones produce rapid subjective improvement, relief of symptoms is also achieved by placebos (Solomon, Dickerson and Eisenberg 1960).

In animals calcium deficiency is known to be one cause of osteoporosis, this subject being reviewed by Nordin (1960a). In the same paper this author revived earlier theories in suggesting that osteoporosis in the human may also be due to calcium deficiency. Although this theory has gained considerable acceptance there is, as with Albright and Reifenstein's theory, little evidence for it. Nordin estimated that the daily dietary intake of 8% of the population of the United Kingdom was less than the figure generally accepted as being necessary to achieve calcium balance. However, as Malm (1958) pointed out, most subjects will adapt to a low calcium intake, while Exton-Smith and Stanton (1965) in a dietary survey of elderly women living alone concluded that the evidence did not implicate calcium deficiency in the development of osteoporosis.

Nordin (1961) found that, unlike normal persons, osteoporotic subjects could not decrease their urinary calcium output when placed on a low calcium diet, and suggested that this relative hypercalciuria and calcium loss from the body might bring about depletion of calcium from the skeleton, and osteoporosis. It is, of

course, equally possible that this inability to lower urinary calcium output, and calcium loss, is the result of rather than the cause of osteoporosis. In the development of osteoporosis bone resorption must be greater than bone formation, leading to a net loss of calcium from the skeleton, and a negative calcium balance. If osteoporosis is progressive, then a continued calcium loss from the body should be seen, and there is no reason why this calcium loss should be reduced by a low calcium intake.

Nordin later suggested (Jasani, Nordin, Smith and Swanson 1965) that decreased oestrogenic activity after the menopause caused a raised plasma calcium, with consequent hypercalciuria and negative calcium balance. Nordin (1960b) and Spencer, Menczel, Lewin and Samachson (1964) suggested that impaired absorption of calcium from the gut might be the cause of idiopathic osteoporosis. However, Rose (1964) showed that if calcium absorption was increased in an osteoporotic subject, the additional calcium did not pass to the bone but was excreted in the urine and the overall calcium balance was unchanged. Impaired calcium absorption from the gut is thus unlikely to be the cause of the osteoporosis.

If calcium deficiency is the cause of osteoporosis it should be possible to reverse the process of osteoporosis by increasing the calcium intake. The theories mentioned above, although attractive, prove

inadequate in that it appears impossible to reverse the process of idiopathic osteoporosis by calcium supplements. Nordin (1961) stated that calcium supplements produced subjective improvement in osteoporosis, and later Nordin (1962) claimed that such supplements produced positive calcium balances as large as 34.6 mg/kg. body weight/day for periods up to one year.

Using more sensitive methods of balance study, Rose (1964) however was unable to achieve positive calcium balances for longer than a few weeks when a high calcium intake was given, and pointed out that no definite evidence of increased radiological density of osteoporotic bone had been seen after long periods of high calcium intake, although with positive calcium balances as high as those claimed by Nordin this should certainly have been seen. There is thus no definite evidence that high calcium intakes can reverse the process of idiopathic osteoporosis.

The theories mentioned above are the two main theories of idiopathic osteoporosis but at the present time neither Albright and Reifenstein's nor Nordin's theories can be regarded as proven. It is clear that valuable information regarding these two theories might be given by a study of bone loss in an aging population, by determination of the relationship of bone loss to the menopause, determination of the numbers of the population showing bone loss, determination of the relative incidence

of bone loss in males and females, and by determination of the universality or otherwise of bone loss.

METHODS OF DETERMINING AMOUNT OF BONE PRESENT

Most studies have employed one of two approaches:-

1. Measurement of weight, volume of solid bone, radiographic density or calcium content of a given volume of bone, all these quantities thought to be measures of the amount of solid bone tissue present.
2. Measurement of thickness of cortical bone. These methods have been employed both in the laboratory and clinically.

Ingalls (1931) weighed the bones from the entire skeleton in 100 male dissecting room subjects. A general fall with age was seen, but it must be stressed that weight and not density was measured. A bone may be lighter because it is more porous, or simply because it is smaller. In fact the material showed a secondary rise in weight extending into the sixth decade. This was attributed to a degenerative process, due to new bone formation as osteophytes around articular surfaces.

This approach was elaborated by Trotter, Broman and Peterson (1960) who measured the density of whole bones, weighing them and determining the volume by displacement. This method, of course, measures the density of the bone as an organ, including bone marrow contents. For these results to be relevant to the amount of bone tissue present at various ages, bone marrow, which increases in volume as the bone becomes more porous, must differ markedly in density from bone

tissue, while actual solid bone tissue must show no change in density with age.

This method employing whole bones can have no application in clinical practice. Several workers have measured the apparent density of small bone samples. Lindahl and Lindgren (1962) studied cancellous bone specimens from vertebrae and tibia at autopsy. The dried, defatted specimens were weighed, the overall dimensions measured, and the overall volume, and thus the apparent density calculated.

Saville (1965) employed iliac crest trephine biopsies, consisting of both cancellous and cortical bone. The weight of a cylinder of bone of standard dimensions was recorded. A similar approach was made by Arnold (1964) who measured the ash weight per unit volume of lumbar cancellous bone. Such approaches are useful, but suffer from the disadvantage that no further studies (e.g., histological) are possible on the specimens.

Caldwell and Collins (Caldwell and Collins 1961; Caldwell 1962) measured the radiographic density of 1 cm thick post-mortem vertebral bone slabs, using this as an index of the amount of calcium salt, and consequently amount of bone, present.

Radiographic methods have been employed frequently in the clinical detection and measurement of bone loss with age. Authors have made radiographic comparison of

selected areas of the skeleton (e.g., ulna, calcaneum and spine) with a standard of known composition (Doyle 1961; Mayo 1961; Nordin, Barnett, McGregor and Nisbet 1962). However the accuracy of such methods has not been fully established. Overlying soft tissues may completely mask any radiographic changes due to loss of cancellous bone. Even if soft tissue is not interposed, loss of bone substance and therefore mineral from the bone as a whole may not be reflected in the intensity of the radiographic image. Ardran (1951) showed that cancellous bone representing 30% of the thickness of the bone may disappear before the loss is visible radiographically. Cobb (1952) demonstrated that 25% of the bone mineral must be lost from a phalanx (radiologically the most accessible bone) before a just detectable difference is seen on radiographs of the finger, while up to 60% of bone mineral can be lost from the spine before this is radiographically detectable in the living subject.

Another clinical method of estimating bone loss is to measure the cortical thickness of a bone from radiographs (Barnett and Nordin 1960). It may not be easy to define the limits of the cortical bone accurately; as the measurements made are small a distinct possibility of error exists, particularly if cortical thickness is expressed as a ratio of the total diameter of the shaft.

Such measurements of cortical thickness have also

been performed in laboratory specimens. Atkinson, Weatherell and Weidmann (1962) measured the cortical thickness in biopsy samples of femoral cortex. The method is valid, but will take no account of changes in porosity which may occur in a cortex unchanged in thickness.

Vost (1963) indirectly estimated the amount of bone in vertebra and iliac crest photometrically, measuring the percentage of light transmitted by a section stained black with iron haematoxylin.

In studying variations in amount of bone with age, the most logical method to use is one which measures this directly (either as the relative volume of a bone specimen occupied by solid bone, or in a histological section, as the proportion of total area occupied by solid bone). Published studies of this nature are very few in number.

Beck and Nordin (1960) studied a large series of cases based on iliac crest material from general hospital autopsies. Photomicrographs of iliac crest sections were matched visually with photomicrographs from a series of nine standard sections, in which the area occupied by bone varied from 6% to 27%. The method is thus largely subjective, and the possible errors are large. Lindahl and Lindgren (1962) measured the area of bone in specimens from tibia, by cutting out and weighing the areas representing bone from a photomicrograph.

In these studies, however, no attempt was made to distinguish between cortical and cancellous bone, and measurements were made on specimens containing both types of bone in varying proportions. Thus any changes occurring in the porpus cancellous bone might be obscured by the much denser cortical bone present, and by the variable amounts of the two types of bone.

From a review of published studies it must be concluded that there is a dearth of quantitative studies of the effect of age on cancellous bone.

THE CONCEPT OF CONTINUOUS TURNOVER AND REMODELLING OF BONE

In 1727 Stephen Hales observed that two drill holes bored in the diaphysis of a growing bone remained equidistant from each other, despite considerable increase in length of the bone. He concluded that bones increased in length only at the epiphyses and not by interstitial expansion of the bone as a whole.

Using the dye madder as an intravital stain of newly formed bone, Duhamel (1742) and Hunter (1837) confirmed this observation, and clearly demonstrated that bones grow by a process of surface accretion, by apposition of new bone on the surface of the old. From these findings and his anatomical observations, Hunter deduced that to maintain the shape of a bone during its growth, deposition of new bone at one site must be associated with absorption of pre-existing bone from another site. Thus during growth the bone is continuously remodelled. Hunter further suggested that this process of remodelling of bone continued throughout life after growth had ceased. In his view bone was not inert, but subject to a continuous turnover throughout life, by the processes of bone formation and resorption. The subsequent demonstration of the cellular processes of bone formation and resorption which were shown to continue throughout life confirmed this view.

The changes occurring in human cancellous bone during the process of remodelling were studied by

Amprino (1937) who demonstrated that in early post natal life the recently formed trabeculae are coarse and stout, and composed of woven bone. These are soon resorbed and replaced by orientated slim trabeculae. The process of turnover and remodelling continues, and these trabeculae show evidence of continuing formation and resorption. This continues throughout adult life.

Such morphological studies were purely descriptive and did not provide information on the order of magnitude of the remodelling process. Despite the fact that in bone, unlike other tissues, the possibility of interstitial expansion or growth is excluded by the rigidity of the tissue, and that therefore all bone formation and resorption must occur at bone surfaces where it may readily be observed, little interest was taken in the possibilities of quantitative studies of this aspect of bone activity until Sissons (1960) re-emphasised the concept of continuous turnover of bone, stressing the importance of quantitative study of bone forming and resorbing activity, and discussing how such data might be expressed.

Since this time several quantitative studies of bone formation and resorption have been made, a stimulus to such studies being the fact that knowledge of changes in bone formation and resorption rates with age might elucidate the pathogenesis of idiopathic osteoporosis. Albright and Reifenstein's theory presupposes a reduced

bone formation rate, while Nordin's calcium deficiency theory implies, on the other hand, an increased rate of bone resorption.

METHODS OF MEASURING BONE ACTIVITY AND TURNOVER

Most methods are morphological and depend on the identification of sites of bone formation and resorption. This is the only practicable approach in a large scale autopsy study such as that to be described. Other methods will be described since the results obtained may be compared with those derived from morphological investigations.

Radioactive Isotope Studies

These are based on the principle that when a radioisotope of calcium is administered intravenously it disappears from the blood, and is taken up mainly by bone, and to a very much smaller extent by soft tissues; a certain amount is excreted in urine and faeces. Numerous workers have employed such studies, their techniques mainly being based on that described by Bauer, Carlsson and Lindquist (1961). If the rate at which the isotope disappears from the blood after a single injection is analysed mathematically then the rate of uptake of isotope by the skeleton or "skeletal accretion rate" may be calculated. The skeletal accretion rate was equated with new bone formation rate. Bone resorption rate has been calculated by further analysis of the rate of fall of isotope specific activity from the blood, or indirectly by combining the radioisotope study with a calcium balance.

Unfortunately it is now known that skeletal accretion rate cannot be directly equated with new bone formation rate. It has been shown that a variable amount of isotopic tracer is taken up diffusely in older bone by exchange with non-labelled calcium in the old bone and by secondary mineralization. Lee, Marshall and Sissons (1965) showed that in adult dogs the bone formation rate was only one third to one half as great as the observed skeletal accretion rate. Also working on dogs, Jowsey, Lafferty and Rabinowitz (1965) showed that the amount of isotope deposited diffusely in areas other than those of bone formation varied with the age of the animal. Only in a young growing animal was bone formation more important than diffuse deposition in skeletal retention of the isotope; in an adult animal only 20% of the administered isotope was retained as a result of bone formation. In addition controversy and dispute still exists over the method of mathematical analysis to be employed in the calculations from the experimental data. (Heaney 1964; Anderson 1963).

Bearing in mind these factors the results of such studies should be interpreted and accepted with some reserve.

Urinary Hydroxyproline Estimations

The amino-acid hydroxyproline is found almost exclusively in collagen, a large proportion of which

is found in bone. It has therefore been suggested that most of the urinary hydroxyproline is derived from bone collagen (Klein and Curtis 1964), and measurement of urinary hydroxyproline has been put forward as an index of bone collagen metabolism (Dull and Henneman 1963). It is not yet clear, however, whether an increase in urinary hydroxyproline is associated with bone collagen synthesis, bone collagen breakdown, or both (Klein and Curtiss 1964; Klein 1966), and it is felt that the test required further evaluation before definite conclusions may be drawn.

Morphological Studies

These are based on methods of identification of sites of bone formation and resorption.

A) Intravital Markers of Bone Formation

1) Autoradiography

The site of uptake of the radioisotope of a bone seeking element may be determined by the application of a sensitive photographic emulsion to the tissue. Isotopes such as Ca^{45}, Sr^{90}, and P^{32} have been shown to be taken up intensely at sites of bone formation, and have been used as intravital markers of bone formation (Leblond, Wilkinson, Bélanger and Robichon 1950). The method is best suited to animal experiments.

2) Other Intravital Markers

Other substances have also been shown to be deposited at sites of bone formation, and have been

used as intravital markers of bone formation. Vincent (1957) used lead for this purpose, and Schour (1936) used alizarin. In both cases however the toxicity of these substances restricts their use to experimental animals.

3) Tetracycline

Tetracycline has been found to be an intravital marker of sites of bone formation, of high precision, and suitable for use in human studies (Harris 1960). If administered on one occasion, tetracycline is deposited permanently at current sites of bone formation (Ghosez 1959) and may be recognised there by its golden yellow fluorescence. Like all intravital markers it has the advantage that if administered on two occasions, the bone formed in the intervening period may be recognised (being that bone lying between the two tetracycline labels). From the linear separation of the two labels, the rate of appositional growth (i.e., the thickness of bone laid down per day at a forming surface) may be calculated, and related to the total amount of bone present, allowing the turnover rate to be calculated.

The method has limitations. The method of double labelling with consequent calculation of the bone formation rate is difficult to apply to cancellous bone as, owing to the irregular nature and orientation of this type of bone, the plane of section through the

tetracycline labelled area is frequently oblique. The tetracycline deposit appears widened and "flared", definition of the label becoming impaired (Sissons and Lee 1964). The linear separation of the labels will be artificially increased by the obliquity of section.

B) Quantitative Morphology

In the type of study envisaged in this work, using large specimens of bone (as against small biopsies) from individuals in normal health until sudden death, tetracycline labelling is clearly impracticable; indeed a single label of tetracycline gives no more information than that derived from accurate identification of bone forming surfaces by other means. Several such methods are available, but again the nature of cancellous bone limits their application.

1) Microradiography

Amprino and Engström (1952) studied the mineral distribution in bone by the already described technique of microradiography. Their results showed that the mineral distribution is not uniform. Recently formed bone is of lower mineral density than the remainder of the bone, the newly formed bone being incompletely calcified, and a surface where bone formation is occurring may be recognised by its low mineral density. Old bone and inert surfaces are of high mineral density. Howship's lacunae may be recognised by their sharp crenated

edge, which may cut across bone of varying mineral density.

This technique is most useful in, and has been extensively employed in, the study of cortical bone. Technical considerations render it of dubious value in the study of cancellous bone. Interpretation of the characteristics of bone surfaces is complicated by the fact that microradiographs are prepared from relatively thick sections (70 - 100 μ); particularly in cancellous bone, oblique surfaces are very frequent in sections of this thickness. In a microradiograph an oblique surface shows a gradual fall of mineral density to the free edge of the bone, due to the gradual reduction in thickness of bone tissue to this point. Such an appearance can simulate or obscure the appearance of bone formation, which also shows a fall in mineral density towards the surface where bone formation is occurring (Sissons 1962), although in the case of bone formation, this gradient is due to incomplete calcification of the tissue rather than to changes in thickness of bone tissue in the section.

2) Osteoblast and Osteoclast Counts

As these are responsible for bone formation and resorption respectively, their numbers might be used as an index of bone formation and resorption (Bauer, Carlsson and Lindquist 1961). Such observations can only be expressed in descriptive terms. Quantitative

measurements are impossible because osteoblasts form a continuous spectrum ranging from the plump active form to the flattened inconspicuous, inactive cell (Pritchard 1956b) and no absolute dividing line can be drawn between the two forms.

3) Histological Methods

In Section 2 it was pointed out that if osteoid can be accurately identified it may be used as an indicator of bone formation. In carefully processed and stained tissue osteoid may be recognised in decalcified sections (Meyer 1956) but for quantitative studies undecalcified material is more reliable. In undecalcified material osteoid may be recognised in unstained sections (Meyer 1956) where it appears relatively translucent, calcified bone appearing granular, or it may be demonstrated by a variety of stains (Löe 1959).

The method most commonly employed is the von Kossa silver nitrate stain (von Kossa 1901) which stains insoluble phosphates and carbonates and certain other insoluble salts of calcium and certain other metals. (Cameron 1930). In osseous tissue it may be regarded as a specific stain for calcified bone, osteoid being unstained, and it should therefore be suitable for quantitative demonstration of osteoid. Personal experience suggests that the von Kossa method lacks precision, in that silver is deposited not only in

calcified tissue but also around it in the surrounding marrow and osteoid in granular form, obliterating detail, and interfering with the identification of osteoid.

In undecalcified preparations, calcified bone stains deeply with haematoxylin, while that which is regarded as osteoid is virtually unstained, affording a clear distinction between the two. The method has not been used in quantitative demonstration of osteoid : from the results of earlier workers it was not certain whether the method reliably and quantitatively demonstrated bone salt. Cameron (1930) concluded that the staining of bone with haematoxylin depended not on the presence of bone salt, but mainly on the presence of a special ground substance associated with calcification.

If this were so the stain might not afford reliable distinction between bone and osteoid, and would not be suitable for the quantitative identification of osteoid. However, the profound change in the staining characteristics of the bone on decalcification with either acids or chelating agents, leading frequently to complete disappearance of any distinction between calcified and non calcified tissue strongly suggests that the material staining with haematoxylin in undecalcified bone sections is bone salt, or some substance most intimately associated with it, and likewise removed by all agents removing calcium salts.

A series of preliminary experiments (v. Section 4) showed that the results obtained by haematoxylin staining of undecalcified bone agreed with those obtained by von Kossa staining. Comparison with microradiographs showed that material staining deeply with haematoxylin in undecalcified sections was calcified, while that material recognised in the stained section as osteoid was consistently uncalcified.

Thus haematoxylin staining of undecalcified sections is a reliable means of demonstrating osteoid, and it is justifiable to use this method for the quantitative demonstration of osteoid and bone formation.

In such stained sections bone resorption is recognised by the morphological characteristics of Howship's lacunae; the bone stains deeply to the edge of the lacunae. The sections are thinner than those used for microradiography and the identification of such crenated surfaces is therefore easier.

SECTION THREE

PRESENT STUDY

PRESENT STUDY

This section is in several parts, describing the materials used and the methods employed.

MATERIALS

It was intended in this study that all measurements should be collected from as large an area of cancellous bone as possible so that this might be considered representative of the bone as a whole; bone formation and resorption in particular are focal processes and distributed unevenly in bone (Jowsey, Owen and Vaughan 1953). In certain cases the amounts of variation to be expected within a given block and at various sites along the iliac crest were to be studied. Thirdly it was important that subjects should have been in good health and active until the time of sampling or death, as preliminary studies showed that considerable reduction in extent of bone formation may be seen in bed-ridden subjects, confirming an observation made by Jowsey (1963).

These considerations precluded the use of biopsies or material from general hospital autopsies, and specimens were obtained from coroners' autopsies on cases dying within 48 hours of accident or the onset of acute illness. No material was taken from cases where more than 48 hours had elapsed between accident, or onset of illness, and death, to exclude possible changes due to immobilization. To avoid cases which

might have been complicated by metabolic bone disease (such as osteomalacia or renal osteodystrophy) all cases with evidence of renal, hepatic, alimentary or pancreatic disease were excluded.

All cases of sudden death in chronic illness, cases of malignant tumours, and any cases showing evidence of Paget's disease were also excluded.

In the youngest age groups (0 - 19) trauma was the commonest cause of death. Acute infections (chiefly pneumonia) and haemorrhage (e.g., subarachnoid haemorrhage) provided smaller numbers of cases. In the 20 - 39 age groups, 75% of deaths were due to trauma or poisoning. A smaller number were due to acute infection. A thrombotic episode (chiefly coronary thrombosis) was the commonest cause of death in the 40 - 59 age groups, followed closely by trauma. Haemorrhagic episodes and drug overdosage caused smaller numbers of deaths. In the over 60 age groups the commonest cause of death (45%) was thrombosis (mainly coronary). Haemorrhagic episodes (cerebral haemorrhage, and ruptured aneurysms) formed 25% of the cases, the remainder being due to acute illnesses and accidental death.

At autopsy, a large slab of iliac crest bone approximately four inches in length and including the anterior superior iliac spine was removed, and fixed in buffered 10% formol-saline. After fixation the

specimen was cut transversely on a Burgess band saw into blocks 5 - 6 mm. in thickness. At least one block from each specimen was selected for perspex embedding and grinding. The blocks for perspex embedding were taken from a point about 2.5 cm. behind the anterior superior iliac spine. In certain cases further blocks for perspex embedding were taken at intervals along the iliac crest to determine the extent of variation of the measured parameters along the iliac crest. For comparison blocks were taken from the adjacent bone for routine haematoxylin and eosin staining after decalcification.

PREPARATION OF UNDECALCIFIED SECTIONS

Sections were prepared from undecalcified blocks by a process of grinding after methyl methacrylate embedding. The method used was modified from that described by Jowsey (1955):-

Dehydration

After adequate fixation (at least 3 days) blocks were dehydrated in ascending grades of alcohol as follows:-

70% alcohol	24 hours
85% alcohol	24 hours
95% alcohol	24 hours
Absolute alcohol I	24 hours
Absolute alcohol II	24 hours
Absolute alcohol III	24 hours

The blocks were then infiltrated with unpolymerized methyl methacrylate by treatment with a 1 : 1 absolute alcohol/methyl methacrylate monomer mixture for 24 hours, followed by methyl methacrylate monomer for 72 hours.

Preparation of Methyl Methacrylate

As supplied methyl methacrylate contains an inhibitor (hydroquinone) to prevent polymerization. This was removed before use by washing the monomer with an equal quantity of 5% sodium hydroxide in a separating funnel. After vigorous shaking the dark brown solution which collected at the bottom after absorption of the inhibitor was discarded. The process was repeated three times. The monomer was then freed from the sodium hydroxide by washing three times with equal volumes of distilled water. After this treatment the monomer was dried by filtration through dried calcium chloride. This is essential as any residual moisture interferes with the process of polymerization, and impairs the quality of the final sections.

Partially polymerized methyl methacrylate was used in preference to methyl methacrylate monomer. This decreases the time needed for hardening of the methyl methacrylate. During polymerization the originally liquid methacrylate thickens and hardens to produce the final rigid block.

Partially polymerized methacrylate was prepared by

adding a catalyst to dried monomer; 1 G. dried benzoyl peroxide was added to 100 ml. of dried monomer, and the solution heated in a water bath to 80 - 85°C, with continuous stirring. The monomer gradually polymerized and thickened and when it reached a thick syrupy consistency the container was immediately cooled in running cold water to arrest the process. Polymerization is an exothermic reaction, and care was necessary to prevent the reaction proceeding to completion, resulting in rapid expansion and bubbling of the methacrylate followed by solidification within a few seconds.

Embedding

After infiltration with monomer the bone blocks were embedded in plastic or aluminium foil moulds. The moulds were half filled with partially polymerized methacrylate, the blocks placed upon this, and the moulds then filled with methacrylate. The moulds were placed in a sealed container to avoid evaporation, and polymerization allowed to take place at 30 - 35°C, the process of hardening being complete in 4 - 8 days. The hardened methacrylate was removed from its mould, and trimmed with a band saw.

Sectioning

The block was sectioned on a modified milling machine (essentially a sophisticated circular saw), the

specimen being advanced automatically. The block was cooled by an oil and water spray to prevent damage and artefacts due to heating. Sections, as cut from the milling machine, varied from 100 - 200 μ in thickness.

Grinding

For microscopical examination sections should be not greater than 20 μ in thickness. (It was earlier pointed out that in thicker sections artefacts from oblique surfaces are common; sections thinner than 20 μ are no easier to interpret than those of 20 μ). Sections of this thickness were prepared by grinding the 100 - 200 μ thick milled sections between two sheets of roughened $\frac{3}{4}$" thick plate glass. A large rectangular sheet served as a base, and a smaller circular plate was moved by hand in a circular fashion, with the section between the two. The glass plates were roughened and "sharpened" by preliminary grinding together with a paste of silicon carbide (Carborundum 220) and water. All traces of abrasive were removed from the plates by brushing under running water, to prevent any tearing of the sections, and the sections then ground by the abrasive properties of the glass alone, using 70% alcohol as a lubricant. This technique is delicate, and enabled the preparation of sections of embedded cancellous bone, of section thickness as little as 15 μ, and free from artefact. Grinding was interrupted at 70 μ, for microradiography of the sections.

Staining

After grinding to 20μ, sections may be examined unstained or stained by a variety of procedures. In the present study, the undecalcified sections were stained with haematoxylin and eosin. This allows the recognition of the morphological characteristics of the different types of bone surface, and in sections of this thickness affords a reasonable degree of cellular detail.

Sections were washed in distilled water, and stained in Cole's iodine ripened haematoxylin(Cole (1943). This contains no acid, and hence prevents any decalcification of the section, with subsequent failure of differential staining of bone and osteoid. Sections were stained for 2 - 6 hours at room temperature, the length of staining time being determined by examination of the sections during the process. They were then "blued" and counterstained lightly in 0.5% aqueous eosin for 30 seconds. Clearing of plastic embedded sections by xylol causes buckling and distortion of the sections. This was avoided, after dehydration in ascending grades of alcohol (70% - 95%), by clearing the sections in a 2 : 1 mixture of 95% alcohol and terpineol, and mounting in Euparal (Flatters and Garnett Ltd., Manchester).

Decalcified Sections

Comparable blocks from each specimen were treated

by conventional histological methods, being decalcified, embedded, sectioned and stained by routine haematoxylin and eosin methods (Drury and Wallington 1967).

PARAMETERS MEASURED

After a preliminary general examination of the morphology of the bone, sections were studied systematically so that several parameters might be measured.

1. Amount of Bone Present in the Section

This is expressed as the percentage of section area occupied by bone as opposed to marrow space, and is termed the bone area.

2. Bone Surface Area

(Not including canalicular or osteocyte lacunar area). The available surface area was expressed in two ways:- The absolute value (surface area per unit volume of tissue i.e., solid bone and marrow), measured in sq.mm/cubic mm., was calculated, and this was then expressed as a relative value in terms of the amount of solid bone present in the areas studied (surface area per unit volume of solid bone) again measured in sq.mm/cubic mm.

3. The Extent of Bone Formation and Resorption

Such measurements may be made and expressed in several different fashions. In cancellous bone, in particular, the most logical method is to determine the percentage of bone surface occupied by bone formation

and resorption. From these percentage figures the absolute and relative surface areas of bone formation and resorption may be calculated.

An alternative method is to count the numbers of osteoid seams present. This may be expressed in relation to the numbers of vascular channels present (applicable only to cortical bone) or as the number of seams in a unit volume of bone (Frost and Villanueva 1960). This method may be valid, but the first described method appears preferable. The counting of numbers of osteoid seams is impracticable in cancellous bone, since what appear in a section to be separate osteoid seams may be no more than parts of a continuous surface of bone formation; furthermore it seems preferable to use a method which is also applicable to the measurment of surfaces of resorption.

MEASUREMENT OF BONE AREA

This was measured by a system of point sampling, with an array of points laid on the tissue, after the method described by Chalkley (1943). The principle of the method is that, if a large number of points is projected randomly on a section of tissue, in the limit the proportion of total points falling on any component of the tissue will equal the proportion of total area occupied by that component. If the section is representative of the tissue from which it is taken, then this proportion is also equal to the proportion

of total volume occupied by the component.

The method was extended by Hennig (1958) and the array used (Zeiss Integrating Eyepiece I) is based on his work. It consists of a Zeiss 8x microscope eyepiece provided with a graticule of 25 points asymmetrically arranged within a circle. The points are joined by a series of straight lines to facilitate their recognition. The integrating eyepiece replaces one 8x eyepiece of a binocular microscope and when a section is viewed the image of the array is superimposed on that of the section (Figure 5), and the number of points falling on any component (in this case calcified bone and osteoid) may readily be determined. The eyepiece may be turned and used in any random position, its orientation being immaterial.

MEASUREMENT OF SURFACE AREA AND ITS COMPONENTS

This was carried out by a method of line sampling. Here an array of random points is replaced by an array of linear paths, which are sufficiently short to spread repeated observations randomly over the section studied. The number of intercepts made by the paths with the surface of any component is noted (N), and if the total length (L) of the paths is known it may be proved (Rogers in a paper by Short 1950) that in a unit volume:-

$$\text{Surface area of component } (S) = \frac{2 \times \text{Number of intersections}}{\text{Total length of Paths}}$$

$$\text{or} \qquad S = \frac{2N}{L}$$

Figure 5. Bone area measurement. An array of 25 points is superimposed on a microscope field. In this instance 6 of the 25 points fall on bone and osteoid; 5 on bone and 1 on osteoid.

The proof of this formula is given in Appendix 1. The results can be expressed in this fashion if the section is considered representative of the tissue from which it is taken.

The proportion of the total intercepts made with any given component is equal, in the limit, to the proportion of surface occupied by this component. Thus the extent of surface occupied by bone formation or resorption may readily be measured and from this their absolute and relative surface areas.

The method was again developed by Hennig (1958) whose work led to the design of the array used in these studies (Zeiss Integrating Eyepiece II). This consists of a Zeiss 8x eyepiece containing a graticule furnished with six parallel lines (Figure 6). Each of the long lines is one fifth of the combined length of the lines, and each of the short lines one tenth of the total length. Used in conjunction with a 10x objective in a Cooke, Troughton & Simms microscope the lines represented a total length of 3.8mm. on the section. In the case of this eyepiece orientation is important and unless the object examined is completely unorientated the eyepiece is rotated to a new random position before each observation.

METHODS OF COUNTING

As prepared, the ground sections were about 2.5 - 3cm.

Figure 6. Surface area measurement. An array of 6 parallel lines is superimposed on a microscope field. In this instance the lines make 9 intercepts with the bone surface.

in length. All cancellous bone down to a depth of 2cm. from the periosteum of the cortical bone cap of the iliac crest was surveyed. This is an arbitrary figure but ensures that a large area is counted so as to be accurate and representative, and also ensures that the bone surveyed corresponds in depth to that of a standard iliac crest biopsy.

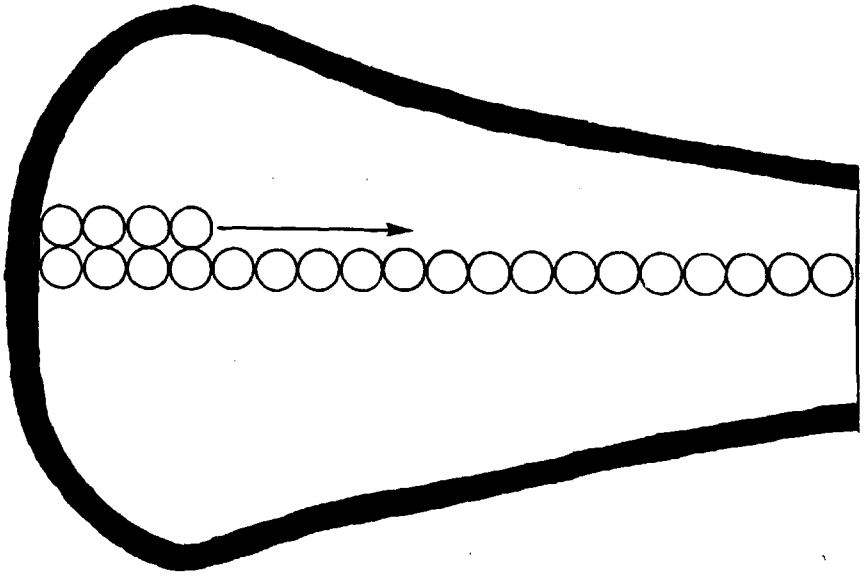
Cortical bone was excluded from the studies, to ensure that changes occurring in a small amount of dense cortical bone did not obscure changes occurring in the cancellous bone. There is no absolute distinction between cortical and cancellous bone so that any distinction is to some extent arbitrary, but it was found that separation of the bone present into a dense bounding shell, giving way abruptly in almost all cases to a lighter spongy network of cancellous bone could be made without difficulty. (Figures 8 and 9). Such distinction was most easily made on gross examination.

In children, cortical bone, the cap of epiphyseal cartilage, and the coarse primary spongiosa were similarly excluded from the studies.

The chosen area of bone was surveyed systematically. The section was mounted on a turntable attached to a mechanical stage of a microscope, and was turned to align the long axis of the section with the direction of lateral travel of the microscope stage. Counting was then carried out, starting adjacent to the "midline"

of the specimen (previously determined by inspection, and approximately bisecting the specimen along its long axis). The section was moved a distance of 1mm. along its long axis between examination of each field, until a full row of fields, extending to 2cm. from the periosteum of the cortical bone cap, had been surveyed. (Figure 7). The next row was then surveyed, the section being moved 1mm. along its short axis between each row, and the process repeated until the whole area of cancellous bone had been surveyed. The diameter of each graticule was 0.9mm.; thus this procedure ensured systematic examination of the section, without overlapping of fields. At the edges of the section, where cortical bone was encountered, partial fields of cancellous bone were counted when measuring bone area. In this case the total number of points falling on cancellous bone and marrow was less than 25. When measuring surfaces partial fields could not be surveyed (as the length of the linear paths falling on the portion of the field surveyed would be unknown). However, because the section was moved 1mm. between each measurement and the diameter of the graticule was 0.9mm. it follows that a further movement of the section of 0.1mm. may be made in order to obtain a full field of cancellous bone, if possible, without overlapping of fields surveyed. This was carried out where necessary. If a full field of cancellous bone could not be obtained by this technique

Figure 7. Diagram to show method of counting, and areas surveyed. The dense black line represents cortical bone.



then it was excluded.

It has been mentioned that in certain cases, counts were made on further sections at intervals along the iliac crest, to study the variations seen.

To enable comparison between the results of this study and those of Beck and Nordin (1960), and Saville (1965) who used trephine biopsies, or sections of similar size, containing both cancellous and cortical bone, an area corresponding in size and position with that which would be sampled by a trephine biopsy was surveyed on a number of sections in this study. This consisted of an area 10 x 6mm. including the cortical bone cap, and bone area was measured on all bone, cortical and cancellous, within this area.

To determine whether loss of bone occurred selectively from any site, in a series of cases separate measurements were made on central and peripheral areas of cancellous bone (v. Results; Experiment 5).

In a series of cases the variation in bone area with increasing depth from the cortical bone cap of the iliac crest was studied.

Number of Counts Made

It is desirable that counts be made over a wide area. The actual number of counts necessary for statistical significance may be calculated (Appendix 2), the theoretical standard error depending on the total

number of counts made (points or intercepts) and the number of counts falling on the sought component. When measuring bone area one set of observations (each of 25 points) was made for each field surveyed. This practice gave a small theoretical standard error, varying from 2% of the bone area where much bone was present and sections large, to 5% of the bone area where little bone was present and sections small. In very large sections alternate fields were counted.

When measuring formation and resorption surface a smaller number of counts (in this case intercepts) is made per field (generally between 0 and 6 as against the 25 counts per field made when measuring bone area). Thus to achieve a satisfactory degree of accuracy more than one set of observations per field is necessary unless the percentage of formation or resorption surface is high, or the section large. The number of counts (intercepts) falling on any component required to produce any given theoretical standard error may be calculated (Appendix 2). In practice it was found that two sets of observations per field produced a theoretical standard error between 5 and 10% of the measured value. In a few cases (where little formation or resorption were present, or the section was small) four sets of observations were necessary. Two sets of observations per field were obtained by rotating the eyepiece and graticule through 90° between first and second

measurements on each field. As cancellous bone shows planes of orientation, between each field the eyepiece was rotated to give a new random orientation of the graticule.

Initially the eyepiece was turned through 90° between first and second sets of observations on each field with the aid of a marker on the other eyepiece. This obliterated part of the microscope field, and it was found that accurate results could be obtained simply by judging the angle of 90° by inspection (Appendix 3).

The theoretical standard error obtained was larger than that for bone area, but the acceptable standard error must be a compromise between the desired degree of accuracy, and the time available. Two such sets of observations on all fields of a section take between three and six hours to perform.

Correction Factors

A number of correction factors have been described to correct bone area or surface area for finite section thickness (Gränkö 1955), and to correct the extent of formation or resorption surface seen for the depth of focus of the microscope (Frost, Villanueva and Roth 1962). Frost argues that because of finite depth of focus of the microscope a greater extent of formation and resorption area is seen than that present in an infinitely thin section. It is dubious whether the correction proposed is valid; its use would in any case

appear to be unnecessary. Measurements will tend to underestimate formation and resorption surface, since very thin osteoid seams and small Howship's lacunae may not be recognised.. Use of the correction decreases the observed values for formation and resorption surface, and would thus tend to accentuate observer error. Therefore this correction was not used.

When measuring bone area or surface area a correction should be made for finite section thickness unless the borders of tissues are perpendicular to the plane of the slide, since an oblique surface will increase the apparent bone area or surface area (Appendix 4).

The size of the error will be decreased by using very thin sections, and in these experiments was reduced by using a thickness of section (20μ) small in relation to average trabecular width (approximately 165μ).

A mathematical correction may be made (Eränkö 1955) if the bodies counted are portions of spheres but this is not applicable to cancellous bone where the structures counted are portions of rods and elongated plates of bone. A simple correction was made when measuring bone area by counting points falling on an oblique surface bounding the left and superior margins of a trabecula, and ignoring those falling on an oblique surface bounding the right and inferior margins of the trabecula (Appendix 4).

When measuring surface area an oblique surface will increase the apparent circumference of a section of a trabecula, thus increasing the measured surface area, (Appendix 4) but the increase is negligible in a thin section, and no correction need be applied.

SECTION FOUR

RESULTS

RESULTS

GENERAL MORPHOLOGY

Cancellous bone consists of a honeycomb like arrangement of thin plates. This pattern, readily appreciated when bone is studied as a macerated gross specimen, cannot be appreciated in the thin sections used in this study, where the thin plates of bone are cut through, and appear as thin elongated structures presenting the more familiar pattern of an interlacing network of thin trabeculae (Figures 8 and 9).

In the young adults studied (Figure 8) trabeculae are numerous and fairly uniform in size and width in any specimen. In a thin section, the trabeculae vary in width from 20 to 500μ, and are arranged in a regular manner. Many trabeculae take origin from medial or lateral cortex, and run obliquely, interlacing to form a series of ogives. The trabeculae are somewhat stouter near their origin from the thick, well defined, cortex.

The appearances in children are similar to those described by Amprino (1937) in the femur. The bone initially formed from the epiphyseal cartilage (the so called primary spongiosa) is coarse, irregular, contains large cartilage remnants, and consists largely of woven bone. A short distance from the epiphyseal cartilage this bone is resorbed and replaced by more

Figure 8. Outline drawing of section of iliac crest from male aged 21 years. (x5).

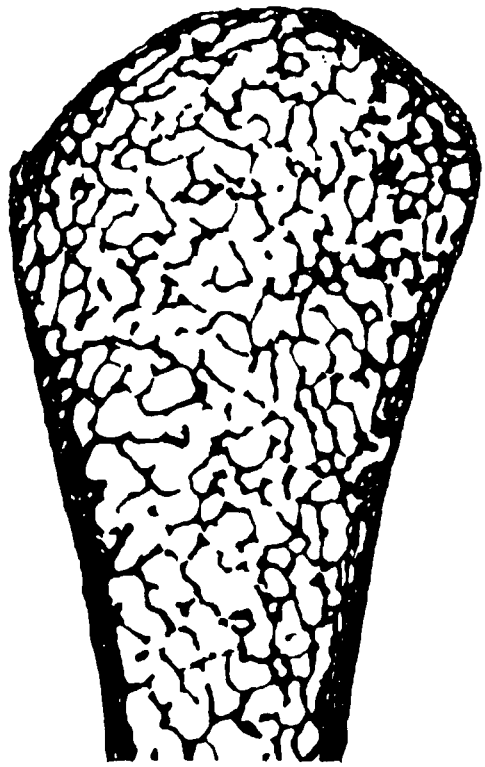
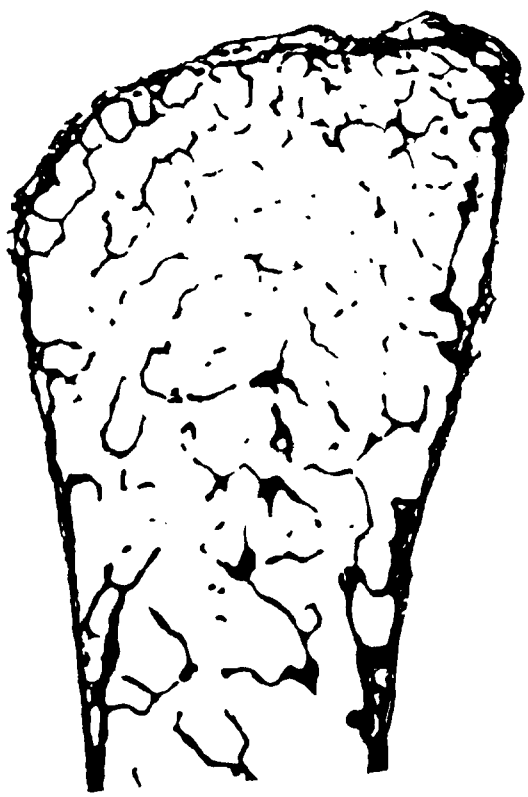


Figure 9. Outline drawing of section of iliac crest from female aged 84 years. (x5).



regular, orientated, trabeculae of lamellar bone (the secondary spongiosa). This resembles adult cancellous bone in its regular arrangement, but the individual trabeculae are sometimes considerably finer.

Considerable changes are evident in sections from aged individuals. (Figure 9). Many trabeculae have disappeared entirely, the remaining trabeculae thus being more widely separated. A similar finding was noted by Eder in vertebral cancellous bone (1960). Many of the remaining trabeculae are extremely thin, while the cortex is reduced to a thin shell.

There is, in addition, a certain coarsening of structure, such that occasional very stout and irregular trabeculae and masses of bone are seen. The pattern seen on microscopical examination is now irregular, some fields being devoid of bone, others showing thin trabeculae, and yet others containing coarse trabeculae. On gross examination, loss of bone is most obvious in the central portion of the spongiosa, but quantitative examination (Experiment 5) showed no selective loss from any part of the spongiosa. No obvious pattern of loss of individual trabeculae was seen, contrasting with the findings in cancellous bone of vertebral body and head of femur. In vertebrae the non weight bearing horizontal trabeculae are lost before the weight bearing vertical

trabeculae (Caldwell and Collins 1961 ; Caldwell 1962). A similar pattern of loss is seen in the femoral head (Hall 1961).

Bone Surfaces

In sections processed by the method described, calcified bone stains deeply and appears purple or purple brown in colour. Portions of the surface are covered by osteoid tissue which in these preparations is virtually unstained by haematoxylin, and appears pink (Figure 10). The surface of the osteoid is smooth, and conspicuous osteoblasts can sometimes be recognised thereon (Figures 10, 11, 12, 13). Neither osteoclasts nor Howship's lacunae were ever seen on the surface of the osteoid, confirming the widely held belief that osteoclasts never attack osteoid.

The osteoid seams appear as discrete structures, although the occasional presence of apparently separate seams on closely related surfaces suggests that these may be parts of a continuous surface of bone formation.

Allowing for artefacts due to obliquity of sectioning, the thickness of an individual osteoid seam is fairly uniform until the ends of the seam, where the osteoid narrows and appears to end abruptly, the adjacent bone showing no obvious layer of uncalcified matrix.

The osteoid seams cover a variable part of the bone surface. The length of individual seams varies

Figure 10. Iliac crest. Undecalcified section stained with haematoxylin and eosin. The calcified bone is stained purplish brown. The upper surface of the trabecula is covered by a layer of relatively unstained, pink, osteoid. Much of the colour has been lost in the photographic processing, and in the original section the calcified bone is more intensely stained. (x 160).

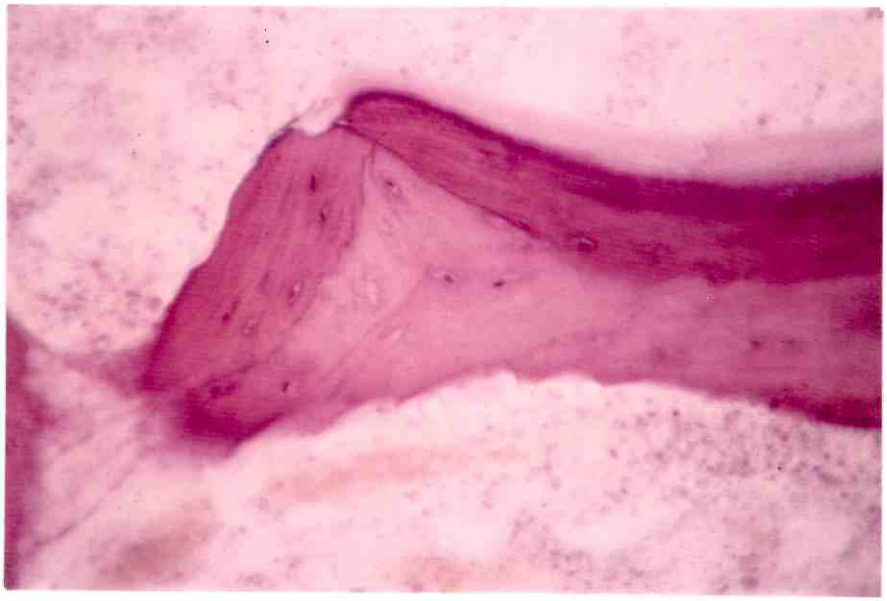


Figure 11. Cancellous bone of iliac crest. The right hand margin of the vertical trabecula is covered by a layer of osteoid tissue; the left hand margin shows an area of bone resorption. Undecalcified section. Haematoxylin and eosin. (x80).

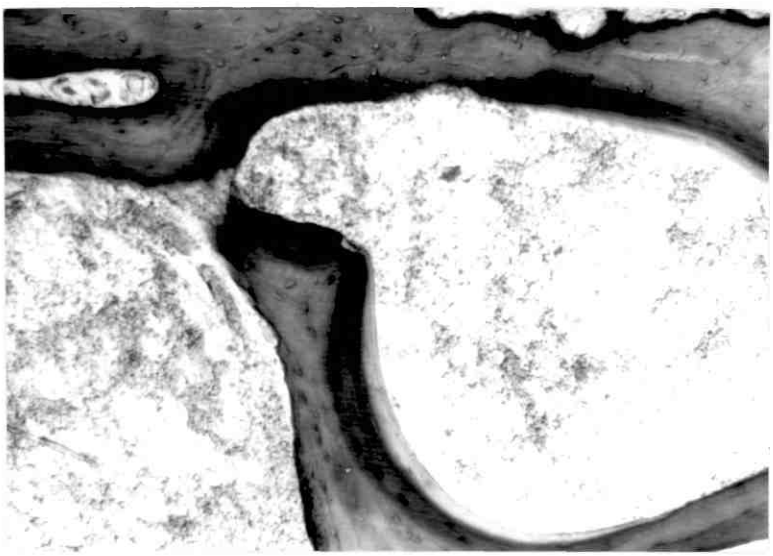


Figure 12. Cancellous bone of iliac crest. The upper surface of the trabecula is covered by a thick osteoid seam; the lower surface has a smooth outline and is considered to be inert. Undecalcified section. Haematoxylin and eosin. (x258).

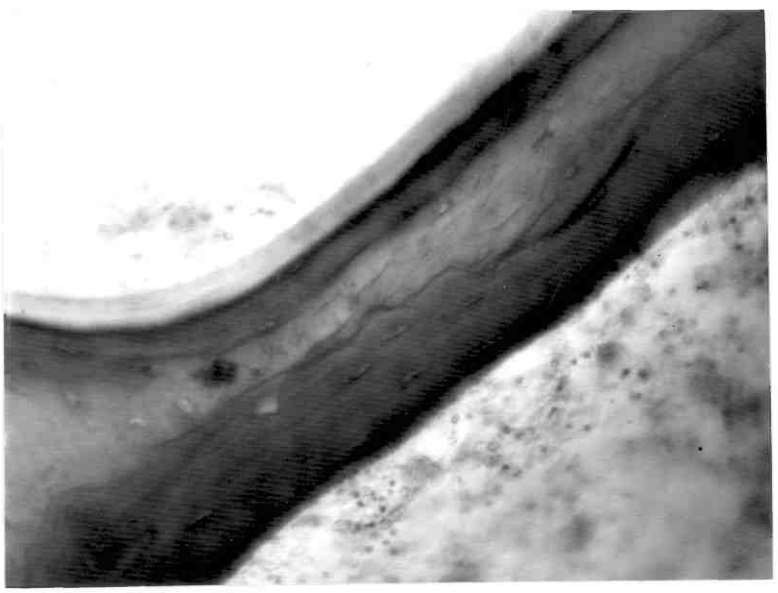
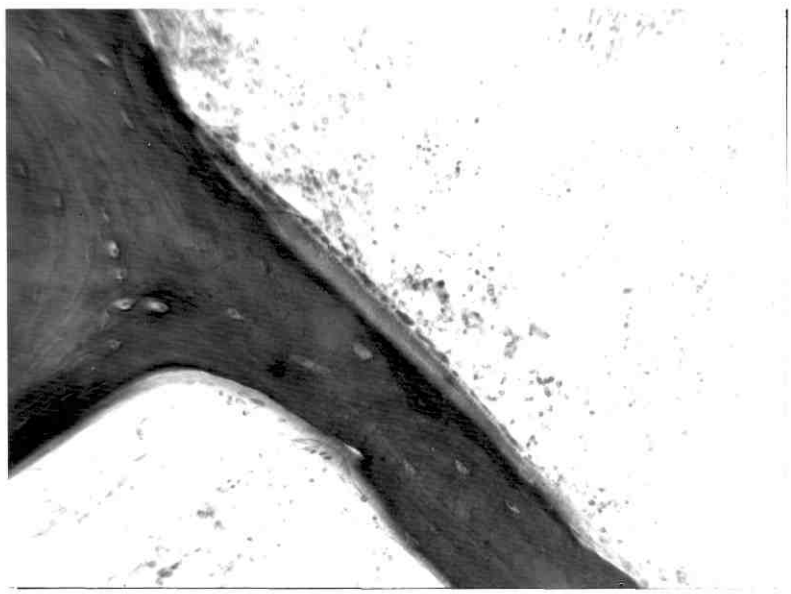


Figure 13. The upper surface of the trabecula is covered by an osteoid seam, on the surface of which is a layer of osteoblasts. Iliac crest. Undecalcified section. Haematoxylin and eosin (x160).



widely in any section, lengths from 70μ or less to 1mm. being seen. Both the length of individual seams and the number of seams varied from case to case. No attempt was made to relate this to extent of surface coverage. Inspection suggested that the peripheral portion of the spongiosa showed a greater surface coverage by osteoid than the central portion. Quantitative measurements were made to confirm this impression (Experiment 5).

Identification of the osteoid was facilitated by the general presence of a line of granular basophilic material, at the junction of the osteoid and calcified bone, the so-called "calcification front". (Robinson and Watson 1955). This is thought to mark the onset of calcification. It is seen nowhere else, and in these preparations stains bright blue.

Oblique surfaces are easily identified. Continuous refocussing is necessary to obtain sharpness of field. They cannot be confused with osteoid seams.

A portion of the trabecular surface shows the irregular, crenated or scalloped, eroded outline of Howship's lacunae, indicating bone resorption (Figures 14 and 15). The appearances vary from deeply punched out classical Howship's lacunae to solitary shallow depressions, which correspond to the individual eroded bays of the scalloped Howship's lacunae. On occasion osteoclasts may be seen in the eroded bays of

Figure 14. Cancellous bone of iliac crest. The upper surface of the trabecula shows an irregular scalloped area indicating bone resorption. Undecalcified section. Haematoxylin and eosin. (x258).

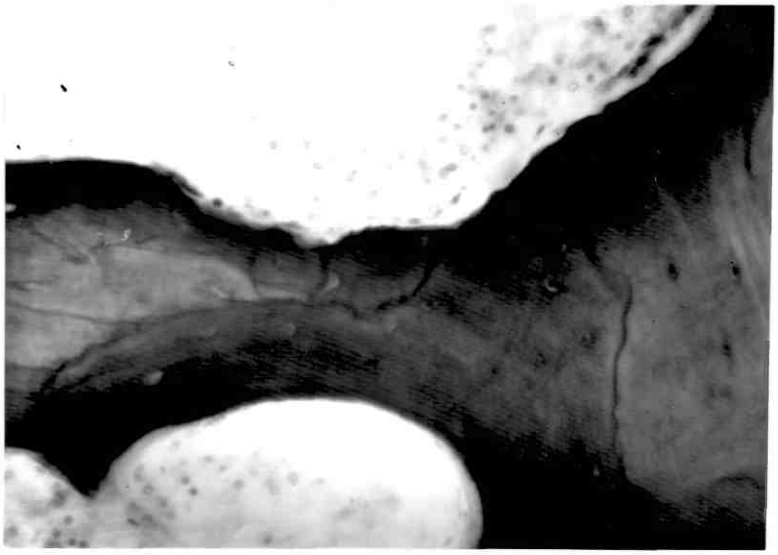
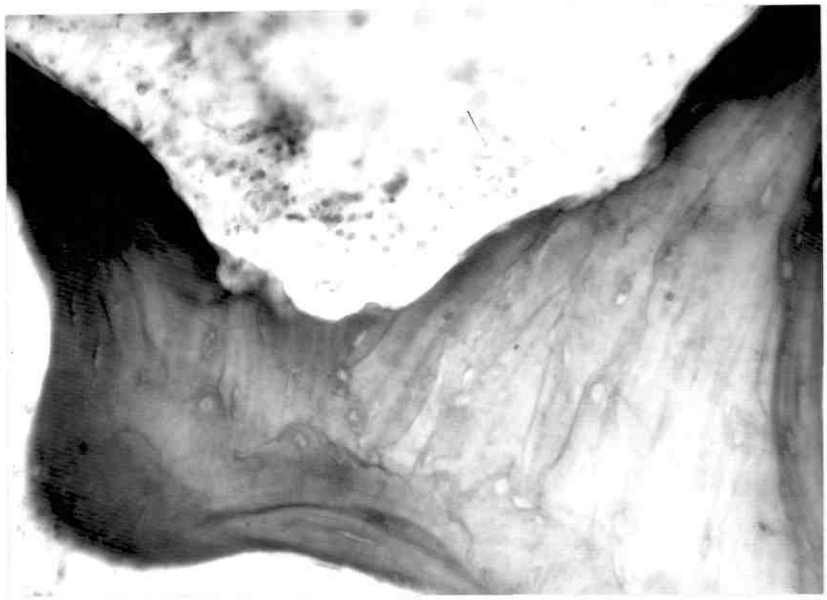


Figure 15. Cancellous bone of iliac crest. The upper surface of the trabecula shows another eroded area of bone resorption. Several Howship's lacunae are visible. Undecalcified section. Haematoxylin and eosin. (x258).



such surfaces.

To exclude the possibility that such surfaces might be artefactual, due to trabecular damage during grinding, sections were examined in water prior to clearing and mounting. Scratches due to grinding are still visible at this stage (Figures 16 and 17). The scratches are very fine, and far narrower than the smallest resorption surface. Where scratches cross trabecular surfaces no irregularity or disruption of surface is ever seen. Gross artefacts due to tearing of the section are rare, and when present are easily distinguished from resorption surfaces. The surface is irregular, but scalloping is absent; the bone edge is not sharp but has a ragged torn appearance (Figure 18).

Areas of resorption are frequently found near areas of bone formation. Sometimes bone resorption on one side of a trabecula is associated with bone formation on the other side (Figure 19), indicative of remodelling of bone.

The remaining portions of the bone surface have a smooth regular outline, without any demonstrable layer of osteoid tissue (Figure 12). These parts of the surface are regarded as inert as far as the processes of bone formation and resorption are concerned.

Figure 16. Ground perspex embedded bone section mounted in water. A scratch due to grinding is apparent. There is no irregularity where this crosses a bone surface. (x160).

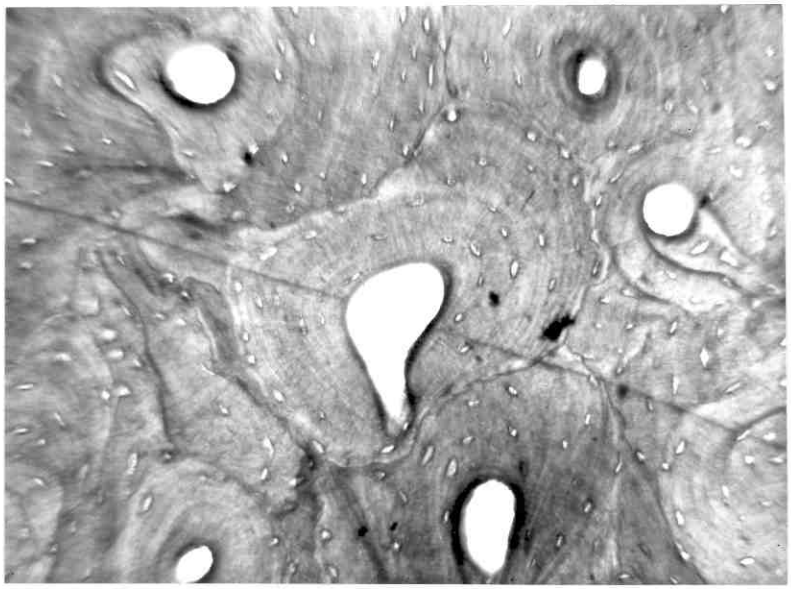


Figure 17. Ground, undecalcified bone section mounted in water. Several grinding scratches are seen. There is no surface irregularity where these cross bone surfaces. (x160).

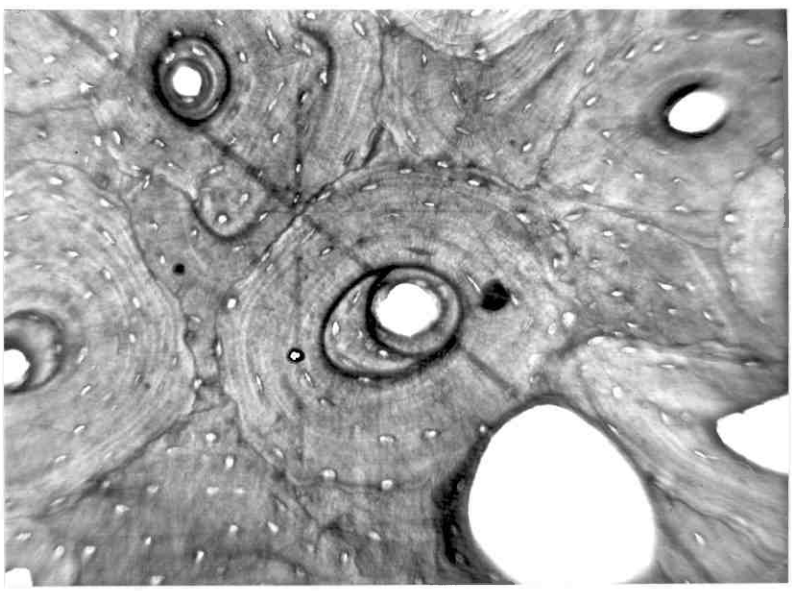


Figure 18. Iliac crest. Gross artefact due to tearing of section. The bone surface is irregular, but the ragged appearance cannot be confused with the "punched out" appearance of bone resorption. (x160).

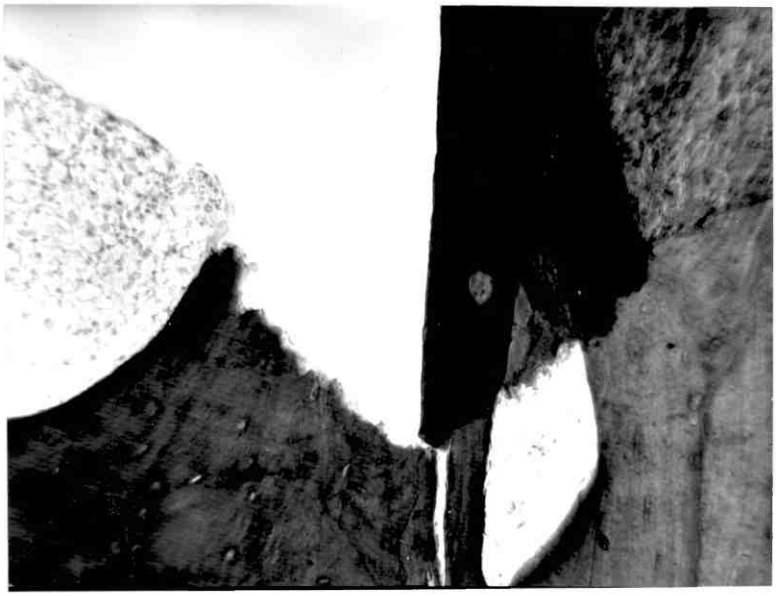


Figure 19. Bone remodelling. On the upper surface of the trabecula is a layer of bone covered by osteoid tissue; on the opposite side of the trabecula is an area of bone resorption. An oblique surface is apparent on the lower trabecular surface to the left. Undecalcified section. Haematoxylin and eosin. (x160).

PRELIMINARY QUANTITATIVE STUDIES

In a study of the present type it is essential that the degree of reproducibility obtained in practice should be known, and should be high. In practice several possibilities of error exist, and several problems of measurement must be considered.

It is of the utmost importance that sampling is adequate, and in this connection the area studied must be of adequate size. It is also essential to be aware of any systematic variation in anatomy, involving any of the parameters measured, within the iliac crest, particularly with regard to increasing distance from the anterior superior iliac spine.

Some of the measurements made involve an element of subjectivity and the possibility of observer error, possibly changing throughout the period of experimentation, must be considered. Finally the inherent accuracy of the counting methods employed should be known.

Some of these factors are amenable to theoretical calculation. To obtain a measure of the others a series of preliminary quantitative experiments (1 - 7) was carried out, the results of which follow.

RESULTS OF PRELIMINARY QUANTITATIVE STUDIES

1. THE DETERMINATION OF THE ACCURACY AND REPRODUCIBILITY OF AREA MEASUREMENTS USING THE ZEISS EYEPIECE I.

The theoretical standard error of bone area measurements may be calculated (Appendix 3). In practice several possible sources of error are apparent:-

a) Any errors inherent in the design of the sampling array.

b) Observer errors.

c) Errors due to inefficient or inadequate sampling of the section, due to the irregular nature of cancellous bone.

This theoretical standard error takes no account of observer error or of inadequate sampling. To obtain an estimate of the degree of accuracy and reproducibility likely to be attained in practice, measurement of bone area was carried out seven times on an area of cancellous bone smaller than that counted in the main survey. Systematic counts were made over this area, varying the section position slightly each time, so that different fields were counted, or changing the orientation of the counting array between series of observations. The results are shown in Table 1.

TABLE 1

Results

a) Original Count

ROW	POINTS ON BONE	TOTAL POINTS COUNTED
A	112	650
B	122	650
C	107	650
D	134	650
TOTAL	475	2600

Bone area = 18.27%

b) Count made after turning grid through 90° .

ROW	POINTS ON BONE	TOTAL POINTS COUNTED
A	113	650
B	120	650
C	104	650
D	139	650
TOTAL	475	2600

Bone area = 18.30%

c) Count made after turning grid through 180°

ROW	POINTS ON BONE	TOTAL POINTS COUNTED
A	137	650
B	104	650
C	126	650
D	104	650
TOTAL	471	2600

Bone area = 18.12

d) Position of area counted moved along long axis of section

ROW	POINTS ON BONE	TOTAL POINTS COUNTED
A	119	650
B	114	650
C	121	650
D	117	650
TOTAL	471	2600

Bone area = 18.12

e) Position of area counted moved along long axis of section

ROW	POINTS ON BONE	TOTAL POINTS COUNTED
A	113	650
B	117	650
C	120	650
D	113	650
TOTAL	463	2600

Bone area = 17.81%

f) Position of area counted moved along short axis of section

ROW	POINTS ON BONE	TOTAL POINTS COUNTED
-	124	650
-	106	650
-	117	650
TOTAL	347	1950

Bone area = 18.04%

c) Position of area counted moved along long and short axes of section

ROW	POINTS ON BONE	TOTAL POINTS COUNTED
-	128	650
-	108	650
-	118	650
TOTAL	354	1950

Bone area = 18.15%

Calculation

Mean bone area	= 18.09%
Standard deviation	= ± 0.20%
Standard error	= ± 0.08%
Coefficient of variation	= 1.1%

Theoretical standard error for a figure of 18.09% derived from a sample of 2600 is ± 0.76%

Conclusion

For section areas and amounts of bone such as this the method is highly accurate and replicable. In counts a), b) and c) the figures for repeat counts on individual rows vary widely but the totals are similar. This shows that the sampling is adequate.

In practice all cancellous bone to a depth of 2cm. from the periosteum of the cortical bone cap was surveyed. This was in almost all cases a considerably

greater area than that surveyed here with a consequent increase in counts, and therefore accuracy.

2. THE DETERMINATION OF THE ACCURACY AND REPRODUCIBILITY OF MEASUREMENTS OF BONE SURFACE AND ITS COMPONENTS USING THE ZEISS EYEPIECE II.

A theoretical standard error cannot be assigned to measurements of the total surface of the bone, and determination of the actual standard deviation and error found is of importance. This is also important for measurements of formation and resorption surface, as although a theoretical standard error may be calculated the identification of formation and resorption surfaces is to some extent subjective, and thus a source of observer error is introduced.

Counts of the total surface area and of formation and resorption surface were carried out five times on a section area comprising 102 fields. The first three counts were made with different random orientations of the counting array in each case, and the other two counts made with the section position altered slightly, i.e., on different fields. The results are shown in Table 2.

TABLE 2

Results

a) Initial Counts

ROW	NO OF FIELDS COUNTED	INTERCEPTS			TOTAL INTERCEPTS (F+R+I)
		WITH FORMATION SURFACE (F)	WITH RESORPTION SURFACE (R)	WITH INERT SURFACE (I)	
A	6	18	18	57	93
B	20	12	28	102	142
C	17	26	47	166	239
D	14	12	40	139	191
E	13	24	11	180	215
FF	12	6	20	154	180
G	8	21	9	77	107
H	7	24	7	76	107
I	5	16	11	67	94
TOTAL	102	159	191	1018	1368

b) 2nd Count. Identical Fields. Altered array positions.

ROW	NO OF FIELDS COUNTED	INTERCEPTS			TOTAL INTERCEPTS
		WITH FORMATION SURFACE (F)	WITH RESORPTION SURFACE (R)	WITH INERT SURFACE (I)	(F+R+I)
A	6	20	19	55	94
B	20	14	31	97	142
C	17	24	47	152	223
D	14	13	36	138	187
E	13	25	11	165	201
F	12	7	27	158	192
G	8	21	9	81	111
H	7	26	9	72	107
I	5	14	10	66	90
TOTAL	102	164	199	984	1347

c) 3rd Count. Identical Fields. Altered array positions.

ROW	NO OF FIELDS COUNTED	INTERCEPTS			TOTAL INTERCEPTS
		WITH FORMATION SURFACE (F)	WITH RESORPTION SURFACE (R)	WITH INERT SURFACE (I)	(F+R+I)
A	6	20	17	49	86
B	20	13	30	101	144
C	17	23	46	155	224
D	14	12	38	141	191
E	13	22	11	175	208
F	12	5	22	155	182
G	8	20	7	72	99
H	7	26	10	79	115
I	5	15	11	61	87
TOTAL	102	156	192	988	1336

d) 4th Count. Area counted moved 0.5 mm. along long axis.

ROW	NO OF FIELDS COUNTED	INTERCEPTS			TOTAL INTERCEPTS
		WITH FORMATION SURFACE (F)	WITH RESORPTION SURFACE (R)	WITH INERT SURFACE (I)	(F+R+I)
A	6	9	13	65	87
B	20	18	25	112	155
C	17	23	61	157	241
D	14	21	29	154	204
E	13	24	13	131	168
F	12	9	19	167	195
G	8	22	7	83	112
H	7	26	8	77	111
I	5	14	8	52	74
TOTAL	102	166	183	998	1347

e) 5th Count. Area counted moved 0.5 mm. each along long and short axes.

ROW	NO OF FIELDS COUNTED	INTERCEPTS			TOTAL INTERCEPTS
		WITH FORMATION SURFACE (F)	WITH RESORPTION SURFACE (R)	WITH INERT SURFACE (I)	(F+R+I)
A	6	8	4	72	84
B	20	36	42	197	275
C	17	35	29	158	222
D	14	15	41	114	170
E	13	20	20	130	170
F	12	19	24	141	184
G	8	6	18	90	114
H	7	36	12	53	101
I	5	9	6	53	68
TOTAL	102	184	196	1008	1388

Surface area

Total length surveyed in each count

= 3.8 x 2 x 102 mm.

Surface area = $\frac{2 \times \text{Total Intercepts}}{\text{Length counted}}$

COUNT	SURFACE AREA (in sq.mm/cu.mm.)
a	3.529
b	3.475
c	3.447
d	3.475
e	3.581

Mean value = 3.501 sq.mm/cu.mm.

Standard deviation = ± 0.054

Coefficient of variation = 1.53%

Standard error = ± 0.024

Formation surface

COUNT	FORMATION SURFACE (as % of total surface)
a	11.62
b	12.17
c	11.68
d	12.32
e	13.26

Mean value = 12.21%

Standard deviation = ± 0.66

Coefficient of variation = 5.41%

Standard error = ± 0.295

or 2% of mean value.

Theoretical standard error for a value of 12.21% from a sample of 1357 = ± 0.89 or 7.3% of mean value.

Resorption surface

COUNT	RESORPTION SURFACE (as % of total surface).
a	13.96
b	14.77
c	14.37
d	13.59
e	14.12

Mean value = 14.16%
Standard deviation = ± 0.35
Coefficient of variation = 2.47%
Standard error = ± 0.16
or 1.1% of mean value

Theoretical standard error for a value of 14.16% from a sample of 1357 = ± 0.95 or 6.7% of mean value

Discussion and Conclusion

Results obtained with the Zeiss eyepiece II are sensitive to the orientation of the eyepiece. The agreement between the figures for corresponding rows in the first three counts shows that the method of randomization of the orientation of the array is adequate, and that the method is valid and accurate.

The results show wide variation between one row and another and demonstrate that the bone is not uniform in structure and activity; the results from one small area (e.g., of biopsy size) may not be representative of the section as a whole. In the

experiments in this study the whole area of the section was sampled, a larger area being counted in most cases than the present area.

The agreement of the totals from the various counts shows that the method of sampling is adequate.

The results show that measurements of total surface area are highly accurate and replicable. The results of formation and resorption surface show a greater but still acceptable variation.

3. THE ACCURACY AND RELIABILITY OF DEMONSTRATION OF OSTEOID BY HAEMATOXYLIN AND EOSIN STAINING IN UNDECALCIFIED SECTIONS.

1. Osteoid was identified in unstained undecalcified sections by its translucent appearance and birefringence. The sections were then stained with haematoxylin and eosin. In all cases the pale staining tissue on the trabecular surface was found to have the same limits and thickness as the material identified as osteoid in the unstained section.

2. A series of undecalcified sections was bisected. One half was stained by von Kossa's method and the other with haematoxylin and eosin. On general examination, the amounts of osteoid tissue were similar in both preparations, both in length of surface covered, and in length and thickness of individual seams. In one case an osteoid seam on a trabecular surface was bisected and the thickness was identical (7μ) in each preparation.

3. Microradiographs were prepared from 70μ thick undecalcified sections which were subsequently stained with haematoxylin and eosin. Comparison of the two preparations showed that what was recognised as osteoid in the stained section was uncalcified, while the boundaries of the calcified bone were identical in the two preparations. This was confirmed by a series of measurements of trabecular thickness at corresponding points in the two preparations using a micrometer eyepiece.

The results are shown in Table 3.

The correspondence between the width of tissue staining deeply in the stained section and therefore presumed calcified and the width of calcified tissue in the microradiograph will be noted.

Conclusion

The pale staining material recognised as osteoid in haematoxylin and eosin stained undecalcified sections is not calcified. The dark staining material recognieed as bone is calcified. The junction between the two corresponds to the junction of calcified and non calcified tissue. This method agrees with other techniques for the demonstration of osteoid and is reliable for use in its quantitative demonstration.

TABLE 3

Agreements of measurements in stained section and corresponding microradiograph.

STAINED SECTION			MICRORADIOGRAPH
Width of presumed calcified tissue (i.e., bone) in μ	Width of presumed uncalcified tissue (i.e., osteoid) in μ	Total width of bone and osteoid in μ	Width of calcified tissue (bone) in μ
85.8	15.0	100.8	85.8
78.0	20.0	98.0	78.0
124.8	19.5	144.3	124.8
121.6	13.5	135.1	121.6
230.0	30.0	260.0	230.0
113.5	19.0	132.5	113.5
185.0	20.0	205.0	185.0
195.0	20.0	215.0	190.0
130.0	20.0	150.0	130.0
50.0	10.0	60.0	50.0

4. TO CHECK THAT CRITERIA OF RECOGNITION OF FORMATION AND RESORPTION SURFACES DID NOT CHANGE THROUGHOUT THE PERIOD OF EXPERIMENTATION.

The recognition of surfaces of formation and particularly resorption involves an element of subjectivity. Occasional osteoid seams are very thin, while Howship's lacunae are recognised solely by the irregularity of the bone surface, which may be slight. As a safeguard that no gross subjective change in the criteria of recognition of these surfaces occurred during the period of experimentation, counts were repeated at varying intervals on random sections, or parts of sections (not all of which were suitable for use in the main survey) without reference to the original results. The results obtained are shown in Table 4.

Conclusion

From the table it is seen that changes observed are slight and within the limits of experimental error. There is no evidence of any subjective change in recognition of formation and resorption surfaces during the period of experimentation.

Table 4. Repeated counts after varying time intervals.

Case No.	Date of 1st Count	Intercepts with Formation Surface	Intercepts with Resorption Surface	Intercepts with Inert Surface	Total	Date of 2nd Count	Intercepts with Formation Surface	Intercepts with Resorption Surface	Intercepts with Inert Surface	Total
5591	Jan.1964	113 12.80%	74 8.38%	696 78.82%	883	May 1964	120 13.70%	78 8.90%	678 77.40%	876
5360	Apr.1964	Not Counted	284 10.35%	Not Counted	2743	March 1965	Not Counted	262 9.47%	Not Counted	2757
10738Q	Apr.1964	75 5.81%	70 5.42%	1146 88.77%	1291	March 1965	64 5.32%	69 5.74%	1069 88.94%	1202
10898	May 1964	73 4.81%	Not Counted	Not Counted	1687	May 1965	86 4.33%	Not Counted	Not Counted	1790
7020	Sept. 1964	80 4.60%	Not Counted	Not Counted	1739	Nov. 1964	83 4.60%	Not Counted	Not Counted	1742
11342	Dec. 1964	162 8.44%	198 10.31%	1560 81.25%	1920	Jan. 1965	156 8.12%	190 9.89%	1575 81.99%	1921
6215	Oct 1964	331 20.32%	256 15.72%	1042 63.96%	1629	March 1965	319 19.08%	249 14.89%	1056 66.03%	1672
11255	Jan.1965	50 17.61%	25 8.81%	209 73.58%	284	March 1965	51 18.95%	25 9.98%	193 71.07%	269

All percentage figures given are as percentages of the total counts in each instance.

5. THE VARIATION IN BONE AREA AND FORMATION AND RESORPTION SURFACE BETWEEN PERIPHERAL AND CENTRAL AREAS OF SPONGIOSA OF ILIAC CREST.

General microscopical examination suggested that with aging bone might be lost first from the central portion of the spongiosa, and that osteoid coverage was greatest in the peripheral portion. This was investigated by quantitative examination of the central and peripheral areas of cancellous bone in eight individuals aged 20 - 29, and nine individuals aged 70 - 79. A division of the bone into these areas was made by a line parallel to, and 3mm. from the superior cortex, and lines running midway between the midline of the section and the lateral and medial cortices. The bone enclosed by these three lines was adjudged to be central. In practice results from individual fields were already available, and in each horizontal row between midline and lateral or medial cortex, the dividing line was drawn so as to divide the number of fields equally into central and peripheral groups. If a row contained an odd number of fields, then the greater number of fields was included with the central group.

The results are tabulated in Table 5, and shown graphically in Figure 20.

Table 5(a). VARIATION IN BONE AREA BETWEEN PERIPHERAL AND CENTRAL AREAS OF ILIAC CREST CANCELLOUS BONE.

BONE AREA

CASE	PERIPHERAL ZONE (P) (in %)	CENTRAL ZONE (C) (in %)	DIFFERENCE (P - C) (in %)
10748	26.72	29.40	-2.68
5590	30.32	24.95	5.37
7160	22.61	22.34	0.27
10762	24.03	21.76	2.27
6144	26.21	20.97	5.24
10788	22.17	18.82	3.35
7114	18.35	15.00	3.35
5591	16.13	14.10	2.03
7396	21.68	18.63	3.05
6145	17.35	16.54	0.81
6087	13.75	14.13	-0.38
5385	16.68	12.47	4.21
10898	10.30	11.42	-1.12
5733	10.70	7.69	3.01
10841P	11.06	6.44	4.62
6146	9.97	5.59	4.38
10711	12.60	5.34	7.26

Table 5(b). VARIATION IN FORMATION SURFACE BETWEEN PERIPHERAL AND CENTRAL AREAS OF ILIAC CREST CANCELLOUS BONE

VARIATION SURFACE

CASE	PERIPHERAL ZONE (P) (in %)	CENTRAL ZONE (C) (in %)	DIFFERENCE (P - C) (in %)
10748	6.51	3.53	2.98
5590	-	-	-
7160	-	-	-
10762	5.28	2.55	2.73
6144	16.72	11.40	5.32
10783	12.79	9.71	3.08
7114	4.66	4.44	0.22
5591	15.07	10.54	4.53
7396	57.83	54.92	2.91
6145	1.59	1.71	-0.12
6087	36.63	24.06	12.57
5385	15.87	5.33	10.54
10898	5.29	3.15	2.14
5733	19.04	16.84	2.20
10841	8.56	6.46	2.10
6146	3.58	1.19	2.39
10711	10.0	5.34	4.66

Table 5(c). **VARIATION IN RESORPTION SURFACE BETWEEN PERIPHERAL AND CENTRAL AREAS OF ILIAC CREST CANCELLOUS BONE**

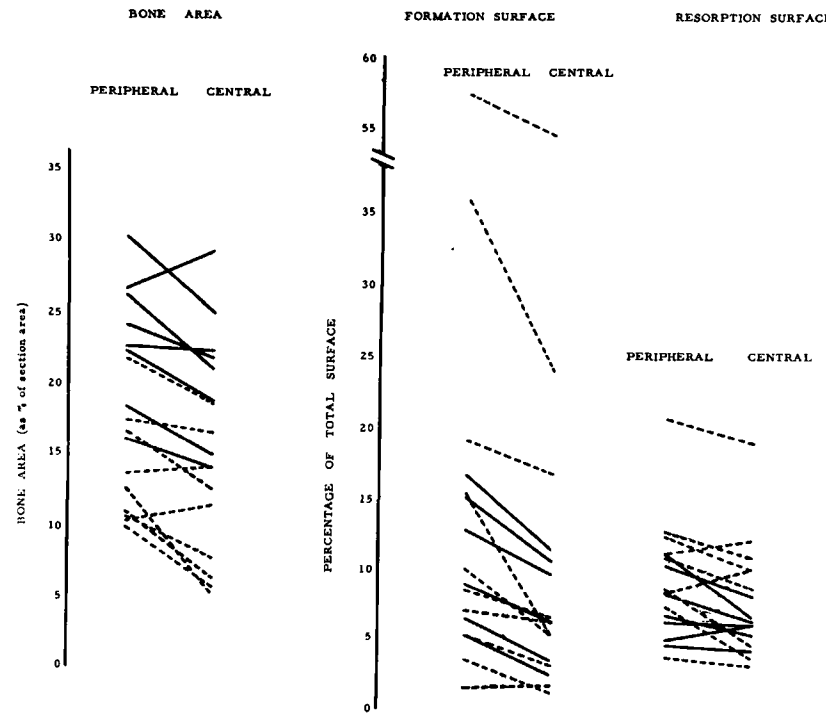
RESORPTION SURFACE

CASE	PERIPHERAL ZONE (P) (in %)	CENTRAL ZONE (C) (in %)	DIFFERENCE (P - C) (in %)
10748	5.14	6.40	-1.26
5590	-	-	-
7160	-	-	-
10762	6.84	5.49	1.35
6144	10.28	8.48	1.80
10788	8.52	6.65	1.87
7114	7.03	6.25	0.78
5591	9.93	6.71	3.22
7396	12.46	10.33	2.13
6145	3.94	3.18	0.76
6087	12.73	10.97	1.76
5385	8.41	10.21	-1.80
10898	10.91	8.80	2.11
5733	20.75	15.60	5.15
10841	11.20	12.08	-0.88
6146	7.39	3.85	3.54
10711	8.65	4.70	3.95

Figure 20. Variations in bone area, and formation and resorption surfaces, between peripheral and central areas of iliac crest cancellous bone. Each line represents one case.

———————— 20 - 29 age group

– – – – – – – 70 - 79 age group



A test of the significance of these differences was carried out (Appendix 5).

1. Peripheral bone area > Central bone area

$t = 4.21$. Degrees of freedom 16 .

This is highly significant ($P < 0.001$).

2. Formation surface - Peripheral > Central

$t = 4.35$. Degrees of freedom 14.

This is highly significant ($P < 0.001$).

3. Resorption surface - Peripheral > Central

$t = 3.192$. Degrees of freedom 14.

This is significant ($P < 0.01 > 0.005$)

Discussion

The results show that the peripheral area of cancellous bone is significantly denser than the central core in the combined group of young and old adults. The graph indicates that the difference is not greater in the elderly. Thus, although on macroscopical examination of a section, loss of bone is more obvious in the central portion of the cancellous bone, this is not because bone is preferentially lost from this area, but because there is less bone in this area initially.

The peripheral shell similarly shows a greater formation and resorption surface than the central area. This does not necessarily mean higher bone formation and resorption rates at the periphery, as these depend upon the linear rate at which bone is formed at, or removed from, any given site as well as on the extent

of bone formation or resorption, but it is very likely that this is in fact the case.

These variations in bone area and formation and resorption surface are most important in the interpretation of small diagnostic bone biopsies which may consist only of the peripheral shell of cancellous bone.

6. THE VARIATION IN BONE AREA OF CANCELLOUS BONE OF ILIAC CREST WITH INCREASING DEPTH OF SAMPLE.

Most iliac crest biopsies are taken with a trephine, introduced vertically through the crest. This investigation was planned to study the variation in amount of bone in such a core sample with increasing depth of the specimen.

Counts were made of the amount of cancellous bone present in four areas of each iliac crest, corresponding to the area sampled by a biopsy trephine, (Figure 21). Divisions were made at 5mm. intervals and the area sampled was 6mm. in width.

The results are shown in Table 6.

An analysis of variance showed significant differences between the four areas of bone ($0.05 > P > 0.01$). The mean figures show a significant fall from area A to B, with a subsequent increase from B to C to D. The individual figures show considerable variations from one area to another. These variations and their magnitude should be borne in mind when interpreting a biopsy sample.

Figure 21. Diagram to show areas counted in Experiment 6.

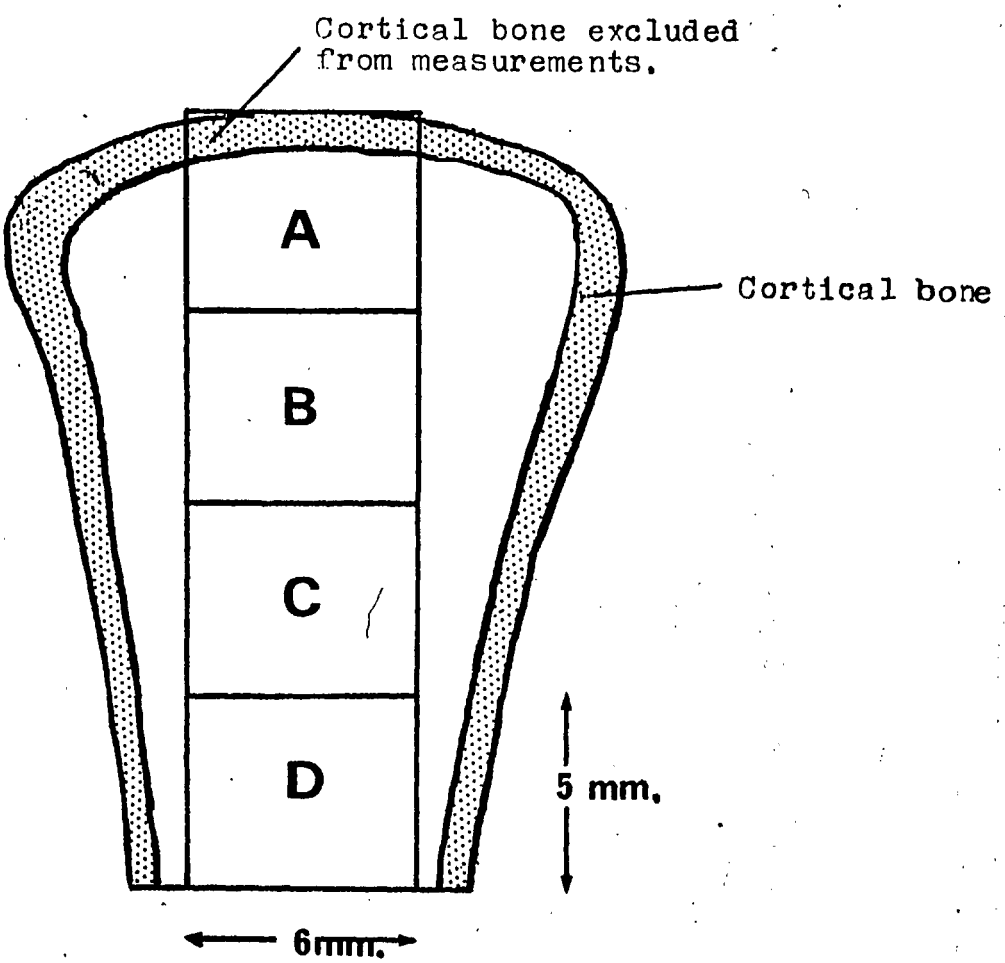


TABLE 6. VARIATION OF BONE AREA WITH INCREASING DEPTH FROM ILIAC CREST

CASE NUMBER	AREA A			AREA B			AREA C			AREA D		
	POINTS ON BONE	TOTAL COUNTED	BONE AREA (%)	POINTS ON BONE	TOTAL COUNTED	BONE AREA (%)	POINTS ON BONE	TOTAL COUNTED	BONE AREA (%)	POINTS ON BONE	TOTAL COUNTED	BONE AREA (%)
7114	94	550	17.1	104	750	13.9	87	750	11.6	145	660	22.0
5590	101	450	22.4	176	750	23.5	188	750	25.1	111	392	28.3
6144	129	575	22.4	137	750	18.3	173	750	23.1	155	675	23.0
5733	49	575	8.5	57	750	7.6	84	750	11.2	87	750	11.6
6846	65	375	17.3	127	750	16.9	165	750	22.0	152	750	20.3
7160	109	550	19.8	149	750	19.9	155	750	20.7	160	750	21.3
5591	123	575	21.4	105	750	14.0	116	750	15.5	92	750	12.3
6145	118	575	20.5	95	750	12.7	107	750	14.3	151	750	20.1
6608	56	250	22.4	123	750	16.4	157	750	20.9	118	750	15.7
6087	74	425	17.4	116	750	15.5	119	750	15.9	103	550	18.7
MEAN			18.92%			15.87%			18.03%			19.33%

7. THE DETERMINATION OF THE VARIATION IN MEASURED PARAMETERS.

a) WITHIN AN INDIVIDUAL BLOCK

b) ALONG THE ILIAC CREST

Counts were carried out to determine bone area, and percentage of formation and resorption surface a) on multiple sections from individual blocks.

b) From sections taken at varying intervals from each other along the iliac crest.

The results are shown in Tables 7 - 12.

Discussion

Within a block the mean variation of bone area observed was 2.7% (as a percentage of the mean figure for each block), that of formation surface 6.0%, and that of resorption surface 7.9% (each of these also expressed as a percentage of the mean figure for each block.) These figures are small, and expressed in absolute terms become very small indeed. It may be concluded that a single section is representative of the block (standard thickness 0.75 cm.) from which it is taken.

As would be expected greater variations are seen between blocks taken along the whole length of the anterior iliac crest, at distances up to 7 cm. behind the anterior superior iliac spine. The variation seen in bone area is less than that seen in formation and resorption surfaces. On occasion quite wide variation was seen in these parameters, although in other cases no significant difference was seen. Generally the variation

Table 7. Variation of bone area in multiple sections from individual blocks.

CASE NO.	BONE AREA	MEAN VALUE	PERCENTAGE VARIATION FROM MEAN
11276	24.24		1.0%
	23.73		3.1%
	24.47 N.S.D.	24.48	0%
	25.03		2.2%
	24.93		1.9%
11576	24.44		0.2%
	24.61 N.S.D.	24.48	0.5%
	24.59		0.5%
	24.27		0.9%
6215	12.38		13.4%
	14.78	14.30	3.4%
	14.96		4.6%
	15.07		5.4%
10779	18.25 N.S.D.	18.52	1.5%
	18.79		1.5%

Mean variation = 2.7%

Standard deviation = 4.6%

There is a significant ($P < 0.05$) difference between a pair of observations linked thus :-]

N.S.D = No significant difference in this group of observations.

Table 3. Variation of formation surface in multiple sections from individual blocks.

CASE NO.	FORMATION SURFACE	MEAN VALUE	PERCENTAGE VARIATION FROM MEAN
11276	19.01]		11.9%
	24.31]		12.7%
	21.98	21.58	1.9%
	20.19		6.4%
	22.39		3.8%
11576	10.96		2.8%
	11.25 N.S.D.	11.27	0.2%
	10.77		4.4%
	12.11		7.5%
6215	19.08		11.6%
	17.49 N.S.D.	17.09	2.3%
	15.96		6.6%
	15.81		7.5%
10779	14.50 N.S.D.	13.76	5.4%
	13.02		5.4%

Mean variation = 6.0%

Standard deviation = 7.3%

There is a significant ($P < 0.05$) difference between a pair of observations linked thus :-]

N.S.D = No significant difference in this group of observations.

Table 9. Variation of resorption surface in multiple sections from individual blocks.

CASE NO.	RESORPTION SURFACE	MEAN VALUE	PERCENTAGE VARIATION FROM MEAN
11276	10.72		7.9%
	12.71		9.2%
	11.05 N.S.D.	11.64	5.1%
	10.34		11.2%
	13.37		14.9%
11576	7.01		25.5%
	9.35	9.41	0.6%
	11.14		18.4%
	10.14		7.8%
6215	14.89		3.4%
	14.04 N.S.D.	14.40	2.5%
	14.81		2.8%
	13.87		3.7%
10779	8.06 N.S.D.	8.31	2.9%
	8.55		2.9%

Mean variation = 7.9%

Standard deviation = 10.8%

There is a significant ($P < 0.05$) difference between a pair of observations linked thus :-

N.S.D = No significant difference in this group of observations.

Table 10. Variation of bone area in sections from different sites along iliac crest.

CASE & BLOCK NO.		DISTANCE BEHIND ANT. SUP. ILIAC SPINE (in cm.)	BONE AREA	MEAN VALUE	PERCENTAGE VARIATION FROM MEAN
10701	C	2.5	25.86	22.76	13.6%
	R	3.25	24.33		6.9%
	P	4.75	21.86		3.9%
	O	5.5	18.99		16.6%
10766	N	1.75	17.23	18.62	7.5%
	O	2.5	15.72		15.6%
	P	3.25	17.24		7.4%
	Q	4.0	19.77		6.2%
	R	4.75	23.13		24.2%
11275	B	3.25	13.73	14.51	5.4%
	C	4.0	15.29 N.S.D.		5.4%
11008 Right	R	2.5	12.54	11.62	7.9%
	S	3.25	10.70		7.9%
11008 Left	M	2.5	14.14	13.96	1.3%
	N	3.25	13.78 N.S.D.		1.3%
10711	S	2.0	14.48	11.11	30.3%
	Q	4.0	11.08		0.3%
	O	6.0	9.36		15.8%
	N	7.0	9.52		14.3%
10748	N	1.5	27.80	26.19	6.2%
	O	2.25	23.92		8.7%
	P	3.0	26.85		2.5%
10738	N	1.5	12.66	14.58	13.2%
	Q	3.75	16.49		13.2%
			Mean variation		9.8%
			Standard deviation		12.4%

There is a significant ($P < 0.05$) difference between a pair of observations linked thus :-]

N.S.D = No significant difference in this group of observations.

Table 11. Variation of formation surface in sections from different sites along iliac crest.

CASE & BLOCK NO.	DISTANCE BEHIND ANT.SUP. ILIAC SPINE (in cm.)	FORMATION SURFACE	MEAN VALUE	PERCENTAGE VARIATION FROM MEAN
10701 S	2.5	9.56	8.40	13.8%
R	3.25	7.85		6.5%
P	4.75	9.78		16.4%
Ø	5.5	6.40		23.8%
10766 N	1.75	8.21	8.61	4.6%
O	2.5	7.47		13.2%
P	3.25	6.46		25.0%
Q	4.0	12.34		42.7%
R	4.75	-		
11275 B	3.25	5.05	5.37	5.9%
C	4.0	5.69 N.S.D.		5.9%
11008 R	2.5	10.17	8.76	16.1%
Right S	3.25	7.35		16.1%
11008 M	2.5	8.12	8.14	0.1%
Left N	3.25	8.15 N.S.D		0.1%
10711 S	2.0	11.87	9.98	18.9%
Q	4.0	11.35		13.7%
O	6.0	8.55		14.3%
N	7.0	8.14		18.4%
10748 N	1.5	5.12	6.92	26.1%
O	2.25	7.16		3.5%
P	3.0	8.48		22.5%
10738 N	1.5	8.45	7.02	20.3%
Q	3.75	5.58		20.3%
		Mean variation		15.1%
		Standard deviation		18.3%

There is a significant ($P < 0.05$) difference between a pair of observations linked thus :-]

N.S.D = No significant difference in this group of observations.

Table 12. Variation of resorption surface in sections from different sites along iliac crest.

CASE & BLOCK NO.	DISTANCE BEHIND ANT. SUP. ILIAC SPINE (in cm.)	RESORPTION SURFACE	MEAN VALUE	PERCENTAGE VARIATION FROM MEAN
10701 S	2.5	9.78	8.43	16.0%
R	3.25	7.14		15.3%
P	4.75	9.13		8.3%
O	5.5	7.67		9.0%
10766 N	1.75	5.91	7.86	24.8%
O	2.5	7.81		0.6%
P	3.25	6.91		12.1%
Q	4.0	10.80		37.4%
R	4.75	-		
11275 B	3.25	5.94 N.S.D.	6.75	12.0%
C	4.0	7.55		12.0%
11008 R	2.5	9.84	9.88	0.4%
Right S	3.25	9.92 N.S.D.		0.4%
11008 M	2.5	9.24	9.11	1.4%
Left N	3.25	8.97 N.S.D		1.4%
10711 S	2.0	8.05	6.64	21.2%
Q	4.0	6.53		1.7%
O	6.0	4.79		27.9%
N	7.0	7.20		8.4%
10748 N	1.5	5.73	6.15	0.7%
O	2.25	5.73 N.S.D		0.7%
P	3.0	6.99		1.4%
10738 N	1.5	8.53	7.06	20.8%
Q	3.75	5.58 N.S.D		20.8%
		Mean variation		11.1%
		Standard deviation		15.4%

There is a significant ($P < 0.05$) difference between a pair of observations linked thus :-]

N.S.D = No significant difference in this group of observations.

seen was greater with increasing distance between blocks. No consistent pattern of variation was seen in bone area. In some cases the density of the cancellous bone decreased from before backwards, in other cases the densest bone was found in the posterior block, while in others no pattern emerged. The extent of formation and resorption surface similarly showed no consistent pattern of variation along the iliac crest.

These results suggest that within the limits examined, the site in iliac crest from which a block or biopsy is taken is immaterial.

The mean variation seen in all three parameters is greater than that seen within an individual block. The mean variation in bone area was 9.8%, in formation surface 15.1%, and in resorption surface 11.1% (as percentages of the mean figure) but these are again small in absolute terms, amounting, for example, to a variation of $\pm$ 2% in a bone area of 20%.

These variations are small in relation to those which may be seen between individual cases (Tables 14, 22 and 25. Figures 22, 30 and 33.) even in the same age group. Thus significant information may be derived from a single section, which may be said to be representative in a general fashion of the individual from which it is taken. The results from a single specimen should however be interpreted with caution, particularly if the results are at the limit of the normal range for the age group.

This should be borne in mind in the interpretation of diagnostic bone biopsies.

Conclusion

Small variations in bone area, and in percentage of formation and resorption surface are seen within an individual block, and larger variations between sites along the iliac crest. The variations seen are smaller than those which may be seen between individual cases, but should be borne in mind in the interpretation of an individual specimen (e.g., biopsy).

RESULTS OF QUANTITATIVE STUDY OF AGE CHANGES.

Table 13. MEAN VALUES IN EACH DECADE

AGE (in years)		0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80 +
BONE AREA (as % of section area)		18.48	20.45	21.58	21.73	19.16	18.12	16.73	14.01	14.38
SURFACE AREA/UNIT VOLUME OF TISSUE (in sq.mm/cu.mm)		4.19	3.50	3.85	3.61	3.70	3.43	3.20	2.63	2.72
SURFACE AREA/UNIT VOLUME OF SOLID BONE (in sq.mm/cu.mm)		22.68	17.31	18.14	16.75	20.11	19.37	19.78	19.77	19.22
FORMATION SURFACE	PERCENTAGE OF TOTAL SURFACE	14.23	14.70	11.06	13.80	9.30	8.31	11.83	14.42	12.11
	SURFACE AREA/UNIT VOLUME OF TISSUE (in sq.mm/cu.mm)	0.60	0.51	0.42	0.49	0.33	0.29	0.38	0.42	0.32
	SURFACE AREA/UNIT VOLUME OF SOLID BONE (in sq.mm/cu.mm)	3.29	2.61	1.97	2.28	1.87	1.57	2.35	2.71	2.37
RESORPTION SURFACE	PERCENTAGE OF TOTAL SURFACE	19.76	12,38	8.43	12.02	9.31	11.62	12.71	10.21	12.55
	SURFACE AREA/UNIT VOLUME OF TISSUE (in sq.mm/cu.mm)	0.86	0.44	0.33	0.44	0.35	0.40	0.40	0.27	0.34
	SURFACE AREA/UNIT VOLUME OF SOLID BONE (in sq.mm/cu.mm)	4.67	2.21	1.51	2.01	1.89	2.20	2.52	2.03	2.40

AGE CHANGES IN BONE AREA

All parameters studied show considerable variation between cases, even in the same age group. The significance of an individual result may be limited, and more significance may be ascribed to mean results, and to the trend of individual results.

Individual results from 93 normal persons aged from 3 months to 93 years are given in Table 14, and shown graphically in Figure 22. Considerable biological variation is evident from the graph of individual results, where a wide scatter of values is seen, at all ages, the extent of variation being broadly similar at all ages.

Both mean figures and trend of individual results show that bone area rises from childhood to adulthood, the cancellous bone becoming stouter and denser. The highest individual value found is 30.03% in a 16 year old girl, and the lowest figure 8.25% in a 72 year old man. After reaching a maximum in early adult life the amount of bone falls with age. The mean value for the 4th decade is 21.73%, and that for the 8th decade is 14.01%. Thus over 40 years, nearly 40% of the bone originally present has been lost. No further loss of bone appears to occur after the age of 80 years. It has been suggested that there may be a limit to the amount of

Figure 22. Bone area. Individual results plotted against age.

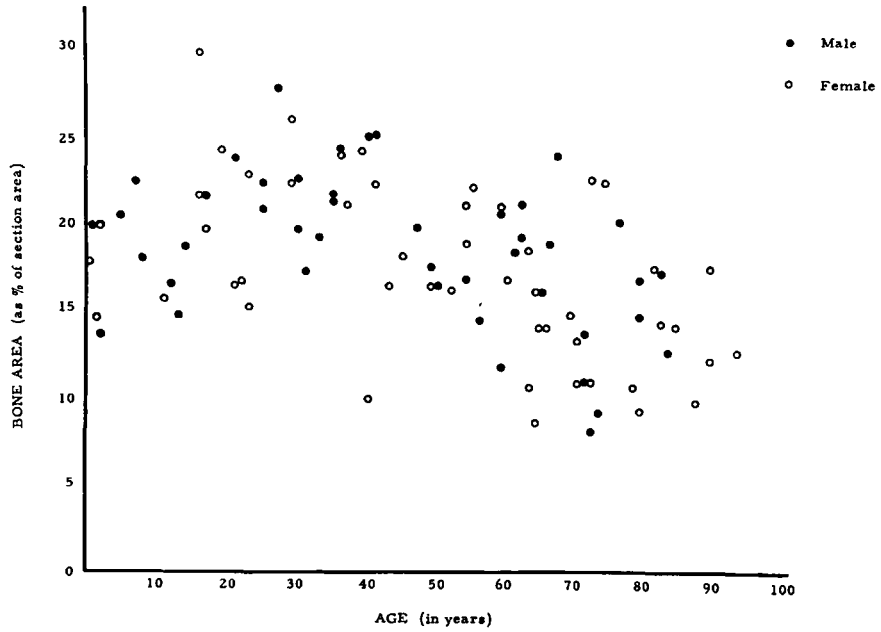


Table 14(a) BONE AREA. INDIVIDUAL RESULTS. AGES 0 - 29

CASE NO.	AGE (in yrs)	SEX	BONE AREA (in %)
5916	8	M	18.14
7156	2	M	13.82
6607	5	M	20.64
7018	11mos	M	20.02
6102	7	M	22.63
MEAN			19.05
7157	1	F	14.68
11922	3mos	F	17.87
5862	1	F	20.00
MEAN			17.52
MEAN FOR DECADE 18.48			

CASE NO.	AGE (in yrs)	SEX	BONE AREA (in %)
5391	17	M	21.73
10969	12	M	16.74
7162	14	M	18.79
6782	13	M	14.89
MEAN			18.03
10794	17	F	19.80
11263	11	F	15.77
11731	16	F	30.03
11576	19	F	24.44
11719	16	F	21.83
MEAN			22.37
MEAN FOR DECADE 20.45			

CASE NO.	AGE (in yrs)	SEX	BONE AREA (in %)
5590	27	M	27.88
6144	21	M	23.98
5591	23	M	15.28
11155	25	M	22.53
10788	25	M	20.98
MEAN			22.13
7114	22	F	16.79
10748	29	F	26.19
10762	23	F	23.05
10820	21	F	16.58
7160	28	F	22.50
MEAN			21.02
MEAN FOR DECADE 21.58			

Table 14(b) BONE AREA. INDIVIDUAL RESULTS. AGES 30 - 59

CASE NO.	AGE (in yrs)	SEX	BONE AREA (in %)
6846	30	M	19.80
5359	33	M	19.43
10701	30	M	22.76
11117	35	M	21.77
11110	35	M	21.69
6011	31	M	17.45
6009	36	M	24.56
MEAN			21.07
6608	39	F	24.35
12080	37	F	21.29
11387	36	F	24.20
MEAN			23.28
MEAN FOR DECADE 21.73			

CASE NO.	AGE (in yrs)	SEX	BONE AREA (in %)
5732	40	M	25.23
6278	47	M	20.02
6152	49	M	17.74
6148	41	M	25.35
MEAN			22.09
10766	45	F	18.33
7373	40	F	10.04
10980	41	F	22.52
11256	49	F	16.61
11091	43	F	16.58
MEAN			16.82
MEAN FOR DECADE 19.16			

CASE NO.	AGE (in yrs)	SEX	BONE AREA (in %)
6012	50	M	16.57
6150	59	M	11.86
12022	59	M	20.93
5674	54	M	16.98
10738	56	M	14.58
MEAN			16.18
5592	54	F	21.33
10970	54	F	19.06
11342	52	F	16.42
11904	59	F	21.20
12021	55	F	22.31
MEAN			20.06
MEAN FOR DECADE 18.12			

Table 14(c) BONE AREA. INDIVIDUAL RESULTS. AGES 60+

CASE NO.	AGE (in yrs)	SEX	BONE AREA (in %)
11118	66	M	19.10
11978	62	M	21.37
6101	62	M	19.46
12027	67	M	24.21
5360	65	M	16.29
10779	61	M	18.57
MEAN			19.82
5881	63	F	18.60
6215	65	F	14.30
6785	65	F	14.27
6147	69	F	14.97
11061	60	F	16.92
11088	63	F	10.67
11154	64	F	16.88
11923	64	F	8.71
MEAN			14.42
MEAN FOR DECADE 16.73			

CASE NO.	AGE (in yrs)	SEX	BONE AREA (in %)
5733	73	M	9.33
6145	79	M	16.99
6087	71	M	13.91
6146	72	M	8.25
10711	71	M	11.11
5385	79	M	14.92
7396	76	M	20.42
MEAN			13.56
10898	78	F	10.77
10841	79	F	9.41
11102	70	F	11.04
11276	72	F	22.88
11313	72	F	10.99
6013	74	F	22.68
6149	70	F	13.50
MEAN			14.47
MEAN FOR DECADE 14.01			

CASE NO.	AGE (in yrs)	SEX	BONE AREA (in %)
5675	82	M	17.43
11461	83	M	12.78
MEAN			15.11
11008	93	F	12.79
11109	89	F	17.68
11503	89	F	12.29
11264	87	F	9.89
11275	82	F	14.51
11977	84	F	14.33
11293	81	F	17.68
MEAN			14.17
MEAN FOR DECADE 14.38			

bone substance that can be lost from any site, and the results support this contention.

Examination of the individual figures shows that the values for the two sexes are intermingled. They appear similar in overall pattern, in size of individual values, in the extent of scatter, in the extent of bone loss with age, and in regard to the age at which the loss of bone begins. There is no evidence of any clear sex difference at any age.

This conclusion is reinforced by comparison of the mean values for adult males and females by the "t" test (Table 15).

Table 15. TESTS OF SIGNIFICANCE OF DIFFERENCES BETWEEN MEAN BONE AREA IN ADULT MALES AND FEMALES.

a) INDIVIDUAL DECADES (Students "t" test employed)

DECADE	BONE AREA	t	DEGREES OF FREEDOM	SIGNIFICANCE
20-29	M > F	0.395	8	N.S.D.
30-39	F > M	1.462	8	N.S.D.
40-49	M > F	1.856	7	N.S.D.
50-59	F > M	2.12	8	$p < 0.10 > 0.05$
60-69	M > F	3.31	12	$p < 0.01$
70-79	F > M	0.33	12	N.S.D.
80+	Insufficient male values for analysis.			

b) OVERALL MEANS

To avoid bias due to the relative preponderance of young males and elderly females in this study, the overall mean figures used are derived from the decade means:-

	ADULT MALES	ADULT FEMALES
Overall mean bone area	18.57%	17.75%
No. of cases	36	40
Variance of mean	0.6056	0.7875

To determine significance of difference between means the following formula is used:-

$$d = \frac{\text{Difference between means}}{\text{S.D. of difference between means}}$$

$$= \frac{\text{Difference between means}}{\sqrt{\text{Sum of variances of the two means}}}$$

$$= \frac{0.82}{\sqrt{1.3931}} = \frac{0.82}{1.18} = 0.69$$

This is not significant, i.e., there is no significant difference between the means.

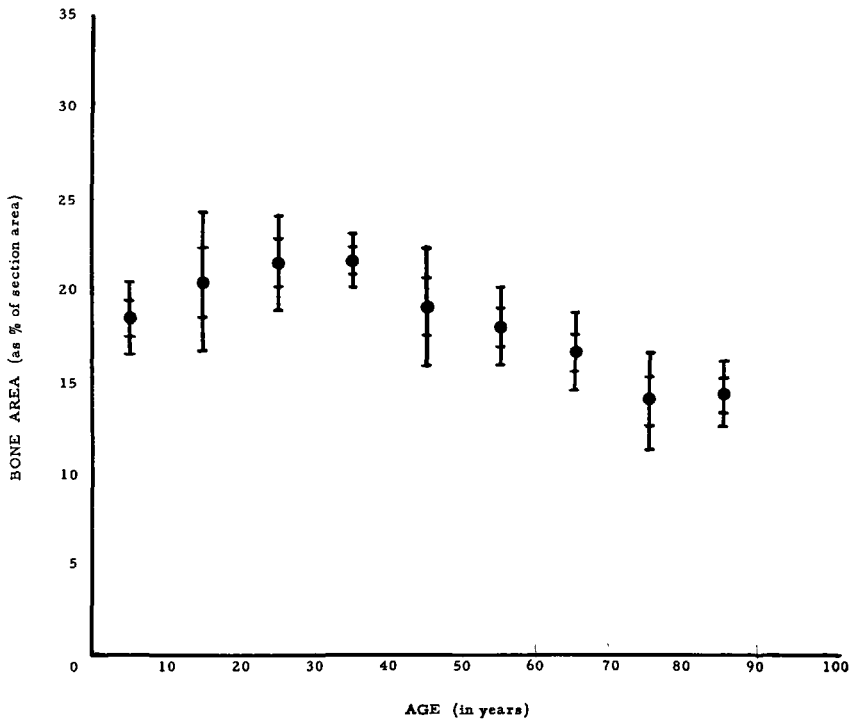
The mean bone area in males is significantly higher than that in females in only one age group (60 - 69). In the preceding decade the mean value in females is higher but only at a 10% level of significance than that in males. In no other decade is there any significant difference between male and female mean values, nor do the means of all male and female results differ significantly from each other. It is probable that the differences seen in the 50 - 59 and 60 - 69 age groups represent sampling variations rather than any true sex differences.

As no significant difference can be seen between the values for males and females, results from both sexes have been combined to obtain mean values for each decade (Tables 13 and 14 and Figure 23).

It is well known that loss of bone is marked in old age. My results confirm this, but also suggest that the process of bone loss starts earlier than is generally recognised. In the graph of individual results (Figure 22) the bulk of high values are seen between the ages of 20 and 40. This graph forms a band, parabolic in shape, and fairly uniform in width. The shape of the graph suggests a plateau between the ages of 20 and 40 with a subsequent fall.

Mean values confirm this. The mean values for the 3rd and 4th decades are virtually identical (21.58% and 21.73% respectively). The mean value then falls to 19.16% in the 5th decade. The trend of individual results shows

Figure 23. Mean bone area for each decade. The bars represent 1 and 2 standard errors respectively.



that this fall affects both sexes.

If polynomial regression lines of successively higher orders, (Fisher 1950, Smith 1954) are fitted to the graph of mean values (Figure 24), a quadratic polynomial regression line produces a significantly better fit than a linear regression line, while a further significant improvement of fit is obtained with a cubic polynomial regression line. The peak of such a cubic polynomial regression occurs in the 20 - 29 age group.

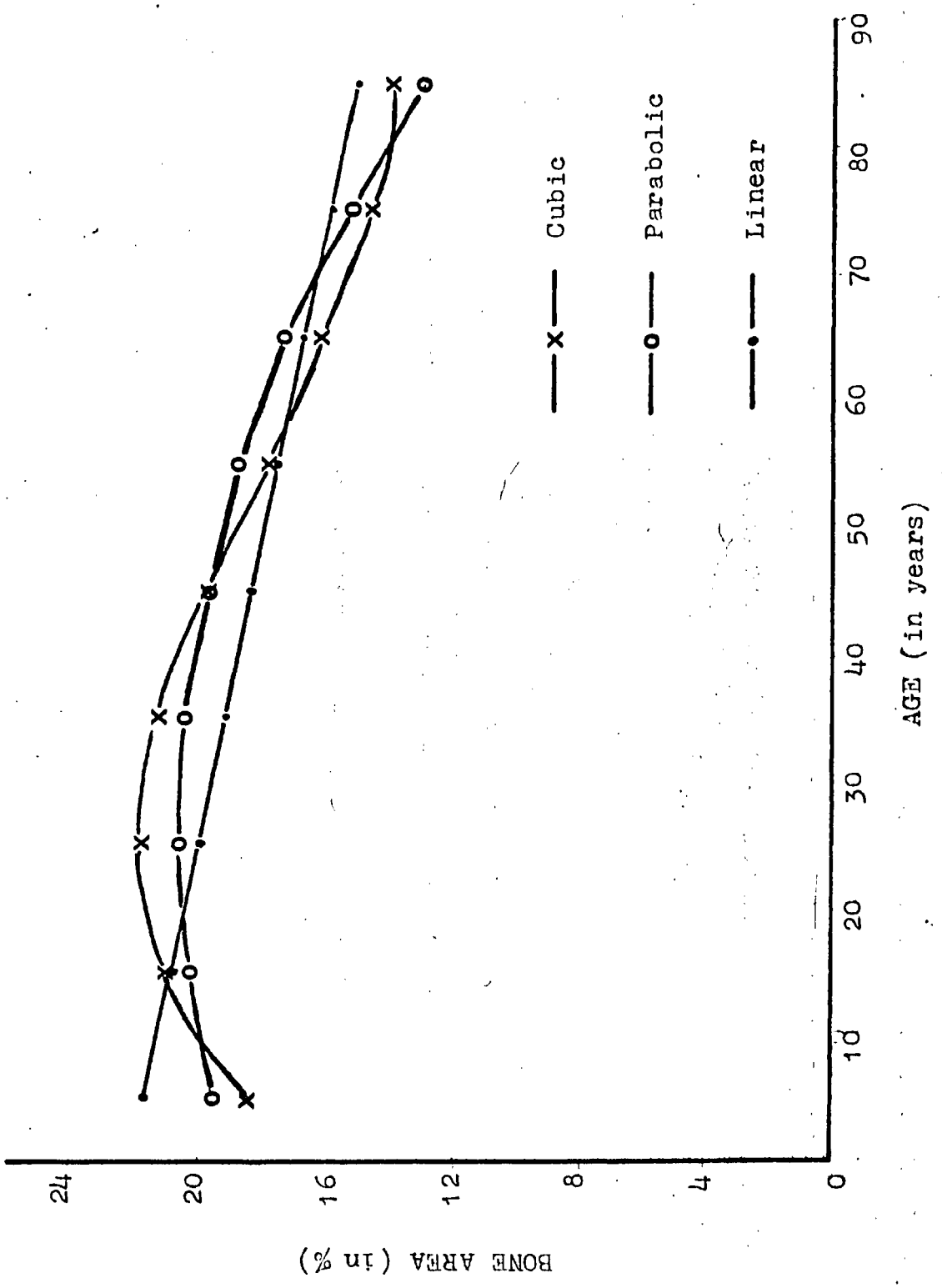
All these expressions strongly suggest that although bone loss is most apparent in old age, the process atarts much earlier, possibly from the age of 30.

The significance of the differences between the mean values in different decades was evaluated by the "t" test (Table 16) the mean figure for each decade being compared with that for the decades 20 - 29 and 30 - 39 combined.

Table 16. "t" TESTS OF SIGNIFICANCE OF DIFFERENCES BETWEEN MEAN BONE AREA IN VARIOUS AGE GROUPS.

AGE GROUPS TESTED	t	DEGREES OF FREEDOM	SIGNIFICANCE (P)
20-39 > 40-49	1.635	27	< 0.10 > 0.05
20-39 > 50-59	2.748	28	< 0.01
20-39 > 0-9	2.37	26	< 0.02
20-39 > 10-19	0.70	27	N.S.D.
80 + > 70-79	0.20	19	N.S.D.

Figure 24. Polynomial regression lines fitting mean bone area figures.



It will be seen that the loss of bone in the 40 - 49 group is significant at a level of 10% while in the 50 - 59 group the loss is highly significant ($P<0.01$). There is no significant difference between the mean values of the 70 - 79 and over 80 groups.

The rise in mean values between children aged 0 - 9 and adults aged 20 - 39 is significant ($P<0.02$). There is no significant difference between the 10 - 19 and 20 - 39 mean values.

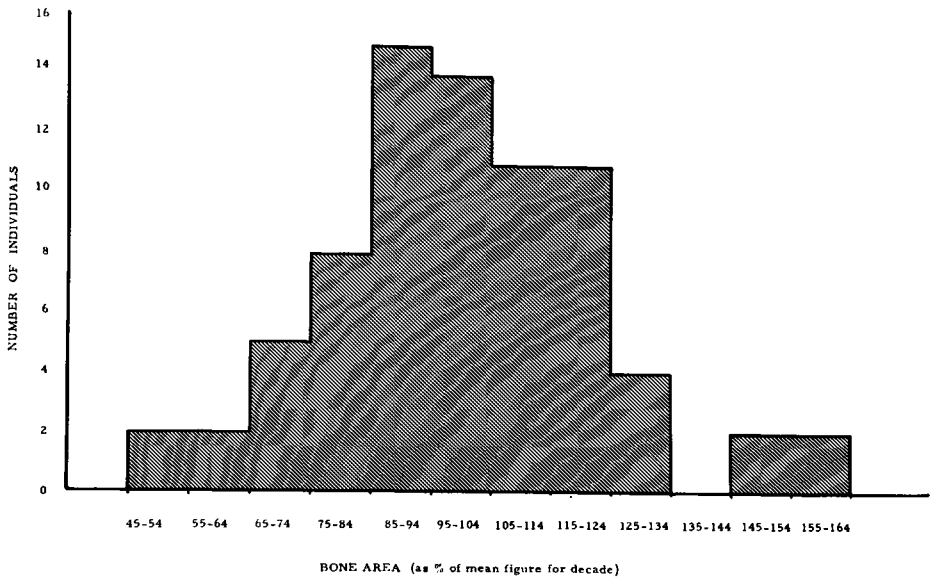
On inspection of the individual results there is no evidence at any age of two populations - one normal and one showing a greater loss of bone substance. There is a wide scatter of results, but at all ages they appear to be evenly spread.

This is confirmed by a histogram of individual results (Figure 25) each result being expressed as a percentage of the mean value for the corresponding decade. This avoids the possible masking of any group of young individuals with lower than normal bone area by normal elderly individuals showing the same absolute bone area. The distribution of results approximates to a normal distribution and there is no evidence whatsoever of a dual population.

NORMAL RANGE OF RESULTS.

It has been noted that individual results form a band of parabolic shape, and of fairly uniform width. There is little tendency for the spread of results to

Figure 25. Histogram of individual results of bone area.



increase with age. It is therefore possible to calculate a standard deviation for the series as a whole, the deviation of each result from the mean for the decade being employed in the calculation. A normal range at any age may then be established from the mean figure for the decade ± 2 S.D., and this is shown in Table 17 and Figure 26.

Table 17. NORMAL RANGE OF BONE AREA. CALCULATION OF STANDARD DEVIATION FOR SERIES AS A WHOLE.

Sum of squares = 1447.7

Number of observations = 92 = n

$$\text{Variance} = \frac{\text{Sum of squares}}{n - 1}$$

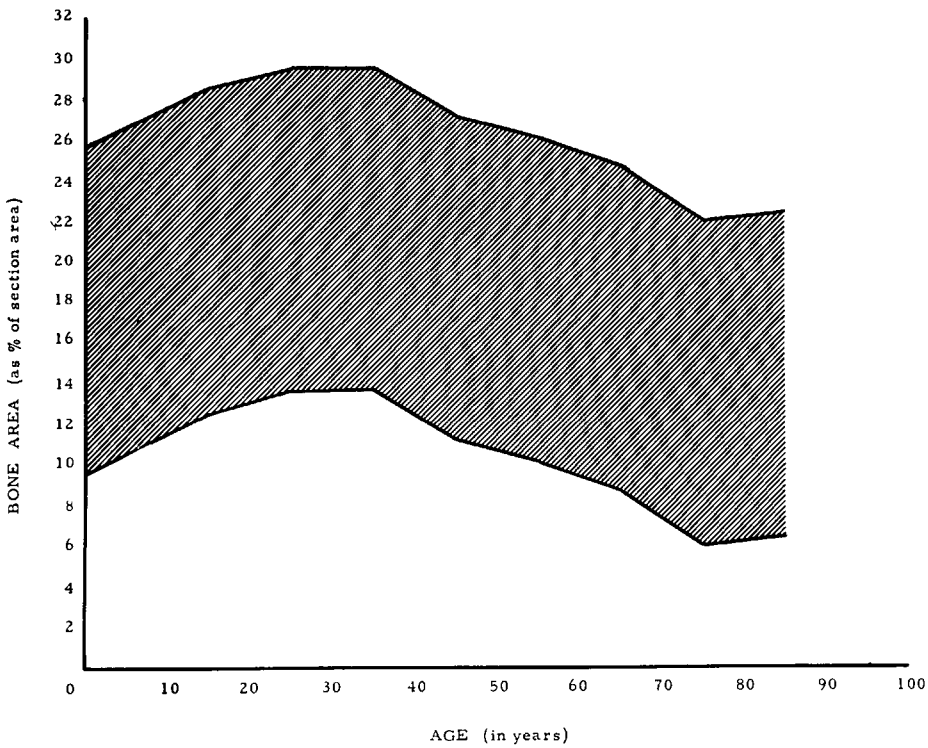
$$= \frac{1447.7}{91} = 15.735$$

$$\text{Standard deviation} = \sqrt{\text{Variance}}$$

$$= \underline{3.967}$$

DECADE (yrs.)	MEAN BONE AREA (in %)	NORMAL RANGE (MEAN ± 2 S.D.) in %
0 - 9	18.48	10.55 - 26.41
10 - 19	20.45	12.52 - 28.38
20 - 29	21.58	13.65 - 29.51
30 - 39	21.73	13.80 - 29.66
40 - 49	19.16	11.23 - 27.09
50 - 59	18.12	10.19 - 26.05
60 - 69	16.73	8.80 - 24.66
70 - 79	14.01	6.08 - 21.94
80 +	14.38	6.45 - 22.31

Figure 26. Normal range of bone area.



Such a range would be expected to embrace approximately 96% of normals, and all but two of the results in this survey fall within it. Such a range may be used in the interpretation of diagnostic biopsy material, although as already noted, single biopsies should be interpreted with caution.

BONE AREA IN BIOPSY SITE

This was measured in 54 cases. The specimens contained both dense cortical and porous cancellous bone, and the relative proportions of the two varied considerably. Individual results are plotted in Figure 27, and shown in Table 18.

The individual values are, of course, somewhat higher than the corresponding values for cancellous bone alone but to a variable degree. Considerable variation is seen between one case and another, but it is of interest and importance that the overall pattern is essentially similar to that of cancellous bone alone. A fall in amount of bone with age is seen. There is no obvious difference between males and females, and there is again no evidence of two populations. The highest mean values were seen in the 20 - 29 and 30 - 39 age groups. No statistical evaluation was attempted because of the small number of samples, and because the area counted cannot be considered a uniform sample. A measure of the total amounts of cortical and cancellous bone tissue in a complete cross section of a bone, such as a vertebra

Figure 27. Individual results of bone area in iliac crest biopsy site against age.

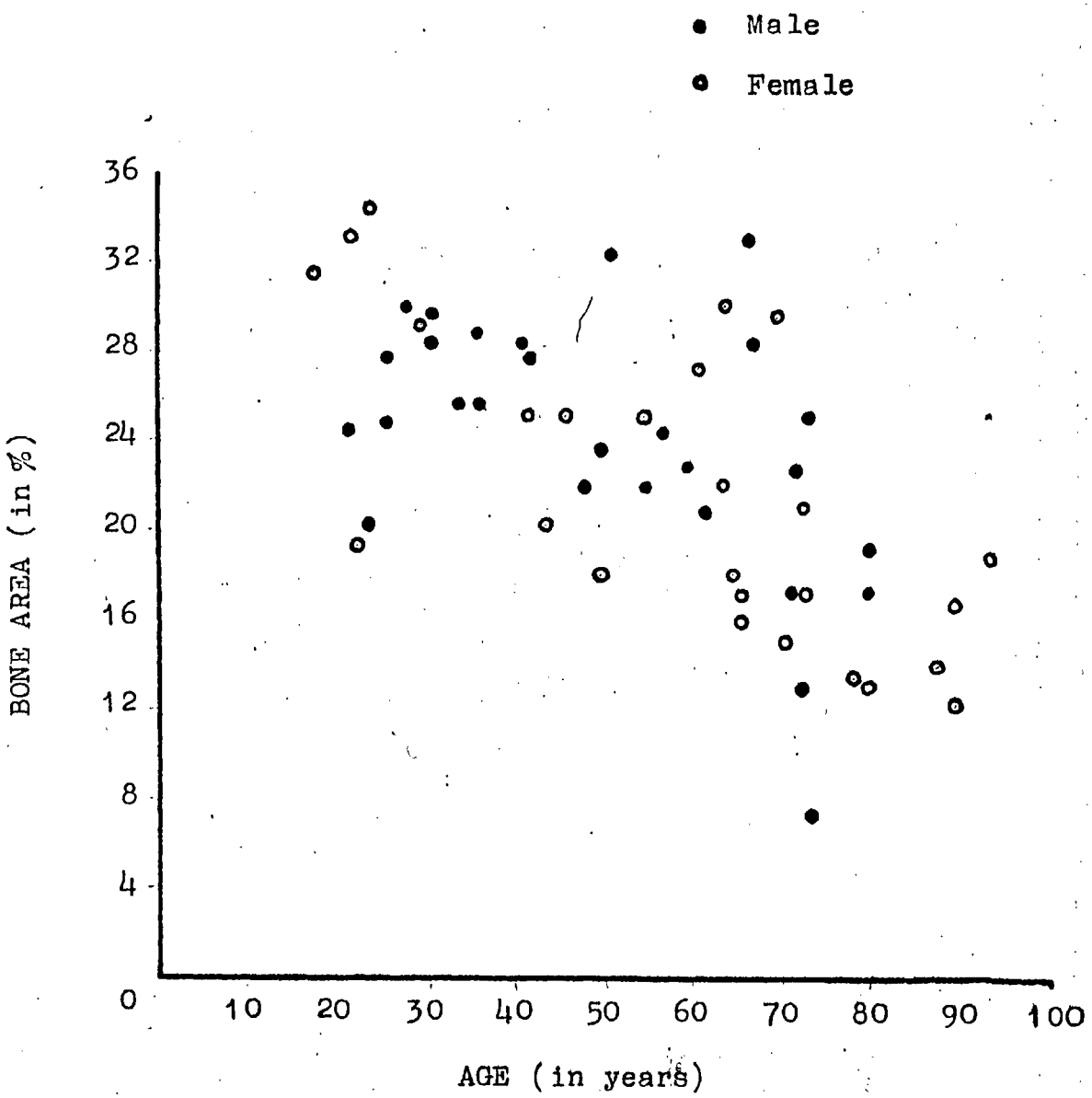


Table 18(a) BONE AREA IN BIOPSY SITE

AGE GROUPS 10 - 59

CASE NO.	AGE (in years)	SEX	BONE AREA (in %)
10794	17	F	31.6
7114	22	F	19.4
5590	27	M	29.9
6144	21	M	24.6
5591	23	M	20.4
10748	29	F	29.1
10788	25	M	24.7
10762	23	F	34.4
11155	25	M	27.7
10820	21	F	33.2
6846	30	M	28.4
5359	33	M	25.4
10701	30	M	29.4
11117	35	M	28.7
11110	35	M	25.6
5732	40	M	28.3
10766	45	F	24.9
6278	47	M	21.8
6152	49	M	23.6
6148	41	M	27.9
10980	41	F	25.1
11256	49	F	18.0
11091	43	F	20.2
5674	54	M	21.8
10738	56	M	24.3
10970	54	F	24.9
6150	59	M	22.9
6012	50	M	32.4

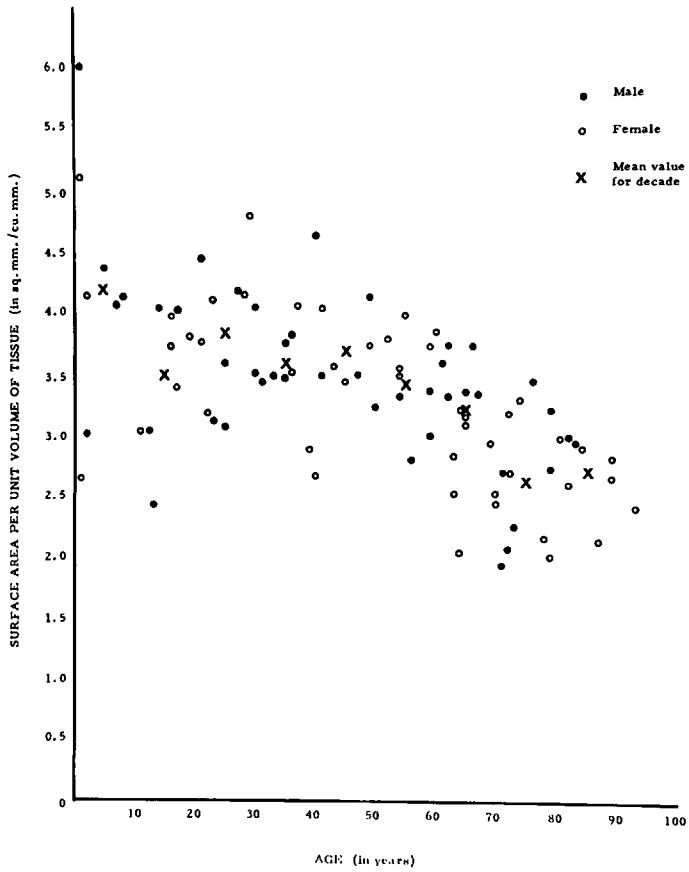
Table 18(b) BONE AREA IN BIOPSY SITE.

AGE GROUPS 60 +

CASE NO.	AGE (in years)	SEX	BONE AREA (in %)
5360	65	M	32.6
5881	63	F	29.7
6215	65	F	17.0
6785	65	F	16.0
6147	69	F	29.4
10779	61	M	20.6
11061	60	F	27.3
11088	63	F	22.0
11154	64	F	18.0
11118	66	M	28.4
5733	73	M	7.4
6145	79	M	17.3
6087	71	M	17.2
6146	72	M	13.3
10711	71	M	22.5
10898	78	F	13.2
10841	79	F	13.1
5385	79	M	19.3
7396	72	M	24.9
11102	70	F	15.2
11276	72	F	21.2
11313	72	F	17.3
11008	93	F	18.6
11109	89	F	16.7
11503	89	F	12.2
11264	87	F	14.0

or rib, will have validity, but such a measurement is clearly impossible in the iliac crest where the position of the inferior margin of the specimen is always arbitrary. It seems most unwise to draw too definite a conclusion from a small biopsy containing variable proportions of dense cortical and porous cancellous bone.

Figure 28. Surface area per unit volume of tissue. Individual and mean values plotted against age.



SURFACE AREA

1. ABSOLUTE SURFACE AREA - SURFACE AREA PER UNIT VOLUME OF TISSUE.

Individual and mean values are shown in Tables 19 and 13 and Figure 28. Highest individual values are seen in childhood, the highest individual value being 6.02 sq.mm. of surface/cu.mm. of tissue. The scatter of results is also greatest in childhood. The surface area falls with age. The fall with age does not exactly parallel that of bone area; the graphs suggest that the fall in surface area may start from infancy, but any changes in youth are small, and no significant decrease in surface area is seen before the 5th decade. The total extent of the fall with age is considerable, the mean surface area decreasing by 39% between the 1st and 8th decades, and 31% between the 4th and 8th decades, compared with a 40% mean loss of bone area during the same period.

No significant difference in surface area between the two sexes is seen at any age, and no evidence of two populations is apparent.

Table 19(a) SURFACE AREA/UNIT VOLUME OF TISSUE. INDIVIDUAL RESULTS. AGES 0 - 29

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5916	8	M	4.14
7156	2	M	3.01
6607	5	M	4.38
7018	11mos	M	6.02
6102	7	M	4.08
MEAN			4.33
7157	1	F	2.64
11922	3mos	F	5.12
5862	1	F	4.16
MEAN			3.97
MEAN FOR DECADE 4.19			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5391	17	M	4.03
10969	12	M	3.04
7162	14	M	4.05
6782	13	M	2.43
MEAN			3.39
10794	17	F	3.41
11263	11	F	3.03
11731	16	F	3.97
11576	19	F	3.81
11719	16	F	3.74
MEAN			3.59
MEAN FOR DECADE 3.50			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5590	27	M	4.21
6144	21	M	4.47
5591	23	M	3.12
11155	25	M	3.60
10788	25	M	3.07
MEAN			3.69
7114	22	F	3.18
10748	29	F	4.82
10762	23	F	4.12
10820	21	F	3.78
7160	28	F	4.17
MEAN			4.01
MEAN FOR DECADE 3.85			

Table 19(b) SURFACE AREA/UNIT VOLUME OF TISSUE. INDIVIDUAL RESULTS. AGES 30 - 59

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
6846	30	M	4.06
5359	33	M	3.50
10701	30	M	3.52
11117	35	M	3.77
11110	35	M	3.48
6011	31	M	3.44
6009	36	M	3.83
MEAN			3.66
6608	39	F	2.89
12080	37	F	4.07
11387	36	F	3.52
MEAN			3.49
MEAN FOR DECADE 3.61			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5732	40	M	4.65
6278	47	M	3.51
6152	49	M	4.14
6148	41	M	3.50
MEAN			3.95
10766	45	F	3.45
7373	40	F	2.67
10980	41	F	4.06
11256	49	F	3.75
11091	43	F	3.57
MEAN			3.50
MEAN FOR DECADE 3.70			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
6012	50	M	3.24
6150	59	M	3.01
12022	59	M	3.37
5674	54	M	3.33
10738	56	M	2.80
MEAN			3.15
5592	54	F	3.52
10970	54	F	3.54
11342	52	F	3.89
11904	59	F	3.74
12021	55	F	3.99
MEAN			3.72
MEAN FOR DECADE 3.43			

Table 19(c) SURFACE AREA/UNIT VOLUME OF TISSUE, INDIVIDUAL RESULTS. AGES 60+ .

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in.sq.mm/cu.mm)
11118	66	M	3.74
11978	62	M	3.75
6101	62	M	3.33
12027	67	M	3.35
5360	65	M	3.37
10779	61	M	3.59
MEAN			3.52
5881	63	F	2.83
6215	65	F	3.18
6785	65	F	3.13
6147	69	F	2.94
11061	60	F	3.86
11088	63	F	2.53
11154	64	F	3.22
11923	64	F	2.03
MEAN			2.97
MEAN FOR DECADE 3.20			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5733	73	M	2.27
6145	79	M	2.73
6087	71	M	2.71
6146	72	M	2.08
10711	71	M	1.94
5385	79	M	3.22
7396	76	M	3.46
MEAN			2.63
10898	78	F	2.16
10841	79	F	2.02
11102	70	F	2.44
11276	72	F	3.19
11313	72	F	2.71
6013	74	F	3.30
6149	70	F	2.52
MEAN			2.62
MEAN FOR DECADE 2.63			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5675	82	M	2.98
11461	83	M	2.96
MEAN			2.97
11008	93	F	2.42
11109	89	F	2.82
11503	89	F	2.67
11264	87	F	2.15
11275	82	F	2.59
11977	84	F	2.91
11293	81	F	2.98
MEAN			2.65
MEAN FOR DECADE 2.72			

2. RELATIVE SURFACE AREA - SURFACE AREA PER UNIT VOLUME OF SOLID BONE.

In cancellous bone a low surface area may be due to little bone being present (i.e., a low bone area) or to the bone present being arranged in large masses, presenting a relatively small surface; similarly a high surface area may be due to much bone being present, or due to the bone present having a fine structure so that it presents a relatively large surface.

These alternatives may be distinguished if the surface area is related to the amount of bone present and expressed as surface area per unit volume of solid bone. Results expressed in this fashion are shown in Table 20 and Figure 29. Again a considerable scatter of individual results is seen, the highest individual results being 30.01 sq.mm./cu.mm. solid bone and the lowest being 11.86 sq.mm./cu.mm. solid bone. No progressive rise or fall with age can be seen, but instead three fairly distinct groups may be discerned - respectively the 0 - 9, 10 - 39 and over 40 groups. The value for the first group is 22.68 sq.mm./cu.mm. ; in the second group means for each decade vary from just under 17 to just over 18 sq.mm./cu.mm. : the overall mean being 17.33 sq.mm./cu.mm., while in the third group the means for the various decades lie between 19 and just above 20 sq.mm./cu.mm. with an overall mean of 19.67 sq.mm./cu.mm.

The differences between these groups are highly significant.

Figure 29. Surface area per unit volume of solid bone. Individual and mean values plotted against age.

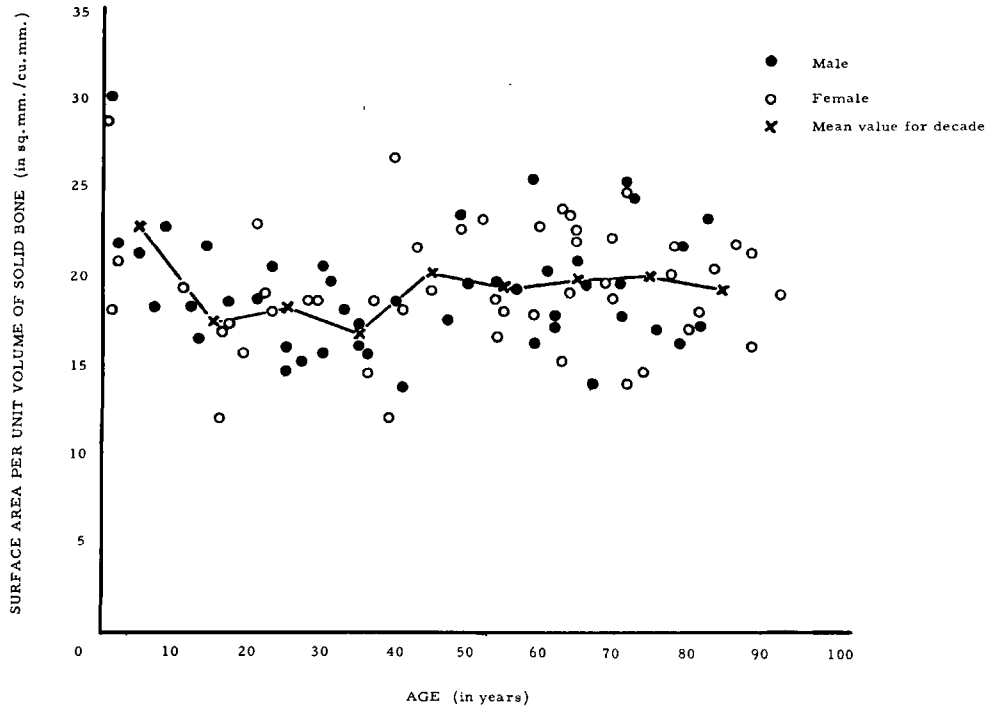


Table 20(a) SURFACE AREA/UNIT VOLUME OF SOLID BONE. INDIVIDUAL RESULTS. AGES 0 – 29

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5916	8	M	22.84
7156	2	M	21.78
6607	5	M	21.21
7018	11mos.	M	30.01
6102	7	M	18.22
MEAN			22.81
7157	1	F	18.00
11922	3mos.	F	28.63
5862	1	F	20.78
MEAN			22.47
MEAN FOR DECADE 22.68			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5391	17	M	18.53
10969	12	M	18.17
7162	14	M	21.56
6782	13	M	16.37
MEAN			18.66
10794	17	F	17.20
11263	11	F	19.26
11731	16	F	11.93
11576	19	F	15.60
11719	16	F	17.14
MEAN			16.23
MEAN FOR DECADE 17.31			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5590	27	M	15.10
6144	21	M	18.64
5591	23	M	20.42
11155	25	M	15.98
10788	25	M	14.64
MEAN			16.96
7114	22	F	18.94
10748	29	F	18.49
10762	23	F	17.87
10820	21	F	22.80
7160	28	F	18.52
MEAN			19.32
MEAN FOR DECADE 18.14			

Table 20(b) SURFACE AREA/UNIT VOLUME OF SOLID BONE. INDIVIDUAL RESULTS. AGES 30 - 59

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in.sq.mm/cu.mm)
6846	30	M	20.52
5359	33	M	18.01
10701	30	M	15.57
11117	35	M	17.22
11110	35	M	16.03
6011	31	M	19.70
6009	36	M	15.59
MEAN			17.52
6608	39	F	11.86
12080	37	F	18.51
11387	36	F	14.53
MEAN			14.97
MEAN FOR DECADE 16.75			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5732	40	M	18.44
6278	47	M	17.51
6152	49	M	23.37
6148	41	M	13.81
MEAN			18.28
10766	45	F	19.13
7373	40	F	26.59
10980	41	F	18.03
11256	49	F	22.57
11091	43	F	21.55
MEAN			21.57
MEAN FOR DECADE 20.11			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
6012	50	M	19.55
6150	59	M	25.38
12022	59	M	16.08
5674	54	M	19.61
10738	56	M	19.33
MEAN			19.99
5592	54	F	16.53
10970	54	F	18.55
11342	52	F	23.14
11904	59	F	17.66
12021	55	F	17.88
MEAN			18.75
MEAN FOR DECADE 19.37			

Table 20(c) SURFACE AREA/UNIT VOLUME OF SOLID BONE. INDIVIDUAL RESULTS. AGES 60+

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
11118	66	M	19.57
11978	62	M	17.54
6101	62	M	17.09
12027	67	M	13.85
5360	65	M	20.71
10779	61	M	20.32
MEAN			18.18
5881	63	F	15.21
6215	65	F	22.21
6785	65	F	21.93
6147	69	F	19.64
11061	60	F	22.79
11088	63	F	23.69
11154	64	F	19.05
11923	64	F	23.33
MEAN			20.98
MEAN FOR DECADE 19.78			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5733	73	M	24.31
6145	79	M	16.04
6087	71	M	19.48
6146	72	M	25.22
10711	71	M	17.70
5385	79	M	21.61
7396	76	M	16.94
MEAN			20.19
10898	78	F	20.01
10841	79	F	21.55
11102	70	F	22.07
11276	72	F	13.95
11313	72	F	24.67
6013	74	F	14.55
6149	70	F	18.66
MEAN			19.35
MEAN FOR DECADE 19.77			

CASE NO.	AGE (in yrs)	SEX	SURFACE AREA (in sq.mm/cu.mm)
5675	82	M	17.09
11461	83	M	23.14
MEAN			20.12
11008	93	F	18.86
11109	89	F	15.95
11503	89	F	21.17
11264	87	F	21.74
11275	82	F	17.89
11977	84	F	20.29
11293	81	F	16.87
MEAN			18.94
MEAN FOR DECADE 19.22			

Table 21. t TESTS OF DIFFERENCES BETWEEN MEANS OF SURFACE AREA/UNIT VOLUME OF SOLID BONE

AGE GROUPS COMPARED	DIFFERENCE BETWEEN MEANS	t	DEGREES OF FREEDOM	SIGNIFICANCE (P)
0 - 9>10 - 39	5.35	4.03	35	<0.001
0 - 9>40+	3.01	2.41	62	<0.02>0.01
40+ >10 - 39	2.34	3.37	83	<0.001

There are no significant differences within the 10 - 39 and over 40 age groups.

The meaning of these differences is discussed later.

FORMATION SURFACE

Individual values of extent of bone surface involved in bone formation are shown in Table 22, and Figure 30, mean values being shown in Table 13. It is apparent from these that, at all ages, a relatively high proportion of the bone surface is occupied by sites of bone formation, a higher proportion than is obvious in a conventional decalcified section. The mean of all values is 13.45%.

Considerable variation is seen between individual cases, even in the same age group, the extent of variation being greater than that seen in bone area. Nevertheless, certain trends are apparent. Highest individual results, and greatest scatter of results are seen in the young and elderly; lower individual results are seen between the ages of 40 and 60.

Study of individual figures reveals no obvious differences between results for males and females at any age. The overall mean figures for males and females do not differ significantly from each other. Therefore, mean figures for each decade were calculated from the combined results of each sex.

The mean figures reflect the trend of individual results. In childhood a mean figure approaching 15% of the trabecular surface is occupied by bone formation. A lower figure is seen once adult life is reached, and the mean figure falls to about 8% in the sixth decade. The mean value then rises again to almost 15% between the ages of 70 and 79.

Figure 30. Formation surface. Individual results plotted against age.

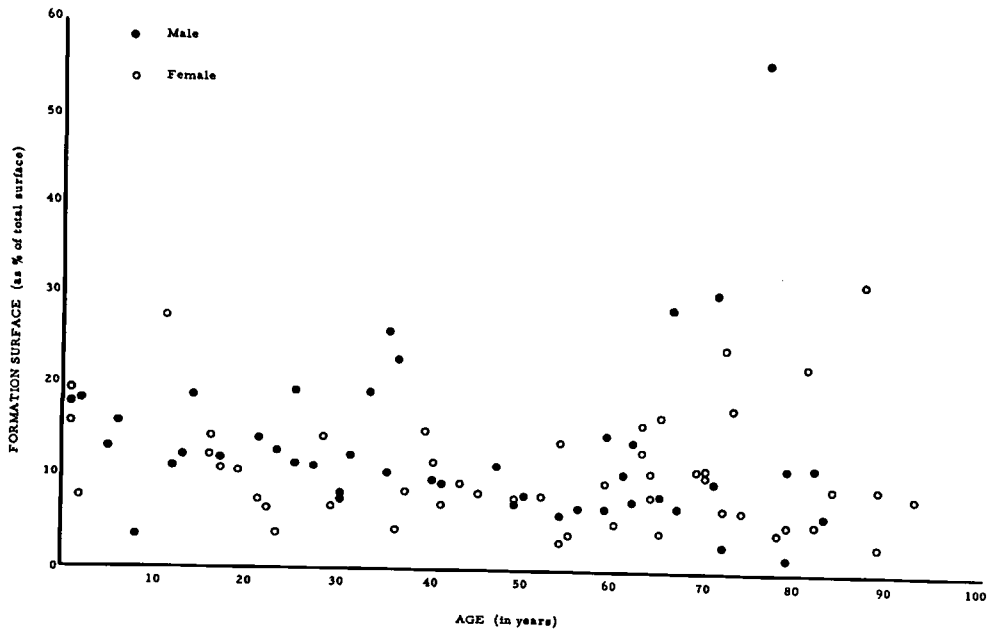


Table 22(a) FORMATION SURFACE. INDIVIDUAL RESULTS. AGES 0 - 29.

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE (in %)
5916	8	M	3.60
7156	2	M	18.61
6607	5	M	13.53
7018	11mos	M	18.25
6102	7	M	16.05
MEAN			14.01
7157	1	F	16.10
11922	3mos	F	19.81
5862	1	F	7.92
MEAN			14.61
MEAN FOR DECADE 14.23			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE (in %)
5391	17	M	12.28
10969	12	M	11.29
7162	14	M	19.13
6782	13	M	12.63
MEAN			13.83
10794	17	F	11.10
11263	11	F	27.87
11731	16	F	14.72
11576	19	F	10.96
11719	16	F	12.72
MEAN			15.47
MEAN FOR DECADE 14.70			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE (in %)
5590	27	M	11.43
6144	21	M	14.59
5591	23	M	13.22
11155	25	M	19.84
10788	25	M	11.66
MEAN			14.15
7114	22	F	6.69
10748	29	F	6.92
10762	23	F	3.97
10820	21	F	7.60
7160	28	F	14.64
MEAN			7.96
MEAN FOR DECADE 11.06			

Table 22(b) FORMATION SURFACE. INDIVIDUAL RESULTS. AGES 30 - 59

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE (in %)
6846	30	M	8.09
5359	33	M	19.60
10701	30	M	8.40
11117	35	M	10.78
11110	35	M	26.52
6011	31	M	12.65
6009	36	M	23.39
MEAN			15.63
6608	39	F	15.47
12080	37	F	8.59
11387	36	F	4.52
MEAN			9.53
MEAN FOR DECADE 13.80			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE (in %)
5732	40	M	9.86
6278	47	M	11.61
6152	49	M	7.50
6148	41	M	9.56
MEAN			9.63
10766	45	F	8.62
7373	40	F	12.13
10980	41	F	7.21
11256	49	F	7.68
11091	43	F	9.57
MEAN			9.04
MEAN FOR DECADE 9.30			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE (in %)
6012	50	M	8.24
6150	59	M	6.91
12022	59	M	15.15
5674	54	M	6.22
10738	56	M	7.02
MEAN			8.71
5592	54	F	14.33
10970	54	F	3.13
11342	52	F	8.28
11904	59	F	9.87
12021	55	F	3.92
MEAN			7.91
MEAN FOR DECADE 8.31			

Table 22(c) FORMATION SURFACE. INDIVIDUAL RESULTS. AGES 60+

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE (in %)
11118	66	M	29.29
11978	62	M	14.54
6101	62	M	7.85
12027	67	M	7.15
5360	65	M	8.42
10779	61	M	10.84
MEAN			13.02
5881	63	F	13.41
6215	65	F	17.40
6785	65	F	4.25
6147	69	F	11.41
11061	60	F	5.28
11088	63	F	16.25
11154	64	F	8.36
11923	64	F	11.10
MEAN			10.93
MEAN FOR DECADE 11.83			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE (in %)
5733	73	M	18.30
6145	79	M	1.64
6087	71	M	31.10
6146	72	M	2.86
10711	71	M	9.99
5385	79	M	11.46
7396	76	M	56.65
MEAN			18.86
10898	78	F	4.33
10841	79	F	5.20
11102	70	F	10.64
11276	72	F	24.60
11313	72	F	7.05
6013	74	F	6.82
6149	70	F	11.29
MEAN			9.99
MEAN FOR DECADE 14.42			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE (in %)
5675	82	M	11.69
11461	83	M	6.30
MEAN			9.00
11008	93	F	8.45
11109	89	F	3.08
11503	89	F	9.52
11264	87	F	32.25
11275	82	F	5.37
11977	84	F	9.20
11293	81	F	23.09
MEAN			12.99
MEAN FOR DECADE 12.11			

In the elderly a number of low individual values is seen, the smallest figure being 1.64%, but there is a group of high values of great interest, both males and females being represented in this group. Of particular interest is a value of 56.65% in an apparently normal man of 75. (Figure 37). This high figure is not due to the sampling of an isolated and unrepresentative focus of high osteoid coverage, as multiple sections from other sites from the iliac crest, and from cancellous bone elsewhere (lumbar vertebral body) gave values of similar magnitude, although the value for femoral cortical bone (5.9%) was not high. The significance of these high results is discussed later.

The possible significance of the differences between the mean values of the various decades was evaluated by the "t" test. No significant difference in extent of formation surface is seen between the decades 0 - 9, 10 - 19, 20 - 29 and 30 - 39. The reduction in extent of formation surface after this time is significant however, the differences between mean values for the 10 - 19 and 50 - 59 age groups and 10 - 19 and 40 - 49 age groups being highly significant ($P < 0.01$ and $< 0.02 > 0.01$ respectively), while a significant fall is seen between the 30 - 39 and 50 - 59 groups ($P < 0.05$). The difference between the 30 - 39 and 40 - 49 groups approaches significance at the 5% level ($P < 0.10 > 0.05$). In middle and old age no significant differences are seen between the individual decades, but a highly significant difference is seen

between the two groups aged 40 - 59, and over 60. ($P < 0.05$).

FORMATION SURFACE AREA PER UNIT VOLUME OF TISSUE (ABSOLUTE SURFACE AREA OF BONE FORMATION).

The measurements described above indicate the proportion of surface occupied by bone formation, but give no idea of the total area occupied by sites of bone formation.

To show this the surface area occupied by bone formation per unit volume of tissue (i.e., bone and marrow) was calculated, and individual results expressed in sq.mm. of formation surface area per cubic mm. of tissue are shown in Table 23, and Figure 31. These show very considerable variation even within the same age group. High values are seen in early life and the lowest values are found in old age, although a group of high values is also seen in the elderly.

No obvious sex difference can be seen in the individual values; therefore mean values were calculated for each decade from the combined male and female results (Table 13 and Figure 31). From these it is seen that a 50% fall occurs in the mean formation surface area with aging, the fall being seen between the first and sixth decades. There is no further fall in old age; indeed after the sixth decade the mean figures show a slight rise to the eighth decade, but this is not significant.

Figure 31. Formation surface area per unit volume of tissue. Individual and mean values plotted against age.

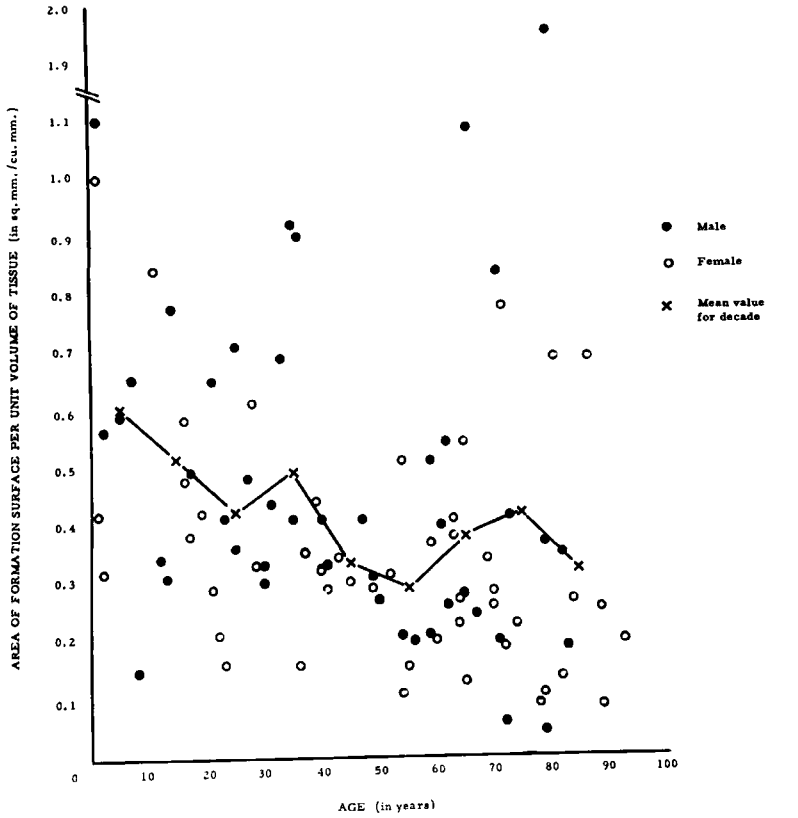


Table 23(a) FORMATION SURFACE AREA PER UNIT VOLUME OF TISSUE. INDIVIDUAL RESULTS. AGES 0 - 29

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5916	8	M	0.15
7156	2	M	0.56
6607	5	M	0.59
7018	11mos	M	1.10
6102	7	M	0.65
MEAN			0.61
7157	1	F	0.43
11922	3mos	F	1.01
5862	1	F	0.33
MEAN			0.59
MEAN FOR DECADE 0.60			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5391	17	M	0.49
10969	12	M	0.34
7162	14	M	0.78
6782	13	M	0.31
MEAN			0.48
10794	17	F	0.38
11263	11	F	0.84
11731	16	F	0.58
11576	19	F	0.42
11719	16	F	0.48
MEAN			0.54
MEAN FOR DECADE 0.51			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5590	27	M	0.48
6144	21	M	0.65
5591	23	M	0.41
11155	25	M	0.71
10788	25	M	0.36
MEAN			0.52
7114	22	F	0.21
10748	29	F	0.33
10762	23	F	0.16
10820	21	F	0.29
7160	28	F	0.61
MEAN			0.32
MEANFOR DECADE 0.42			

Table 23(b) FORMATION SURFACE PER UNIT VOLUME OF TISSUE. INDIVIDUAL RESULTS. AGES 30 - 59

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
6846	30	M	0.33
5359	33	M	0.69
10701	30	M	0.30
11117	35	M	0.41
11110	35	M	0.92
6011	31	M	0.44
6009	36	M	0.90
MEAN			0.57
6608	39	F	0.44
12080	37	F	0.35
11387	36	F	0.16
MEAN			0.32
MEAN FOR DECADE 0.49			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5732	40	M	0.41
6278	47	M	0.41
6152	49	M	0.31
6148	41	M	0.33
MEAN			0.37
10766	45	F	0.30
7373	40	F	0.32
10980	41	F	0.29
11256	49	F	0.29
11091	43	F	0.34
MEAN			0.31
MEAN FOR DECADE 0.33			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
6012	50	M	0.27
6150	59	M	0.21
12022	59	M	0.51
5674	54	M	0.21
10738	56	M	0.20
MEAN			0.28
5592	54	F	0.51
10970	54	F	0.11
11342	52	F	0.31
11904	59	F	0.37
12021	55	F	0.16
MEAN			0.29
MEAN FOR DECADE 0.29			

Table 23(c) FORMATION SURFACE AREA PER UNIT VOLUME OF TISSUE. INDIVIDUAL RESULTS. AGES 60+

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
11118	66	M	1.09
11978	62	M	0.54
6101	62	M	0.26
12027	67	M	0.24
5360	65	M	0.28
10779	61	M	0.40
MEAN			0.46
5881	63	F	0.38
6215	65	F	0.54
6785	65	F	0.13
6147	69	F	0.34
11061	60	F	0.20
11088	63	F	0.41
11154	64	F	0.27
11923	64	F	0.23
MEAN			0.31
MEAN FOR DECADE 0.38			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5733	73	M	0.42
6145	79	M	0.04
6087	71	M	0.84
6146	72	M	0.06
10711	71	M	0.20
5385	79	M	0.37
7396	76	M	1.96
MEAN			0.56
10898	78	F	0.09
10841	79	F	0.11
11102	70	F	0.26
11276	72	F	0.78
11313	72	F	0.19
6013	74	F	0.23
6149	70	F	0.28
MEAN			0.28
MEAN FOR DECADE 0.42			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5675	82	M	0.35
11461	83	M	0.19
MEAN			0.27
11008	93	F	0.20
11109	89	F	0.09
11503	89	F	0.25
11264	87	F	0.69
11275	82	F	0.14
11977	84	F	0.27
11293	81	F	0.69
MEAN			0.33
MEAN FOR DECADE 0.32			

FORMATION SURFACE AREA PER UNIT VOLUME OF SOLID BONE (RELATIVE SURFACE AREA OF BONE FORMATION).

When the area of bone formation is related to the amount of bone present, individual results again show considerable variation (Table 24 and Figure 32). No obvious sex difference can be seen. When mean values for each decade derived from combined male and female results are plotted (Figure 32), the pattern is that of a fall from infancy to the sixth decade, and a subsequent rise. The decrease in relative surface area of bone formation from infancy onwards is significant by the third decade ($P < 0.05$).

As in the case of the formation surface area per unit volume of tissue, the mean values show an upward trend after the sixth decade but in this instance the rise is far more pronounced. From the fifth decade the differences between mean values for individual decades are not significant, but the difference between the two groups, aged 40 - 59 and over 60 is highly significant ($P < 0.05 > 0.02$).

Figure 32. Formation surface area per unit volume of solid bone. Individual and mean values plotted against age.

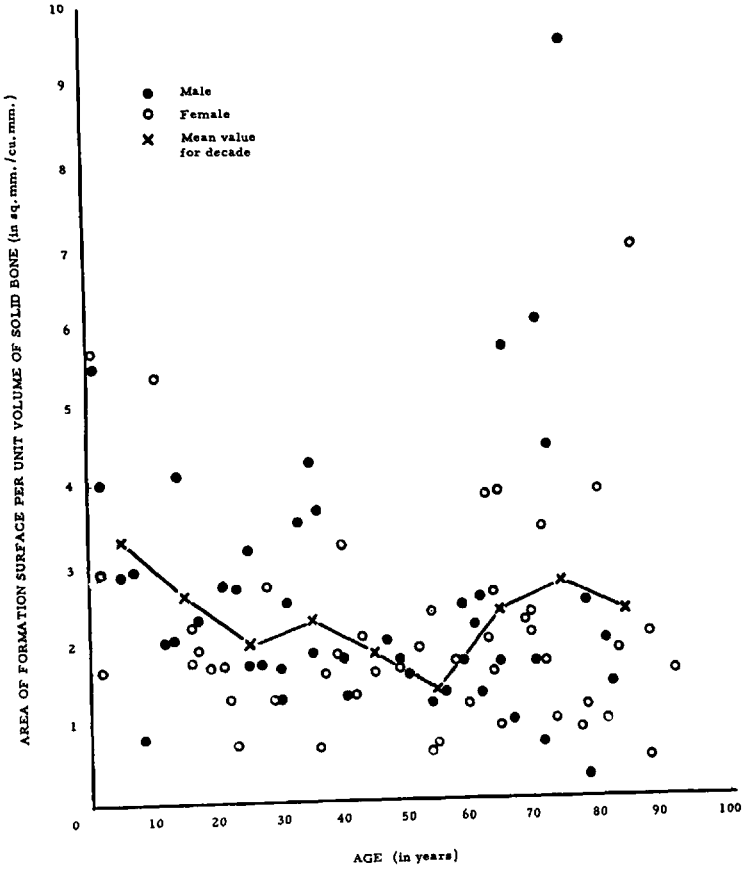


Table 24(a) FORMATION SURFACE AREA PER UNIT VOLUME OF SOLID BONE. INDIVIDUAL RESULTS. AGES 0 - 29

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5916	8	M	0.82
7156	2	M	4.05
6607	5	M	2.87
7018	11mos	M	5.48
6102	7	M	2.92
MEAN			3.23
7157	1	F	2.88
11922	3mos	F	5.67
5862	1	F	1.65
MEAN			3.40
MEAN FOR DECADE 3.29			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5391	17	M	2.28
10969	12	M	2.05
7162	14	M	4.12
6782	13	M	2.07
MEAN			2.63
10794	17	F	1.91
11263	11	F	5.37
11731	16	F	1.76
11576	19	F	1.71
11719	16	F	2.18
MEAN			2.59
MEAN FOR DECADE 2.61			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5590	27	M	1.73
6144	21	M	2.72
5591	23	M	2.70
11155	25	M	3.17
10788	25	M	1.71
MEAN			2.40
7114	22	F	1.27
10748	29	F	1.28
10762	23	F	0.71
10820	21	F	1.73
7160	28	F	2.71
MEAN			1.54
MEAN FOR DECADE 1.97			

Table 24(b) FORMATION SURFACE AREA PER UNIT VOLUME OF SOLID BONE. INDIVIDUAL RESULTS. AGES 30 - 59

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
6846	30	M	1.66
5359	33	M	3.53
10701	30	M	1.30
11117	35	M	1.86
11110	35	M	4.25
6011	31	M	2.49
6009	36	M	3.65
MEAN			2.68
6608	39	F	1.83
12080	37	F	1.59
11387	36	F	0.66
MEAN			1.36
MEAN FOR DECADE 2.28			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5732	40	M	1.82
6278	47	M	2.04
6152	49	M	1.75
6148	41	M	1.32
MEAN			1.73
10766	45	F	1.60
7373	40	F	3.23
10980	41	F	1.30
11256	49	F	1.73
11091	43	F	2.06
MEAN			1.98
MEAN FOR DECADE 1.87			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
6012	50	M	1.61
6150	59	M	1.75
12022	59	M	2.44
5674	54	M	1.22
10738	56	M	1.38
MEAN			1.68
5592	54	F	2.37
10970	54	F	0.58
11342	52	F	1.92
11904	59	F	1.74
12021	55	F	0.70
MEAN			1.46
MEAN FOR DECADE 1.57			

Table 24(c) FORMATION SURFACE AREA PER UNIT VOLUME OF SOLID BONE. INDIVIDUAL RESULTS. AGES 60+

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
11118	66	M	5.73
11978	62	M	2.55
6101	62	M	1.34
12027	67	M	0.99
5360	65	M	1.74
10779	61	M	2.19
MEAN			2.42
5881	63	F	2.04
6215	65	F	3.88
6785	65	F	0.93
6147	69	F	2.24
11061	60	F	1.20
11088	63	F	3.85
11154	64	F	1.59
11923	64	F	2.59
MEAN			2.29
MEAN FOR DECADE 2.35			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5733	73	M	4.45
6145	79	M	0.26
6087	71	M	6.06
6146	72	M	0.72
10711	71	M	1.75
5385	79	M	2.48
7396	76	M	9.60
MEAN			3.62
10898	78	F	0.87
10841	79	F	1.66
11102	70	F	2.35
11276	72	F	3.44
11313	72	F	1.74
6013	74	F	0.99
6149	70	F	2.11
MEAN			1.81
MEAN FOR DECADE 2.71			

CASE NO.	AGE (in yrs)	SEX	FORMATION SURFACE AREA (in sq.mm/cu.mm)
5675	82	M	2.00
11461	83	M	1.46
MEAN			1.73
11008	93	F	1.60
11109	89	F	0.49
11503	89	F	2.07
11264	87	F	7.01
11275	82	F	0.96
11977	84	F	1.87
11293	81	F	3.90
MEAN			2.56
MEAN FOR DECADE 2.37			

RESORPTION SURFACE

Individual results of extent of trabecular surface showing resorption cavities are shown in Table 25 and Figure 33, and mean values in Table 13. It is seen that at all ages, as with formation surface, a relatively high proportion of the surface is occupied by sites of bone resorption, again a higher proportion than is obvious in a conventional decalcified section. This is due largely to the essential rigidity of perspex embedded undecalcified sections, which preserves the sharp outline of small resorption cavities. Due to distortion and softening of the bone in decalcification these sharp outlines are blurred, and the outlines of many resorption cavities lost in decalcified sections. The overall mean percentage of resorption surface is 11.91%. In numerical value, both individual and mean figures are in most cases quite similar to those of formation surface.

As with formation surface the individual figures show a considerable scatter, even within the same age group. The greatest scatter and highest individual results are seen in childhood before the age of 10 years, in which group all the results lie between 14 and 32%. The highest individual value seen is one of 31.62% in a three month old child. Individual values are lower in the 10 - 19 age group, lying between 7 and 21%. In adult life between 20 and 50, with one exception, individual results are relatively low and show the least variation,

Figure 33. Resorption surface. Individual results plotted against age.

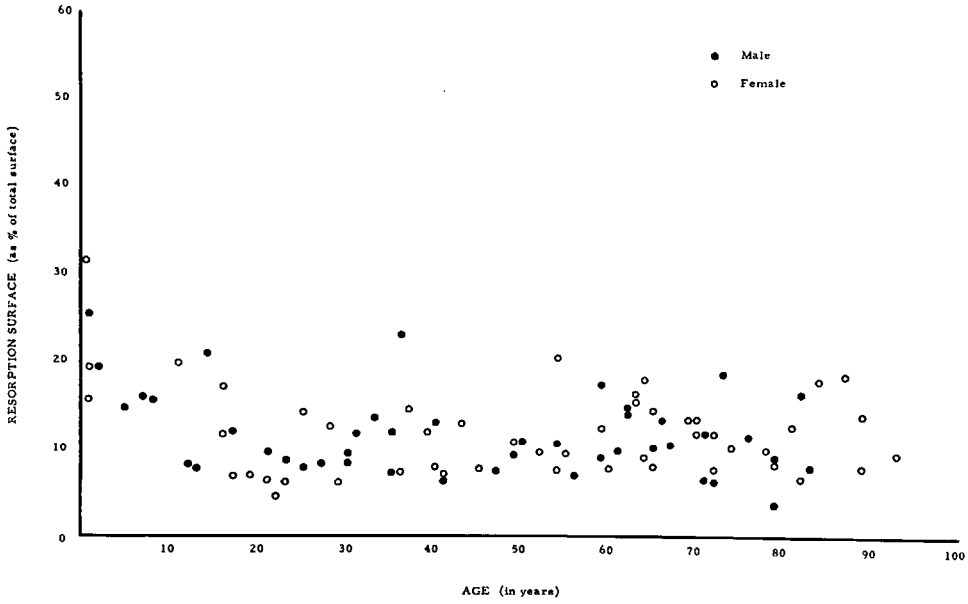


Table 25(a) RESORPTION SURFACE. INDIVIDUAL RESULTS. AGES 0 - 29

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE (in %)
5916	8	M	15.78
7156	2	M	19.25
6607	5	M	14.72
7018	11mos	M	25.48
6102	7	M	16.05
MEAN			18.26
7157	1	F	15.68
11922	3mos	F	31.62
5862	1	F	19.53
MEAN			22.28
MEAN FOR DECADE 19.76			

CASE NO	AGE (in yrs)	SEX	RESORPTION SURFACE (in %)
5391	17	M	12.02
10969	12	M	8.15
7162	14	M	21.00
6782	13	M	7.62
MEAN			12.20
10794	17	F	6.76
11263	11	F	19.96
11731	16	F	11.70
11576	19	F	7.01
11719	16	F	17.19
MEAN			12.52
MEAN FOR DECADE 12.38			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE (in %)
5590	27	M	8.20
6144	21	M	9.56
5591	23	M	8.61
11155	25	M	14.40
10788	25	M	7.84
MEAN			9.72
7114	22	F	4.46
10748	29	F	6.15
10762	23	F	6.21
10820	21	F	6.32
7160	28	F	12.59
MEAN			7.15
MEAN FOR DECADE 8.43			

Table 25(b) RESORPTION SURFACE. INDIVIDUAL RESULTS. AGES 30 - 59.

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE (in %)
6846	30	M	9.45
5359	33	M	13.59
10701	30	M	8.43
11117	35	M	7.40
11110	35	M	11.96
6011	31	M	11.84
6009	36	M	23.34
MEAN			12.29
6608	39	F	12.08
12080	37	F	14.75
11387	36	F	7.37
MEAN			11.40
MEAN FOR DECADE 12.02			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE (in %)
5732	40	M	13.18
6278	47	M	7.71
6152	49	M	9.29
6148	41	M	6.76
MEAN			9.24
10766	45	F	7.86
7373	40	F	7.89
10980	41	F	7.12
11256	49	F	11.05
11091	43	F	12.94
MEAN			9.37
MEAN FOR DECADE 9.31			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE (in %)
6012	50	M	10.96
6150	59	M	9.17
12022	59	M	17.56
5674	54	M	10.88
10738	56	M	7.06
MEAN			11.13
5592	54	F	20.81
10970	54	F	7.48
11342	52	F	9.89
11904	59	F	12.72
12021	55	F	9.69
MEAN			12.12
MEAN FOR DECADE 11.62			

Table 25(c) RESORPTION SURFACE. INDIVIDUAL RESULTS. AGES 60+

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE (in %)
11118	66	M	13.51
11978	62	M	14.92
6101	62	M	14.33
12027	67	M	10.57
5360	65	M	10.35
10779	61	M	10.09
MEAN			12.30
5881	63	F	15.70
6215	65	F	14.61
6785	65	F	8.11
6147	69	F	13.59
11061	60	F	7.88
11088	63	F	16.73
11154	64	F	9.20
11923	64	F	18.31
MEAN			13.02
MEAN FOR DECADE 12.71			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE (in %)
5733	73	M	19.02
6145	79	M	3.63
6087	71	M	11.95
6146	72	M	6.32
10711	71	M	6.64
5385	79	M	9.16
7396	76	M	11.60
MEAN			9.76
10898	78	F	9.96
10841	79	F	8.64
11102	70	F	12.23
11276	72	F	12.05
11313	72	F	7.76
6013	74	F	10.35
6149	70	F	13.67
MEAN			10.67
MEAN FOR DECADE 10.21			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE (in %)
5675	82	M	16.73
11461	83	M	7.95
MEAN			12.34
11008	93	F	9.49
11109	89	F	14.15
11503	89	F	8.05
11264	87	F	18.79
11275	82	F	6.75
11977	84	F	18.15
11293	81	F	12.87
MEAN			12.61
MEAN FOR DECADE 12.55			

forming a closely packed group, ranging from 5 - 15%.

In later life an appreciable number of higher individual values is seen, the highest being approximately 20%. The degree of scatter is also greater, and the lowest individual results of the study is seen; that of 3.63% in a 79 year old man.

No obvious difference can be seen at any age between male and female results and mean figures for each decade were therefore plotted from the combined results.

The mean figures agree with the trends of individual values. The pattern of the mean values reflects the high values seen in infancy and childhood. Subsequently the pattern is not regular, but the lowest results are seen in adult life, with a general trend towards higher values with increasing age. The highest value of 19.76% is seen in the 0 - 9 group. A highly significant fall ($P < 0.02$) is seen between this group and the 10 - 19 group, followed by a further fall, which approaches significance at the 5% level ($P < 0.10 > 0.05$), between the 10 - 19 and 20 - 29 groups. The lowest mean value (8.43%) is seen in this latter group. An increase is seen between the 20 - 29 and 30 - 39 groups, which again approaches significance at the 5% level ($P < 0.10 > 0.05$).

No significant differences are seen between the 30 - 39, 40 - 49 and 50 - 59 decades. A higher mean is seen in the 60 - 69 group; significantly higher than the mean value for the 40 - 49 group ($P < 0.01$). A significant

fall is seen between the 60 - 69 and 70 - 79 groups ($P < 0.05$). No further significant change is seen. If the value for the 40 - 49 group is compared with that of the combined over 50 group, then the latter is significantly higher ($P < 0.05$).

RESORPTION SURFACE PER UNIT VOLUME OF TISSUE (ABSOLUTE SURFACE AREA OF BONE RESORPTION.)

This parameter is a measure of the total surface area of bone resorption per unit volume of bone and marrow. Individual results are shown in Table 26, and graphically in Figure 34, which also shows mean values for each decade.

Individual results again show considerable scatter. The bulk of values lie between 0.2 and 0.5 sq.mm./cu.mm. The highest individual values, up to 1.62 sq.mm./cu.mm. are seen in infancy, while the lowest values are seen in the eighth decade, the lowest value observed being 0.1 sq.mm./cu.mm.

Values for males and females are intermingled at all ages, and no obvious difference can be seen between them. As before, therefore, mean values for each decade were calculated from combined male and female values (Table 13).

On examination of these mean values, an abrupt and highly significant ($P < 0.05 > 0.02$) fall is seen between the first and second decades. Subsequently no significant variation is seen, apart from a significant fall from the seventh to the eighth decade.

Figure 34. Resorption surface area per unit volume of tissue. Individual and mean values plotted against age.

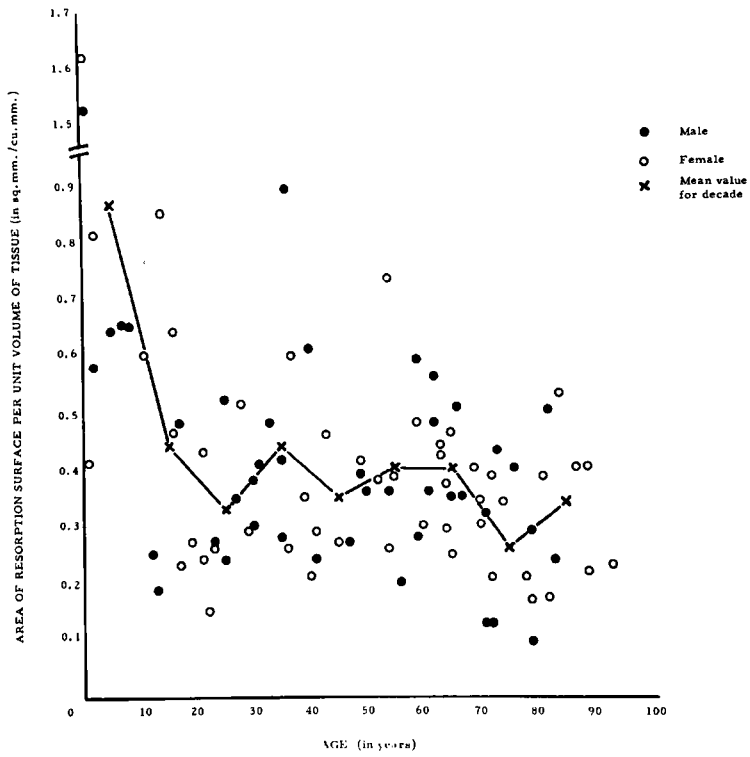


Table 26(a) RESORPTION SURFACE AREA PER UNIT VOLUME OF TISSUE. INDIVIDUAL VALUES. AGES 0 - 29.

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5916	8	M	0.65
7156	2	M	0.58
6607	5	M	0.64
7018	11mos	M	1.53
6102	7	M	0.65
MEAN			0.81
7157	1	F	0.41
11922	3mos	F	1.62
5862	1	F	0.81
MEAN			0.95
MEAN FOR DECADE			0.86

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5391	17	M	0.48
10969	12	M	0.25
7162	14	M	0.85
6782	13	M	0.19
MEAN			0.44
10794	17	F	0.23
11263	11	F	0.60
11731	16	F	0.46
11576	19	F	0.27
11716	16	F	0.64
MEAN			0.44
MEAN FOR DECADE			0.44

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5590	27	M	0.35
6144	21	M	0.43
5591	23	M	0.27
11155	25	M	0.52
10788	25	M	0.24
MEAN			0.36
7114	22	F	0.15
10748	29	F	0.29
10762	23	F	0.26
10820	21	F	0.24
7160	28	F	0.52
MEAN			0.29
MEAN FOR DECADE			0.33

Table 26(b) RESORPTION SURFACE AREA PER UNIT VOLUME OF TISSUE. INDIVIDUAL RESULTS. AGES 30 - 59

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
6846	30	M	0.38
5359	33	M	0.48
10701	30	M	0.30
11117	35	M	0.28
11110	35	M	0.42
6011	31	M	0.41
6009	36	M	0.89
MEAN			0.45
6608	39	F	0.35
12080	37	F	0.60
11387	36	F	0.26
MEAN			0.40
MEAN FOR DECADE 0.44			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5732	40	M	0.61
6278	47	M	0.27
6152	49	M	0.39
6148	41	M	0.24
MEAN			0.38
10766	45	F	0.27
7373	40	F	0.21
10980	41	F	0.29
11256	49	F	0.41
11091	43	F	0.46
MEAN			0.33
MEAN FOR DECADE 0.35			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
6012	50	M	0.36
6150	59	M	0.28
12022	59	M	0.59
5674	54	M	0.36
10738	56	M	0.20
MEAN			0.36
5592	54	F	0.73
10970	54	F	0.26
11342	52	F	0.38
11904	59	F	0.48
12021	55	F	0.39
MEAN			0.44
MEAN FOR DECADE 0.40			

Table 26(c) RESORPTION SURFACE AREA PER UNIT VOLUME OF TISSUE. INDIVIDUAL RESULTS. AGES 60+

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
11118	66	M	0.51
11978	62	M	0.56
6101	62	M	0.48
12027	67	M	0.35
5360	65	M	0.35
10779	61	M	0.36
MEAN			0.44
5881	63	F	0.44
6215	65	F	0.46
6785	65	F	0.25
6147	69	F	0.40
11061	60	F	0.30
11088	63	F	0.42
11154	64	F	0.30
11923	64	F	0.37
MEAN			0.37
MEAN FOR DECADE 0.40			

CASE NO	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5733	73	M	0.43
6145	79	M	0.10
6087	71	M	0.32
6146	72	M	0.13
10711	71	M	0.13
5385	79	M	0.29
7396	76	M	0.40
MEAN			0.26
10898	78	F	0.21
10841	79	F	0.17
11102	70	F	0.30
11276	72	F	0.38
11313	72	F	0.21
6013	74	F	0.34
6149	70	F	0.34
MEAN			0.28
MEAN FOR DECADE 0.27			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5675	82	M	0.50
11461	83	M	0.24
MEAN			0.37
11008	93	F	0.23
11109	89	F	0.40
11503	89	F	0.22
11264	87	F	0.40
11275	82	F	0.17
11977	84	F	0.53
11293	81	F	0.38
MEAN			0.33
MEAN FOR DECADE 0.34			

RESORPTION SURFACE PER UNIT VOLUME OF SOLID BONE (RELATIVE SURFACE AREA OF BONE RESORPTION.)

Here the surface area of bone resorption is related to the actual amount of bone present. Individual results are shown in Table 27 and Figure 35. The majority of results lie between 1 sq.mm./cu.mm. and 3 sq.mm./cu.mm. but as with all the parameters measured, a considerable scatter of results is seen at all ages. The highest individual results are seen in infancy, the highest figure being that of 9.05 sq.mm./cu.mm. in a three month old child.

The values for males and females are again completely intermingled, and as before mean values for each decade (Table 13) were calculated from the combined values from each sex. If these means are plotted graphically (Figure 35) a clearer pattern emerges:- that of a pronounced fall from the first to second decade, continued to the third decade. The difference between the values of first and second decades is highly significant ($P < 0.02 > 0.01$). After the third decade the mean values suggest a rise with age. Numerically the increase is not very great, but the differences between the results of the third decade and those of the sixth and seventh decades are highly significant ($P < 0.02 > 0.01$ and < 0.01 respectively).

The significance of the changes in formation and resorption surface and their surface area are discussed in the following section.

Figure 35. Resorption surface area per unit volume of solid bone. Individual and mean values plotted against age.

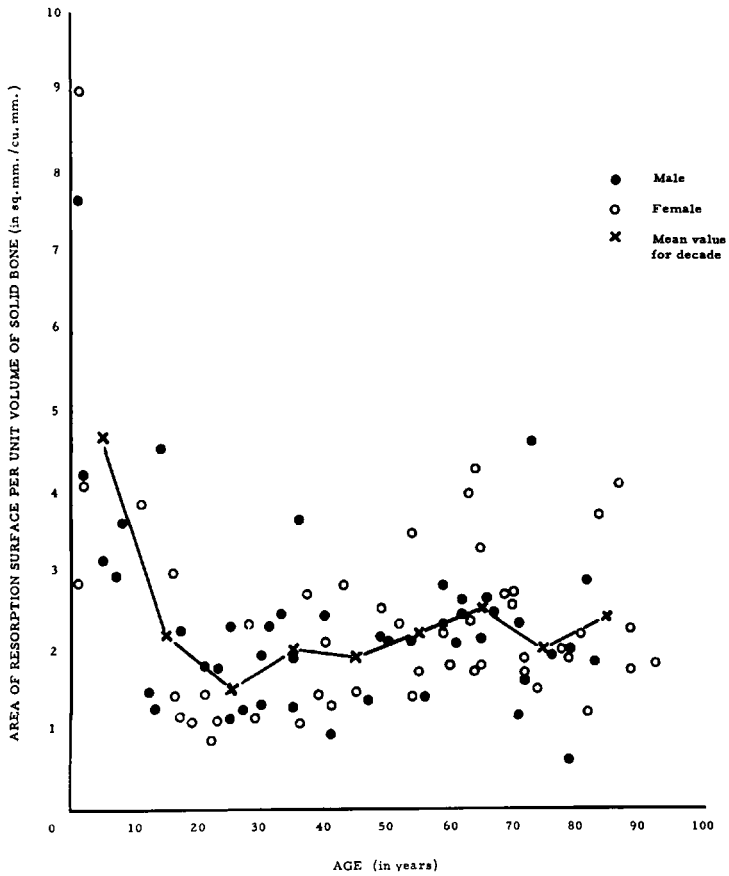


TABLE 2(a) RESORPTION SURFACE AREA PER UNIT VOLUME OF SOLID BONE. (MEDIAL WALL).

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5916	8	M	3.60
7156	2	M	4.19
6607	5	M	3.12
7018	11 mos	M	7.65
6102	7	M	2.92
MEAN			4.30
7157	1	F	2.82
11922	3 mos	F	9.05
5862	1	F	4.06
MEAN			5.31
MEAN FOR DECADE 4.67			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5391	17	M	2.23
10969	12	M	1.48
7162	14	M	4.53
6182	13	M	1.25
MEAN			2.37
10794	17	F	1.16
11263	11	F	3.84
11731	16	F	1.40
11576	19	F	1.09
11716	16	F	2.95
MEAN			2.09
MEAN FOR DECADE 2.21			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5590	27	M	1.24
6144	21	M	1.78
5591	23	M	1.76
11155	25	M	2.30
10788	25	M	1.15
MEAN			1.65
7114	22	F	0.86
10748	29	F	1.14
10762	23	F	1.11
10820	21	F	1.44
7160	28	F	2.33
MEAN			1.38
MEAN FOR DECADE 1.51			

Table 27(b) RESORPTION SURFACE AREA PER UNIT VOLUME OF SOLID BONE. INDIVIDUAL VALUES. AGES 30 - 59

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
6846	30	M	1.94
5359	33	M	2.45
10701	30	M	1.31
11117	35	M	1.27
11110	35	M	1.92
6011	31	M	2.33
6009	36	M	3.64
MEAN			2.12
6608	39	F	1.43
12080	37	F	2.72
11387	36	F	1.07
MEAN			1.74
MEAN FOR DECADE 2.01			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5732	40	M	2.43
6278	47	M	1.36
6152	49	M	2.17
6148	41	M	0.93
MEAN			1.72
10766	45	F	1.47
7373	40	F	2.10
10980	41	F	1.28
11256	49	F	2.49
11091	43	F	2.79
MEAN			2.03
MEAN FOR DECADE 1.89			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
6012	50	M	2.14
6150	59	M	2.33
12022	59	M	2.82
5674	54	M	2.13
10738	56	M	1.39
MEAN			2.16
5592	54	F	3.44
10970	54	F	1.39
11342	52	F	2.34
11904	59	F	2.25
12021	55	F	1.73
MEAN			2.23
MEAN FOR DECADE 2.20			

Table 27(c) RESORPTION SURFACE AREA PER UNIT VOLUME OF SOLID BONE. INDIVIDUAL RESULTS. AGES 60+

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
11118	66	M	2.64
11978	62	M	2.62
6101	62	M	2.45
12027	67	M	1.46
5360	65	M	2.14
10779	61	M	2.07
MEAN			2.23
5881	63	F	2.39
6215	65	F	3.25
6785	65	F	1.78
6147	69	F	2.67
11061	60	F	1.80
11088	63	F	3.96
11154	64	F	1.75
11923	64	F	4.27
MEAN			2.73
MEAN FOR DECADE 2.52			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5733	73	M	4.62
6145	79	M	0.58
6087	71	M	2.33
6146	72	M	1.59
10711	71	M	1.16
5385	79	M	1.98
7396	76	M	1.97
MEAN			2.03
10898	78	F	1.99
10841	79	F	1.90
11102	70	F	2.70
11276	72	F	1.68
11313	72	F	1.91
6013	74	F	1.51
6149	70	F	2.55
MEAN			2.03
MEAN FOR DECADE 2.03			

CASE NO.	AGE (in yrs)	SEX	RESORPTION SURFACE AREA (in sq.mm/cu.mm)
5675	82	M	2.86
11461	83	M	1.84
MEAN			2.35
11008	93	F	1.79
11109	89	F	2.26
11503	89	F	1.75
11264	87	F	4.08
11275	82	F	1.20
11977	84	F	3.68
11293	81	F	2.17
MEAN			2.42
MEAN FOR DECADE 2.40			

SECTION FIVE

DISCUSSION

DISCUSSION

Discussion will be concentrated upon consideration of bone area results, since of all parameters measured in this morphological study of cancellous bone, bone area is the most accurately measurable and most easily interpretable, and therefore most meaningful and of most practical significance.

BONE AREA

Several important points emerge from this study. No sex difference can be seen. Values from males and females are intermingled and each appears to be part of the same homogenous group. Individual results plotted against age form a band of fairly uniform width, parabolic in shape. The bone area rises throughout childhood, and reaches a maximum in early adult life, the bulk of high individual results occurring between 20 and 40 years of age. The bone area subsequently falls with age, the fall being highly significant by the sixth decade. No further fall is seen after the eighth decade. In all age groups individual results form a continuous series. There is no evidence at any age of a dual population - a normal group and a pathological group with less bone. Loss of bone with age appears to be a universal process, affecting all individuals.

Comparison with other published studies on cancellous bone is difficult because it is unusual for results to be expressed in absolute terms, and frequently no distinction has been drawn between cancellous and cortical bone.

Beck and Nordin (1960) studied a large series based on iliac crest bone from general hospital autopsies. Loss of bone substance with age was considered to be more frequent in women than men. A virtual absence of cases under 40 years of age prevents assessment of the age at which loss of bone begins. Frequency distributions of the results were asymmetrical and it was concluded (Nordin 1963) that two populations were represented at all ages - a normal group and an abnormal group with less bone. These results may be criticised on several grounds, The cases studied were not normal before death since all were hospitalized. The method of measurement was subjective, the amount of bone present in a section being determined by visual comparison with a standard sample. Results were expressed in a somewhat arbitrary fashion, scores of 1 - 9 representing percentage areas of bone from 6 - 27%. Specimens showing a percentage area of bone greater than 27% were given a score of 9 only, a system which seems likely to encourage an asymmetrical frequency distribution. For these reasons it is felt unwise to draw firm conclusions from these results.

Saville (1965) measured the weight/volume ratio of iliac crest trephine samples, and concluded that loss of bone was related to the climacteric in both sexes, but examination of his figures shows that in females the amount of bone diminished from the third decade while in men the amount of bone fell from the third to the fifth decades, this fall being highly significant ($P < 0.01$). Thus these

figures tend to support the thesis that loss of bone with aging may start at an early period in life.

More closely comparable is the study of Garner and Ball (1966), who measured the percentage of section area occupied by bone in a number of iliac crest trephine specimens. The figures lie in the same range as those of the present study. Although the conclusion is drawn that no appreciable fall in bone area is seen before the age of 50, examination of the scattergram of individual results shows that they form a parabolic band, similar to that of the present study. Results are limited, but the pattern suggests that a peak is seen at the age of 30 - 35 with a subsequent fall before the age of 50, although naturally the fall is more obvious in later years. Although results from females in particular are limited, values from males and females are completely intermingled, and no sex difference can be seen. Again the figures appear to form one continuous series.

The results are not completely comparable, as those from the present study are derived from section areas five to ten times as large as those of Garner and Ball's study, but they are closely related. If mean figures are calculated for each decade from the combined results of the two studies, a rise in bone area is seen from childhood. Very little difference is seen between the mean figures of the second, third and fourth decades, that for the fourth decade being fractionally higher, but

from this time a progressive decrease with age is seen.

Comparison may be made with results from other sites of cancellous bone. Studying vertebral body slabs by radiographic densitometry Caldwell (1962) showed a progressive decrease with aging in the amount of bone present, the process appearing to start from youth. No significant difference was seen between males and females.

Somewhat similar results were obtained by Arnold (1964) using the ash content per cubic centimetre of vertebral cancellous bone as a measure of the amount of bone tissue present. A progressive decrease in amount of bone as a function of age was seen, a marked linear fall being seen between 35 and 85 years of age. No sex difference can be seen. Vost (1963) studying vertebral bone concluded that a bimodal population was present in both sexes. There is no evidence of this in Arnold's data, nor is any sex difference apparent.

Lindahl and Lindgren (1962) studied the bone area in tibial cancellous bone, and also measured the weight/volume ratio of samples of vertebral and tibial cancellous bone. In both sites, and with both methods, they showed a progressive fall in the amount of bone present, the fall starting from the 14-19 age group, the extent and rate of loss being essentially the same in men and women.

Cortical bone has also been studied. The percentage of section area occupied by bone in femoral cortex was

also measured in the cases from which the present study was drawn (Sissons, Holley and Heighway 1967), the results suggesting loss of bone from the age of 20 years (the earliest age studied). No difference was seen between the sexes until after the age of 70 years when values from males appeared to be slightly higher than those from females.

Atkinson, Weatherell and Weidmann (1962) measured the weight/volume ratio and cortical thickness of femoral cortical trephine samples, both being parameters of the amount of bone present. They concluded that the weight/volume ratio began to decrease in both sexes during the fifth decade while cortical thickness diminished in a linear fashion throughout the age range studied (from 20 years onwards). This work was elaborated by Atkinson (1965), grouping the cases according to the percentage of vascular spaces in the cortex (the remaining area consisting of bone). He established a relation of increasing porosity of bone to aging, this trend becoming definite after the 30 - 39 age group. The individual figures of this study show no obvious sex difference.

In rib Sedlin (1964) used the area of cortical bone in a cross section of the bone, and the ratio of this area to the total cross-section area as indices of the amount of bone present. The former index declines from early adulthood in both males and females, while the latter index declines from infancy in a roughly linear fashion

in both sexes. There is no obvious relation to the menopause. Takahashi and Frost (1966) confirmed these results with a larger series. These authors stated that their results showed that women tend to retain bone better than men until after the age of 50, and thereafter lose bone more quickly, but if the published figures are plotted and examined, the pattern of fall appears similar in the two sexes, and this conclusion appears unjustified.

Trotter and her associates (Broman, Trotter and Peterson 1958, Trotter, Broman and Peterson 1960) measured the density of whole bones as an index of the amount of bone present, long bones, ribs and vertebral column being studied. They concluded that a decline in amount of bone present was seen with age, and that this proceeded at a uniform rate. No cases younger than 25 years were examined.

These laboratory studies show varying ages of onset of bone loss. This is not surprising as different bone sites were used, and all studies were on relatively small populations. Almost all of the studies, however, suggest that bone loss with aging starts from an early age, earlier than that recognised clinically, and that in these respects there is no obvious sex difference.

The results of the present study are fully in keeping with those of other studies reviewed above. Again, the thesis from this study of the universality of bone loss with aging, bone loss with increasing age being a process apparently affecting all individuals, rather than a

process affecting a group of individuals while the majority of elderly persons show little or no bone loss, is supported by several of the other studies reviewed.

RELATION OF ONSET OF BONE LOSS TO MENOPAUSE

Of more importance than the actual chronological age of onset of bone loss is the relation this bears to the menopause. The relevance of Albright's theory of osteoporosis depends on this point. Albright originally related the onset of osteoporosis to the menopause using clinical and simple radiological criteria for the recognition of bone loss. Both of these are insensitive. Two recent clinical radiological studies (Nordin, MacGregor and Smith 1966, Meema, Bunker and Meema 1965) claim to relate the onset of bone loss to the menopause. Nordin and co-workers data relate to spine, metacarpal and femur. The greatest fall occurs between five and fifteen years after the menopause in each case, but in the case of spine and metacarpal the loss actually appears to start before the menopause. These metacarpal results are somewhat similar to those of Morgan, Pulvertaft and Fourman (1966) who concluded that cortical thinning (and therefore bone loss) occurred progressively, certainly starting from the age of 40 in both sexes. Changes possibly occurring before 40 years were not investigated. Nordin and co-workers did not study males. Femoral cortical thickness showed practically no change before the age of 60 years, but this takes no account of bone loss in the

form of increasing porosity which may have occurred at an earlier age. It is felt that the study of Nordin and co-workers does not demonstrate any conclusive relation between the menopause and bone loss.

Meema, Bunker and Meema (1965) studied total hydroxyapatite content of a cross section of the radius. Their results suggested that bone loss from the radius starts at about the age of the menopause, but this does not prove a causal relationship. Before this could be done it should be shown that no changes in males occur at the same age. Males were not investigated. Their results suggested a relation between onset of bone loss and induction of artificial menopause, although clinically Donaldson and Nassim (1954) and Moon and Urist (1962) could find no evidence of this. Theseresults of Meema and co-workers await confirmation by other workers.

The results of the present study lead to a different conclusion. Frommer (1964) estimated the modal and median age of the female menopause to be 50.1 years. In the present study, although considerable bone loss in the female is seen after the menopause, the figures suggest that the time of onset is before the menopause. Furthermore bone loss in the male appears to start at the same age, and to occur to the same extent. There is no evidence to suggest that bone area in males remains constant until a later age.

By definition osteoporosis is a reduction in the amount of bone present. Thus a reduction in bone area is

a valid measure of osteoporosis. The present study therefore does not support Albright and Reifenstein's theory of pathogenesis of osteoporosis, as at no time is there any evidence of any difference between males and females as regards loss of bone.

Although the present study was based on as large a series of cases as was practicable, this conclusion is still based on a relatively small sample, and further results are necessary for this conclusion to be definitely established. It finds support however in the laboratory studies of other workers discussed earlier. Employing various methods for measuring bone loss, and studying various bone sites, almost all show that loss of bone starts from an earlier age than is generally realised and that the onset of bone loss appears to be unrelated to the menopause.

The individual results of this study form a band of fairly uniform width. At no age is there any evidence of a bimodal population; of a normal group, and of an abnormal osteoporotic group with less bone. Therefore, neither are the results in keeping with Nordin's theory that senile osteoporosis is a pathological phenomenon, seen in a group of aging people and due to calcium deficiency. They are not against the concept that bone loss with aging is mediated by calcium removal from the skeleton, but there is no evidence of a group of osteoporotic individuals, the remainder of the population

showing less or no bone loss, as would be the case if calcium deficiency were the cause of osteoporosis.

Rather than senile and postmenopausal osteoporosis being regarded as a pathological condition, the rise in bone area throughout childhood reaching a peak in early adult life, the subsequent loss of bone substance from a relatively early age (all individuals apparently being affected), and the similarities in age of onset and degree of loss of bone between males and females suggest that much if not all loss of bone is a normal aging process. It is suggested that involutional (i.e., senile and post-menopausal) osteoporosis is essentially a physiological manifestation of aging, rather than a pathological process seen in old age.

Strehler, Mark, Mildvan and Gee (1959) laid down criteria for a change in body structure to be considered part of the aging process. The change should be "universal, progressive, deleterious to the organism and intrinsic". Loss of bone with aging would certainly appear to meet the first three criteria, and probably the fourth.

Shock (1960) has peported that many physiological functions show a decrement after the age of thirty years and Ruger and Stoesigger (1927) reported that in males certain muscular functions such as strength of pull and power of grip, after rising to a maximum in adulthood, gradually fell away after the ages of 20 - 40 years.

The amount of bone present appears to behave likewise.

This does not mean that bone loss is related to change in any of the functions mentioned. What determines loss of bone is at present unknown; the causation may be multifactorial, but the change appears to be inevitable and universal.

SITES OF PREDILECTION OF OSTEOPOROSIS

Osteoporosis was originally regarded (Albright, Bloomberg and Smith, 1940) as affecting predominantly the axial skeleton, composed largely of cancellous bone, and as being seen later and to a lesser degree in the appendicular skeleton, composed mainly of cortical bone.

Comparison of the changes demonstrated in this study with those of the femoral cortex of the same cases (Sissons, Holley and Heighway 1967) shows however that loss of bone starts as early, possibly earlier, from femoral cortical bone as from iliac crest cancellous bone. In absolute terms, the amount of bone lost from each site is similar but the relative loss is much greater from the iliac crest. In the light of these results it is suggested that radiologically bone loss is recognised earlier, and more readily, from sites containing predominantly cancellous bone, rather than actually affecting these earlier.

INCIDENCE OF BONE LOSS IN MALES AND FEMALES

No sex difference was seen in the present study, and this is in agreement with most laboratory studies where amount of bone substance or bone ash content per unit

volume of bone and marrow has been measured. Most clinical studies have suggested a predominance of female cases of osteoporosis (Cooke 1955) although it is of interest that in surveys of asymptomatic patients using radiological criteria for the recognition of osteoporosis, Gershon-Cohen, Rechtman, Schraer and Blumberg (1953) and Vincent and Urist (Vincent and Urist 1961, Urist 1960) concluded that the indicence of osteoporosis in elderly males was little different to that in females. This difference between clinical and laboratory studies is as yet unexplained (Nordin 1964b). Clinical recognition of osteoporosis depends mainly on symptomatology, due largely to crush fracture of the vertebrae. It is possible that other factors besides bone loss play a part in the causation of bone failure and pathological crush fractures of the vertebrae, seen in osteoporotic patients. More work is necessary on this subject.

SURFACE AREA

The measurement of surface area of bone is a neglected subject. Surface area has been measured in femoral cortical bone of the cases forming the present study (Sissons, Holley and Heighway 1967), and a study of surface area of vertebral cancellous bone has also been published by Bromley, Dockum, Arnold and Jee (1966) but, other than these, no measurements have been reported.

The vertebral results are expressed in different numerical units (cm./sq.cm.) to those used in this study (sq.mm./cu.mm.), but if converted to the same units the results of the two studies are similar both as regards actual values obtained and pattern of change with age, both studies showing a decline from childhood.

The relationship of surface area to amount of bone tissue present (surface area per unit volume of solid bone or relative surface area) has not been studied by other workers.

Comparison of surface area of cancellous bone of iliac crest and cortical bone of femoral shaft reveals that, in absolute terms, despite their different structural arrangement, the two types of bone provide a similar extent of surface area per unit volume of tissue, the mean values varying during adult life from 3.9 to 3.0 sq.mm./cu.mm. in iliac crest, and from 3.0 to 4.3 sq.mm./cu.mm. in femoral cortical bone. It will be noted that, while loss of surface area roughly parallels loss of bone in

cancellous bone, in cortical bone loss of bone and increasing porosity serves to increase the bone surface area.

A different situation is seen if the figures for surface area are expressed in relation to the amount of bone present. Expressed in this fashion the surface area of iliac crest cancellous bone is considerably greater per unit volume of solid bone than that of cortical bone, by a factor of five to one.

As metabolic interchanges such as bone formation and resorption and much of mineral exchange will occur at the trabecular and Haversian surface, it is likely that bone with a high surface area relative to the amount of bone will be metabolically more active, and will show a more rapid turnover, than bone with a low surface area. Cancellous bone has long been regarded as possessing a more rapid turnover rate than cortical bone (Bauer, Aub and Albright, 1929; Amprino and Engström 1952; Bryant and Loutit 1961); the considerably greater surface area of cancellous bone per unit volume of solid bone provides a morphological basis for this, and is undoubtedly a factor concerned in the higher turnover rate.

As has been seen the relative surface area of cancellous bone is significantly greater in childhood and the over 40 age group than in the 10 - 39 age group, this being a reflection of the delicate bone trabeculae seen in childhood and the preponderance of slender

trabeculae amongst those which remain in old age.

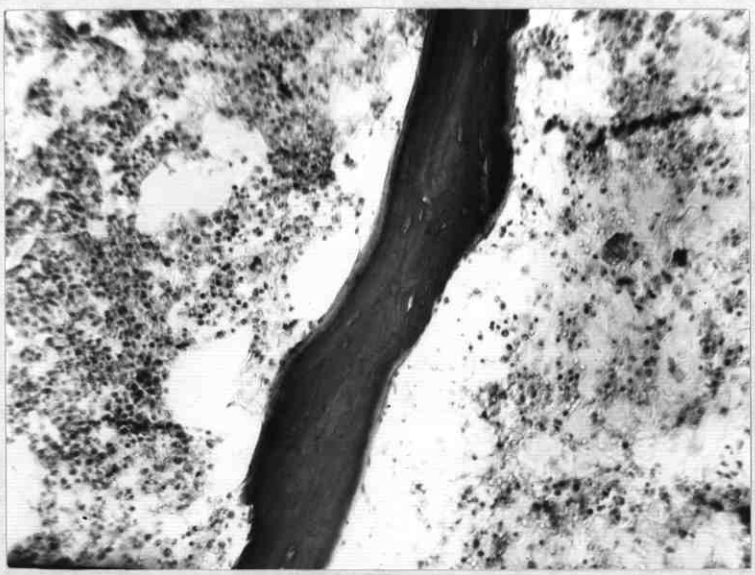
In particular the relative surface area is significantly higher in infants and young children than in any other age group. Experimental studies using radio-active isotopes have suggested (Bauer, Carlsson and Lindquist 1961) that bone turnover is more rapid in infants and the young than in older individuals and it is suggested that the greater relative surface area in this group may well play an important causative part.

BONE FORMATION AND RESORPTION

Bone formation and resorption are related topics and will therefore be discussed together. The high values of formation surface seen in the elderly are of considerable interest. No values above 20% were found between 40 and 60 years of age, although a few were seen in adults below 40. In cases aged 60 years and over, however, virtually one sixth show values above 20%. Several explanations are possible. These values tend to be rather separate from the main group, and might be pathological, There is however no evidence to suggest this. The bone was taken from normal persons dying suddenly. In every case autopsy showed no skeletal abnormality, nor any pathological lesion which might affect the skeleton. In these cases the bone structure is normal, the osteoid is regular in distribution, individual osteoid seams are regular and well defined, resorption appears normal, and there is no evidence of the disorderly, irregularly increased, bone formation and resorption surface, and mosaic pattern of the bone characteristic of Paget's disease, (Collins 1956; Sissons 1966). There is no evidence whatsoever of osteitis fibrosa to suggest hyperparathyroidism (Sissons 1966). Florid osteomalacia is marked by increased surface coverage by osteoid as well as by the presence of abnormally thick osteoid seams (Sissons 1966). (Figure 36). In cases of clinical osteomalacia studied personally, virtually all the bone surface is covered by osteoid of approximately

Figure 36. Iliac crest bone from case of clinical osteomalacia. The bone surface is covered by a very thick layer of deeply staining osteoid, with little distinction between bone and osteoid, and blurring of the calcification front. Undecalcified section. Haematoxylin and eosin. (x160).

Figure 37. Iliac crest bone from 75 year old male with high osteoid coverage. The osteoid seam thickness is not increased, and there is no evidence of osteomalacia. Undecalcified section. Haematoxylin and eosin. (x160).



double the normal thickness. The possibility arises that the high values of formation surface obtained in an apparently normal population represent examples of early or subclinical osteomalacia. There is no evidence to support this view; in the cases under discussion the thickness of osteoid seams is normal. (Figure 37). The morphology and staining reaction of individual seams are normal, and the appearance of the calcification front is normal (abnormalities of osteoid seams and calcification fromt being seen in osteomalacia (Johnson 1964).) (Figure 36).

It is more likely that these values are physiological and part of the normal group, and they have been accepted as so, and used in calculating mean figures for the relevant decades, which may be compared with other published results. Such published studies are sparse, and comparison is hampered by the fact that in some instances findings have not been expressed in absolute terms.

Results from the femoral cortical bone of the present group of cases were presented in an earlier study (Sissons, Holley and Heighway 1967). Comparison of the two sites indicates that iliac crest cancellous bone possesses a considerably greater extent of formation surface at all ages than femoral cortical bone. In the discussion of surface area it has been pointed out that cancellous bone has long been regarded as possessing a higher turnover rate than cortical bone, and that some of this greater

turnover rate is due to the greater surface area (per unit volume of solid bone) of cancellous bone. The present results suggest that the greater percentage of surface occupied by bone formation is also a factor.

Frost and his co-workers studied bone formation surface in cortical bone of ribs and clavicles (Villaneuva, Sedlin and Frost 1963). Direct comparison of the present results and those of Frost and colleagues is not possible, as their results are expressed in terms of the number of seams present, and not the extent or area of surface covered. The technique of surface measurement is preferable to that of counting osteoid seams, as what appear in a section to be separate osteoid seams are frequently no more than parts of a continuous surface of bone formation.

Although direct comparison is impossible it is of interest that the pattern of results is similar to that seen in this study. A sharp fall in the number of osteoid seams was observed from infancy through childhood to a minimum between the ages of 35 and 40, a subsequent increase being seen in the elderly.

Using microradiographs, Jowsey, Kelly, Riggs, Bianco, Scholz and Gershon-Cohen (1965) measured the extent of bone formation in a number of sites of cancellous and cortical bone. Their values from iliac crest are similar in pattern to those of the present study, although the extent of formation surface as judged by osteoid coverage

is consistently higher than the extent of such surfaces as judged from microradiographs by Jowsey and her colleagues. Much of the discrepancy is attributable to technical factors. Interpretation of the characteristics of bone surfaces in microradiographs is complicated by the fact that microradiographs are prepared from thick sections. Jowsey and co-workers used sections of 100 μ thickness. In cancellous bone, oblique surfaces which may simulate or obscure the appearances of bone formation abound in sections of this thickness.

The solitary other published study of extent of formation surfaces in iliac crest cancellous bone confirms the numerical values found in the present study. Van der Sluys Veer, Smeenk and Van de Heul (1964) measured the extent of formation surfaces in iliac crest biopsy specimens by tetracycline labelling. Their mean value of 12% (range 6 - 22%) in 17 control subjects agrees well with my figures.

The pattern of results is of more importance than the numerical values obtained, and in this respect the studies of Jowsey and co-workers and myself are in general agreement, both showing a marked fall in formation surface from infancy to childhood, the values then levelling off and tending to rise again in later life.

Bromley, Dockum, Arnold and Jee (1966), using a von Kossa stain, measured the extent of osteoid coverage in human vertebral cancellous bone. Their results are very

similar both in magnitude and pattern to those derived from iliac crest in the present study.

Thus studies agree that a profound fall in formation surface is seen from infancy to adulthood. Values then level off, and in most cases a subsequent rise is seen in middle or old age.

With the exception of studies on the femurs of the cases used in the present investigation there are no other published studies on formation surface per unit volume of bone or tissue.

Published data on extent of resorption surface in cancellous bone are also scanty. Jowsey, Kelly, Riggs, Bianco, Scholz and Gershon-Cohen (1965) also measured resorption surface from microradiographs of 100μ thick sections of iliac crest. The mean values in the present study are higher in all decades except possibly the second. This is probably an expression of the greater accuracy of identification of small Howship's lacunae in thin sections, as opposed to microradiographs from sections five times as thick. Again, however, the pattern of results is of more importance, and here the two studies agree. After very High values in childhood the lowest mean figure is seen in the 20 - 29 decade, with a subsequent trend towards high values.

Sedlin, Villaneuva and Frost (1963) measured resorption surface in cortical bone of ribs, values being expressed in terms of resorption surface area per unit volume of tissue. The values obtained are similar in magnitude to

those of this study, and in both a sharp fall was seen from infancy through childhood to maturity.

SIGNIFICANCE OF PATTERN OF FORMATION SURFACE AND RESORPTION SURFACE CHANGES

Most published studies agree in showing highest values of formation and resorption surface in infancy and lowest values in early adult life with a subsequent increase, and my results also show that the area of formation surface, (both in absolute terms as square mm. per cubic mm. of tissue and in relative terms as square mm. per cubic mm. of solid bone) is greatest in infancy.

The meaning of these changes, at least in adult life, is not entirely clear, and depends upon the relationship between extent of formation and resorption surface, and bone formation and resorption rate. Bone formation rate is the product of extent of formation surface and the linear rate at which bone is laid down at any site (appositional growth rate). This latter parameter can only be measured by in vivo techniques, such as multiple labelling with tetracycline, or similar marker, and very little information exists on its variation (if any) with age (Frost 1963; Taylor, Epker and Frost 1966).

Most workers (Jowsey, Kelly, Riggs, Bianco, Scholz and Gershon-Cohen 1965; Bromley, Dockum, Arnold and Jee 1966) tacitly assumed that changes in bone formation rate are mediated exclusively or almost entirely by changes in the extent of bone formation i.e., in formation surface.

From this point of view an increase in formation surface can be equated with an increase in bone formation rate and vice versa.

It is probable that this is the case in increased bone formation seen in pathological conditions, such as Paget's disease, in which condition Lee (1967) showed that the bone formation rate was ten times greater than normal but that the appositional growth rate was only slightly, if at all, increased. The increase in bone formation rate was due almost entirely to increase in extent of formation surface.

It is by no means certain, however, that this state of affairs applies to the process of aging. Indeed, recent experimental results suggest that the appositional growth rate of bone decreases with age. Frost's initial results (1963) employing tetracycline labelling were equivocal, but in a more recent study, using cortical bone of ribs (Taylor, Epker and Frost 1966) it was found that the appositional growth rate fell throughout life, being approximately halved between the ages of twenty and seventy. It seems likely that a similar reduction will be seen in cancellous bone. Lee, Marshall and Sissons (1965) measured appositional growth rate in both cortical and cancellous bone in two dogs. The rate was less in the older animal in both cortical and cancellous bone.

Further work on this subject is necessary, but of the two conflicting possibilities such evidence as is available

suggests that the appositional growth rate falls with age, instead of remaining constant as other workers have assumed.

According to either of these two views, the appositional growth rate in infancy is not lower than in adult life. Thus it seems certain that the fall in formation surface, formation surface area per unit volume of tissue, and formation surface area per unit volume of solid bone from infancy to adulthood represent a fall in bone formation rate; bone formation rate being highest in infancy. This in association with the high resorption surface seen in infancy suggests that bone turnover rate will also be highest in infancy. This finds support from data obtained from isotopic studies by Bauer, Carlsson and Lindquist (1961) who calculated that the rate of skeletal turnover was highest in infancy.

With regard to the increased formation surface seen in old age, several possible explanations exist. If the appositional growth rate remained constant the increased formation surface might compensate for the reduced bone surface area; the smaller area of bone present being covered more fully by sites of bone formation, the bone formation surface area remaining constant. It has been shown that the formation surface area per unit volume of tissue and per unit volume of solid bone in fact tend to rise in later life. Such a process would be seen as a factor tending to preserve calcium homeostasis.

It is suggested, however, that the most probable cause

of the increased formation surface seen in old age is a lowered appositional growth rate, bone formation being seen over a greater surface at any given time as bone is laid down more slowly at any site, and changes in formation surface area would be secondary to this.

Turning now to interpretation of changes of resorption surface throughout life, it is seen that formation surface and resorption surface both fall from infancy to childhood. The similarity of the patterns suggests that just as the bone formation rate, so the bone resorption rate falls from infancy to childhood.

The increase in mean resorption surface seen in old age might be related to a slowed rate of bone resorption at any site, or to an absolute increase in bone resorption as an imbalance between bone formation and resorption must occur to produce the bone loss of aging.

No experimental work has been carried out, however, on any possible change in the linear rate of bone resorption with age, and at present, therefore, such discussion of the significance of changes of resorption surface with aging must remain speculative.

In view of such imponderable factors it is felt that, at the present time, formation surface and resorption surface measurements should be considered more as general indicators of the extent of bone formation and resorption, rather than as accurate measures of bone formation and resorption rates as Jowsey and her co-workers have assumed

in their calculations. (Jowsey, Kelly, Riggs, Bianco, Scholz and Gershon-Cohen 1965). It is probably unjustified, and may well be fallacious to compare directly the extent of formation and resorption surface throughout life, in order to determine whether bone loss is mediated by an increase in bone resorption, a decrease in bone formation, a combination of the two, or, indeed, any combination of changes giving an imbalance between bone formation and resorption rates. The morphological methods used in this study need to be supplemented by dynamic methods such as tetracycline labelling for such information to emerge.

Such studies should be made throughout adult life; in seeking to establish the mechanism of bone loss with age, there is limited value in studying, as other workers have done, bone formation and resorption surfaces or bone mineral dynamics in established and clinically evident cases of osteoporosis, where bone loss has already occurred.

It is evident that by their nature morphological studies of the present type cannot provide full information as to the mechanisms of metabolic bone diseases, and for this to be obtained they must be supplemented by dynamic morphological methods in selected cases. Studies of the present kind do form, however, an indispensable background to the practical study of metabolic bone diseases.

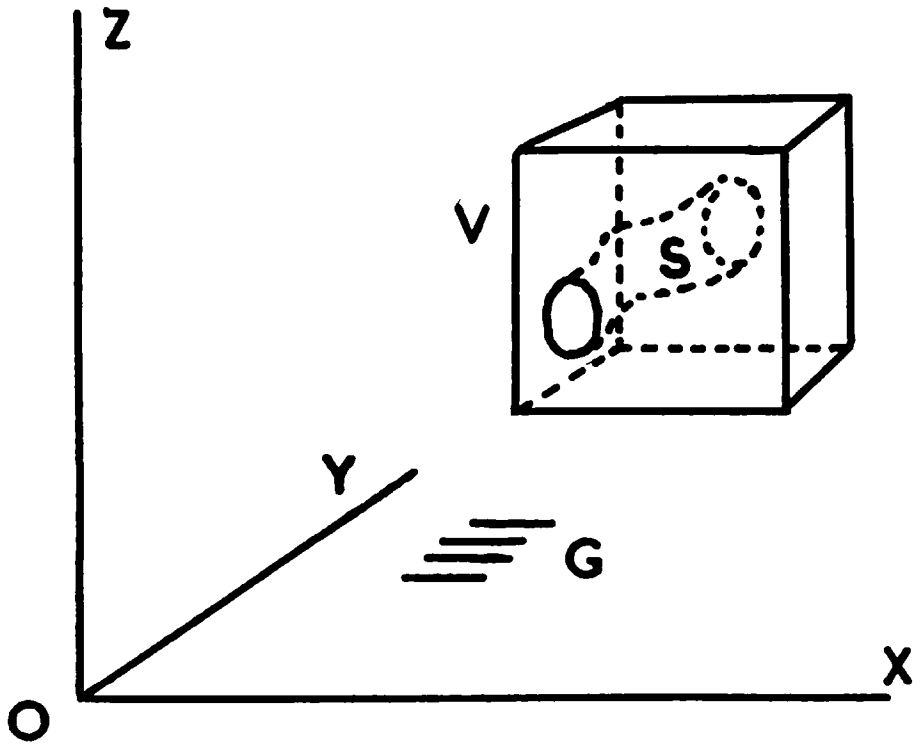
APPENDICES

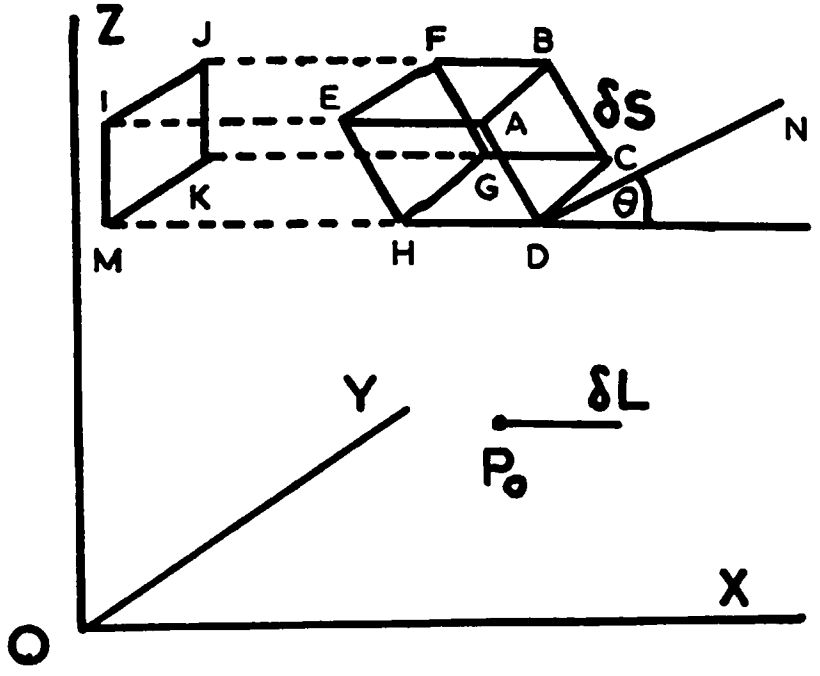
Figure 38. (On following page).

Figures to illustrate proof

of formula S.A. = $\frac{2N}{L}$

(v. appendix 1.).


Z
V
S
O
Y
G
X
O


Z
J
F
B
I
E
A
δS
N
C
K
G
θ
M
H
D
Y
δL
P₀
X
O

Appendix 1.

PROOF OF FORMULA S.A. = $\frac{2N}{L}$

after Rogers, C.A. in Short, R.H.D. Phil.Trans.B 1950 - 51 235, 35. (p.67)

Consider volume V of bone. Let S be the surface area of a trabecula contained in the volume V. Let G be a grid of lines of total length L, the size of the grid being small in comparison to V. G is moved by a simple translation to a random position in the volume V (each position being equally likely).

First consider the case when all lines of G are parallel to some fixed direction. For convenience we choose an origin O, and rectangular Cartesian co-ordinate axes OX, OY and OZ with OX in the direction of the lines of G. Consider an element of length δ l of one of the lines of G. Let P_o be the end point of this element with the lesser x co-ordinate. The element is moved by a translation to a random position in V.

Consider an element of the surface S with area δ s, the element being so small that it may be regarded as flat. Let θ be the acute angle between the normal to this element and the OX axis.

Now when the element δ l of G is moved into V it intersects the element δ s of S if, and only if, the point P_o is moved into the skew prism formed by moving

the element δs by a translation in the direction from X to O through a distance δl.

The volume of this prism is the product of δl and the area of the projection of the element δs on the plane OYZ

The area of projection is $\delta s \cos \theta$

$\therefore$ The volume $= \delta l \, \delta s \cos \theta$

As P is equally likely to be at any point in the volume V, its chance of being in this prism of volume $\delta l \, \delta s \cos \theta$ is $\frac{\delta l \, \delta s \cos \theta}{V}$

This is consequently the chance that the element δl of G intersects the surface element δs. We may assume that δl is so small that the chance of intersecting the surface S more than once is negligible.

Then the chance that δl intersects the surface S at some point is obtained by summing the chances of δl intersecting surface elements such as δs or by evaluating the integral

$$\int_S \frac{\delta l \quad \delta s \quad \cos \theta}{V} = \frac{\delta l}{V} \int_S \cos \theta \, \delta s$$

taken over the surface S.

As the surface S is highly convoluted, and as the normals to the surface are equally likely to point in any direction then

$$\int_S \cos \theta \, \delta s = CS$$

where C is the mean value of $\cos \theta$.

To find C we consider a sphere centre O with unit radius. The surface area of this sphere is 4π ; the area of projection of the surface on the plane OYZ is 2π (π from the hemisphere on one side of the plane OYZ and π from the hemisphere on the other side). The mean value for cos θ is the ratio of these 2 areas

$$\therefore C = \frac{1}{2}$$

The chance that the element δl intersects the surface at some point is

$$\frac{\delta l}{V} c S$$

$$= \frac{\delta l S}{2V}$$

On the average the element δl intersects the surface S, $\frac{\delta l S}{2V}$ times.

Summing over the whole length of the grid G, the grid will intersect the surface $\frac{LS}{2V}$ times on the average. Although for convenience it has been assumed that all the lines of G are parallel to the x axis it is clear that this assumption is not necessary.

$$N = \frac{LS}{2V}$$

$$S = \frac{2NV}{L}$$

If $V = 1$

then $S = \frac{2N}{L}$ per unit volume

Appendix 2.

DERIVATION OF THEORETICAL STANDARD ERROR FOR USE WITH AREA AND SURFACE AREA MEASUREMENTS USING ZEISS EYEPIECES I and II.

Let n be the total number of counts (points or intercepts) made on any section, and c be the percentage of total (surface or surface area) occupied by the sought component.

Then c will also be the percentage of counts falling upon the sought component. This figure will be a mean figure derived from many separate observations, which in themselves have a binomial distribution.

c will also have a binomial distribution (approaching a normal distribution as n rises) and as such has a theoretical standard error (expressed as a % of the total) of

$$\sqrt{\frac{c(100 - c)}{n}}$$

Expressing this as a fraction of the sought component

$$S.E = \frac{\sqrt{\frac{c(100 - c)}{n}}}{c}$$

As a percentage this becomes

$$S.E = \frac{100\sqrt{\frac{c(100 - c)}{n}}}{c} = 100\sqrt{\frac{100 - c}{nc}}$$

$$(S.E.)^2 = 100^2 \times \frac{100 - c}{nc}$$

Multiplying both sides by $\frac{nc}{100}$ and dividing by $(S.E.)^2$

then $$\frac{nc}{100} = \frac{100}{(S.E.)^2} \times (100 - c)$$

Now $\frac{nc}{100}$ equals no. of counts falling on sought component

To give a S.E. of 10% (of sought component)

$\frac{nc}{100}$ = No. of points on component = $\frac{100}{10^2} \times 100 - c$

= 100 - c.

∴ For a standard error of 10, 100 - c counts must have fallen on the sought component.

Similarly for a standard error of 5, 4 (100 - c) points on component c are necessary.

Appendix 3.

AGREEMENT OF SURFACE MEASUREMENTS USING DIFFERENT METHODS OF TURNING ZEISS EYEPIECE II THROUGH 90°.

Sample counts (20 fields counted in each case).

A. Original count, using new random orientation of eyepiece for each field.

Intersections with formation surface	=	12
Intersections with resorption surface	=	14
Intersections with inert surface	=	96
Total intersections	=	122

B. Count made with eyepiece at right angles to orientation in A (Angle being determined by inspection only.)

Intersections with formation surface	=	11
Intersections with resorption surface	=	11
Intersections with inert surface	=	95
Total intersection	=	117

C. Count made with eyepiece at right angles to orientation in A (Angle being determined with markers.)

Intersections with formation surface	=	11
Intersections with resorption surface	=	12
Intersections with inert surface	=	97
Total intersections	=	120

All three results are similar, although measurements from individual fields in A showed considerable differences

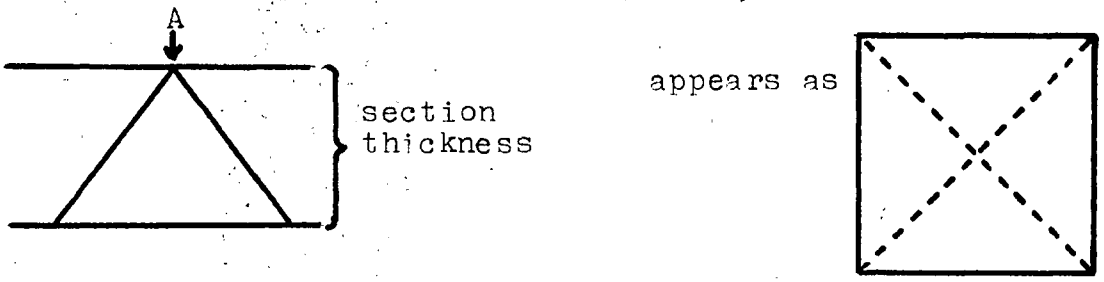
from those in B and C. The results of B and C (individual fields and totals) are virtually identical, showing that method C is accurate.

Appendix 4

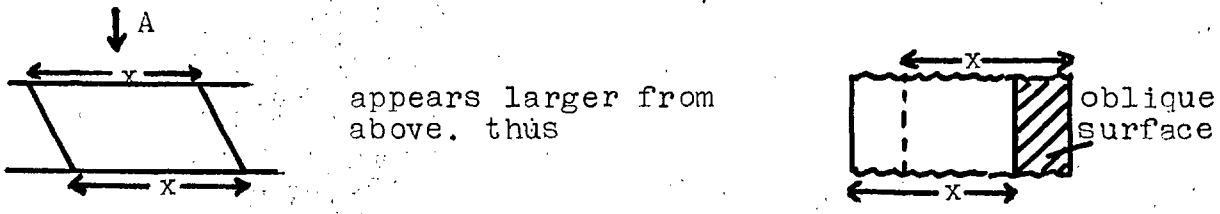
CORRECTIONS FOR OBLIQUE SURFACES

An opaque structure, of finite thickness, and with oblique surfaces appears relatively larger when viewed, with an increased area and circumference, since only the greatest diameter is apprcciated.

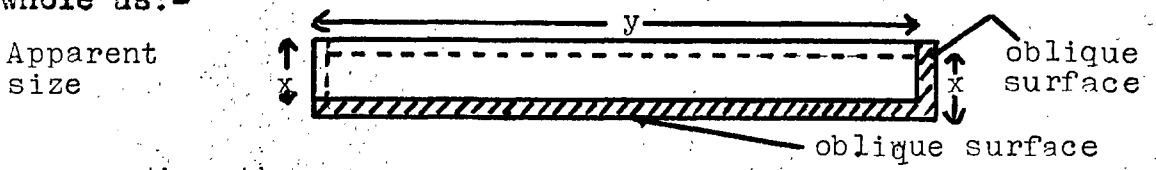
For example a four sided pyramid, viewed from A



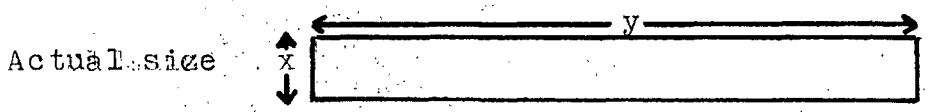
Similarly a trabecula with oblique surfaces:-



The trabecula is elongated and therefore appears as a whole as:-



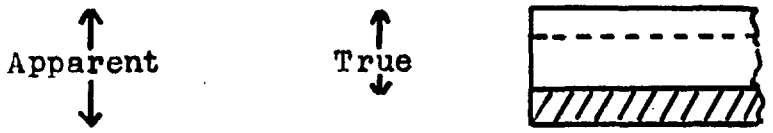
rather than:-



although in sections of this thickness the bone is not opaque, and the oblique surfaces to the left and at the top of the trabecula can be recognised.

The apparent circumference will be greater than the true circumference. At ends of trabeculae this error

will be relatively large



but in absolute terms any error here contributes little to the final result. Similarly the bulk of the circumference is along the sides of trabeculae and here the error is relatively and absolutely minute.

Thus the error is minimal as regards circumference (the actual quantity measured in surface counting with the Zeiss Eyepiece II.).

It will be seen that the error is of greater magnitude and importance in bone area measurements (since the apparent area of a trabecula with oblique surfaces is increased far more than the apparent circumference) and a correction must be applied.

Mathematical corrections (Eränkö 1955) suitable if the bodies counted are portions of spheres are not applicable to a complex structure such as cancellous bone, but reference to the diagrams will show that a full correction for this error is made by the method of counting points on oblique surfaces bounding the left and superior margins of trabeculae only. In sections of this thickness the bone is not opaque and <u>all</u> oblique surfaces can be recognised and this correction applied.

Appendix 5.

STATISTICAL METHODS

All formulae used in the statistical evaluation of this study have been taken from

1. Statistical methods in biology by N.T.J.Bailey. 1959. English Universities Press, London.

2. Biomathematics by C.A.B.Smith, 1954. Charles Griffin, London.

3. Statistical methods for research workers by R.A.Fisher. 11th edition 1950. Oliver and Boyd, London.

ACKNOWLEDGMENTS

My thanks are due to Dr.H.A.Sissons, Institute of Orthopaedics, London, at whose suggestion this work was carried out, for his advice and encouragement throughout the period of this study, and for the provision of excellent technical facilities.

I also wish to thank Dr.Sissons for reading the manuscript, and to thank Dr.P.D.Byers of the Institute of Orthopaedics for his advice and encouragement.

I wish to acknowledge with thanks the technical assistance given by Mr.E.A.Wallington and the staff of the Department of Morbid Anatomy, Institute of Orthopaedics.

I wish to thank Professor Francis Camps, and H.M.Coroner for Inner North London, Dr.R.I.Milne for allowing me access to some of the cases studied.

I am grateful to Mr.C.Davenport and Mr.R.J.Whitley, of the Institute of Orthopaedics, and their staffs for assistance with references and illustrations respectively.

My thanks are finally due to Miss Susan Brady for typing the manuscript.

BIBLIOGRAPHY

ALBRIGHT,F. (1947). Osteoporosis. Ann.intern.Med. 27, 861.

ALBRIGHT,F., BLOOMBERG,E. & SMITH,P.H. (1940). Post-menopausal osteoporosis. Trans.Assoc.Amer. Phycns. 40, 298.

ALBRIGHT,F. & REIFENSTEIN,E.C.Jr. (1948). The Parathyroid Glands and Metabolic bone disease. Williams and Wilkins, Baltimore.

AMPRINO,R. (1937). Transformations histologiques pendant l'accroisement et le remaniement du col du fémur après la naissance. C.R.Ass.Anat. 32, 19.

AMPRINO,R. & ENGSTRÖM,A. (1952). Studies on X ray absorption and diffraction of bone tissue. Acta anat. (Basel). 15, 1.

ANDERSON,J. (1963). Metabolic bone diseases and diagnostic procedures. In Bone metabolism in relation to clinical medicine. Ed. Sissons,H.A. Pitman Medical, London.

ARDRAN,G.M. (1951). Bone destruction not demonstrable by radiology. Brit.J.Radiol. 24, 107.

AREY,L.B. (1919). The origin, growth and fate of osteoclasts and their relation to bone resorption. Amer.J.Anat. 26, 315.

ARNOLD,J.S. (1964). The quantitation of bone mineralization as an organ and tissue in osteoporosis. In Dynamic studies of metabolic bone disease. Ed. Pearson,O.H. & Joplin,G.F. Blackwell, Oxford.

ATKINSON,P.J. (1965). Changes in resorption spaces in femoral cortical bone with age. J.Path.Bact. 89, 173.

ATKINSON,P.J., WEATHERELL,J.A. & WEIDMANN,S.M. (1962). Changes in density of the human femoral cortex with age. J.Bone Jt.Surg. 44-B, 496.

BAILEY,N.T.J. (1959). Statistical methods in biology. English Universities Press, London.

BAKER,S.L. (1959). The general pathology of bone. In A textbook of X ray diagnosis. Ed. Shanks,S.C. & Kerley,P.J. Vol.III. H.K.Lewis, London.

BALL,J. (1960). Diseases of bone. In Recent advances in pathology. 7th Edition. Ed. Harrison,C.V. Churchill, London.

BALL,J. (1963). Histological diagnosis in metabolic bone disease. In Bone metabolism in relation to clinical medicine. Ed. Sissons,H.A. Pitman Medical, London.

BARNETT,E. & NORDIN,B.E.C. (1960). The radiological diagnosis of osteoporosis: a new approach. Clin.Radiol. 11, 166.

BAUER,G.C. (1962). Comparison of calcium kinetics and skeletal morphology. In Medical uses of Ca^{47} p.71. Technical Reports Series No.10. International Atomic Energy Agency, Vienna.

BAUER,G.C. (1964). Kinetics of bone diseases. In Bone dynamics. p.498. Ed. Frost,H.M. Churchill, London.

BAUER,G.C.H., CARLSSON,A. & LINDQUIST,B. (1961). Metabolism and homeostatic function of bone. In Mineral metabolism. Vol.1. Part B. p.609. Ed. Comar,C.L. & Bronner,F. Academic Press, New York.

BAUER,W., AUB,J.C. & ALBRIGHT,F. (1929). Studies of calcium and phosphorus metabolism. V. A study of the bone trabeculae as a readily available reserve supply of calcium. J.exp.Med. 49, 145.

BEADLE,O.A. (1931). The Intervertebral Discs. Spec. Rep.Ser.med.Res.Coun. (London), No.161.

BECK,J.S. & NORDIN,B.E.C. (1960). Histological assessment of osteoporosis by iliac crest biopsy. J.Path. Bact. 80, 391.

BÉLANGER,L.F., ROBICHON,J., MIGICOVSKY,B.B., COPP,D.H. & VINCENT,J. (1963). Resorption without osteoclasts (osteolysis), In Mechanisms of hard tissue destruction. p.531, Ed. Sognnaes,R.F. American Association for the Advancement of Science, Washington, D.C.

BHASKAR,S.N., MOHAMMED,C.I. & WEINMANN,J.P. (1956). A morphological and histochemical study of osteoclasts. J.Bone Jt.Surg. 38-A, 1335.

BLOOM,W. & BLOOM,M.A. (1940). Calcification and ossification. Calcification of developing bones in embryonic and newborn rats. Anat.Rec. 78, 497.

BLOOM,W., BLOOM,M.A. & McLEAN,F.C. (1941). Calcification and ossification. Medullary bone changes in the reproductive cycle of female pigeons. Anat. Rec. 81, 443.

BROMAN,G.E., TROTTER,M. & PETERSON,R.R. (1958). The density of selected bones of the human skeleton. Amer.J.phys.Anthrop. 16, 197.

BROMLEY,R.G., DOCKUM,N.L., ARNOLD,J.S. & JEE,W.S.S. (1966). Quantitative histological study of human lumbar vertebrae. In Research in radiobiology. p191. Annual report of progress in the internal irradiation program of the radiobiology division of the department of anatomy, University of Utah College of Medicine.

BRYANT,F.J. & LOUTIT,J.F. (1961). Human bone metabolism deduced from strontium assays. A.E.R.E. Report No.3718. H.M.S.O. London.

CALDWELL,R.A. (1962). Observations on the incidence, aetiology, and pathology of senile osteoporosis. J.clin.Path. 15, 421.

CALDWELL,R.A. & COLLINS,D.H. (1961). Assessment of vertebral osteoporosis by radiographic and chemical methods post-mortem. J.Bone Jt.Surg. 43-B, 346.

CAMERON,D.A. (1961). Erosion of the epiphysis of the rat tibia by capillaries. J.Bone Jt.Surg. 43-B, 590.

CAMERON,D.A. (1963). The fine structure of bone and calcified cartilage. Clin.Orthop. 26, 199.

CAMERON,G.R. (1930). The staining of calcium. J.Path. Bact. 33, 929.

CARNEIRO,J. & LEBLOND,C.P. (1959). Role of osteoblasts and odontoblasts in secreting the collagen of bone and dentin as shown by radioautography in mice given tritium-labelled glycine. Exp.Cell Res. 18, 291.

CASPERSSON,T. (1947). The relations between nucleic acid and protein synthesis. Symposia Soc. exptl.Biol. 1, 127.

CHALKLEY,H.W. (1943). Method for the quantitative morphologic analysis of tissues. J.nat.Cancer Inst. 4, 47.

COBB,W.H. (1952). In Cowdry's Problems of ageing. Biological and medical aspects. 3rd.Edn. p.822. Ed. Lansing,A.I. Williams and Wilkins, Baltimore.

COLE,E.C. (1943). Studies on haematoxylin stains. Stain Technol. 18, 125.

COLLINS,D.H. (1956). Paget's disease of bone. Lancet ii, 51.

COOKE,A.M. (1955). Osteoporosis. Lancet i, 877 & 929.

COOPER,Sir A. (1823). A treatise on dislocations and on fractures of the joints. 2nd Edn. p.123. Longman, Hurst, Rees, Orme & Browne, London.

DONALDSON,I.A. & NASSIM,J.R. (1954). The artificial menopause with particular reference to the occurrence of spinal porosis. Brit.med.J. i, 1228.

DOYLE,F.H. (1961). Radiological assessment of bone density. III. Ulnar bone mineral concentration in metabolic bone diseases. Brit.J.Radiol. 34, 698.

DRURY,R.A.B. & WALLINGTON,E.A. (1967). Carleton's histological technique. 4th Edn. Oxford University Press, London.

DUDLEY,H.R. & SPIRO,D. (1961). The fine structure of bone cells. J.biophys.biochim.Cytol. 11, 627.

DUHAMEL,H.L. (1742). Sur le développment et la crue des os des animaux. Mém.Acad.roy.Sci. 55, 354.

DULL,T.A. & HENNEMAN,P.H. (1963). Urinary hydroxyproline as an index of collagen turnover in bone. New Engl.J.Med. 268, 132.

EDER,M. (1960). Der Strukturumbau der Wirbelspongiosa. Virchow's Arch.path.Anat. 333, 509.

ENGSTRÖM,A. (1949). Microradiography. Acta radiol. (Stockh.) 31, 503.

ERANKÖ,O. (1955). Quantitative methods in histology and microscopic histochemistry. p.71. S.Karger, Basle.

ERDHEIM,J. (1914). Rachitis und Epithelkörporchen. Denkschrift Akad.Wiss.Wien. 90, 363.

EVANS,F.G. & KING,A.I. (1961). Regional differences in some physical properties of human spongy bone. In Biomechanical studies of the musculo-skeletal system. p.49. Ed. Evans,F.G. Charles C.Thomas, Springfield.

EVANS,F.G. & LISSNER,H.R. (1955). Studies on pelvic deformations and fractures. Anat.Rec. 121, 141.

EXTON-SMITH,A.N. & STANTON,B.R. (1965). Report of an investigation into the dietary [illegible] women living alone. p.39. King Edward's Hospital Fund for London.

FISHER,R.A. (1950). Statistical methods for research workers. 11th Edn. Chap. V, p.147. Oliver and Boyd, London.

FOLLIS,R.H.Jr. (1951). Histochemical studies on cartilage and bone. Bull.Johns Hopkins Hosp. 89, 9.

de FOREST,A.V. & ELLIS,G. (1940). Brittle lacquer as an aid to stress analysis. J.aeronaut.Sc. 7, 205. Cited by Evans,F.G. in Stress and strain in bones. p.12. Charles C.Thomas, Springfield.

FROMMER,D.J. (1964). Changing age of the menopause. Brit.med.J. 11, 349.

FROST,H.M. (1963a). Bone remodelling dynamics. Charles C.Thomas, Springfield.

FROST,H.M. (1963b). Measurement of human bone formation by means of tetracycline labelling. Canad.J. Biochem. 41, 31.

FROST,H.M. & VILLANUEVA,A.R. (1960). Observations on osteoid seams. Henry Ford Hosp.Bull. 8, 212.

FROST,H.M., VILLANUEVA,A.R. & ROTH,H. (1962). Qualitative method for measuring osteoclastic activity. Henry Ford Hosp.Bull. 10, 217.

GAILLARD,P.J. (1955). Parathyroid gland tissue and bone in vitro. II and III. Proc.kon.med.Akad.Wet. C58, 279 and 286.

GAILLARD,P.J. (1957). Parathyroid gland and bone in vitro. Schweiz.med.Wschr. 87, 447.

GARDNER,W.U. & PFEIFFER,C.A. (1943). Influence of estrogens and androgens on the skeletal system. Physiol.Rev. 23, 139.

GARNER,A. & BALL,J. (1966). Quantitative observations on mineralised and unmineralised bone in chronic renal azotaemia and intestinal malabsorption syndrome. J.Path.Bact. 91, 545.

GEGENBAUER,C. (1864). Ueber die Bildung des Knochengewebes. Jena Z.Med.Naturwiss. 1, 345.

GEGENBAUER,C. (1867). Ueber die Bildung des Knochengewebes, Ibid. 3, 206.

GERSHON-COHEN,J., RECHTMAN,A.M., SCHRAER,H. & BLUMBERG,N. (1953). Asymptomatic fractures in osteoporotic spines of the aged. J.Amer.med.Ass. 153, 625.

GHOSEZ,J.P. (1959). La microscopie de fluorescence dans l'étude du remaniemant haversien. Arch.Biol. (Liege) 70, 169.

GLIMCHER,M.J. (1959). Molecular biology of mineralized tissues with particular referance to bone. In Biophysical science - a study program. p.359. Ed. Oncley,J.L. John Wiley & Sons, New York.

GLIMCHER,M.J. (1960). Specificity of the molecular structure of organic matrices in mineralization. In Calcification in biological systems. Ed. Sognnaes,R.F. American Association for the Advancement of Science, Washington, D.C.

GOLDHABER,P. (1958). The effect of hyperoxia on bone resorption in tissue culture. Arch.Path. 66, 635.

GOLDHABER,P. (1960). Behaviour of bone in tissue culture. In Calcification in biological systems. Ed. Sognnaes,R.F. American Association for the Advancement of Science, Washington, D.C.

GOLDHABER,P. (1963). Some chemical factors influencing bone resorption in tissue culture. In Mechanisms of hard tissue destruction. p.609. Ed. Sognnaes,R.F. American Association for the Advancement of Science, Washington, D.C.

GOODSIR,J. (1845). In Anatomical and pathological observations. Ed. Goodsir,J. & Goodsir,H.D.S. McPhail, Edinburgh.

HALES,S. (1727). Statical essays. Vol.1. p.339. Innys, Woodward, London.

HALL,M.C. (1961). The trabecular patterns of the neck of the femur with particular reference to changes in osteoporosis. Canad.med.Ass.J. 85, 1141.

HALL,M.C. (1965). The locomotor system - functional histology. p.130. Charles C.Thomas, Springfield.

HAM,A.W. (1952). Some histophysiological problems peculiar to calcified tissues. J.Bone Jt.Surg. 34-A, 701.

HAM,A.W. (1965). Histology. 5th Edn. p.394p Pitman Medical, London.

HANCOX,N.M. (1949). The osteoclast. Biol.Rev. 24, 448.

HANCOX,N.M. (1956). The osteoclast. In The biochemistry and physiology of bone. Ed. Bourne,G.H. Academic Press, New York.

HANCOX,N.M. & BOOTHROYD,B. (1964). Ultrastructure of bone formation and resorption. In Modern trends in orthopaedics. No.4. Chap. 3. Ed. Clark,J.M.P. Butterworths, London.

HARRIS,W.H. (1960). A microscopic method of determining rates of bone growth. Nature (Lond.) 188, 1038.

HEANEY,R.P. (1964). Interpretation of calcium kinetic data. In Dynamic studies of metabolic bone disease. Ed.Pearson,O.H. & Joplin,G.F. Blackwell, Oxford.

HELLER,M., McLEAN,F.C. & BLOOM,W. (1950). Cellular transformations in mammalian bones induced by parathyroid extract. Amer.J.Anat. 87, 315.

HENNEMAN,P.H. & WALLACH,S. (1957). A review of the prolonged use of estrogens and androgens in postmenopausal and senile osteoporosis. Arch. intern.Med. 100, 715.

HENNIG,A. (1958). Kritische Betrachtungen zur Volumen - und Oberflächenmessung in der Mikroskopie. Zeiss Werkzeitschrift 30, 78.

HIRSCH,C. & BRODETTI,A. (1956). The weight bearing capacity of structural elements in femoral necks. Acta orthop.scand. 26, 15.

HOWSHIP,J. (1819). Observations on the morbid appearances and structure of bones. Med-chir. Trans. 10, 176.

HUNTER,J. (1837). The works of John Hunter, F.R.S. with notes. Ed. Palmer,J.F. Longman, Rees, Orme, Brown, Green & Longman, London.

INGALLS,N.W. (1931). Observations on bone weights. Amer.J.Anat. 48, 45.

IRVING,J.T. & HANDELMAN,S.C. (1963). Bone destruction by multinucleated giant cells. In Mechanisms of hard tissue destruction. p.515. Ed. Sognnaes,R.F. American Association for the Advancement of Science, Washington, D.C.

JAFFE,H.L. (1930). The resorption of bone. A consideration of the underlying processes particularly in pathological conditions. Arch.Surg. 20, 355.

JAFFE,H.L. (1933). Hyperparathyroidism (Recklinghausens's disease of bone). Arch.Path. 16, 63 and 236.

JASANI,C., NORDIN,B.E.C., SMITH,D.A. & SWANSON,I. (1965). Spinal osteoporosis and the menopause. Proc. roy.Soc.Med. 58, 441.

JOHNSON,L.C. (1964). Morphologic analysis in pathology: the kinetics of disease and general biology of bone. In Bone biodynamics. Ed. Frost,H.M. Churchill, London.

JOWSEY,J. (1955). The use of the milling machine for preparing bone sections for microradiography and microautoradiography. J.sci.Instrum. 32, 159.

JOWSEY,J. (1963). Microradiography of bone resorption. In Mechanisms of hard tissue destruction. p.447 Ed. Sognnaes,R.F. American Association for the Advancement of Science, Washington, D.C.

JOWSEY,J., KELLY,P.J., RIGGS,B.L., BIANCO,A.J., SCHOLZ,D.A. & GERSHON-COHEN,J. (1965). Quantitative microradiographic studies of normal and osteoporotic bone. J.Bone Jt.Surg. 47-A, 785.

JOWSEY,J., LAFFERTY,W. & RABINOWITZ,J. (1965). Analysis of distribution of Ca^{45} in dog bone by a quantitative autoradiographic method. J.Bone Jt.Surg. 47-A, 359.

JOWSEY,J., OWEN,M. & VAUGHAN,J. (1953). Microradiographs and autoradiographs of cortical bone from monkeys injected with ^{90}Sr. Brit.J.exp.Path. 34, 661.

KEITH,A. (1919). Menders of the maimed. p.287. Oxford University Press, London.

KIRBY-SMITH,H.T. (1933). Bone growth studies - a miniature bone fracture observed microscopically in a transparent chamber introduced into the rabbit's ear. Amer.J.Anat. 53, 377.

KLEIN,L. (1966). The relationship between urinary hydroxyproline, calcium and serum alkaline phosphatase during fracture healing and acute disuse osteoporosis. In Proceedings of 4th european symposium on calcified tissues. Excerpta Medica, Amsterdam.

KLEIN,L. & CURTISS,P.H.Jr. (1964). Urinary hydroxyproline as an index of bone metabolism. In Dynamic studies of metabolic bone disease. Ed. Pearson,O.H. & Joplin,G.F. Blackwell, Oxford.

KÖLLIKER,A. (1873). Die normale Resorption des Knochengewebes und ihre Bedeutung für die Enstehung der typischen Knochenformen. Vogel, Leipzig.

KÖLLIKER,A. (1889). Handbuch der Gewebelehre des Menschen. Band 1. p.269. Engelmann, Leipzig.

von KOSSA,J. (1901). Ueber die im organismus künstlich erzeugbaren Verkalkungen. Beitr.path.Anat. 29, 163.

KUMAMOTO,K. & LEBLOND,C.P. (1956). Radioautographic study of mineralization of growing teeth with labeled calcium. J.dent.Res. 35, 147.

LACROIX,P. (1956). The histological remodelling of adult bone. In Ciba Foundation symposium on bone structure and metabolism. Ed. Wolstenholme, G.E.W. & O'Connor,C.M. Churchill, London.

LEBLOND,C.P., WILKINSON,G.W., BÉLANGER,L.F. & ROBICHON,J. (1950). Radioautographic visualization of bone formation in the rat. Amer.J.Anat. 86, 289.

LEE,W.R. (1967) Bone formation in Paget's diaease. J.Bone Jt.Surg. 49-B, 146.

LEE,W.R., MARSHALL,J.H. & SISSONS,H.A. (1965). Calcium accretion and bone formation in dogs. J.Bone Jt.Surg. 47-B, 157.

LE GROS CLARK,W.E. (1945). Bone. In The tissues of the body. 2nd Edn. p.61. Oxford University Press, London.

LÉRICHE,R. & POLICARD,A. (1926). Les problèmes de la physiologie normale et pathologique de l'os. Masson, Paris.

LINDAHL,O. & LINDGREN,A.G.H. (1962). Grading of osteoporosis in autopsy specimens, Acta orth. scand. 32, 85.

LOE,H. (1959). Bone tissue formation. Acta odont. scand. 17, 311.

McLEAN,F.C. & BLOOM,W. (1940). Calcification and ossification. Calcification in normal growing bone. Anat.Rec. 78, 333.

McLEAN,F.C. & BLOOM,W. (1941). Calcification and ossification. Mobilization of bone salt by parathyroid extract. Arch.Path. 32, 315.

MALM,O.J. (1958). Calcium requirement and adaption in adult men. Scand.J.clin.Lab.Invest. 10, Supplement 36, 1.

MAYO,K.M. (1961). Radiological assessment of bone density. II. Quantitative measurement of bone mineral content in normal adult bone. Brit.J.Radiol. 34, 693.

MEEMA,H.E., BUNKER,M.L. & MEEMA,S. (1965). Loss of compact bone due to menopause. Obstet. and Gynec. 26, 333.

MEYER,K., DAVIDSON,E., LINKER,A. & HOFFMAN,P. (1956). The acid mucopolysaccharides of connective tissue. Biochem.biophys.Acta (Amst.) 21, 506.

MEYER,P.C. (1956). The histological identification of osteoid tissue. J.Path.Bact. 71, 325.

MOON,N.F. & URIST,M.R. (1962). Gerontal and gerontoid osteoporosis. Clin.Orthop. 23, 269.

MORGAN,D.B., PULVERTAFT,C.N. & FOURMAN,P. (1966). Effects of age on the loss of bone after gastric surgery. Lancet ii, 772.

NEUBERGER,A. & SLACK,J.G.B. (1953). The metabolism of collagen from liver, bone, skin and tendon in the normal rat. Biochem.J. 53, 47.

NEUMAN,W.F. & NEUMAN,M.W. (1953). The nature of the mineral phase of bone. Chem.Rev. 53, 1.

NORDIN,B.E.C. (1960a). Osteomalacia, osteoporosis and calcium deficiency. Clin.Orthop. 17, 235.

NORDIN,B.E.C. (1960b). Osteoporosis and calcium deficiency. Proc.Nutr.Soc. 19, 129.

NORDIN,B.E.C. (1961). The pathogenesis of osteoporosis. Lancet 1, 1011.

NORDIN,B.E.C. (1962). Calcium balance and calcium requirement in spinal osteoporosis. Am.J. clin.Nutr. 10, 384.

NORDIN,B.E.C. (1963). Osteoporosis. In Bone metabolism in clinical practice. Ed. Sissons, H.A. Pitman Medical, London.

NORDIN,B.E.C. (1964a). Osteoporosis. Advances in metab.Disorders. 1, 125.

NORDIN,B.E.C. (1964b). The application of basic science to osteoporosis. In Bone biodynamics. p.521. Ed. Frost,H.M. Churchill, London.

NORDIN, B.E.C., BARNETT, E. McGREGOR, J. & NISBET, J. (1962). Lumbar spine densitometry. Brit.med.J. 1, 1793.

NORDIN, B.E.C., McGREGOR, J. & SMITH, D.A. (1966). The incidence of osteoporosis in normal women : its relation to age and the menopause. Quart. J.Med. 35, 25.

POMMER, G. (1885). Untersuchungen über Osteomalacie und Rachitis. Vogel, Leipzig.

PRITCHARD, J.J. (1952). A cytological and histochemical study of bone and cartilage formation in the rat. J.Anat. 86, 259.

PRITCHARD, J.J. (1956 a). General anatomy and histology of bone. In The biochemistry and physiology of bone. p.1. Ed. Bourne, G.H. Academic Press, New York.

PRITCHARD, J.J. (1956 b). The osteoblast. Ibid. p.179.

REIFENSTEIN, E.C. Jr. (1957). The relationships of steroid hormones to the development and the management of osteoporosis in aging people. Clin.Orthop. 10, 206.

RETTERER, E. (1906). Évolution du tissu osseux. J.Anat. (Paris). 42, 193.

RIBBERT, H. (1880). Ueber senile Osteomalacie und Knochenresorption im Allgemeinen. Virchow's Arch.path.Anat. 80, 436.

RINDFLEISCH,E. (1873), A manual of pathological histology. Vol.II. p.248. Translated by E.B.Baxter. New Sydenham Society, London.

ROBINSON,R.A. (1952). An electron-microscopic study of the crystalline inorganic component of bone, and its relationship to the organic matrix. J.Bone Jt.Surg. 34-A, 389.

ROBINSON,R.A. & CAMERON,D.A. (1958). Electron microscopy of the primary spongiosa of the metaphysis at the distal end of the femur in the newborn infant. J.Bone Jt.Surg. 40-A, 687.

ROBINSON,R.A. & WATSON,M.L. (1955). Crystal-collagen relationships in bone as observed in the electron microscope. III. Crystal and collagen morphology as a manifestation of age. Ann.N.Y.Acad.Sci. 60, 596.

ROSE,G.A. (1964). The study of osteoporosis and osteomalacia. Postgrad.med.J. 40, 158.

RUGER,H.A. & STOESSIGER,B. (1927). On the growth curves of certain characters in man (males). Ann. Eugen. 2, 76.

SANDISON,J.C. (1928). A method for the microscopic study of the growth of transplanted bone in the transparent chamber of the rabbits' ear. Anat. Rec. 40, 41.

SAVILLE,P.D. (1965). Changes in bone mass with age and alcoholism. J.Bone Jt.Surg. 47-A, 492.

SCHOUR,I. (1936). Measurements of bone growth by alizarine injections. Proc.Soc.exp.Biol.(N.Y.) 34, 140.

SEDLIN,E.D. (1964). The ratio of cortical area to total cross section area in rib diaphysis : a quantitative index of osteoporoses. Clin. Orthop. 36, 161.

SEDLIN,E.D., VILLANUEVA,A.R. & FROST,H.M. (1963). Age variations in the specific surface of Howship's lacunae as an index of human bone resorption. Anat.Rec. 146, 201.

SHOCK,N.W. (1960). Some of the facts of aging. In Aging. p.241. Ed. Shock,N.W. Publication No.65. American Association for the Advancement of Science, Washington, D.C.

SHORT,R.H.D. (1950 - 51). Alveolar epithelium in relation to growth of the lung. Phil.Trans.B 235, 35.

SISSONS,H.A. (1960). Osteoporosis of Cushing's syndrome. In Bone as a tissue. Ed. Rodahl,K., Nicholson,J.T. & Brown,E.M. McGraw-Hill, New York.

SISSONS,H.A. (1962). Age changes in the structure and mineralization of bone tissue in man. In Radioisotopes and bone. Ed. McLean,F.C., Lacroix,P. & Budy,A.M. Blackwell, Oxford.

SISSONS,H.A. (1964). Histological studies of normal and osteoporotic bone. In L'Ostéoporose. Ed. Hioco,D.J. Masson, Paris.

SISSONS,H.A. (1966). Bones. In Systemic Pathology. Chap. 37. Ed. Payling Wright,G. & Symmers,W.St.C. Longmans, London.

SISSONS,H.A., HOLLEY,K.J. & HEIGHWAY,J. (1967). Normal bone structure in relation to osteomalacia. In L'Ostéomalacie. Ed. Hioco,D.J. Masson,Paris.

SISSONS,H.A. & LEE,W.R. (1964). Tetracycline studies of bone turnover. In Bone and tooth. Ed. Blackwood,H.J.J. Pergamon Press, Oxford.

SMITH,C.A.B. (1954). Biomathematics. Charles Griffin, London.

SMITH,R.W.Jr., EYLER,W.R. & MELLINGER,R.C. (1960). On the incidence of senile osteoporosis. Ann. intern.Med. 52, 773.

SOLOMON,G.F., DICKERSON,W.J. & EISENBERG,E. (1960). Psychologic and osteometabolic responses to sex hormones in elderly osteoporotic women. Geriatrics. 15, 46.

SPENCER,H., MENCZEL,J., LEWIN,I. & SAMACHSON,J. (1964). Absorption of calcium in osteoporosis. Amer. J.Med. 37, 223.

STARR,K.W. (1947). Delayed union in fractures. Butterworth, London.

STREHLER,B.L., MARK,D.D., MILDVAN,A.S. & GEE,M.V. (1959). Rate and magnitude of age pigment accumulation in the human myocardium. J.Gerontol. 14, 430.

TAKAHASHI,H. & FROST,H.M. (1966). Age and sex related changes in the amount of cortex of normal human ribs. Acta orthop.scand. 37, 122.

TAYLOR,T.C., EPKER,B.N. & FROST,H.M. (1966). The appositional rate of haversian and endosteal bone formation measured in tetracycline labeled ribs. J.Lab.clin.Med. 67, 633.

TOMES,J. & DE MORGAN,C. (1853). Observations on the structure and development of bone. Phil.Trans. B. 143, 109.

TROTTER,M., BROMAN,G.E. & PETERSON,R.R. (1960). Densities of bones of white and negro skeletons. J.Bone Jt.Surg. 42-A, 50.

URIST,M.R. (1959). The etiology of osteoporosis. J. Amer.med.Ass. 169, 710.

URIST,M.R. (1960). Observations bearing on the problem of osteoporosis. In Bone as a tissue. Ed. Rodahl,K., Nicholson,J.T. & Brown,E.M. McGraw-Hill, New York.

URIST,M.R. (1962). Osteoporosis. Ann.Rev.Med. 13, 273.

VAN DER SLUYS VEER,J., SMEENK,D. & VAN DER HEUL,R.O. (1964). Tetracycline labelling of bone in hyperparathyroidism. In Bone and tooth. Ed. Blackwood,H.J.J. Pergamon Press, Oxford.

VILLANUEVA, A.R., SEDLIN, E.D. & FROST, H.M. (1963). Variations in osteoblastic activity with age by the osteoid seam index. Anat.Rec. 146, 209.

VINCENT, J. (1955). Recherches sur la constitution de l'os adulte. Éditions Arscia, Brussels.

VINCENT, J. (1957). Les remaniements de l'os compact marqué ă l'aide de plomb. Rev.Belge Path. 26, 161.

VINCENT, P.J. & URIST, M.R. (1961). The appearance of osteoporosis in ambulatory institutionalized males. Clin.Orthop. 19, 245.

VIRCHOW, R. (1851). Die Identität von Knochen, Knorpel und Bindegewebskörperchen, sowie über Schleimgewebe. Verhandl.physikalmedic. Gesellsch. im Würzburg. 2, 150.

VIRCHOW, R. (1853). Das normale Knochenwachsthum und die rachitische Störung desselben. Arch.path.Anat. Physiol. 5, 409.

VIRCHOW, R. (1860). Cellular Pathology. 2nd Edn. English translation by F.Chance. J.Churchill, London.

VOST, A. (1963). Osteoporosis : a necropsy study of vertebrae and iliac crests. Amer.J.Path. 43, 143.

WATT, J.C. (1928). The development of bone. Arch.Surg. 17, 1017.

WEIDENREICH, F. (1923a). Knochenstudien. I Teil : Über Aufbau und Entwicklung des Knochens und den Charakter des Knochengewebes. Z.Anat.Entwick-Gesch. 69, 382.

WEIDENREICH,F. (1923b). Knochenstudien. II Teil: Über Sehnenverknöcherungen und Faktoren der Knochenbilding. Ibid. 62, 558.

WIELAND,E. (1909). Klinische und anatomische Untersuchungen über sogen. angeborene und über frühzeitig erworbene Rachitis. II Teil. Jb. Kinderheilk. 70, 539.

YOUNG,R.W. (1963). Histophysical studies on bone cells and bone resorption. In Mechanisms of hard tissue.destruction. p.471. Ed. Sognnaes,R.F. American Association for the Advancement of Science, Washington,D.C.