CHEMICAL PATHOLOGY IN
RELATION TO CLINICAL MEDICINE

BONE METABOLISM

in relation to clinical medicine

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The Proceedings of a Symposium organised by the Association of Clinical Pathologists and the Association of Clinical Biochemists held in London at the Royal College of Surgeons
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Introduction

This is the second of the symposia organised by the Association of Clinical Pathologists and the Association of Clinical Biochemists dealing with the application of chemical pathology to clinical medicine. Partly through the acquisition of new scientific techniques and partly through increasing awareness of the skeleton as a metabolically active system of the first importance, bone in recent years has received much attention from biological and medical scientists. There have already been published a number of valuable symposia on bone and calcium metabolism and on the technical methods of their study, but these carry with them the atmosphere of the physiological rather than of the pathological laboratory. The aim of the symposium here recorded was to provide a reasonably comprehensive review of a very rapidly advancing field of medical biology, having in mind the needs and interests of those who work in hospitals and face the pressing problems of human disease.

In effect, the symposium brought together a considerable amount of information which is not readily available to the general clinical pathologist. Pathologist, biochemists, and clinicians all took part, for it is only by collaboration between these three groups of specialists that real progress in the practice of medicine can be made. The boundary is a shifting one between laboratory methods, which we call routine, and those which at first are held to be research techniques, and one of the important results of this symposium was to clarify the possibilities, and perhaps also the limitations, of present day laboratory procedures.

A large audience attended the symposium and it is the hope that in publishing the proceedings the work will also interest a wider circle of readers concerned with the investigation and management of cases of metabolic bone disease.

D.H. COLLINS, O.B.E., M.D., F.R.C.P.

Basic Considerations

Contents

Structural Aspects of Bone

H. A. SISSONS

Normal Bone Structure

Bone structure may be considered from two points of view - the gross anatomical configuration of the tissue and the micropscopic pattern of arrangement of its constituent fibres. Anatomically, bone can be described as compact or cancellous. This is apparent in Fig.1,

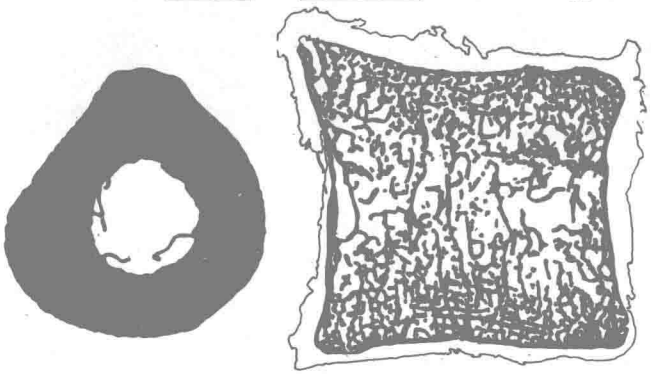


Figure 1. Normal bone (female aged 20 years): compact and cancellous arrangement. Outline drawings from sections of femoral cortex and lumbar vertebral body. The femoral cortex consists of compact bone with small vascular canals, while the vertebral body consists of a network of slender trabeculae separated by relatively wide marrow spaces. 91% of the cortical area of the femur is occupied by bone: the corresponding value for the cancellous bone of the vertebral body varies from 14% (central part) to 21% (upper and lower areas).

which contrasts the appearance of the midshaft of the femur with that of a lumbar vertebral body. The cortical bone of the femur has a compact arrangement, while the vertebral body consists almost entirely of cancellous bone (i.e. of thin trabeculae). On the basis of its fibre pattern, bone can be described as lamellar or non-lamellar (see Baker, 1939). Both types of tissue consist of collagen fibres which are bound together by mucopolysaccharide material. In lamellar bone, the fibres are arranged in sheets or lamellae; in any one of these

lamellae the fibres are parallel to one another, but their direction differs from that of the fibres in adjacent lamellae. Non-lamellar bone fails to show this oriented arrangement of its collagen fibres, which are disposed in an irregular felt-work. The arrangement of collagen fibres is clearly shown when sections are examined in polarized light (Fig. 2).

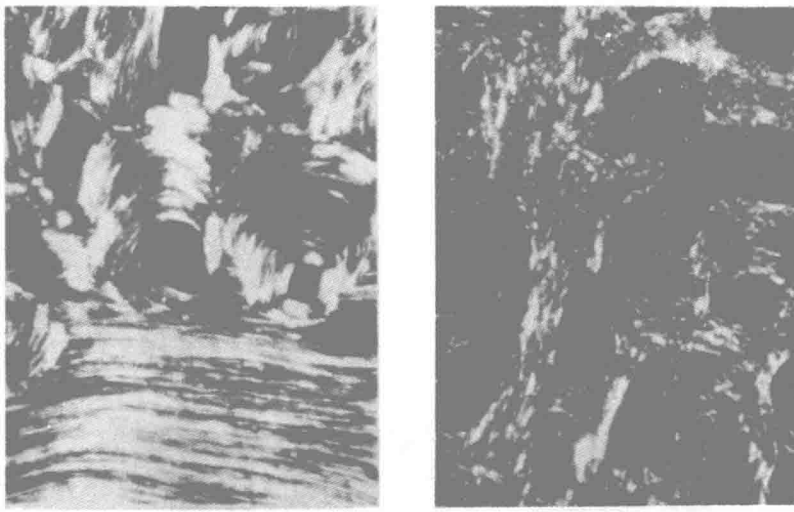


Figure 2. (a) Lamellar bone (normal femoral cortex: male aged 33 years)
(b) Non-lamellar bone (fracture callus).
Both are viewed in polarized light to show the arrangement of collagen fibres.

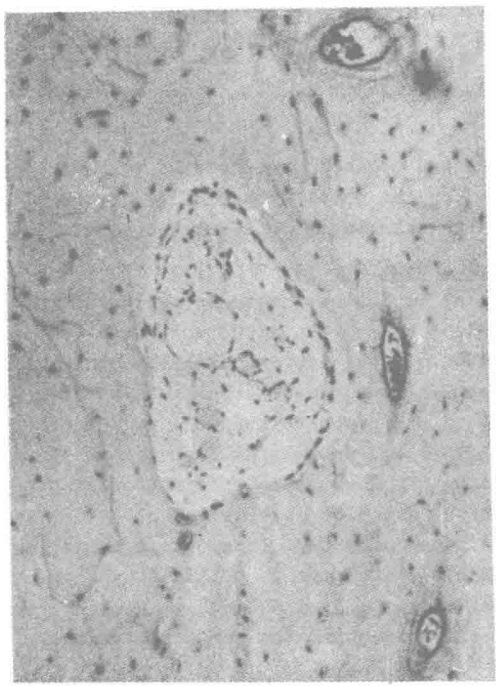
In the normal adult, almost all the skeleton consists of lamellar bone: in the embryo or the young child, however, much of it consists of non-lamellar bone, and similar tissue can develop under pathological conditions in adult life, particularly when bone is formed rapidly. In adult cortical bone, the constituent lamellae are arranged either parallel to the endosteal or periosteal surfaces, or concentrically around penetrating vascular channels to form more or less cylindrical structures which are known as Haversian systems or osteones.

Bone remodelling

Bone is a rigid structure and can consequently undergo changes of size and shape only by the addition and removal of material at its surface. Throughout life, bone is deposited and resorbed as a result of the activity of the connective tissue cells covering its surfaces - the periosteum, the endosteum, and the surfaces of the trabeculae of

cancellous bone and of the vascular spaces of compact bone. An adult bone is the result of a complex series of remodelling activities, and its histological structure provides a certain amount of information with regard to the pattern and timing of these activities. As long ago as 1853, Tomes and de Morgan were aware that adult bone tissue was subject to continuous remodelling activity, and realised that each osteone had been preceded by a resorption cavity, and had indeed been formed by the deposition of successive concentric lamellae on the inner wall of such a cavity.

The histological appearance of each part of the bone surface indicates the type of activity occurring there. In decalcified and stained preparations, surfaces where bone formation is actively taking place are characterised by the presence of osteoblasts, and by a layer of palely-staining osteoid tissue (bone matrix without mineral material) on the surface of the calcified bone (Fig. 3a). Similarly, surfaces of resorption are characterised by their irregular crenated outline and by the presence of multinucleated osteoclasts (Fig. 3b). In the normal skeleton, however, much of the bone surface is inactive: these areas have a smooth outline, but are devoid of conspicuous osteoblasts or appreciable osteoid borders, and in decalcified sections stained with haematoxylin and eosin they show a superficial line of basophilic staining (Fig. 3b).



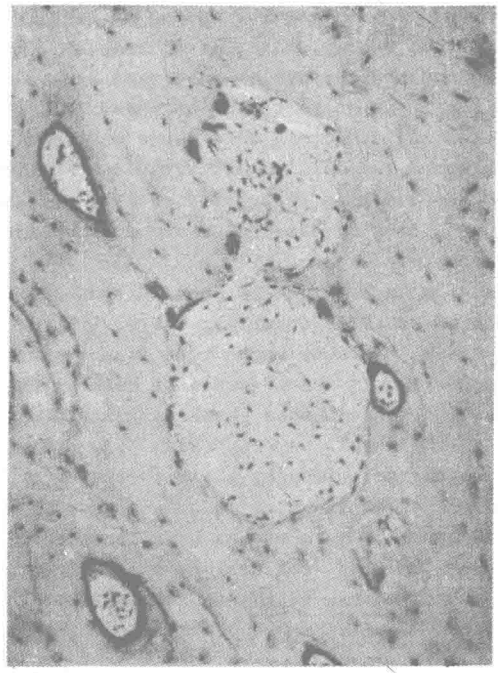


Figure 3. (a) **H. & E. section from cortex of femoral shaft in a child, showing a developing osteone with its lining of palely-staining osteoid tissue covered by osteoblasts.**

(b) **Another field from the same preparation, showing an area of active osteoclastic resorption.**

In both (a) and (b) several small vascular canals are lined by inactive darkly staining surfaces.

Normally, the two opposed processes of bone deposition and resorption are balanced, so the total amount of bone present in any part of the skeleton remains relatively constant (Sissons, 1960).

Changes in metabolic bone disease

In pathological conditions, changes can occur in the total amount of bone tissue present and in the gross anatomical configuration of any part of the skeleton. The average skeleton contains about 5 kg. (dry weight) of actual bone tissue (Ingalls, 1931) of which about 1 kg. is calcium. It is important to know the amount of bone tissue normally present in different parts of the skeleton: this information is necessary in connection with the recognition of osteoporosis, which is, by definition, a reduction in this amount.

Information on the amount of bone tissue normally present in different parts of the skeleton can be obtained by a variety of different methods, including weighing of individual bones (Trotter, Broman & Peterson, 1960), measurement of the cortical thickness (Barnett &

Nordin, 1960); Bernard & Laval-Jeantet, 1962), determination of radiographic density and calcium content (Caldwell & Collins, 1961; Caldwell, 1962), and measurement of the apparent density of macerated bone specimens (Lindahl & Lindgren, 1962). All these methods show an appreciable decrease of skeletal mass in normal individuals after about 50 years of age, and this is something which complicates the recognition, and indeed the definition, of osteoporosis.

The reduction in bone mass in the normal ageing skeleton, or in osteoporosis, is readily apparent in histological sections: here too, the change is amenable to quantitative study. In normal cortical bone, as much as 95% of the total space can be occupied by bone, while in cancellous bone, the corresponding figure is about 20%, or even less (Fig. 1). In osteoporotic material (Fig. 4), widening of vascular spaces can result in as little as 30% of the total space of the cortex

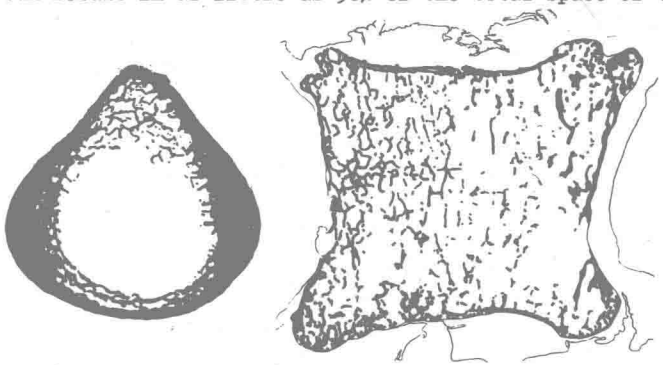


Figure 4. Osteoporosis (female aged 83 years). Outline drawings from sections of femoral cortex and lumbar vertebral body. Comparison with Fig. 1 shows that the femoral cortex is thinned as well as porotic, while the bone trabeculae of the vertebral body are slender and reduced in number. 60% of the (narrowed) cortical area is occupied by bone: the corresponding value for the cancellous bone of the vertebral body varies from 6% (central part) to 10% (upper and lower areas).

being occupied by bone, and figures of only 6 - 10% can be obtained for cancellous bone. Information is accumulating (see references quoted above) on the normal range of values for the amount of bone in various parts of the skeleton, and it is on such information that the practical recognition of osteoporosis must depend. In the living patient, the histological diagnosis of this condition usually depends on iliac crest biopsy, and normal standards for this part of the skeleton have been provided by Beck and Nordin (1960).

In the normal ageing skeleton, and in 'idiopathic' osteoporosis, there is morphological evidence to indicate that the decrease in the amount of cortical bone results from exaggerated osteoclastic resorption, and from the arrest of the development of some osteones at a stage where they have a wide central canal (Sissons, Jowsey & Stewart, 1960; Jowsey, 1960). It should be stated, however, that any local imbalance between bone formation and bone resorption will result in a

reduction in skeletal mass, i.e. in osteoporosis. Either exaggerated resorption or diminished formation can be responsible and it is possible that in some circumstances both these processes may be increased but still out of balance. This, in fact, is the situation in most osteolytic metastatic tumour deposits, where, despite the presence of active bone formation at the margin of the lesion, more bone is being destroyed than is being produced.

When imbalance between bone formation and bone destruction leads to progressive reduction in the amount of bone tissue in a particular part of the skeleton, certain structures may be selectively removed, with consequent change in the general anatomical configuration of the tissue. In Fig.4, for example, it can be seen that the endosteal part of the femoral cortex is selectively involved, with consequent enlargement of the diameter of the marrow cavity. Similarly, the transverse trabeculae of the cancellous bone of the vertebral bodies are more involved than the vertical ones (Caldwell & Collins, 1961; Caldwell, 1962; see also Fig. 4).

The amount of surface present in different types of bone tissue is of interest in connection with the changes in metabolic bone disease. Some approximate data for this parameter, as well as for the amounts of bone present, are given in Table I. The figures for the

TABLE I

(Values are approximate)

Type of bone	% of total space occupied by bone	Surface (Sq.cm. per cu.cm.bone)	Surface (Sq. metres per Kg. bone)
Normal cortical	90-95	2	0.1
Normal cancellous	15-20	300	15
Osteoporotic cortical	30	4	0.2
Osteoporotic cancellous	6-10	600	30

available surface are expressed in terms of the amount of actual bone tissue present: cancellous bone is seen to provide approximately 150 times as much surface area as the same amount of cortical bone. It would be interesting to know the relative amounts of the available surface occupied by bone formation and bone destruction, and by inactive

surfaces, but little information is at present available on the subject. In Paget's disease and hyperparathyroidism, however, the available surface can be greatly increased and almost wholly occupied by formation and resorption: such an appearance is interpreted as indicating a very high rate of skeletal remodelling.

Bone turnover

The extent of involvement of bone surfaces by formation and resorption can give, at best, only a general indication of the activity of the structural remodelling of the tissue, but more precise information on this topic is becoming available through the use of certain vital bone 'markers' which outline the surfaces where bone formation is occurring. Such markers make it possible to measure, in histological preprarations, the actual volume of bone tissue formed during a given period (Sissons, 1960). In recent years, it has been found that tetracycline antibiotics behave in this way (Milch, Rall & Tobie, 1958; Harris, 1960; Frost, 1960; Frost, Villanueva & Roth, 1960) and useful information is accumulating from their use. The tetracycline remains fixed in the tissue and can be demonstrated in undecalcified sections by its fluorescence, as shown in Figs. 5 and 6, which are from material which my colleague Dr. Lee and I have studied at the Institute of Orthopaedics. In Fig. 5, the three fluorescent rings

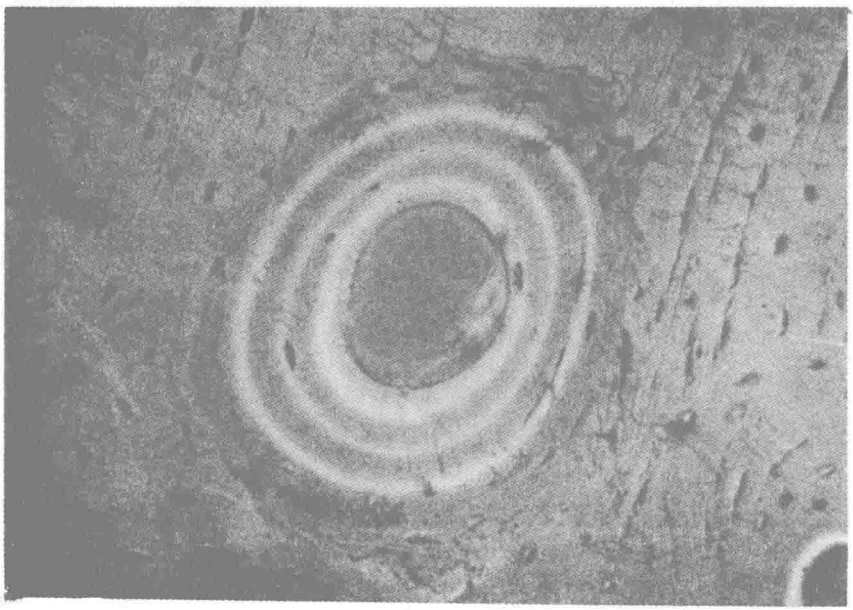


Figure 5. Male aged 37 years. Operation specimen following 3 doses of tetracycline at approximately monthly intervals. Undecalcified bone section showing 3 fluorescent tetracycline rings in a developing osteone.

indicate the situation of the growing surface at intervals of approximately one month. The rate at which bone is being deposited can be determined by measuring the distance between these rings; in a number of cases it has been found to be of the order of 1μ per day. In Fig.6, the complex pattern of marking that can be produced by more prolonged periods of therapeutic tetracycline administration is shown.

When two tetracycline markers are used, determination of the area of bone between them gives a measurement of the rate of bone formation for the part of the skeleton concerned. This is usually expressed as a percentage of the total amount of bone present. Values of about 0.01 to 0.02% per day have been obtained for normal adult cortical bone by ourselves and by other workers using this technique (Frost, 1960; Frost, Villanueva & Roth, 1960): much higher values are obtained in children. The average adult rate of bone formation (or of 'bone turnover' for a system where formation and resorption are balanced), if envisaged as being evenly and randomly distributed, would correspond to a mean life for the tissue of about 17 years. It must be emphasised, however, that bone does not appear to behave as a homogeneous

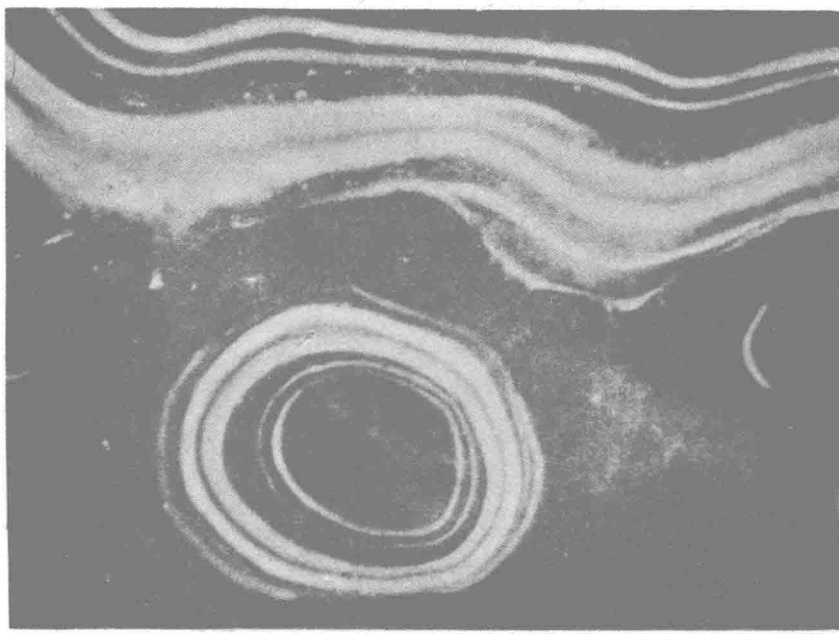


Figure 6. Female aged 43 years. Autopsy specimen in case of poliomyelitis with respiratory paralysis treated intermittently with tetracycline for approximately 12 months prior to death. Undecalcified bone section showing fluorescent bands corresponding to periods of tetracycline treatment.

tissue when studied in this way: different parts of the skeleton, and even different regions of a single bone, show considerable variation

in their rates of bone formation.

It will be of interest, as information accumulates, to see how tetracycline values for bone formation compare with those for calcium accretion as determined by tracer techniques.

Studies of bone mineral

In describing surfaces of bone formation (p.3) it was assumed that certain structures could be recognised, because of their relatively pale staining in haematoxylin and eosin sections of decalcified bone, as osteoid tissue. In carefully prepared and stained material such an assumption is probably justified (Meyer, 1956), but several less subjective procedures are available for the study of bone mineral and the precise identification of osteoid tissue. The use of these procedures is of particular importance in connection with the histological diagnosis of osteomalacia, which depends on the identification of abnormally large amounts of osteoid tissue. The most usually adopted method is to prepare microtome sections of undecalcified bone, and to identify the mineral material (hydroxyapatite) by its positive von Kossa reaction (Ball, 1963). Another

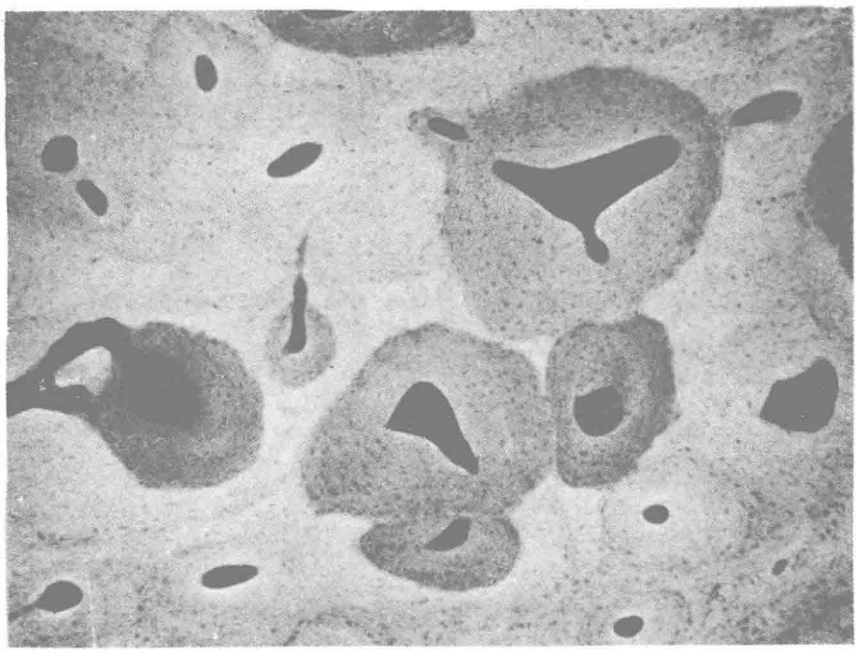


Figure 7. Male aged 33 years. Microradiograph of ground 'section' of normal bone (femoral cortex) showing osteones of varying degrees of mineralization.

technique, which is perhaps more of research than routine interest, is microradiography. This depends on the high X-ray absorption of bone mineral. Osteoid tissue does not absorb X-rays, and is consequently indistinguishable from soft tissue components in microradiographs.

Microradiography is usually applied to relatively thick 'sections' of bone, which have been prepared by grinding (Jowsey, 1955). When bone was first studied in this way (Engström & Amprino, 1950) it was evident that it was not uniformly mineralized, but that different areas of the tissue show different degrees of X-ray absorption (Fig. 7). Evidence has accumulated to show that lamellar bone undergoes a slow increase in mineralization following its formation. Osteones which are in the process of formation, or which have recently completed this stage of development, can be recognised by their low mineral content relative to that of older structures (Fig. 7). Inactive parts of the bone surface show a narrow zone of increased mineral density which appears to correspond with the line of intense haematoxylin staining observed in decalcified preparations. In addition, the comparison, after the experimental administration of bone-seeking radioactive isotopes such as ^{45}Ca, of microradiographs and autoradiographs of bone sections, has demonstrated that the relatively incompletely mineralized areas of bone are the sites of maximum isotope uptake ('hot spots') (Ponlot, 1959): this is because the mineral material they contain can exchange easily with the calcium of the body fluids, while that of the more completely mineralized bone is less readily available for such exchange.

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